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Mild increase in bodyweight due to short-term high fat diet feeding exerts an attenuating impact on the development of experimental allergic asthma in mice

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„Research is to see what everybody has seen and think what nobody has thought.“

Albert Szent-Gyorgi, 1937 Nobel Laureate

To my wife and children

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1 Summary

This dissertation was performed in the frame of the International Research Training Group 1911 “Immunoregulation of Inflammation in Allergy and Infection” as part of the Doctorial Degree Program “Biomedicine” at the university of Lübeck. It aimed at improving the understanding of the complex interactions between obesity and asthma, since accumulating research suggests a close association between these entities. However, the clear nature of this association, as well as the immune processes involved, remain elusive. Moreover, controversial findings exist regarding the impact of mildly increased bodyweight on the development of allergic asthma suggesting a distinct impact of overweight on allergic asthma.

The objective of this thesis was to establish a mouse model of diet-induced mild bodyweight increase associated with the beginning of a systemic inflammation and the subsequent induction of a pulmonary allergy. As key objectives were defined: i) the establishment of an experimental mouse model of high fat diet (HFD) induced overweight/obesity; ii) the dissection of the impact of feeding on allergic asthma outcome, such as airway inflammation, mucus production, and airway hyperresponsiveness (AHR); iii) the analysis of the impact of the feeding on the major asthma-relevant pulmonary immune cells, such as eosinophils, alveolar macrophages, neutrophils, dendritic cells (DCs), and T cells; iv) the evaluation of alteration of the crosstalk between DCs and T cells in this model; and v) the identification of possible changes in adipokines or complement system that may occur and be involve in this model.

Female C57BL/6JRJ mice were fed with a HFD or a chow diet (CD) for 12 weeks. Alternatively, an HFD feeding with higher levels of calories and different lipid sources was used. Allergic asthma was induced by intraperitoneal ovalbumin (OVA)/Alum sensitization followed by repeated OVA airway-challenges. As central asthma indicating parameters, AHR, airway and pulmonary recruitment of inflammatory cells, and mucus production were assessed. Histology, measurement of systemic metabolic parameters, and flow cytometric analyses of immune cells were performed. In addition, the immunological crosstalk of DCs and T cells was analyzed. To evaluate a possible role of leptin and the anaphylatoxin C5a receptor 1 (C5aR1) in this model, preliminary experiments addressing these issues were performed.

Mice fed with a HFD showed an increase in bodyweight associated with several metabolic alterations. In comparison to various reports about experimental obesity models, these metabolic alterations were consistently between what to expect in lean and obese mice, thus, proves this feeding model suitable to induce what is here called “mild increase” in

bodyweight mimicking a model for overweight. Upon OVA challenge, CD-fed mice developed AHR, airway inflammation, and mucus production, clearly demonstrating a strong allergic asthma induction. Furthermore, HFD mice also developed such allergic asthma, however, it was markedly reduced as compared to CD mice. HFD feeding alone without OVA challenges did not induce an asthma-like phenotype. Moreover, OVA-induced increases in pulmonary neutrophils, DCs, and $CD4^+$ T cells were significantly attenuated by HFD feeding. Importantly, MHCII and CD40 expressions in pulmonary $CD11b^+$ DCs were lower in HFD fed than in CD fed mice, which was associated with a decreased T helper (Th) 1/17 differentiation and regulatory T cell formation, but no change in Th2 differentiation. Preliminary results obtained from leptin receptor expression analyses in lung DCs and T cells, T cell differentiation experiments with leptin stimulation, C5aR1 receptor expression analysis in lungs and airway neutrophils, and use of C5aR1 deficient mice , do not point towards an involvement of these systems in this model.

Collectively, my findings suggest that mild bodyweight gain attenuates the development of allergic asthma through reducing pulmonary DC recruitment and MHCII/CD40 expression leading to a diminished Th1/17 but unchanged Th2 differentiation. In conclusion, this dissertation provides new evidences suggesting that multiple metabolic and immunological pathways are cross-talking during the development of obesity-associated asthma and that, in contrast to obesity, mild bodyweight gain may protect from allergic asthma development. As overweight and asthma prevalence increase worldwide, we face a clear medical need to elucidate the impact of overweight on the early development of allergic asthma. Future studies should include a direct comparison of lean, overweight and obese conditions on the development of allergic asthma.

2 Zusammenfassung

Diese Dissertation wurde im Rahmen der *International Research Training Group 1911 "Immunoregulation of Inflammation in Allergy and Infection"* als Teil des Promotionsstudienganges „Biomedizin" an der Universität zu Lübeck durchgeführt. Das übergeordnete Ziel dieser Arbeit ist es, das Verständnis über den Zusammenhang von Adipositas und Asthma zu verbessern, da zunehmende Daten auf eine enge Assoziation dieser beiden Erkrankungen hinweisen. Dennoch ist weiterhin unklar, wie genau diese beiden Erkrankungen zusammenhängen und welche immunologischen Vorgänge dabei beteiligt sind. Zusätzlich existieren widersprüchliche Daten, die andeuten, dass eine leichte Gewichtszunahme einen differentiellen Einfluss auf das allergische Asthma haben könnte und dieses Zusammenspiel andere Mechanismen beinhaltet.

Die Zielsetzung dieser experimentellen Arbeit war es, ein Mausmodel einer Hochfettdiät-induzierten Gewichtszunahme mit beginnenden systemischen metabolischen Veränderungen zu entwickeln und die Einflüsse auf die Entwicklung einer pulmonalen Allergie zu untersuchen. Als Schlüsselschritte wurden definiert: I. Entwicklung eines experimentellen Mausmodells einer Hochfettdiät-induzierten Gewichtszunahme; II. die Untersuchung der Einflüsse auf Asthma-Schlüsselmerkmale wie Atemwegsentzündung, Mukusproduktion und bronchiale Hyperreagibilität; III. die Analyse der zentralen Asthma-relevanten Immunzellen wie Eosinophile, Makrophagen, Neutrophile, dendritische Zellen und T Zellen; IV. die genauere Aufgliederung der Interaktion von dendritischen Zellen und T Zellen; und V. Hinweise zu überprüfen, ob Adipokine oder Komponenten des Komplementsystems in diesem Model eine relevante Rolle spielen könnten.

Weibliche C57BL/6JRJ Mäuse wurden für 12 Wochen mit einer Hochfettdiät oder einer Kontrolldiät gefüttert. Eine alternative Hochfettdiät wurde ebenfalls angewendet, die mehr Kalorien und andere Fettquellen aufwies. Ein allergisches Asthma wurde mit intraperitonealer Applikation von Ovalbumin (OVA) und einem Aluminium-haltigen Adjuvans gefolgt von intratrachealen OVA-Gaben induziert. Als zentrale Asthma-Schlüsselmerkmale wurden Atemwegsentzündung, Mukusproduktion und bronchiale Hyperreagibilität analysiert. Histologische Untersuchungen, Analysen systemischer metabolischer Parameter und Durchflusszytometrie von Immunzellen wurden durchgeführt. Zusätzlich wurde die Interaktion von dendritischen Zellen und T Zellen genauer analysiert und eine mögliche Beteiligung von Leptin oder dem Komplementrezeptor C5aR1 untersucht.

Die Hochfettdiät-gefütterten Mäuse wiesen eine leichte Gewichtszunahme gegenüber den Kontrolldiät-gefütterten Mäusen auf. Die Gewichtszunahme sowie damit einhergehenden metabolischen Veränderung waren konsistent zwischen den Erwartungen von schlanken und

adipösen Mäusen. Daher wurden diese Hochfettdiät-induzierten Veränderungen im Kontext dieser Arbeit zusammengefasst als „milde Gewichtszunahme“ bezeichnet sowie als experimenteller Prototyp für Übergewicht verstanden. Nach OVA-Behandlung entwickelten die Kontrolldiät-gefütterten Mäuse eine Zunahme der Atemwegsentzündung, Mukusproduktion und bronchiale Hyperreagibilität und wiesen damit eine Entwicklung eines schweren allergischen Asthmas auf. Hochfettdiätfütterung allein war nicht in der Lage, einen Asthma-ähnlichen Phänotypen zu induzieren. In Kombination mit einer OVA-Behandlung konnte jedoch ebenfalls eine Zunahme der Atemwegsentzündung, Mukusproduktion und bronchiale Hyperreagibilität festgestellt werden, die Ausprägung dieser Asthma-Schlüsselmerkmale war jedoch deutlich geringer ausgeprägt als bei den asthmatischen Kontrolldiät-gefütterten Mäusen. Im Speziellen war die Asthma-assoziierte pulmonale Rekrutierung von Neutrophilen, dendritischen Zellen und $CD4^+$ T Zellen geringer nach der Hochfettdiätfütterung. Von besonderer Bedeutung war, dass die Expression von MHCII und CD40 auf konventionellen $CD11b^+$ dendritischen Zellen in Hochfettdiät-gefütterten Mäusen geringer war als in Kontrolldiät-gefütterten Mäusen; dies war zudem mit einer geringeren Th1/Th17 T-Helferzell-Differenzierung und Bildung regulatorischer T Zellen, jedoch keiner veränderten Th2-Differenzierung, vergesellschaftet. Erste Ergebnisse nach Analyse der Leptinrezeptorexpression in pulmonalen dendritischen Zellen und T Zellen, T-Helferzell-Differenzierungsexperimenten mit Leptinstimulation, Analyse von der C5aR1-Expression in pulmonalen und Atemwegsneutrophilen sowie der Verwendung von C5aR1-Knockout-Mäusen ergaben keine Hinweise, dass diese biologischen Systeme in diesem Modell involviert sind.

Zusammengefasst ergaben die Resultate dieser Arbeit deutliche Hinweise darauf, dass eine milde Gewichtszunahme die Entwicklung eines allergischen Asthmas abschwächen kann und dass dafür eine veränderte Interaktion von dendritischen Zellen und T Zellen via MHCII und CD40 verantwortlich ist, die dann zu einer verminderten Th1/Th17- aber unveränderten Th2-Differenzierung führt. Schlussfolgernd liefert diese Dissertation neue Daten für das Forschungsfeld Adipositas-assoziiertes Asthma und zudem wissenschaftliche Belege dafür, dass es verschiedene metabolische und immunologische Phänotypen geben könnte, die wiederum differentiell die Entwicklung eines allergischen Asthmas beeinflussen. Ein leichtes Übergewicht könnte, im Gegensatz zu Adipositas, einen protektiven Einfluss auf die Entwicklung eines allergischen Asthmas haben. Aufgrund der weltweit stetig zunehmenden Prävalenz von Übergewicht und Asthma, liegt eine klare medizinische Notwendigkeit vor, mehr über die klinischen und immunologischen Zusammenhänge zu erforschen. Zukünftige Studien sollten einen direkten Vergleich von Normalgewicht, Übergewicht und Adipositas einbeziehen.

3 Introduction

The basic aim of this dissertation was to improve the understanding of the complex interactions between obesity and asthma as accumulating research points towards a close connection. This dissertation was performed within the International Research Training Group 1911 “Immunoregulation of Inflammation in Allergy and Infection” as part of the Doctoral Degree Program “Biomedicine” at the university of Lübeck. However, given my background in medicine with, to date, more than 10 years of experience in clinical work, this work was also characterized by a clinician’s perspective and motivation, although it mainly focused on molecular and cellular aspects of the disease. Nowadays, a whole body of research focuses on obesity and its impact on non-allergic asthma. However, some research suggests a distinct impact of overweight on allergic asthma, hence, this was chosen for the closer angle of this dissertation.

To introduce the overarching topics and the relevant background information, firstly, I will introduce obesity including adipokines as the possible link between metabolism and immunity. Then I will introduce asthma, especially allergic asthma with its relevant clinical features and immune mechanisms. Furthermore, I will present the currently dominating view on obesity-associated asthma, and the data supporting shared and complementary pathological mechanisms of obesity and asthma. Lastly, I will introduce the complement system as an additional possible link between metabolism and asthma and the role possibly taken by the C5aR1.

3.1 Obesity

3.1.1 Obesity prevalence

Obesity is tremendously increasing all over the world (Blüher, 2019; WHO, 2018). As an example, in the U.S. the prognosis is that half of the American adult population will be obese in 2030 (Finkelstein et al., 2012; Malik et al., 2013). Besides, between the 70’s and today, in every country obesity prevalence has risen (NCD Risk Factor Collaboration (NCD-RisC), 2017), consequently, obesity cannot merely be viewed as a problem of the “western world” but rather has to be appreciated as a global health care burden, with health care consequences of overnutrition being more influential than the consequences of undernutrition (WHO, 2018). Although a global problem, studies revealed tremendous regional differences, for instance in Southeast Asia, the Caribbean, and Latin America a profound increase in bodyweight happened, whereas almost no change in bodyweight was noted in Eastern Europe (NCD Risk Factor Collaboration (NCD-RisC), 2017).

Although the rise in obesity prevalence started in countries of higher economic wealth, such as the U.S., Australia, and Western Europe, it was later followed by middle-income and then low-income countries (OECD, 2017; Swinburn et al., 2011). This pattern clearly suggests that improvements in economy and wealth at a population level are a driving force behind obesity. In contrast, in the last decade, childhood obesity appeared to decrease in some high-income countries, such as the Scandinavian countries, Australia, Japan, and the U.S. (NCD Risk Factor Collaboration (NCD-RisC), 2016; Rokholm et al., 2010). This plateau effect in affluent countries could be explained by the hypothesis that a decrease in economic disparities may lower the heterogeneity in obesity prevalence (Sargent, 2009). However, the worldwide large heterogeneity in obesity prevalence may not only reflect economic differences but also ethnic, social, environmental, and other kinds of differences as well as differences in exposure to obesogenic foods (NCD Risk Factor Collaboration (NCD-RisC), 2016). Of note, even within countries, a high regional variability in obesity prevalence can exist; e.g. in Germany, the prevalence ranges from around 20% in cities in the northwest up to over 28% in Saxony-Anhalt (Blüher, 2019; Hauner et al., 2008).

Bottom line is that the constant and tremendous increase in obesity prevalence underlines the overall medical significance and that so far no country has succeeded in reversing this trend and successfully combating obesity (Blüher, 2019; Roberto et al., 2015).

3.1.2 Obesity pathogenesis

Obesity is characterized by an excessive accumulation of body fat (Hill et al., 2012). This is mainly a consequence of a long-term imbalance of energy intake and energy expenditure, meaning a situation in which too many calories are taken up in relation to the calories burned. From an evolutionary standpoint, humans had to frequently face periods of uncertain food supply, which may have contributed to a genotype favoring overeating, saving energy expenditure, and a propensity for physical inactivity (Blüher, 2019). This may have led to an overrepresentation of genetic variants promoting highly efficient calorie extraction from foods as well as efficient energy storage in adipose tissue rather than fat oxidation. Indeed, data from twin studies (Börjeson, 1976; Stunkard et al., 1990), familial clustering of BMI (Reilly et al., 2005), and the identification of congenital forms of obesity (Farooqi et al., 1999) suggested that there is a genetic susceptibility to develop obesity. In contrast, more recent studies, encompassing gene-environment-metabolism interactions have reported opposing data (Rothschild et al., 2018; Weissbrod et al., 2018). In addition, the rapid increase in the prevalence of obesity itself (NCD Risk Factor Collaboration (NCD-RisC), 2017; Yanovski, 2018) and studies about genetically related populations living in different environments (Hsueh et al., 2018; Schulz et al., 2006) suggested that obesity is rather a product of the

environment and an inappropriate lifestyle. In line, obesity increased in the last decades, together with major changes in lifestyle, such as a reduction in home cooking, industrial production of convenience foods, sedentary activity at work and recreation, and a growing habit of snacking of calorie-dense foods (Blüher, 2019; Swinburn et al., 2011). This so called "Westernization of lifestyle" comes along with the postulate that an energy flipping point was reached during the 1960's (Blüher, 2019). This time period saw major changes in food supply and increased the accessibility and cost effectiveness of energy-dense refined carbohydrates and fats due to technical revolution (Carden and Carr, 2013). As a consequence, the increased and easier availability of energy-dense foods led to an enormous increase in overall calorie intake. After this energy flipping point, the weight gain phase began, and resulted in the worldwide obesity pandemic of today (McGinnis and Nestle, 1989). Finally, the major contribution of the microbiome to the obesity pandemic is intensively scrutinized and may provide an explanation of the high variability of obesity within a population (Korem et al., 2017; Mendes-Soares et al., 2019; Valdes et al., 2018; Zeevi et al., 2015)

3.1.3 Obesity as a risk factor

Obesity is the major contributor to non-communicable diseases, which are associated with an estimated decline in life expectancies of 5-20 years (Fontaine et al., 2003; Prospective Studies Collaboration et al., 2009). More than 70% of early deaths are attributed to obesity and associated complications, making obesity the number one cause of premature disability and death (Blüher, 2019; WHO, 2017). Obesity profoundly increases the risk of metabolic diseases, such as type 2 diabetes and fatty liver disease, cardiovascular complications like myocardial infarction and stroke, musculoskeletal diseases, such as osteoarthritis and joint trouble, psychoneurological disease like Alzheimer's and depression, and some types of cancer like breast and colon cancer (Blüher, 2019). Furthermore, it is increasingly reported that obesity impacts on allergic diseases such as asthma (Bach, 2002; Julia et al., 2015).

3.1.4 Systemic and adipose tissue alterations in obesity

The excessive accumulation of body fat is the central feature of the obesity pathogenesis (Hill et al., 2012). However, a chronic low-grade inflammation in the adipose tissue, in particular the visceral adipose tissue (VAT), is known to be a main driver of obesity-associated sequelae (Mathis, 2013). Furthermore, immune cells of innate immune system accumulate in VAT, in particular macrophages increase along the with gain of bodyweight regulating the local and systemic metabolic homeostasis (Osborn and Olefsky, 2012). More specifically, it was demonstrated that in lean and metabolically healthy state adipose tissues,

macrophages exert anti-inflammatory properties, while in obese and metabolically unhealthy state macrophages of the classically activated M1 subset are primed to exert pro-inflammatory functions (Chawla et al., 2011). This formation of this obesity-associated subset is driven by lipopolysaccharide or IFNγ, express CD11c, and produce pro-inflammatory mediators like Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-6, and IL-1β (Mathis, 2013).

Interestingly, in humans, high accumulation of adipose tissue M1 macrophages is a typical finding in “metabolic unhealthy” obesity (Antonopoulos and Tousoulis, 2017), which is the obesity type with highest risk for medical complications, such as insulin resistance, diabetes mellitus, cardiovascular complications, and even cancer (Dobson et al., 2016; Eckel et al., 2016; Engin, 2017). Accumulation of ectopic fat in liver tissue is an additional finding in “metabolic unhealthy” obesity (Antonopoulos and Tousoulis, 2017). The higher the lipid accumulation the higher the hepatic insulin resistance defining the progress to systemic insulin resistance (Antonopoulos and Tousoulis, 2017). Next to insulin resistance, increases in serum lipids, such as cholesterol and triglycerides, are also characterizing the clinical picture of “metabolic unhealthy” obesity (Gilardini et al., 2018; Saklayen, 2018).

In addition, this clinical picture of “metabolic unhealthy” obesity involves a change in adipokines. Such molecules participate to a molecular system in which the adipose tissue impacts on the whole body and may mediate negative metabolic effects (Fasshauer and Blüher, 2015). The two major adipokines are leptin and adiponectin (Fasshauer and Blüher, 2015). Leptin is increasingly secreted in the obese state and promotes the secretion of IL-6 and TNF-α by adipocytes, triggering the differentiation of circulating $CD4^+$ lymphocytes into a Th1 phenotype characterized by IL-2, IFNγ and TNF-α secretion (Ali and Ulrik, 2013; Martín-Romero et al., 2000). Furthermore, the fact that leptin can activate eosinophils and decrease eosinophil apoptosis (Leiria et al., 2015a), led to the speculation that leptin contributes to a relocation of adipose tissue eosinophils to the lung parenchyma in terms of obesity (Lloyd and Saglani, 2013).

In contrast to leptin, adiponectin, which blood levels are inversely correlated with BMI, exerts anti-inflammatory effects, mainly the inhibition of IL-6 and TNF-α signaling and induction of IL-10 and IL-1 receptor antagonist (Ali and Ulrik, 2013).

Of interest, the two major adipokines, namely leptin and adiponectin seem to act in an opposite manner in asthma; with leptin acting as an asthma-aggravator (Bruno et al., 2009; Loffreda et al., 1998; Procaccini et al., 2012a; Wong et al., 2007), whereas adiponectin improves the allergic asthma phenotype (Medoff et al., 2009; Shore et al., 2006; Yamamoto et al., 2013). Furthermore, leptin and changes in bronchial epithelial cell leptin receptor expression have been implicated with the development and the severity of asthma and increased allergen-associated AHR and airway inflammation (Shore et al., 2005). In contrast,

adiponectin attenuates allergen-induced AHR, while adiponectin-receptor deficiency promotes airway eosinophilia (Medoff et al., 2009; Shore et al., 2006). Finally, a possible role for adiponectin in asthma pathology is additionally supported by the clinical association of low adiponectin levels of asthmatics in population studies (Nagel et al., 2009).

3.2 Asthma

Asthma is clinically characterized by attacks of breathlessness, wheezing, and cough (Locksley, 2010), caused by a chronic inflammation of the airways (Lambrecht and Hammad, 2015). Asthma is associated with high disease burden as well as high mortality (Masoli et al., 2004). The burden of asthma is especially high in children with 10-14 years of age and in the elderly with 75-79 years of age (The Global Asthma Network, 2018). Furthermore, asthma is associated with high socioeconomic burden as related direct and indirect health care costs reach more than 80 billion dollar in the U.S. (Nunes et al., 2017; Nurmagambetov et al., 2018). Furthermore, asthma prevalence is increasing worldwide, in particular in developed western countries, reaching nearly 340 million people affected in 2018 (The Global Asthma Network, 2018). Due to these numbers, asthma is considered as one of the most prevalent diseases of the world with 15-20% prevalence in many countries (Masoli et al., 2004; Sears, 2014).

The core pathological feature of asthma is a constant inflammation of the airways leading to mucus hypersecretion, airway remodeling, airway narrowing and hyperresponsiveness (AHR). Collectively, these features induce repeated periods of coughing, shortness of breath, wheezing and chest tightness (Lambrecht and Hammad, 2015; Locksley, 2010). Asthma is a chronic, thus, not curable disease which central immunopathological aspect is a dysregulation of pulmonary T helper (Th) 2/Th17 immunity (European Academy of Allergy and Clinical Immunology, 2013; Pelaia et al., 2012).

As first line of symptomatic therapy, inhalable short- and long-acting $ß_2$-adrenergic agonists are used (Bundesärztekammer (BÄK) et al., 2018) to dilate the airways, allowing a good disease control in the majority of patients. In addition, local or systemic corticosteroids administration is used in order to unspecifically suppress inflammation (Bundesärztekammer (BÄK) et al., 2018). However, 5-10% of patients remain symptomatic and are then considered steroid-resistant, a condition which is frequently related to increased disease severity and higher hospitalization rate (Ciepiela et al., 2015; Pelaia et al., 2012). Since the course of the disease upon steroid-resistance is aggravated and the knowledge about its causes and underlying pathological processes is still limited, new biological and targeted treatments are needed to mitigate the symptoms and disability in these patients (Pelaia et al., 2012).

3.2.1 Allergic versus non-allergic asthma

The traditional conception is that two different subtypes of asthma can be distinguished, an allergic (extrinsic, atopic) and a non-allergic (intrinsic, non-atopic) forms.

Allergic asthma is primarily dominated by allergen-associated immunoglobulin E (IgE) reactivity and a maladaptive Th2 immune response (Passalacqua and Ciprandi, 2008). The cause of atopy, is the sensitization to harmless environmental antigens, such as feces of house dust mite, allergenic molecules covering animal dander, fungal spores, pollen, or peanuts in susceptible individuals. The nature of this allergic sensitization can be verified using skin-prick test to the proteins of common allergens and increased serum levels of IgE (Bundesärztekammer (BÄK) et al., 2018). Furthermore, this sensitization often happens during childhood and is often associated with eczema and comorbid allergic rhinitis (Lambrecht and Hammad, 2015). Therefore, a basic clinical rule is: the more allergies a person has, the higher the risk to develop allergic asthma later on (Simpson et al., 2010).

In contrast, non-allergic asthma, frequently accompanied by chronic rhinosinusitis, nasal polyposis, or obesity, is more prevalent in steroid-resistant patients (Lambrecht and Hammad, 2015). Furthermore, it is not considered to be primarily induced by an allergen or involved maladaptive type 2 immunity (DeKruyff et al., 2014), but is rather associated with physical activity-induced exhaustion, cold air, or air pollutants. Among these pollutants, diesel exhaust particles are well known to induce and exacerbate asthma (Saxon and Diaz-Sanchez, 2005). Other environmental triggers, such as ozone, acting as a reactive oxidant chemicals, and active as well as passive smoking increases the risk of respiratory diseases (Larsson, 1995; Saxon and Diaz-Sanchez, 2005; Withers et al., 1998). Such non-allergic asthma usually occurs in adulthood and is often considered to primarily affect postmenopausal women (DeKruyff et al., 2014). In addition, non-allergic asthma is usually the one subtype, considered to be associated with obesity (Julia et al., 2015). While obesity and asthma are both increasingly prevalent in modern societies (Blüher, 2019; The Global Asthma Network, 2018; WHO, 2018), it appears, in addition, that both entities potentially share pathological factors or may potentiate each other (Ali and Ulrik, 2013; Ford, 2005).

Nevertheless, clinical features, such as family history of atopy or asthma, home tobacco smoke exposure, age, gender, bodyweight, or severity of symptoms, alone are often not sufficient to clearly define whether asthma is allergic or non-allergic (Sinisgalli et al., 2012).

3.2.2 Asthma – concept of phenotypes and endotypes

During the last years, the traditional distinction between allergic and non-allergic asthma has been challenged, and asthma is now increasingly recognized to be a syndrome with several distinct asthma subtypes, which can be characterized by different immunopathologies, genetic associations, clinical presentation, age, and environmental risk factors (Amelink et al., 2013; Haldar et al., 2008; Kim et al., 2016; Lambrecht and Hammad, 2015; Ozdemir et al., 2018; Schleich et al., 2014; Wenzel, 2012).

Originally, all asthmatics were believed to be allergic and driven by a marked Th2 maladaptive response, characterized by a strong eosinophilia in the airways. However, clinical trials revealed that subgroups of patients exist, e.g. with a severe asthma or an asthma without typical eosinophilia (Wenzel, 2012). Moreover, clinical studies showed efficacy of antibody-therapies against the allergy-related factor IgE in only a subgroup of patients (Busse et al., 2001; Solèr et al., 2001; Wenzel, 2012). As a consequence, the term “allergic asthma” began to evolve to specifically describe the patients of this subgroup, which concomitantly led to the definition of several other subtypes (Wenzel, 2012). Herewith, the concept of different asthma phenotypes and endotypes was introduced (Wenzel, 2012). Thus, a phenotype will describe the “observable properties of an organism that are produced by the interactions of the genotype and the environment” (Anderson, 2008; Lötvall et al., 2011; Wenzel, 2012), and an endotype will be defined by a specific biological pathway explaining the properties of the phenotype (Anderson, 2008; Lötvall et al., 2011; Wenzel, 2012). The effort to structure asthma and to clearly define phenotypes and endotypes have led to a much more elaborate but also more complex definition of asthma as an “umbrella term” (Howard et al., 2015; Kuruvilla et al., 2019). Clinical features, such as measures of allergy, lung functions, typical inducers of exacerbations, response to asthma medication, and airway inflammation can be used to define asthma phenotypes (Howard et al., 2015). Furthermore, immunological features allow the distinction of asthma endotypes into Th2-high/dominant asthma or just Th2-asthma and Th2-low asthma or non-Th2-asthma (Kuruvilla et al., 2019; Wenzel, 2012). Moreover, the Th2-asthma would be further divided into atopic, late-onset, and aspirin-exacerbated respiratory disease, while the non-Th2-asthma would be divided into non-atopic, smoking-related, obesity-related, and non-Th2 in elderly patients (Kuruvilla et al., 2019; Wenzel, 2012). Although this concept of Th2- and non-Th2-asthma closely corresponds to the traditional concept of allergic versus non-allergic asthma, one can expect, that in future “-omics” research approaches, such as metabolomics, proteomics and the like, huge amounts of data will be collected, which will help to further unravel the complex pathologies collectively called “asthma” (Kuruvilla et al., 2019).

3.2.3 The many causes leading to allergic asthma

Allergy (atopy) triggers multiple disease conditions such as atopic skin disease, conjunctivitis, rhinitis, food allergies, and asthma. The general characteristics of allergic asthma are reversible airway hyperresponsiveness (AHR), airway inflammation, elevated serum IgE levels, increased eosinophil counts, mucus cell hyperplasia, and increased mucus production (Locksley, 2010; Possa et al., 2013; Schatz and Rosenwasser, 2014). The clinical picture is dominated by cough, wheezing, shortness of breath, and chest tightness (Lambrecht and Hammad, 2015), with symptoms usually occurring as a direct response to contact with the inducing allergen in form of an attack. During the chronic course of asthma, a subepithelial fibrosis and smooth muscle thickening develops as a consequence of recurrent asthma attacks and persistent airway inflammation.

However, the clinical picture of allergic asthma is heterogeneous and the central features AHR, inflammation, and mucus production may be differently present (Amelink et al., 2013; Haldar et al., 2008; Schleich et al., 2014; Wenzel, 2012). This can be a consequence of different disease shapes and various involvements of environmental factors (Lloyd and Saglani, 2010). The concept is that, in addition to a genetically induced susceptibility, allergen exposure as well as environmental factors shape and determine the disease onset and progression (Wills-Karp et al., 2001).

Genetics studies have led to the identification of several susceptibility genes for asthma development. The most robust candidate genes are involved in innate immunity and immunoregulation, T cell differentiation and activation, epithelial and mucosal immunity, and lung function (Vercelli, 2008). To name some prominent examples, polymorphisms in the gene encoding for Toll-like receptor (TLR) 4 as part of innate immunity, in genes of encoding for GATA3, IL4, or IL13, which are important for Th2 differentiation and function, and in genes, which are associated with epithelial cell function and airway remodeling, such as defensin beta, TNF-α and transforming growth factor (TGF) beta 1, have been implicated with the development and the course of allergic asthma (Kabesch et al., 2003; Levy et al., 2005; Moffatt and Cookson, 1997; Pykäläinen et al., 2005; Silverman et al., 2004; Vercelli, 2008; Vladich et al., 2005; Werner et al., 2003).

Despite the identification of such susceptibility genes, environmental factors affect strongly the development and severity of allergic asthma. These factors may alone lead to the development of asthma or impact on the course of the disease through interaction with genetically induced factors. For instance, the presence of typically involved allergens like feces from house dust mite, grass and tree pollens, volatile food components, e.g. from peanuts, or animal dander is often non-determinable and influenced by many factors, such as weather, season, or habits of other people. In addition, environmental factors such as

exposure to infectious agents in early childhood, age, gender, passive exposure to smoking in childhood, nutritional status, rural versus urban living, nutritional status and possibly many others more have to be mentioned (Di Cicco et al., 2020; Kurukulaaratchy et al., 2006). Therefore, environmental factors are more suitable to explain the huge variability of allergic asthma.

The so-called hygiene hypothesis has been proposed to explain how the environment, in particular the environmental changes due to industrialization in modern “western” societies, impact the development of asthma (Wills-Karp et al., 2001). The central idea of the hygiene hypothesis is that low hygienic environmental standards, which favors frequent infections in early in childhood, lead to a better protection against the development of atopy and allergic asthma (Eder and von Mutius, 2004; Strachan, 1989). However, it is more and more appreciated that the central aspect is rather not the lack of contact to microorganism *per se* but the lower diversity of microorganisms the immune systems has to deal with (Ardura-Garcia et al., 2018; Scudellari, 2017). In addition, the microbiome comprising a great diversity of commensal bacteria is known to be reduced in the “western” world and also involved in asthma pathology, which indicates that exposure to bacteria has an impact on allergic asthma (Bello et al., 2018; Milani et al., 2017; Scudellari, 2017; Segata, 2015). Finally, other environmental factors such as diet and especially an inappropriate hypercaloric diet have been described as factors of influence (Julia et al., 2015).

Next to core genetical and environmental influences, prenatal factors may also impact on the risk to develop allergic asthma. Smoking, stress, nutrition with highly-processed foods, and antibiotic treatments during pregnancy, as well as birth modus (cesarean section versus “natural” childbirth) may affect the early development of allergic asthma (Douros et al., 2017; Lapin et al., 2015; Loewen et al., 2018; Miller, 2008; Thavagnanam et al., 2008; Zacharasiewicz, 2016).

Taken together, there is nothing such as “one route to asthma”, but various factors exist which are involved in shaping the individual’s risk for allergic asthma and the course of disease.

3.2.4 Obesity-associated asthma

Since the turn of the millennium, data were reported about a close connection between obesity and asthma. In this regard, in the late 1990s, several publications first reported about obesity and asthma, in particular in a health survey in Canada from 1994-1995 the prevalence of asthma was increased in obese women (Chen et al., 1999), while Camargo et al. reported data from over 60.000 women, showing a positive correlation between increasing

BMI and an increased relative risk of asthma (Camargo et al., 1999). Moreover, these reports were followed by observations that among asthmatic children living in an urban environment, the obese children reported more wheezing and took more medication than their lean counterparts (Belamarich et al., 2000; Dixon and Peters, 2018; Flaherman and Rutherford, 2006). Furthermore, irrespective to confirmed asthma diagnosis, obesity was associated with asthma symptoms such as wheezing in the National Study of Health and Growth in the UK report (Belamarich et al., 2000; Figueroa-Muñoz et al., 2001). Over the last two decades, additional reports evidenced a positive correlation between obesity and asthma diagnosis or asthma disease severity (Akerman et al., 2004; Beuther and Sutherland, 2007; Capelo et al., 2015; Ekström et al., 2018; Flaherman and Rutherford, 2006; Luthe et al., 2018; Papoutsakis et al., 2013; Pinedo et al., 2009; Sybilski et al., 2015; Van Veen et al., 2008). In line, weight loss was reported to decrease asthma disease burden (Beuther and Sutherland, 2007; Camargo et al., 1999; Koyuncuoğlu Güngör, 2014; Sideleva et al., 2013; Vijayakanthi et al., 2016).

However, several epidemiological studies revealed contradictory data, for instance some studies reported that increasing BMI was associated with decreased AHR (Kwon et al., 2012; Schachter et al., 2001). Similarly, no correlation between bodyweight and asthma as well as asthma read-outs were found at all (Bustos et al., 2005; D et al., 2009; Kwon et al., 2012; Sutherland et al., 2009; Toennesen et al., 2018). Some authors try to explain this heterogeneity of epidemiological data in humans, by the fact that in many studies, asthma was defined based on study participant's self-reporting of asthma-typical symptoms or an impact was analyzed on surrogate parameters of asthma, such as wheezing or the use of medication (Kwon et al., 2012; Schachter et al., 2001, 2003; Telenga et al., 2012). In addition, some epidemiological studies provided deeper understanding to this uncertainty as it was increasingly recognized that among the several asthma phenotypes (Amelink et al., 2013; Wenzel, 2012), obesity predominantly increased the risk of non-atopic asthma but not for allergic asthma (Ali and Ulrik, 2013; Chen et al., 2006; Haldar et al., 2008; Holguin et al., 2011; Kelley et al., 2005; Miethe et al., 2018; Sutherland et al., 2008; Visness et al., 2010).

In contrast to obesity, studies analyzing the impact of overweight on asthma are seldom. Interestingly, although still a matter of debate, some studies support overweight as a possible protective factor with respect to all-cause mortality (Flegal et al., 2013; Wang et al., 2016), in particular in chronic obstructive pulmonary disease, which shares pathological aspects with asthma (Galesanu et al., 2014). However, the majority of studies dealing with the impact of bodyweight increase on asthma development do not distinguish between overweight and obesity, e.g. in studies with children usually developing allergic asthma (Barlow and Expert Committee, 2007). As a consequence, the effect of overweight in comparison to normal weight or obesity on the development of allergic asthma is fairly unknown. Yet, this is most

relevant for children frequently born into an obesogenic environment and later on developing allergic asthma (Matsui, 2014).

Taken together, a huge body of epidemiological data exists showing that obesity and asthma, understood as an umbrella term for all asthma phenotypes, are somehow related. Furthermore, accumulating data suggest that obesity increases the risk and severity of non-atopic asthma phenotype, whereas its effect on allergic asthma remains elusive. In addition, some data indicate that overweight as opposed to obesity may exert a differential impact.

3.3 Immune regulation of allergic asthma

The general view is that allergic asthma develops as a consequence of an inappropriate Th2/Th17-adaptive immune response toward normally harmless environmental substances ("non-self proteins") in genetically susceptible individuals (Laumonnier et al., 2017). Importantly, allergic asthma immunopathology comprises the two parts of the immune system, i.e. the innate and adaptive immune systems, and should be distinguished into a sensitization phase and an effector phase.

3.3.1 Immune system

The immune system is a network of mucosal surfaces, immune cells, humoral factors, and lymphoid tissues, defending the host's integrity and protecting him from invading pathogenic microorganisms but also from harmful environmental influences (Murphy et al., 2008). The physical barrier of epithelia and mucosa establishes the first line of defense, supported by glands and their protective secretions including bacteriolytic lysozyme, mucociliary clearance mechanisms, low stomach pH and a huge variety of antimicrobial enzymes and proteins, such as lysozyme, pepsin, α- and ß-defensins, and pulmonary surfactant (Murphy et al., 2008). If these mechanisms are not sufficient to protect the host, more specific immune responses are involved and organized by an innate and an adaptive immune systems. While the innate immunity ensure a quick but rather unspecific response, the adaptive immunity, comprising T and B cell responses, provides a more pathogen-specific, together a specific memory function. Importantly, many features of the adaptive immunity are partly shaped by the innate immune system (Pancer and Cooper, 2006; Rettig et al., 2015; Shishido et al., 2012; Turvey and Broide, 2010).

3.3.2 Innate immunity

The innate immune system was evolutionary the first to evolve and is present in all multicellular organisms (Pancer and Cooper, 2006). Once a pathogen overcomes the physical barrier of epithelia and mucosa, humoral and cellular components of the innate immune system provide a quick and strong response (Rettig et al., 2015; Shishido et al., 2012).

3.3.2.1 Humoral innate immune system

The humoral innate immune system is a wide range of soluble factors aimed at recognizing, labeling and/or destroying dangers. At first, the coagulation system acts as a non-cellular part of the innate immune system, by predominantly protecting the vascular system from injury and invading pathogens and maintaining the vascular system's integrity (Rettig et al., 2015). So far, two coagulation pathways can be discriminated, the intrinsic and extrinsic coagulation system, both joining into the common pathway. The final result of these signaling cascades is the formation of the fibrin clot (Johari and Loke, 2012). An injured endothelial surface activates the intrinsic pathway via Factor XII, then leading to the activation of Factors XIIa, XI, IX, VIII, and X. Damage to the vascular system may also expose the Tissue Factor, which initiates the extrinsic pathway, then leading to the activation of Factors VIIa, IX, and X. The collective activation of Factor X starts the common pathway ending up in activating thrombin, cleavage of fibrinogen and forming the fibrin clot (Rettig et al., 2015). In parallel, another more important system, the complement system, plays a central role in supporting identification and removal of the external and internal dangers. Finally, a third line of the non-cellular part of the innate immune system are natural, polyreactive antibodies (Rettig et al., 2015). These natural antibodies are structurally highly flexible due to recombinations of gene segments coding the variable region of the antibodies. This allows binding to a wide range of microbial surfaces and self-directed antibodies. As such, natural antibodies are considered to constitute a basal level of immunity (Panda and Ding, 2015; Zhou et al., 2007).

3.3.2.2 Cellular innate immune system

Besides these non-cellular components, immune cells of the innates immune system are needed to facilitate the purpose of defense (Turvey and Broide, 2010). Prototypical innate immune cells are macrophages, eosinophils, neutrophils and dendritic cells (DCs), which all induce and regulate downstream inflammatory effector mechanisms (Murphy et al., 2008). To fulfill such task, these immune cells rely on surface expression of pattern recognition receptors that detect specific molecular patterns, which are on the one hand, structures of

pathogens known as pathogen-associated molecular patterns (PAMPs), on the other hand, damage-associated molecular patterns (DAMPs) deriving from the host himself in context of an immune reaction.

Macrophages

Macrophages are one of the main effector cells of the innate immune system. Macrophages are a heterogeneous immune cell population, displaying a variation of inflammatory and anti-inflammatory functions (Gordon, 2003) and as consequence of their historic discoveries have different names such as osteoclasts in the bone, alveolar macrophages in the lungs, Langerhans cells in the skin, or Kupffer cells in the liver (Gosselin et al., 2014; Lavin et al., 2014) among others. The functions of macrophages are diverse and very much dependent on their origin, and range from tissue homeostasis, removal of apoptotic cells of invading pathogens by phagocytosis, reactions to tissue damage, and initiations, orchestration, and resolution of inflammatory responses (Vannella and Wynn, 2017). Therefore, macrophages are involved in many diseases, e.g. asthma, autoimmune diseases, atherosclerosis, or other metabolic disorders (Fricker and Gibson, 2017; McNelis and Olefsky, 2014; Moore et al., 2013).

In the lungs, macrophages are a very abundant and heterogeneous immune cell type (Cai et al., 2014). At least two distinct macrophage populations in the lungs can be distinguished, interstitial and alveolar macrophages. Interstitial macrophages are mainly located in the lung interstitium between alveoli, express MHCII at their surfaces for antigen presentation to T cells and seem to interact with DCs (Gibbings et al., 2017; Sabatel et al., 2017). Furthermore, it was demonstrated that interstitial macrophages produce IL-10 and prevent DCs from promoting a Th2-mediated allergy the airways (Bedoret et al., 2009; Kawano et al., 2016). Therefore, interstitial macrophages are suggested to mainly exert anti-inflammatory properties and maintain immune homeostasis in the lungs.

In contrast, alveolar macrophages are located between the pulmonary mucosa and the external environment; they are thought to display regulatory and tolerogenic functions at steady-state (Soroosh et al., 2013). Collectively, alveolar macrophages may rather play a central suppressive role in terms of asthma (Draijer and Peters-Golden, 2017; Saradna et al., 2018). However, activated alveolar macrophages can recruit other immune cells into the alveolar space by secreting cytokines, such as IL-1ß, TNF-α, IL-6, and reactive oxygen species (Arango Duque and Descoteaux, 2014).

Furthermore, two extreme phenotypic types in the macrophage spectrum exist i) the classically activated (or M1), and ii) the alternatively activated (or M2) phenotypes (Gordon, 2003). While the M1 macrophages are induced by IFNγ and lipopolysaccharide (LPS) and are mainly involved in driving inflammation in response to pathogens, the M2 macrophages,

induced by IL-4 and IL-13, are involved in anti-inflammatory responses (Biswas and Mantovani, 2010; Sica and Mantovani, 2012). Moreover, increased M2 macrophages were observed to participate in allergic inflammation and tissue remodeling in asthma and to produce anti-inflammatory mediators, such as IL-10, TGF-ß, and IL-1 receptor antagonist in asthma (Martinez and Gordon, 2014; Melgert et al., 2011). In the context of allergic inflammation, M1 and M2 macrophages may coexist in a mixed phenotype depending on the balance of activating and inhibiting signals (Moreira and Hogaboam, 2011). However, when the cytokines IL-4 and IL-13 increase, the M2 phenotype is favored (Melgert et al., 2011).

Eosinophils

Although a resident eosinophils exist in the lung as tolerogenic cells (Mesnil JCI 2016), they become prominent in airways, lung tissue and circulation of individuals with allergic asthma disease (Possa et al., 2013; Saglani et al., 2007) and contribute to the allergic asthma phenotype in several ways. Eosinophils act on other cells involved in allergic asthma, such as basophils and mast cells through release of lipid mediators (Kita et al., 1991; Possa et al., 2013). Furthermore, eosinophils can act as antigen-presenting cells and help to recruit T cells into the inflamed tissue, promote airway inflammation, and drive Th2 differentiation (Fulkerson et al., 2006; Shi et al., 2000; Walsh et al., 2008; Wang et al., 2007) and were reported to co-regulate DC-T cell crosstalk (Gundel et al., 1991; Jacobsen et al., 2011; Kanda et al., 2009). Furthermore, eosinophils are central in inducing airway hyperresponsiveness, a characteristic of asthma (Possa et al., 2013), directly through the inhibition of parasympathetic nerve activity (Evans et al., 1997; Possa et al., 2013), or indirectly by secreting IL-5 and IFNγ (Kanda et al., 2009; Leckie et al.).

Neutrophils

Neutrophils are increasingly recognized as a very complex immune cell type, playing an important effector function during acute inflammation, and chronic inflammatory disorders (Vannella and Wynn, 2017). Neutrophils are recruited to the site of inflammation by chemotactic molecules (endo- and exogenous chemokines, bacterial peptides or complement components) through extravasation of the vascular system. Neutrophils can form extracellular traps to bind pathogens, engulf microbes, and secrete anti-microbial peptides (von Köckritz-Blickwede and Nizet, 2009). Furthermore, in an allergic asthma context, neutrophils participate to allergic sensitization and airway inflammation through reactive oxygen species generation and induction of pro-inflammatory cytokines (Hosoki et al., 2016; Sibille and Marchandise, 1993). Neutrophils also participate in activating T and B cells through pathogen presentation (Vannella and Wynn, 2017). In addition, neutrophils are found in the airways, lung tissue, and blood circulation during asthma exacerbation (Ciepiela et al., 2015), and in severe asthma neutrophil numbers are highly increased (Alphonse et al.,

2008). In contrast, in milder forms of asthma, the number of neutrophils in the airways is lower, to levels similar to those observed in healthy controls (Ciepiela et al., 2015). Thus, neutrophils – in concert with increased numbers of Th17 – are considered a marker of severe and usually difficult-to-treat asthma (Cheng and Palaniyar, 2013; Newcomb and Peebles, 2013). In such "neutrophilic asthma", IL-17 and IFNγ are increased in lung tissue, mainly contributing to the severity of the clinical picture (Cheng and Palaniyar, 2013).

Dendritic cells (DCs)

Dendritic cells are the classical allergen-presenting cell type and play a central role in asthma by orchestrating the crosslink between innate and adaptive immunity (Murphy et al., 2008), and are enriched in tissues with contact to the outside, such as skin or mucosal surfaces (Alvarez et al., 2008). As “sentinels of the mucosal surfaces”, DCs take up antigens supported in that task by different pattern-recognition-receptors such as Toll-like receptors or Fcε receptors (Salazar and Ghaemmaghami, 2013). Antigens are processed into smaller peptides and loaded in the groove of the class II Major Histocompatibility Complex (MHCII), to present them to naive T cells. Together with this presentation, co-stimulatory receptors, and cytokines, DCs stimulate naïve T cells and drive polarization of effector T helper (Th) cells such as Th1, Th2, and Th17 cells or promote the rise of tolerogenic regulatory T(reg) cells (Herbert et al., 1995; Roche et al., 2000; Salazar and Ghaemmaghami, 2013; Sung et al., 2006; Wan et al., 2000). Mechanistically, next to an orchestra of cytokines secreted by DCs and the epithelium, and upon pro-inflammatory environment, co-stimulatory receptors participate in the interactions between primed DCs with naïve T cell initiated through MHCII recognition. Although co-stimulatory CD86 and CD80 are required to drive allergy-associated Th2 differentiation (Li et al., 2016), the co-stimulatory molecule CD40 has been associated to allergic asthma as well as obesity-associated inflammation (Kowal et al., 2006; Natal et al., 2008). and is of peculiar importance for Th1, Th17 and Tregs differentiations (Chen et al., 2014; Haase et al., 2004; Ma and Clark, 2010).

In addition to an unusual population of plasmacytoid DCs (pDCs), which appears to be unrelevant for allergic asthma pathogenesis (de Heer et al., 2004; Lewkowich et al., 2008; Plantinga et al., 2013), three different pulmonary DCs subtypes can be distinguished. These DC subtypes share common surface markers, and express specific surface molecules, which can be used for identification via flow cytometry. There are conventional (c) DCs – either positive for CD103 or CD11b -, and $CD64^+$ monocyte derived (mo)DCs,. Upon activation, both $CD11b^+$ and $CD103^+$ cDCs home to the draining lymph nodes; however, the $CD11b^+$ cDCs display a much higher potency to drive Th2 immunity than $CD103^+$ cDCs (Plantinga et al., 2013). In addition, $CD64^+$ moDCs also seem to be potent drivers of Th2 polarization but also exert local pro-inflammatory effects (Plantinga et al., 2013). In contrast, $CD103^+$ cDCs

was seen to rather drive Th1 and Treg formation. Taken together, the functions of the different DC subsets are heterogenous, with $CD11b^+$ cDCs predominantly responsible for maladaptive Th2/Th17 immune responses, moDCs orchestrating the local inflammation in the lung, and $CD103^+$ cDCs seemingly not potent Th2 drivers but rather involved in tolerogenic functions in the lung (Hoffmann et al., 2016).

3.3.3 Adaptive immunity

Compared to the innate immunity, the adaptive immune system is a way more specific and highly specialized part of the immune system (Pancer and Cooper, 2006). The adaptive immunity is needed to complement the wide range of reactions against pathogenic molecular patterns mounted by the innate immune system, and react to pathogens trying to evade the host's innate immune reactions (Pancer and Cooper, 2006). The adaptive immune cells comprise T and B lymphocytes and the humoral immunity by antibodies (Murphy et al., 2008). Furthermore, the adaptive immune system is capable of forming an immunological memory helping to more rapidly react in case of a recurrent infection.

T Lymphocytes

T lymphocytes are highly mobile cells that remain mostly in an inactive, observant mode (Stockinger et al., 2004). Originating from the thymus, they firstly home to peripheral lymphoid organs, where interactions with cells of the innate immunity take place. There, upon binding of a single T cell expressing a specific T cell receptor to an MHC-loaded antigen-presenting cell, a clonal expansion of the T cell occurs. This leads to its activation, proliferation, and differentiation into effector T cells (Pennock et al., 2013). Different T cell subsets can be distinguished by their expression of the CD4 and CD8 co-receptors. These co-receptors determine that $CD4^+$ T cells interact with antigen-presenting cells via MHCII, while $CD8^+$ T cells with MHCI (Murphy et al., 2008). While $CD8^+$ T cells can differentiate into cytotoxic T cells important for defense against viruses or intracellular pathogens, $CD4^+$ T cells differentiate into effector Th1, Th2, Th17, or Treg. Finally, memory T cells can develop in order to enable a rapid immune response in case of re-exposure to the same antigen (Murphy et al., 2008).

Naïve and effector T cells as well as regulatory T cells are positive for the surface markers CD3 and CD4. Naïve T cells and effectors T cells can be divided upon CD62L and CD44 surface expression, the latter can further distinguished by intracellular production of above-mentioned signature cytokines (Ender et al., 2017). The Th1 cells are characterized by the secretion of IFNγ, and Th17 cells by IL-17 as signature cytokines (Swain, 1999). Both cell types are primarily important for host defense of infections In contrast, Th2 cells are

prototypic for allergic asthma and mainly characterized by production of cytokines such as IL-4 and IL-13 (León, 2017). A two-step process is needed for the development of Th2 cells in context of allergy, first the initial allergen exposure – the so called sensitization – and then a second and later subsequent exposures, which lead to allergy-typical symptoms (León, 2017).

B lymphocytes

Compared to T cells, B lymphocytes have a totally different duty. Indeed, antigen-specific B cell receptors allow B cells to act as allergen-presenting cell able to internalize, process, and present antigens via MHCII to activated Th cells. This interaction enhances the T cell reactivity but also lead to the B cell differentiation switch into plasma cells, secreting antigen-specific antibodies. These antibodies are a specific soluble form of the B cell receptor and bind antigen at the surface of pathogens, leading to their opsonization and thus activating the complement system. Importantly, the T cell interaction determines the immunoglobulin type of the antibodies, e.g. a Th2 milieu with high levels of cytokines such as IL-4 and IL-13 leads to a class switch from IgM to IgE production (Murphy et al., 2008).

3.3.4 Allergic sensitization of asthma

Allergic sensitization is an immunological process ultimately leading to the formation of IgE antibodies against common harmless allergens, a critical part of the development of asthma. Common harmless allergens constantly reach the lung epithelium through inhaled air (Perros et al., 2009). The capacity of an allergen to reach the bronchi and induce a local reaction depends on factors such as quantity and size. The bigger the particle, the more likely this particle is filtered in the nose, where it may rather trigger allergic rhinitis but pulmonary allergy (Passalacqua and Ciprandi, 2008), or trapped by the mucus covering the airways. However, if escaping the clearance systems of the airways, these allergens may directly interact with pattern-recognition receptors, such as Toll-like receptors. but also with protease-activated receptors present at the surface of the epithelial mucosa (Hammad and Lambrecht, 2008; Lambrecht and Hammad, 2012) Furthermore, physical damages and epithelial lesions trigger the release of alarmins such as IL-33, IL-1ß, and thymic stromal lymphopoietin (TSLP). In addition, activated epithelial cells secrete growth factors, chemokines, and danger signals, e.g. granulocyte-macrophage colony stimulating factor (GM-CSF), Chemokine (C-C motif) ligand (CCL)-17, CCL-20, Chemokine (C-X-C motif) ligand (CXCL) 2, uric acid, or adenosine triphosphate (Kouzaki et al., 2009; Lambrecht and Hammad, 2014a). These processes promote the recruitment and activation of innate immune cells, such as neutrophils, mast cells, alceolar macrophages, and moDCs (Hammad and Lambrecht, 2008). Moreover, the type 2 innate lymphoid cells (ILC2), an innate cell type similar to T cells,

establish a type 2 cytokine milieu favoring the recruitment eosinophils to the site of inflammation (Gold et al., 2014). Finally, DCs play a central role during allergic sensitization, taking up the antigens either through the epithelial layer (Gold et al., 2014), or by sensing allergens that made their way through the epithelium before processing them and homing to the draining lymph nodes (Hoffmann et al., 2016). Mechanistically, allergic asthma is a condition in which DCs present the processed antigen to naïve $CD4^+$ T cells and promote a Th2 differentiation as well as IL-4 secretion (Gold et al., 2014; Murphy et al., 2008). This occurs via the activation of signal transducer and activator of transcription (STAT) 6 pathway, followed by the binding of the transcription factor GATA binding protein (GATA) 3 (Pai et al., 2004; Swain et al., 1990). Although Th2 dominant, an involvement of Th17 cells in severe form of asthma has been reported (Al-Ramli et al., 2009; Hall et al., 2016; Lewkowich et al., 2008), in particular upon inflammatory conditions characterized by high levels of IL-6, IL-1ß, TGF-β and IL-23, which promote the activation of STAT3 and RORγT driving the differentiation to a Th17 phenotype. Finally, interaction with Th2 cells, B cells are undergoing a class-switch and start to secrete antigen-specific IgE (Fahy, 2015).

3.3.5 Effector phase of allergic asthma

Upon anew exposure to the allergen against which the sensitization took place, or a cross-reactive antigen, an allergic asthma response occurs. The main trigger of this response is the interaction of the allergen with IgE, usually bound to the surface of mast cells. Upon allergen binding, mast cells degranulate and the release biologically active products stored in vesicles, such as histamine, serotonin, proteases, proteoglycans, leukotrienes, and cytokines such as IL-4 and IL-5 (Fahy, 2015; Passalacqua and Ciprandi, 2008). This rapid cascade of events is responsible for the acute symptoms, such as cough, wheezing and shortness of breath as a consequence of mucus hyperproduction and airway inflammation (Hayashi et al., 2012; Williams and Galli, 2000). The activated mast cells also account for the extravasation of other inflammatory cells, in particular eosinophils, neutrophils and lymphocytes, leading to an inflammatory infiltration into the lung tissue and airways.

Furthermore, DCs uptake and process the allergen, and recruit effector T cells to the site of inflammation by secreting chemokines, such as CCL17/22 (Perros et al., 2009). T lymphocytes, especially Th2 cells, secrete high amounts of type 2 cytokines, such as IL-4, IL-5, and IL-13 (Romagnani, 2006), B cells switch class upon IL-4, airway smooth muscle cell undergo hyperplasia and goblet-cell metaplasia happen upon IL-13, and IL-5 helps mobilizing eosinophils (Kuperman et al., 2002; Murphy et al., 2008). Moreover, eosinophils contribute with toxic substances and radicals, prostaglandins and leukotrienes, and pro-

inflammatory cytokines (i.e., IL-3, IL-5, CSF-2) (Amin et al., 2016; Dombrowicz and Capron, 2001).

Additionally, recurrent allergen exposures harm the integrity of the epithelial cell barrier, enforcing the release of alarmins triggering the recruitment of other immune cells, such as eosinophils, neutrophils, Th17 cell, macrophages, innate lymphoid cells, and DCs (Fahy, 2015; Galli et al., 2008). All these immune cells act in concert and contribute to the pathogenesis of asthma and the acute asthma attack (Chen et al., 2016; Ciepiela et al., 2015; Hoffmann et al., 2016; Kim et al., 2012; Yoshihara et al., 2006; Yu et al., 2014).

Altogether, the extent of the tissue inflammation determines the severity of asthma (Louis et al., 2000) and participate to the typical asthma symptoms, such as cough, wheezing, bronchial hyperreactivity, and shortness of breath. Moreover, chronic exposure to the allergen and consequently recurrence of inflammatory processes leads to chronic airway inflammation including presence of large numbers of innate and adaptive immune cells and remodeling of airways.

3.4 The complement system: Integration of metabolism and immunity

The complement system was first discovered in the late 19th century as a defense mechanism against pathogens, leading to their coating with antibodies and complement proteins (opsonization) for better removal by phagocytic cells (Murphy et al., 2008). Nowadays, the complement system is predominantly considered as the first line of defense of the innate immunity for eliminating invading microorganisms, apoptotic cells and cellular debris (Zipfel and Skerka, 2009). The complete complement system comprises more that 50 soluble, membrane-bound, and intracellular proteins and is found in many body fluids such as blood, lymph, and interstitial fluids (Arbore et al., 2017). There are three ways to initiate the complement system via complement cascades initiated either by i) classical pathway (through complement protein C1), ii) the Lectin pathway or iii), the alternative pathway, all converge to the activation of the complement factor C3.

In the classical pathway, the C1 complex either detects a microbial surface directly or binds to antibodies that have already bound to pathogen's surface. Mechanistically, C1 complex consists of C1q, an homohexamer with each subunit displaying a globular head and a long collagen-like tail, and associated with pairs of inactive proteases (C1s and C1r) to form the C1q:C1r$_2$:C1s$_2$ complex. Upon pathogen binding, C1r and C1s are activated, and the complex triggers the cleavage of C4 to C4b, which can then binds and cleaves C2 forming C2a. That later complex, C4bC2a, forms the C3 convertase which initiates the cleavage of C3 (Murphy et al., 2008).

Similarly, the same C2 cleavage and C4bC2a complex formation occurs upon activation, the lectin pathway is initiated when the soluble pattern recognition receptors mannose-binding lectin (MBL) and ficolins, which circulate through the blood and extracellular fluids, get in contact to carbohydrates on microbial surfaces. Both, MBL and ficolins, have a structure similar to C1q and the C1 complex of the classical pathway, and together with MBL-associated serine proteases (MASP-1, MASP-2, and MASP-3), they form shape the MBL-MASP complex. After initiation MASP-1 undergoes a conformational change, activates MASP-2 leading to the cleavage of C4 into C4b (Murphy et al., 2008).

Finally, C3 in its hydrolyzed form C3(H_20) binds to complement factor B which then is cleaved into C3b(H_2O)Bb by the plasma protease factor D. This complex acts as a C3 convertase and can activate more C3 molecules by cleavage. This results in the generation of activated C3b, which is either directly inactivated or binds to a cell surfaces of microbes and then is moving downstream the complement pathways (Murphy et al., 2008).

The complement system, as a “master alarm system”, has to funnel danger sensing, i.e. recognition of potential harmful structures, into danger transmission, i.e. translation into adequate immunological actions (Köhl, 2006). Importantly, all three initiation pathways converge into the activation of C3 acting either as sensor of the alternative pathway or as substrate for proteolytic cleavage by C3 convertases formed in the classical or the lectin pathways (Köhl, 2006). Once activated, C3 splits into C3a and C3b, the later binding to a microbial surface, triggers even more generation of C3b via the cleavage of factor B Bb, and the shaping the C3 convertase C3bBb (Murphy et al., 2008). This last step is the common start into the downstream part of the complement pathway leading to final complement activation. After the C3 convertase assembly and C3 cleavage, a C5 convertase is generated leading in a similar way to the cleavage of the complement C5 into C5a and C5b. This event is followed by the formation of a complex containing C5b and later complement components such as C6, C7, C8, and C9 into the microbial cell membrane, the so-called membrane attack complex (MAC) (Zipfel and Skerka, 2009). The MAC disrupts the cell membrane integrity which results into destruction of the respective pathogen (Kolev et al., 2014). However, the exact role of MAC, especially in defense against non-gram-negative bacteria, is still not fully elucidated (Bayly-Jones et al., 2017; Bloch et al., 1997; Tomlinson et al., 1989; Würzner, 2003).

Although historically considered a part of the humoral innate immune system, it is now clear that the complement system is also part of the adaptive immune system and acts as a bridge between innate and adaptive immunity (Köhl, 2006; Reis et al., 2019). Indeed, complement proteins are present in almost all cells of the body, including B and T cells (Kolev et al., 2014; Morgan and Gasque, 1997). In particular, it was shown that allergen-presenting cells and T cells, which home their target tissue, do contain key complement components such as C3,

C5, factors B and D, thus providing a functional machinery for local immune-cell derived complement activation at the surface of cells (Kolev et al., 2014; Strainic et al., 2008), but also intracellularly (Kolev et al., 2014; Liszewski et al., 2013), thus, challenging the traditional appreciation of the complement system as a solely extracellular defense mechanism.

Furthermore, the complement system was shown to exert crucial functions for tissue homeostasis, lipid metabolism, bodyweight regulation, insulin resistance, and hematopoietic cell mobilization (Hajishengallis et al., 2017). In particular, in the liver, hepatocytes, represent the major source of complement proteins of all three activation pathways (Qin and Gao, 2006). Further, hepatocytes and the hepatic immune cell fraction express receptors for the key components C1q, C3a, and C5a (Dembitzer et al., 2012; Rezvani et al., 2014). In addition, a widespread activation of the complement system in the liver has been reported in patients with obesity and fatty liver as obesity-associated downstream pathological sequelae (Rensen et al., 2009), together with higher plasma levels and higher deposition of complement factors in liver tissues in humans and mouse models (Botelho et al., 2014; Rensen et al., 2009; Wlazlo et al., 2013; Xu et al., 2016). In contrast, mice deficient either for C1q, C3a receptor (C3aR) or for the C5a receptors (C5aR1 and C5aR2) are protected from diet-induced obesity and its seqaelae (Hillian et al., 2013; Lim et al., 2013; Mamane et al., 2009; Phieler et al., 2013a; Poursharifi et al., 2014a; Roy et al., 2013). Collectively, these data imply that the activated complement system contributes to the pathogenesis of obesity and associated complications and that antagonizing these signaling pathways may be protective. Although the key role of the complement system remains to orchestrate innate and adaptive immune responses as well as initiates general inflammatory reactions (Köhl, 2006; Ricklin et al., 2010), emerging evidence points towards the complement system bridging the innate immune system with metabolic disorders (Phieler et al., 2013a; Vlaicu et al., 2016), and participates to the concept of “metaflammation” (Hotamisligil, 2006; Ricklin et al., 2010).

3.4.1 The complement anaphylatoxin receptor C5aR1

The anaphylatoxins C3a and C5a are important parts of the complement system serving as central drivers of inflammation and bridging innate and adaptive immunity (Ricklin et al., 2010; Zipfel and Skerka, 2009). The anaphylatoxins act on many immune cells but also on non-immune cells, e.g. smooth muscle cells, endothelial cells, and platelets (Fischer and Hugli, 1997; Fischer et al., 1999; Fukuoka and Hugli, 1988; Klos et al., 2009). Next to their potent pro-inflammatory actions, anaphylatoxin are also involved in tissue regeneration, neuroprotection, and fibrogenesis (Bénard et al., 2004; Hillebrandt et al., 2005; Mastellos et al., 2001; Strey et al., 2003).

Since anaphylatoxins are highly effective for host defense, misdirected activation could be of potential harm to the host, e.g. in context of a septic shock (Yan and Gao, 2012).

For complement regulation, complement receptors, such as complement receptor C3a receptor, are involved in controlling and regulating the effector functions exerted by anaphylatoxin C3a (Köhl, 2006). C3aR is widely expressed in human innate and adaptive immune cells including neutrophils, eosinophils, basophils, dendritic cells, monocytes, mast cells and activated B- and T-lymphocytes and predominantly exert pro-inflammatory effects, such as secretion of proinflammatory cytokines. (Wang et al., 2019).

C5a signaling is transduced through two cognate receptor C5aR1 (CD88) and C5aR2 (C5L2, GPR77) (Laumonnier et al., 2017). Both C5a receptors are (G)-protein-coupled receptors (GPCRs) and their genes cluster with other chemoattractant receptors including the formyl peptide receptor family, bradykinin receptors, ChemR23, chemokine receptors CXCR1 and CXCR2 and several orphan receptors (Laumonnier et al., 2017). Interestingly, C5aR2 is often expressed at a lower level than C5aR1 (Bamberg et al., 2010; Chen et al., 2007), and is traditionally considered to predominantly exert anti-inflammatory and regulatory properties, whereas C5aR1 is the pro-inflammatory prototype, although, this dichotomous picture has recently been challenged (Chen et al., 2007; Gao et al., 2005; Karsten et al., 2017; Zaal et al., 2019; Zhang et al., 2010).

Binding of C5a to C5aR1 leads to intracellular signal transduction via pertussis-toxin-sensitive and -insensitive G proteins (Braun et al., 2003; Nilsson et al., 1996). Activation of C5aR1 is followed by downstream activation of different signaling pathways like phosphatidylinositol 3-kinase gamma (PI3K-γ), phospholipase C, and phospholipase D, and involves intracellular Ca^{2+} mobilization and phosphorylation of ERK1/2, p38-mitogen activated protein kinase (MAPK) and Akt (Chiou et al., 2004; Jiang et al., 1996; Karsten et al., 2015; Mullmann et al., 1990; Perianayagam et al., 2002; Zaal et al., 2017). The C5aR1 is widely expressed in human and mice, however, focus is on immune cells of the myeloid lineage, such as leucocytes and DCs, and probably also T cells (Bosmann et al., 2012; Karsten et al., 2015; Kirchhoff et al., 2001; Köhl et al., 2006; Lalli et al., 2008; Solomkin et al., 1981; Werfel et al., 1992).

3.4.2 C5aR1 and allergic asthma

As one of the complement system functions is to link innate and adaptive immunity it has early been consider to play a role in allergic asthma (Murphy et al., 2008) and indeed, complement activation is a relevant pathological feature of allergic asthma pathology (Murphy et al., 2008). More specifically, C5 can be cleaved and activated by asthma-specific processes, in particular via proteases released from mast cells and other inflammatory cells in the lung as a consequence of IgE-mediated degranulation (Wills-Karp and Koehl, 2005), or

alveolar macrophages (Huber-Lang 2005). Moreover, exogenous proteases derived from allergens can directly cleave and activate anaphylatoxins (Maruo et al., 1997; Wills-Karp and Koehl, 2005). In consequence, C5a has been closely correlated with disease severity, and is found in BAL and serum of asthma patients (Abdel Fattah et al., 2010; Krug et al., 2001). In addition, C5a signaling is relevant for many aspects of asthma exacerbation, such as smooth muscle contraction, mucus hypersecretion, recruitment of inflammatory cells, release of histamine and leukotrienes from mast cells, and degranulation of eosinophils (Murphy et al., 2008; Takafuji et al., 1994). Furthermore, Many studies have shown a strong involvement of the C5a-C5aR1 signaling axis in allergic asthma and, the current understanding is that the role is different in allergen sensitization and in the effector phase of allergic asthma. While C5a/C5aR1 displays a pro-allergic function during the effector phase of allergic asthma, it shows an anti-allergic, protective effect during allergen sensitization. Indeed, ablation of C5aR1 signaling increases AHR, airway inflammation and mucus production in experimental allergic asthma (Drouin et al., 2006; Köhl et al., 2006; Krug et al., 2001; Lajoie et al., 2010; McKinley et al., 2006). In contrast, ablation of C5aR1 signaling in already established allergic asthma leads to a reduction of airway inflammation and AHR (Köhl et al., 2006).

3.4.3 C5aR1 and obesity

The complement system is involved in many obesity-related pathologies, such as lipid metabolism, bodyweight regulation, and insulin resistance (Hajishengallis et al., 2017), and is highly active in obesity and associated sequelae (Rensen et al., 2009). The complement system is relevant for metabolic organ physiology (Phieler et al., 2013a), in particular, C5aR1 which is expressed by adipocytes together with other complement proteins (Rouaud et al., 2017; Schäffler et al., 2007). Furthermore, the production of anaphylatoxins and the expression of their receptors correlate with adipocyte insulin resistance, lipid disorder, and visceral obesity (van Greevenbroek et al., 2012; Lim et al., 2013; Vlaicu et al., 2016). In human, a study with obese women showed a close correlation between parameters of obesity and adipose tissue C5aR1 but not C5aR2 expression suggesting an adipose-immune interaction specifically via C5aR1 signaling (Poursharifi et al., 2014b). Interestingly, while a key step in defining obesity as a metabolic disturbance is the local and systemic adipose tissue inflammation (Wensveen et al., 2015), C5aR1-deficient mice were protected from diet-induced adipose tissue inflammation and even presented with an anti-inflammatory secretion profile (McCullough et al., 2018). Furthermore, C5aR1 signaling was also implicated with metabolic systemic inflammation and with obesity-related neoplasia (Doerner et al., 2016), and C5aR1-deficient mice are fairly protected from diet-induced obesity (Phieler et al., 2013a; Poursharifi et al., 2014a; Roy et al., 2013). Finally C5aR1 signaling has been shown

to be involved in pancreatic beta cell functions, highlighting the close connection between the complement system and metabolic functions beyond the adipose tissue (Atanes et al., 2018).

In conclusion, the C5a/C5aR1 signaling axis contributes to the pathogenesis of obesity and related complications and may present a molecular bridge between overweight and obesity on the one side and allergic asthma on the other.

3.5 Objectives of the thesis

The main purpose of this thesis was to establish a mouse model of diet-induced mild bodyweight increase associated with a beginning systemic inflammation and subsequent induction of pulmonary allergy.

The following was defined as key objectives:

I. Establish an experimental mouse model of HFD-induced overweight but not obesity.
II. Dissect the impact of diet on allergic asthma outcome.
III. Analyze the major asthma-relevant pulmonary immune cells, such as eosinophils, macrophages, neutrophils, DCs, and T cells.
IV. Dissect whether the crosstalk of DCs and T cells may be altered in this model.
V. Dissect whether changes in adipokines or complement protein expression are involved in this model.

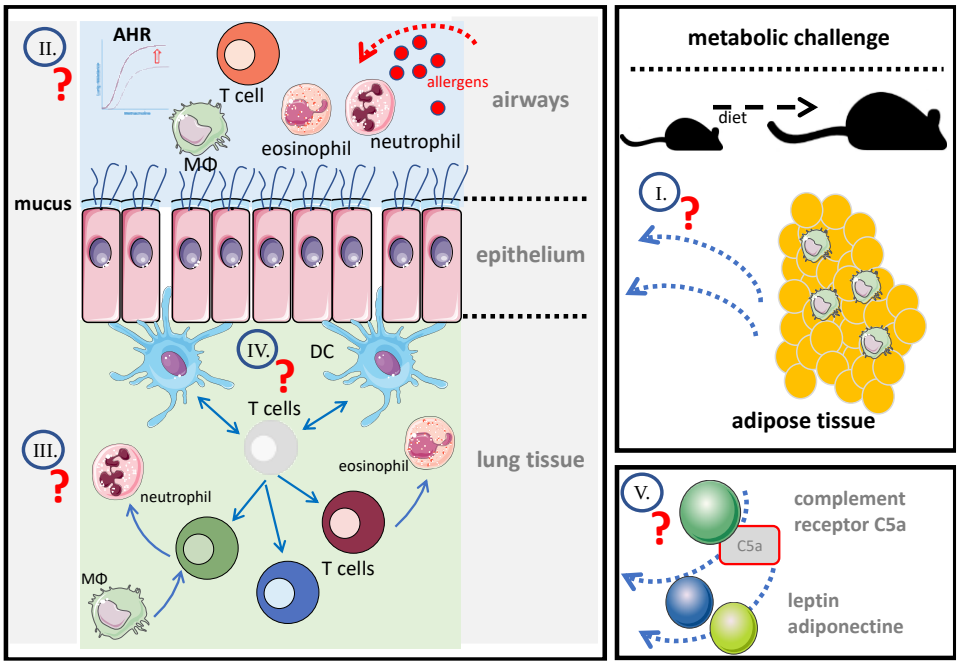

Figure 1: Key objectives of the thesis. I. Establish mouse model of HFD-induced overweight. II. Analyze asthma outcome. III. Analyze major pulmonary immune cell composition. IV. Dissect DC-T cells crosstalk. V. Check possible involvement of adipokine and complement system in the disease development or severity.

Material and Methods

3.6 Material

3.6.1 Mice

For this thesis, female C57BL/6JRj wild type mice (obtained at 4 weeks of age from Janvier, France) were used for the majority of experiments. In addition, C5aR1 knockout mice (genotype C57BL/6-129S4-C5ar1^{tm1Cge}, internal breeding, “Gemeinsame Tierhaltung”, University of Lübeck, Germany) were used. Both mouse strains were included in projects approved by the Schleswig-Holstein state authorities (*Ministerium für Landwirtschaft, Energiewende, Umwelt und Ländliche Räume*, Kiel, Germany; acceptance no.: 79-6/15).

OT-II mice (genotype C57BL/6-Tg(TcraTcrb)425Cbn/Crl, were bred internally, and also approved by the Schleswig-Holstein state authorities (*Ministerium für Landwirtschaft, Energiewende, Umwelt und Ländliche Räume*, Kiel, Germany; acceptance no.: 39_2013-08). T cell polarization experiments were performed with C57BL/6 wild type mice from inhouse breeding of Prof. Divanovic’ lab at the Division of Immunobiology of Cincinnati Children’s Hospital Medical Center in Cincinnati, Ohio, USA. The use of these mice was approved by the local Institutional Animal Care and Use Committee (IACUC 2017-0044).

3.6.2 Diets

The chow diet (CD) was obtained from Altromin (#1324). The high fat diets were supplied by Ssniff (HFD, EF R/M acc. TD88137 mod.; HFD60, E15741-34, EF acc. D12492 (II) mod.).

Table 1: Used diets for feeding with macronutrient and energy specifications.

	CD	HFD	HFD60
ID	Altromin 1324	Ssniff E15721-34, EF R/M acc. TD88137 mod.	Ssniff E15741-34, EF acc. D12492 (II) mod.
Energy	13 MJ/kg 3188 kcal/kg (100%)	22 MJ/kg 5.255 kcal/kg (165%)	24,4 MJ/kg 5.828 kcal/kg (183%)

Energy intake (mouse/week)	64 kcal	105 – 263 kcal	117– 291 kcal
Carbohydrates	64 kcal%	43 kJ%	21 kJ%
Fat	12 kcal%	42 kJ%	60 kJ%
Protein	24 kcal%	15 kJ%	19 kJ%
Cholesterol	n/a	2.071 mg/kg	290 mg/kg
Fat Source	n/a	Butter, Cholesterol	Tallow

3.6.3 Chemicals

Table 2: Used chemicals in alphabetical order.

Substance	Manufacturer
Acetyl-β-Methyl-Choline (Methacholine)	Sigma-Aldrich Chemie GmbH, Steinheim
Aluminum potassium sulfate ($KAl(SO_4)_2$)	Merck, KGaA, Darmstadt
Ammonium chloride (NH_4Cl)	Sigma-Aldrich Chemie GmbH, Steinheim
Arginine	Thermo Fisher Scientific Inc., Waltham, USA
Asparagine	Thermo Fisher Scientific Inc., Waltham, USA
Atipamezol	Sigma-Aldrich Chemie GmbH, Steinheim
Aqua ad injectabilia	B. Braun Melsungen AG, Melsungen
BD FACS Flow Sheath Fluid	BD Biosciences Europe, Erembodegem, Belgium
β-Mercaptoethanol	Sigma-Aldrich Chemie GmbH, Steinheim
Bovine serum albumin (BSA)	Sigma-Aldrich Chemie GmbH, Steinheim
Carboxyfluoresceine-Succinimidylester (CFSE)	Life technologies Corporation, Carlsbad, USA
CD3, hamster anti-mouse	eBioscience, Vienna, Austria
CD28, hamster anti-mouse	eBioscience, Vienna, Austria
Chloral hydrate ($C_2H_3Cl_3O_2$)	Merck, KGaA, Darmstadt
Citric acid, crystalline	Merck, KGaA, Darmstadt
Compensation beads (anti rat/hamster)	BD Biosciences Europe, Erembodegem, Belgium
CSF-2 (GM-CSF), recombinant murine	Peprotech Corporation, Rocky Hill, USA

DMEM	Life technologies Corporation, Carlsbad, USA
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich Chemie GmbH, Steinheim
DNase I from bovine pancreas	Sigma-Aldrich Chemie GmbH, Steinheim
Entalan	Merck, KGaA, Darmstadt
Eosin	Chroma Technology Corporation, Bellow Falls, USA
Esmeron	Organon, Oss, Niederlande
Ethanol, absolute	J. T. Baker, Deventer, Netherlands
Ethanol, 70% denaturated	Carl Roth GmbH & Co. KG, Karlsruhe
Ethanol, 96% denaturated	Carl Roth GmbH & Co. KG, Karlsruhe
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich Chemie GmbH, Steinheim
Fetal calf serum (FCS) (Lot A04304-0413)	PAA Laboratories GmbH, Pasching, Östereich
Folate	Thermo Fisher Scientific Inc., Waltham, USA
Formaldehyde solution, 37%	Sigma-Aldrich Chemie GmbH, Steinheim
Glacial acetic acid	Merck, KGaA, Darmstadt
GM-CSF, rekombinant murin	Peprotech Corporation, Rocky Hill, USA
Goat anti-hamster IgG, whole molecule	MP Biomedicals
Hematoxylin	Chroma Technology Corporation, Bellow Falls, USA
HEPES	Thermo Fisher Scientific Inc., Waltham, USA
Isopropanol	Otto Fishar GmbH & Ko. KG, Saarbrücken
IFN γ , recombinant murine	eBioscience, Vienna, Austria
IFN γ , anti-mouse	eBioscience, Vienna, Austria
IL-4, recombinant murine	eBioscience, Vienna, Austria
IL-4, anti-mouse	eBioscience, Vienna, Austria
IL-6, recombinant murine	eBioscience, Vienna, Austria
IL-12, recombinant murine	eBioscience, Vienna, Austria
iQ SybrGreen Supermix	Bio-Rad Laboratories GmbH, München
Ketamine hydrochloride	Sigma-Aldrich Chemie GmbH, Steinheim
Leptin, rekombinant murin	Peprotech Corporation, Rocky Hill, USA
L-glutamine (200 mM concentrate)	Life technologies Corporation, Carlsbad, USA
Liberase^{TM} TL Research Grade	Roche Diagnostics International AG, Rotkreuz, Risch, Schweiz
MACS BSA stock solution	Milteny Biotec GmbH, Bergisch Gladbach
MEM essential vitamins	Thermo Fisher Scientific Inc., Waltham, USA
Metedomidin	Sigma-Aldrich Chemie GmbH, Steinheim
Non-essential amino acids (100x)	Sigma-Aldrich Chemie GmbH, Steinheim
Ovalbumin, grade V	Sigma-Aldrich Chemie GmbH, Steinheim
Phosphate Buffered Saline (PBS)	PAA Laboratories GmbH, Pasching, Östereich
Penicillin-Streptomycin, 100x Liquid	Life technologies Corporation, Carlsbad, USA
Phloxine	Chroma Technology Corporation, Bellow Falls, USA
Potassium bicarbonate ($KHCO_3$)	Sigma-Aldrich Chemie GmbH, Steinheim
RPMI 1640	Life technologies Corporation, Carlsbad, USA

Schiff-reagent	Merck, KGaA, Darmstadt
Sodium chloride	Sigma-Aldrich Chemie GmbH, Steinheim
Sodium dihydrogen phosphate (Na_2H2PO_4)	Sigma-Aldrich Chemie GmbH, Steinheim
Sodium hydrogen phosphate (Na_2HPO_4)	Sigma-Aldrich Chemie GmbH, Steinheim
Sodium iodate ($NaIO_3$)	Merck, KGaA, Darmstadt
Sodium pyruvate	Life technologies Corporation, Carlsbad, USA
TGFβ, recombinant murine	eBioscience, Vienna, Austria
Trizol reagent	Life technologies Corporation, Carlsbad, USA
Trypan blue	Life technologies Corporation, Carlsbad, USA
Tween 20	Sigma-Aldrich Chemie GmbH, Steinheim
Xylazine	Sigma-Aldrich Chemie GmbH, Steinheim

3.6.4 Antibodies for flow cytometry

Table 3: Antibodies and reagents used for flow cytometry.

Epitope	Clone	Label	Manufacturer	Species	Concentration (mg/ml)	Dilution
CCR7	4B12	APC	eBioscience, Vienna, Austria	rat	0.2	1:200
CD3	17A2	PE-Cy7	eBioscience, Vienna, Austria	armenian hamster	0.2	1:400
CD3	OKT3	AF488	eBioscience, Vienna, Austria	mouse	0.2	1:200
CD3e	145-2C11	eF450	eBioscience, Vienna, Austria	armenian hamster	0.2	1:300
CD4	RM4-5	PE-Cy7	eBioscience, Vienna, Austria	rat	0.2	1:400
CD4	RM4-5	V450	BD Biosciences, Belgium	rat	0.2	1:400
CD11b	M1/70	BV510	Biolegend, London, UK	rat	0.08	1:800
CD11c	N418	APC	eBioscience, Vienna, Austria	armenian hamster	0.2	1:800
CD11c	N418	FITC	eBioscience, Vienna, Austria	armenian hamster	0.2	1:800
CD16/32	93	-	eBioscience, Vienna, Austria	-	0.1	1:100
CD19	1D3	eF450	eBioscience, Vienna,	rat	0.2	1:300

			Austria			
CD40	IC10	APC	eBioscience, Vienna, Austria	rat	0.2	1:200
CD44	IM7	BV421	Biolegend, London, UK	rat	0.2	1:300
CD49b	DX5	eF450	eBioscience, Vienna, Austria	rat	0.2	1:300
CD62L	MEL-14	APC	Biolegend, London, UK	rat	0.2	1:100
CD64	X54-5/7.1	PE	Biolegend, London, UK	mouse	0.2	1:800
CD103	2E7	Per-CP–Cy5.5	Biolegend, London, UK	armenian hamster	0.2	1:800
C5aR1/CD88	20/70	APC	Biolegend, London, UK	rat	0.2	1:800
Fixable viability dye	-	APC-eF780	eBioscience, Vienna, Austria	-	-	1:1500
Foxp3	FJK-16s	APC	eBioscience, Vienna, Austria	rat	0.2	1:200
F4/80	BM8	PE-Cy7	eBioscience, Vienna, Austria	rat	0.2	1:300
IFN γ	XMG1.2	APC	eBioscience, Vienna, Austria	rat	0.2	1:200
IL-10	JES5-16E3	PE	eBioscience, Vienna, Austria	rat	0.2	1:200
IL-13	eBio13A	PE	eBioscience, Vienna, Austria	rat	0.2	1:200
IL-17	eBio17B7	PE	eBioscience, Vienna, Austria	rat	0.2	1:200
MHCII	M5/144.15.2	APC-eF780	Biolegend, London, UK	rat	0.2	1:1500
MHCII	M5/144.15.2	FITC	Biolegend, London, UK	rat	0.5	1:1500
Ly6G	1A8	V450	BD Biosciences, Belgium	rat	0.2	1:300
Ly6G	1A8	APC	BD Biosciences, Belgium	rat	0.2	1:400
Siglec F	E50-2440	BV421	BD Biosciences, Belgium	rat	0.2	1:300
Siglec F	E50-2440	PE	BD Biosciences, Belgium	rat	0.2	1:300

3.6.5 PCR primer

Table 4: PCR primer and sequences.

Gene	Sequence	Annealing temperature
Actin beta	5'-GCACCACACCTTCTACAATGAG-3' 5'-AAATAGCACAGCCTGGATAGCAAC-3'	60 °C
Leptin receptor	5'-TGTCTCAGCTACATCTCTGC-3' 5'-CTGAACCATCCAGTCTCTTG-3'	60 °C

3.6.6 Consumables

Table 5: Consumables.

Material	Manufacturer
BD Microtainer tube	BD Biosciences Europe, Erembodegem, Belgium
Cell strainer 40 µm	BD Biosciences Europe, Erembodegem, Belgium
Cell strainer 100 µm	BD Biosciences Europe, Erembodegem, Belgium
ELISA-reservoir 25 ml	VWR International GmbH, Darmstadt
Filtertip 10 µl, 100 µl, 1000 µl	Sarstedt AD & Co., Nümbrecht
Microscope slide	Gerhard Menzel GmbH, Braunschweig
MACS Separation LS Column	Milteny Biotec GmbH, Bergisch Gladbach
MACS Separation MS Column	Milteny Biotec GmbH, Bergisch Gladbach
Micro tube 0.5 ml; 1.5 ml; 2 ml	Sarstedt AD & Co., Nümbrecht
Needle 26G	BD Biosciences Europe, Erembodegem,

	Belgium
Nitrile Powder-Free Examination Gloves	Ansell Healthcare GmbH, Munich
PCR tubes	Bio-Rad Laboratories GmbH, München
Petri dish 60x15 mm	Greiner Bio-One GmbH, Frickenhausen
Pipette tip 10 µl, 100 µl, 1000 µl	Sarstedt AD & Co., Nümbrecht
Pipette with tip 5 ml, 10 ml, 25 ml	Greiner Bio-One GmbH, Frickenhausen
Plate 6 wells	Sarstedt Inc., Newton, USA
Plate 48 wells (flat bottom)	Sigma-Aldrich Chemie GmbH, Steinheim
Plate 96 wells (U bottom)	Greiner Bio-One GmbH, Frickenhausen
Pur-Zellin swab	Greiner Bio-One GmbH, Frickenhausen
Spatula	VWR International GmbH, Darmstadt
Single-use syringe 1 ml	B. Braun Melsungen AG, Melsungen
Syringe 5 ml, 10 ml	BD Biosciences Europe, Erembodegem, Belgium
Tracheal cannula for mouse (OD 1.2 mm, L 15mm)	Hugo Sachs, March-Hugstetten
Tube 5 ml	Sarstedt AD & Co., Nümbrecht
Tube 15 ml, 50 ml	Sarstedt AD & Co., Nümbrecht
Weighing dish	Greiner Bio-One GmbH, Frickenhausen

3.6.7 Kits

Table 6: Used commercial kits.

Kit	**Manufacturer**
$CD4^+$ T cell isolation kit	Milteny Biotec GmbH, Bergisch Gladbach

DNase I Kit, RNase frei	Thermo Fisher Scientific Inc., Waltham, USA
Fixation/Permeabilization Solution Kit	BD Biosciences
Foxp3 staining kit	eBioscience, Vienna, Austria
GeneJet Gel Extraction kit	Life Technologies, Darmstadt, Germany
HDL and LDL/VLDL Assay Kit (Biochain)	BioChain Institute Inc., USA
Mouse IFN-γ DuoSet (ELISA)	R&D Systems, Wiesbaden
Mouse Il-10 DuoSet (ELISA)	R&D Systems, Wiesbaden
Mouse IL-13 DuoSet (ELISA)	R&D Systems, Wiesbaden
Mouse metabolic kit (K15124C-1)	Meso Scale Discovery, Gaithersburg, USA
Naïve $CD62L^+$ T cell isolation kit	Milteny Biotec GmbH, Bergisch Gladbach
PowerSoil DNA Isolation kit	MoBio, Canada
PerfeCTa NGS Library Quantification kit	Quanta BioSciences, Beverly, MA, USA
Quant-iT dsDNA BR Assay kit	Life Technologies, Darmstadt, Germany
Revertaid cDNA synthesis kit	Thermo Fisher Scientific Inc., Waltham, USA
RNeasy® Mini Kit	Qiagen, Venlo, Netherlands
Triglyceride Colorimetric Assay Kit	Cayman Chemical, USA

3.6.8 Buffer, solutions, and cell culture media

Table 7: Buffer, solutions, and cell culture media.

Buffer/solution/media	Substance(s)
Anesthetic	Sodium chloride, 0.9% 75 mg/kg Ketamin 0.75 mg/kg Metedomidin
Antagonist	Sodium chloride, 0.9% 0.75 mg/kg Atipamezole
Block buffer	Anti-mouse CD16/CD32 antibody 1:100 dilution in FACS buffer
Digestion medium (lung, VAT)	RPMI 1640 0.25 mg/ml Liberase TL 0.5 DNase I
Eosin solution	40 ml A. dest. 0.4 g Eosin
Eosin-phloxin solution	40 ml Eosin solution 6 ml Phloxin sotion 270 ml ethanol 96% 2 ml Glacial acetic acid
FACS buffer	MACS BSA stock solution 1:20 dilution in PBS
Formaldehyde solution	900 ml A. dest. 7.8 g Na_2HPO_4 1.87 g NaH_2PO_4 100 ml Formaldehyde stock solution (37%)

Full medium	RPMI 1640 10% FBS, heat inactivated 100 units/ml Penicilline 100 µg/ml streptomycine 2 mM L-glutamine
Hemalaun	1000 ml A. dest. 1 g hematoxilline 0.2 g sodium iodate 50 g aluminium potassium sulfate 50 g chloral hydrate 1 g citric acid
Phloxin solution	10 ml A. dest. 0.1 g Phloxin
Pure medium	RPMI 1640
Red blood cell lysis buffer (RBC)	A. dest. 155 mM NH_4Cl 10 mM $KHCO_3$ 0.1 mM EDTA pH 7.2
T cell media (500 ml)	415 ml DMEM 50 ml FBS 5 ml MEM essential vitamins 5 ml 100x non-essential amino acids 5 ml 100x sodium pyruvate 5 ml 100x mix of arginine, asparagine, and folate 5 ml 100x L-glutamine 1M HEPES

	100 units/ml penicilline 100 µg/ml streptomycine 0.05 ml β-Mercaptoethanol
Wash medium	RPMI 1640 10% FBS, heat inactivated 100 units/ml Penicilline 100 µg/ml streptomycine 2 mM L-glutamine 0.5 mg/ml DNaseI

3.6.9 Laboratory equipment

Table 8: Laboratory equipment.

Equipment	**Manufacturer**
BD FACS Aria™ III	BD Bioscience, San Jose, USA
Biological Safety Cabinets	Nuaire Inc., Plymouth, USA
Centrifuge 5424	Eppendorf AG, Hamburg
Centrifuge 5424R	Eppendorf AG, Hamburg
Centrifuge 5810R	Eppendorf AG, Hamburg
Chemical hood	Waldner Laboreinrichtungen GmbH & Co KG, Wangen
Cytospin centrifuge Cellspin I	Tharmac GmbH, Walsolms
CFX96 Touch™ Real-Time PCR Detection System	Bio-Rad Laboratories GmbH, München
Dissecting scissors	WPI Deutschland GmbH, Berlin
ELISA-Reader Fluostar Omega 0415	BMG Labtech GmbH, Ortenberg

ELISA-Washer Nunc-ImmunoTM Wash 12	Thermo Fisher Scientific Inc., Waltham, USA
FlexiVent	SCIREQ Scientific Respiratory Equipment Inc., Montreal, Canada
Flow cytometer BD LSR II	BD Bioscience, San Jose, USA
Flow cytometer BD LSR Fortessa 2	BD Bioscience, San Jose, USA
Forceps	WPI Deutschland GmbH, Berlin
Fridge, 4 °C and -20 °C combined	Liebherr-International Deutschland GmbH, Biberach an der Riß
Hot-air cabinet	Memmert, Schwabach
Incubator	Heraeus, Hanau
IR Direct Heat CO_2 Incubator	Nuaire Inc., Plymouth, USA
MACS Magnetic Cell Separator	Milteny Biotec GmbH, Bergisch Gladbach
Microscope Fluovert FS	Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany
Microscope Leica DM IL LED	Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany
Microscope camera Leica EC3	Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany
Multichannel pipette Biohit M300	Sartorius Biohit Liquid Handling Oy, Helsinki, Finnland
Neubauer counting chamber, improved	VWR International GmbH, Darmstadt
PCR, Cycler Mastercycler®	Eppendorf AG, Hamburg
pH-Meter Seven Easy PH S20-K	Mettler Toledo, Schwerzenbach, Schweiz
Pipetboy	Integra Biosciences AG, Zizers, Schweiz

Pipette (0,1-2,5 µl; 0,5-10 µl; 10-100 µl; 20-200 µl; 100-1000 µl)	Eppendorf AG, Hamburg
Precision balance LC6200S	Sartorius AG, Göttingen
Qubit fluorometer	Life Technologies, Darmstadt
SECTOR imager 6000	Meso Scale Discovery, Gaithersburg, USA
Vortex Reax 2000	Heidolph Instruments GmbH & Co. KG, Schwabach

3.6.10 Computer software

Table 9: Software used for data assembly, analysis, and visualization.

Program	**Company**
BD FACSDiva 7.0	BD Biosciences, San Jose, USA
FlexiVent Software 5.3	SCIREQ Scientific Respiratory Equipment Inc., Montreal, Canada
FlowJo X	FlowJo, LLD, Ashland, USA
GraphPad Prism 5.03 for Windows	Graph Pad Software Inc., San Diego, USA
Microsoft Excel 2011/2016 for Mac	Microsoft Corporation, Redmond, USA
Microsoft Powerpoint 2011/2016 for Mac	Microsoft Corporation, Redmond, USA
Microsoft Word 2011/2016 for Mac	Microsoft Corporation, Redmond, USA
MSD Discovery Workbench analysis software	Meso Scale Discovery, Gaithersburg, USA
R version 3.2	The Comprehensive R Archive Network

3.7 Methods

3.7.1 Mice handling and feeding protocols

Mice were maintained in a regular 12-hour light-dark cycle under standard conditions in a specific pathogen-free facility. For experiments with specific 12 weeks diets, mice reached a final age of 16 weeks; C57BL/6 wild type mice and OT-II mice were at the age of 12-24 weeks. All procedures involving animals and their care were conducted in accordance with national and international laws and policies and, according to protocols, approved by the local authorities of the Animal Care and Use Committee from Schleswig-Holstein state authorities (*Ministerium für Landwirtschaft, Energiewende, Umwelt und Ländliche Räume*, Kiel, Germany; 79-6/15 and 39_2013-08). The diets were started at the age of 4 weeks after weaning and performed over a period of 12 weeks. Mice had free access to the supplied food. Frequent visits were implemented to monitor animal wellbeing, weight development, and food intake.

For injections of substances, the intra-peritoneal (i.p.) route was performed cautiously to ensure mouse wellbeing and proper injection. Mice were removed gently from the animal cage and kept under the sterile working bench. Then they were restrained in a head-down position and the injection was performed in the lower right quadrant of the abdomen. Organ damage was prevented during this process.

Intra-tracheal (i.t.) applications of mice were performed in close collaboration with and under the authority of the experimental animal care facility of the University of Lübeck, Germany. Animals were first anesthetized by i.p. injections of 75 mg/kg Ketamin and 0.75 mg/kg Metedomidin. Then, narcotized mice were held by their incisor teeth on an immunization board with an elastic band. The tongue was carefully pulled out and a maximal volume of 50 µl was given into the throat. To force pulmonal inhalation of the substance, the tongue was pulled out and the nose was covered at the same time. After treatment and not later than 30 minutes after anesthesia, mice received the antagonist Atipamezol, 0.75 mg/kg. They were put back into their cages and placed on bedding material in an upright position to prevent reflux of the administered substance and were kept under red light to prevent them from cooling and monitored until full recovery.

3.7.2 Metabolic parameters

Bodyweight and food consumption were assessed weekly. After sacrificing the mice, perigonadal fat depots, representing the visceral adipose tissue (VAT), were removed and weighted.

For determination of serum leptin and insulin concentration, a commercially available mouse metabolic kit from Meso Scale Discovery (K15124C-1) was used following the manufacturer's instructions. Briefly, leptin and insulin were detected in a multiplexed sandwich immunoassay using plates pre-coated with a capture antibody. The samples were added together with the specific labeled detection antibody (either anti-leptin or anti-insulin). labeled with a proprietary electrochemiluminescent compound. After incubations and washing steps, the proprietary read buffer allowed the appropriate chemical environment for electrochemiluminescence and the intensity of emitted light allowed the quantification against standard solutions using the Meso Scale Discovery SECTOR instrument. Using that technology, the concentration ranges of detection was between 0 and $1x10^7$ for Leptin, and between 0 and $0.5x10^7$ pg/ml for insulin.

Furthermore, blood lipids were detected in plasma samples. Total cholesterol, high-density lipoprotein (HDL) cholesterol, very low and low density lipoprotein (VLDL/LDL) cholesterol were measured using the HDL and LDL/VLDL Assay Kit (BioChain). Triglycerides were analyzed using the Triglyceride Colorimetric Assay Kit (Cayman chemical). Lipids were measured according to the manufacture's instructions.

3.7.3 Gut microbiota

3.7.3.1 Collection, DNA extraction, 16s rRNA PCR, and gene sequencing for intestinal microbiota analysis

Stool samples were freshly collected directly upon defecation and immediately frozen at -80 °C. Later, DNA extraction, 16s rRNA PCR, and gene sequencing for intestinal microbiota analysis was performed with friendly support of M.Sc. Annika Sünderhauf, institute of nutritional medicine, University Hospital Schleswig-Holstein, Campus Lübeck. Briefly, microbial genomic DNA was extracted from each fecal sample using the PowerSoil DNA Isolation kit (MoBio, Canada). The hypervariable regions V1 and V2 of the 16s rRNA genes were PCR amplified using the forward primer 27F 5`-*AATGATACGGCGACCACCGAGATCTACAC*XXXXXXXX**TATGGTAATTG*T***AGAGTTTGATCCTGGCTCAG-3´ (in italics the 5´Illumina adaptor B, the 8 X represent the unique indices, in bold the primer pad, in bold and italics the primer linker and underlined the primer 27F) and the reverse primer 338R 5`-*CAAGCAGAAGACGGCATACGAGAT*XXXXXXXX **AGTCAGTCAG*CC***TGCTGCCTCCCGTAGGAGT (in italics the 3´ reverse complement sequence of the Illumina adaptor, the 8 X represent the unique indices, in bold the primer pad, in bold and italics the primer linker and underlined the primer 338R). Forward and reverse primers were tagged with 16 and 24 unique indices. Then, 20 µl PCR reactions,

containing 1 µl 1:10 diluted template DNA, were performed using Phusion Hot Start DNA Polymerase (Life Technologies, Darmstadt, Germany), with the following cycling conditions: 30 sec at 98 °C, followed by 30 cycles of 9 sec at 98 °C, 60 sec at 55 °C and 90 sec at 72 °C, and final extension for 10 min at 72 °C. PCR products were quantified on a gel via the Quantum ST4 System (Vilber Lourmat, Marne La Vallée, France) and one subpool per gel with PCR products of equimolar ratios was prepared. Subpools were applied on a gel, extracted with the GeneJet Gel Extraction Kit (Life Technologies, Darmstadt, Germany) and quantified with the Quant-iT dsDNA BR Assay Kit on a Qubit fluorometer (Life Technologies, Darmstadt, Germany). Subpools were again pooled in equimolar amounts and purified by Agencourt Ampure Beads (Beckman Coulter, Krefeld, Germany). PerfeCTa NGS Library Quantification Kit (Quanta BioSciences, Beverly, MA, USA) was used for quantification of the final library, which was sequenced using the Illumina MiSeq system (paired-end reads, 2 x 300 base pairs).

3.7.3.2 Sequence processing for intestinal microbiota analysis

Bioinformatical processing was undertaken with friendly support of Dr. rer. nat. Axel Künstner, Institute of Experimental Dermatology, Group of Medical Systems Biology, and Institute for Cardiogenetics, University of Lübeck.

Raw reads were demultiplexed with CASAVA version 1.8.2 and merged using fastq_mergepairs from VSEARCH version 2.0.2 with a maximum number of mismatches of 12 and the merged reads (contigs) between 270 and 330 bp long (Rognes et al., 2016). Subsequently, contigs were quality filtered using fastq_filter command (USEARCH version 8.1.1861) with expected numbers of errors set to 0.5. Finally, chimeras were identified by uchime_ref (USEARCH) with the RDP Gold database version 9 as reference database and were removed from the contig set (Edgar et al., 2011). Chimera free sequences were dereplicated (VSEARCH) and clustered into operational taxonomic units (OTUs) at 97% similarity level using UPARSE (as implemented in the USEARCH suite) (Quast et al., 2013). Taxonomy was assigned to the genus level for the OTUs using SILVA database v123 and Mothur version 1.38.0 with 80% bootstrap support (Schloss et al., 2009).

3.7.3.3 Statistics for intestinal microbiota analysis

For each individual, 8100 contigs were randomly chosen to normalize the different contig numbers between the individuals. Singletons were removed from the OTU table leaving 588 OTUs for downstream analysis. All statistical analyses were performed using R version 3.2.

3.7.4 Experimental ovalbumin (OVA)-driven allergic asthma model

Mice were fed for nine weeks (either CD, HFD, or HFD60) before the OVA sensitization protocol was initiated; the diet was maintained during asthma induction (Figure 2). For asthma induction, OVA was chosen as experimental allergen, following a previously published protocol with minor modifications (Ender et al., 2017; Wiese et al., 2017). Briefly, mice after both feedings were immunized by i.p. injection with OVA/Alum (150µg/1mg) on days 0 and 7 and challenged i.t. with 1.5% OVA (in 50 µl PBS) on days 14, 16, 18, and 20. After 24 hours, airway hyperresponsiveness (AHR) was measured and mice were sacrificed for tissue harvest and further analysis. PBS was used as control for all diets (CD/PBS, HFD/PBS, HFD60/PBS) as compared to OVA-treated mice for all diets (CD/OVA, HFD/OVA, HFD60/OVA).

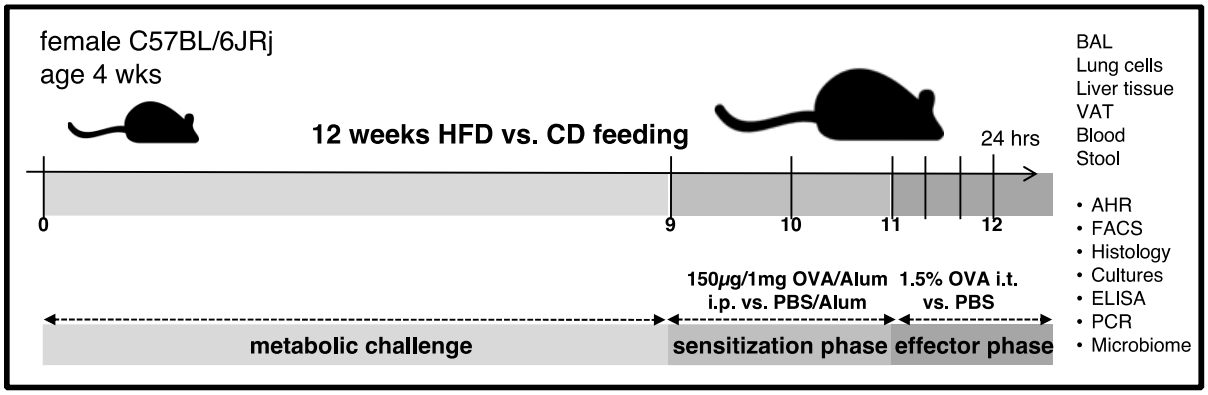

Figure 2: Feeding and treatment scheme. Feeding was started with four weeks old mice and executed over a period of 12 weeks. After nine weeks of feeding, mice were immunized i.p. with OVA/Alum as compared to PBS/Alum for the first time and a week later for the second. In week 12, one week after the second immunization, the airways were challenged with OVA compared to PBS four times every other day. 24 hours after the fourth challenge, mice were either ventilated to assess lung function or sacrificed for further analyses.

3.7.5 Measurement of airway hyperresponsiveness

For assessment of the airway hyperresponsiveness (AHR), the lung resistance was determined using mechanical ventilation and a forced oscillatory technique using a FlexiVent (SciReq) system, as published previously by the laboratory group (Ender et al., 2017; Engelke et al., 2014; Schmudde et al., 2013; Wiese et al., 2017).

Before each experiment, the FlexiVent system was calibrated following the manufacturer's instructions. Mice were anaesthetized by i.p. injection of 50 µl 76 mg/ml Ketamin and 4.8 mg/ml Xylazin, and muscle relaxation was achieved by administration of 50 µl of 10 mg/ml of rocuronium bromide (Esmeron). Under deep narcosis, mice were fixed on worktop and the trachea was dissected, opened at the cricothyreoid ligament, and intubated using a cannula,

which was connected to the endo-tracheal tubing of the flexiVent system. Aerosolized Acetyl-β-Methyl-Choline (methacholine) was generated by an ultrasonic nebulizer and delivered in-line through the inhalation port for 10 seconds in increasing dosages (0, 2.5, 5, 10, 25, and 50 mg/ml). Airway resistance was measured two minutes later by perturbating the lung eight times in a row with three ventilations between each perturbation. Methacholine acts as a bronchoconstrictor due to its parasympathomimetic properties, which leads to an increase of airway resistance, a characteristic feature of asthma.

3.7.6 Collection of bronchoalveolar lavage fluid and airways cells

Bronchoalveolar lavage (BAL) samples were obtained for analysis of influx of cells into the airways. In case of prior assessment of AHR, this was performed after lung ventilation using the canula, with which the trachea was already intubated. Otherwise, the trachea was intubated using a thin plastic catheter. Afterwards, 1 ml of ice-cold PBS was injected and subsequently aspirated. BAL was removed prior to lung tissue collection.

The whole aspirate was centrifuged (4 °C, 250 g, 10 min), the BAL fluid removed, and its volume noted. After red blood cell lysis (100 µl RBC lysis buffer, 1 min, room temperature), BAL cells were washed once in PBS, re-suspended in a final volume of 1 ml PBS. An aliquot of 10 µl was taken for cell counting. Cells numbers are shown per ml BAL fluid. Finally, BAL cells were spun down and re-suspended in 100 µl of block buffer for further determination by flow cytometry.

3.7.7 Lung cell isolation

After sacrifice of mice, the right lung lobe was removed for cell isolation. The lung tissue was carefully chipped into small pieces and placed in a 6 wells plate in which each well has been filled with 5 ml of pure medium and outfitted with a 40 µm cell strainer. The tissue was digested at 37 °C for 45 min with gentle shaking using 0.25 mg/ml Liberase TL and 0.5 mg/ml DNase I. Afterwards the strainer was placed on top of a 50 ml tube. The digested lung tissue was squeezed thoroughly using the stamp of a 5 ml syringe. The cell strainer was repeatedly washed with the digestion medium from the corresponding well and with additional 10 ml washing medium containing FBS and DNase. This 15 ml single cell suspension was centrifuged (4 °C, 350 x g, 10 min) and the supernatant removed. The cell pellet was re-suspended in 3 ml of RBC lysis buffer (3 min, room temperature) and the lysis reaction was stopped by addition of 30 ml PBS. An aliquot of the lung cell suspension (20 µl) was taken for cell counting.

3.7.8 Visceral adipose tissue (VAT) digestion and immune cell isolation

After removal of lung tissue, the perigonadal fat depots, representing the majority of the VAT, were removed. The digestion followed the same procedure as described for the lung tissue. Briefly, the tissue was digested at 37 °C for 45 min with gentle shaking using 0.25 mg/ml Liberase TL and 0.5 mg/ml DNase I. The suspension was filtered and washed through a 40 µm cell strainer and a RBC performed before cells were counted and further processed for flow cytometric analysis.

3.7.9 Determination of cell numbers

An aliquot of each cell suspension was taken and merged with the same volume of trypan blue. For manual enumeration of cell numbers, a Neubauer cell counting chamber was used and living cells were counted under the light microscope.

The number of cells was calculated as follows:

$$\frac{cells}{ml} = \left(\frac{cells\ counted\ in\ complete\ chamber}{number\ of\ counted\ chamber\ squares}\right) \times dillution\ factor \times 10^4$$

3.7.10 Histological analyses

3.7.10.1 Preparation of lung and liver tissue

After sacrifice of mice the left lung lobe was removed for histology analysis. For histological analysis of liver tissue, the whole liver was flushed with PBS before organ removal. The collected tissue samples were placed in an embedding cassette and fixed for approximately six hours in a 3.7% formaldehyde solution. Then, the samples were washed in water over night under continuous shaking. That step was followed by tissue dehydration by using an increasing ethanol-series with final replacement of ethanol by paraffin. This was performed as follows:

- Incubation for 24-72 hours in 70% ethanol
- Incubation for 24-72 hours in 80% ethanol
- Incubation for 24-72 hours in 90% ethanol
- Incubation for 24 hours in 96% ethanol
- Incubation for 24 hours in 100% ethanol (3 times)
- Incubation for 24 hours in paraffin:ethanol 1:2.
- Incubation for 24 hours in paraffin (3 times)

At the end, the sample was embedded in paraffin and cut with a microtome into 5 µm slices layed on glass slides. Then, slides were stained either with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS).

3.7.10.2 PAS staining

Staining with periodic acid-Schiff detects carbohydrate-containing components such as glycoproteins, mucines, and glycogen. Free hydroxyl groups of saccharides are oxidized, which forms violet complexes with the fuchsine sulfate of the Schiff-reagent. With this violet color reaction, the amount of mucus production in the airways can be detected. Before staining, the samples have to be deparaffinized and rehydrated. This was performed as follows including staining and counterstaining steps:

- Incubation for 5 min in 100% ethanol
- Incubation for 5 min in 96% ethanol
- Incubation for 5 min in 90% ethanol
- Incubation for 5 min in 80% ethanol
- Incubation for 5 min in 70% ethanol
- Incubation for 5 min in distilled water
- Incubation for 10 min in 0.8% periodic acid
- Incubation for 5 min in distilled water
- Incubation for 1 hour in Schiff-reagent (in dark)
- 10 min washing with tab water
- Incubation for 5-10 min in hemalaun
- 10 min washing with tab water
- Incubation for 5 min in 70% ethanol
- Incubation for 5 min in 80% ethanol
- Incubation for 5 min in 90% ethanol
- Incubation for 5 min in 96% ethanol
- Incubation for 5 min in 100% ethanol
- Incubation for 5 min in xylol (3 times)

After this, the slices were mounted with entellan. Four slices representing different regions of the lung were selected and counted with regard to mucus production for each mouse. PAS positive and PAS negative airways were counted by light microscopy. The mean percentages of PAS positive airways were used for quantification of mucus production.

3.7.10.3 H&E staining

Lung tissue was H&E stained to determine inflammatory cell infiltration, and liver tissue was H&E stained to determine lipid accumulation. The basic principle of H&E staining is that positively charged hematoxylin binds to negatively charged structures such as DNA, which then appears blue, and that negatively charged eosin counterstains other structures, which then appears red. Before staining, the samples have to be deparaffinized by a decreasing ethanol-series and rehydrated. This was performed as follows including staining with hemalaun and counterstaining eosin-phloxine:

- Incubation for 5 min in 100% ethanol
- Incubation for 5 min in 96% ethanol
- Incubation for 5 min in 90% ethanol

- Incubation for 5 min in 80% ethanol
- Incubation for 5 min in 70% ethanol
- Incubation for 5 min in distilled water
- Incubation for 10 min in hemalaun
- Incubation for 35 sec in eosin-phloxine
- Incubation for 20 sec in 96% ethanol
- Incubation for 1 min in 100% ethanol (twice)
- Incubation for 2 min in 100% ethanol
- Incubation for 5 min in xylol (4 times)

After the replacement of ethanol by xylol the slices were mounted with entellan. Four slices of each mouse were randomly assessed using a light microscope.

3.7.11 Flow cytometric analysis and cell sorting

Flow cytometry was performed to identify and quantify single immune cells in BAL, lung tissue and VAT. Specific flow cytometers provided lasers as light source as well as optics, detectors and analysis systems, enabling single cell analysis based on light and fluorescence behavior. Immune cells were characterized based on how they scatter light at different angles. Forward scatter (FSC) of light correlates with the size of the cell; side scattered (SSC) light is proportional to the granularity and structural complexity of the cell.

In addition, specific fluorophore labeled antibodies were used for deeper characterization of immune cells. For this purpose, labeled antibodies were added to the cell sample in order to bind specific cell surface molecules. Although all fluorophores have specific excitation and emission spectra, when more than one fluorophore is used, emission spectra may overlap, which requires fluorescence compensation. This was done by manual adjustments before analysis with the FlowJo software using the automatically determined compensation matrix calculated by the Diva software after using stained compensation beads. Such compensation bead controls were performed in 50 µl FACS buffer into micro tubes for each single fluorophore used in the staining panel. Then, one drop of negative control beads and one of anti-rat/anti-hamster positive beads were added to each micro tube, together with 0.5 µl of each antibody used for the cell staining.

3.7.11.1 Fluorescence staining

Single cell suspensions were adjusted to $1x10^6$ cells/100 µl. Each sample was incubated with a mouse seroblock FcR, which blocks the surface receptors FcRγIII and FcRγII (CD16 and 32) and thereby reduces unspecific antibody binding. Unbound antibodies were then removed and the appropriate antibodies for the staining were used. To do so, $1x10^6$ cells were incubated at 4 °C for 20 min with 100 µl of antibody master mix prepared using FACS

buffer. After washing repeatedly with PBS, cells were re-suspended in 300 µl FACS/BSA buffer and analyzed by usually using either BD LSRII or ARIA III flow cytometer.

To determine the *in vivo* T cell differentiation, lung cell suspensions were stimulated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA), and 500 ng/ml Ionomycin in the presence of the Golgi apparatus inhibitors monensin and Brefeldine A, to block cytokine secretion. Then, cells were fixed and permeabilized using a Foxp3 staining kit following the manufacturer's instructions before staining. In that case, the permeabilizing/wash buffer was used to prepare the antibodies master mix.

3.7.11.2 Fluorescence-Activated Cell Sorting (FACS)

For cell sorting, the BD FACS Aria III cell sorter equipped with a 100 µm nozzle was used. For the purification of lung DCs and $CD4^+$ T cells the full single cell suspension was used and the antibody master mix upscaled in accordance to the total cell number.

After staining with the appropriate antibody master mix, the lung cell suspension was filtered by using a 40 µm cell strainer before sorting. The cells were collected into full medium containing 20 ng/ml GM-CSF (CSF-2).

3.7.11.3 Immune cell identification

In the following, the gating strategies used for this thesis will be introduced by depicting the cell specific molecules bound with a fluorophore labelled antibody.

BAL cells

BAL fluid was obtained as described above (section 3.7.6) and BAL cells were identified using specific surface markers such as Siglec F, CD4, and Ly6G. Thus, eosinophils were identified as Siglec F^+ cells, macrophages as Siglec F^+ showing an additional autofluorescent signal, T cells as Siglec $F^-/CD4^+$ cells, and neutrophils as Siglec $F^-/Ly6G^+$ (Figure 3).

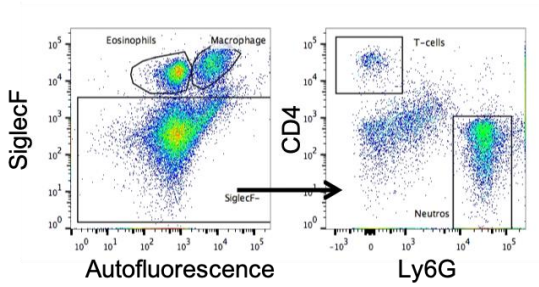

Figure 3: BAL cell gating. BAL cells were identified using autofluorescence, Siglec F, CD4, and Ly6G. Cells were identified as Siglec F^+ eosinophils, Siglec F^+/autofluorescence+ macrophages, Siglec F^-/$CD4^+$T cells, Siglec F^-/$Ly6G^+$ neutrophils.

VAT cells

The perigonadal fat depots, representing the majority of the VAT, were removed and prepared like for lung tissue (see section 3.7.8). The cell suspension staining allows the identification and quantification of VAT macrophages. To do so, cells were stained and analyzed with flow cytometry and VAT macrophages were identified as $F4/80^+CD11b^+CD11c^+$ cells (Figure 4).

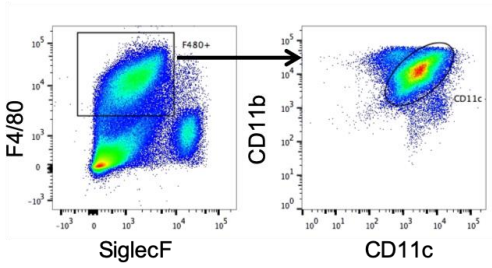

Figure 4: VAT cell gating. Siglec F^-/$F4/80^+$ cells were considered as macrophages. Their identification was further confirmed by measuring their expression of CD11b and CD11c.

Lung cells

After removal and preparation of cell suspension (see section 3.7.7), a wide range of lung cells were identified using three different staining procedure (Figure 5 A, B+C, D+E). Firstly, antibodies raised against Siglec F and Ly6G were used to identify Siglec F^-/$Ly6G^+$ neutrophils (Figure 5 A). Then CD62L, and CD44 were used to identify among the total $CD3^+$ $CD4^+$ T cells, the naïve CD62L+CD44- and effector CD62L-CD44+ T cells (Figure 5 B). Furthermore, intracellular cytokine staining was performed using antibodies for IL-13, IFNγ, IL-17, Foxp3, and IL-10 to further dissect $CD44^+$ effector T cells (Figure 5 C). Finally, further markers allow the identification among lung cells of eosinophils (Siglec F^+) and alveolar macrophages

(Siglec F^+CD11c^+) (Figure 5 D). Furthermore, in the Siglec F^- cell population, and after exclusion of lineage-positive cells ($CD3e^+$ T cells, $CD19^+$ B cells, $Ly6G^+$ neutrophils, and $CD49b^+$ NK cells), dendritic cells (DCs) were identified as $CD11c^+MHCII^+$ cells (Figure 5 E). The conventional (c)DCs were further characterized using CD103 and CD11c expression and monocyte-derived DCs upon additional CD64 expression (Ender et al., 2017; Engelke et al., 2014; Hoffmann et al., 2016; Wiese et al., 2017) (Figure 5 E). Of note, since the flow cytometric analysis of lung cells used only the right lung lobe (see section 3.7.7), number of cells are presented, unless further notice, as number of cells per right lung.

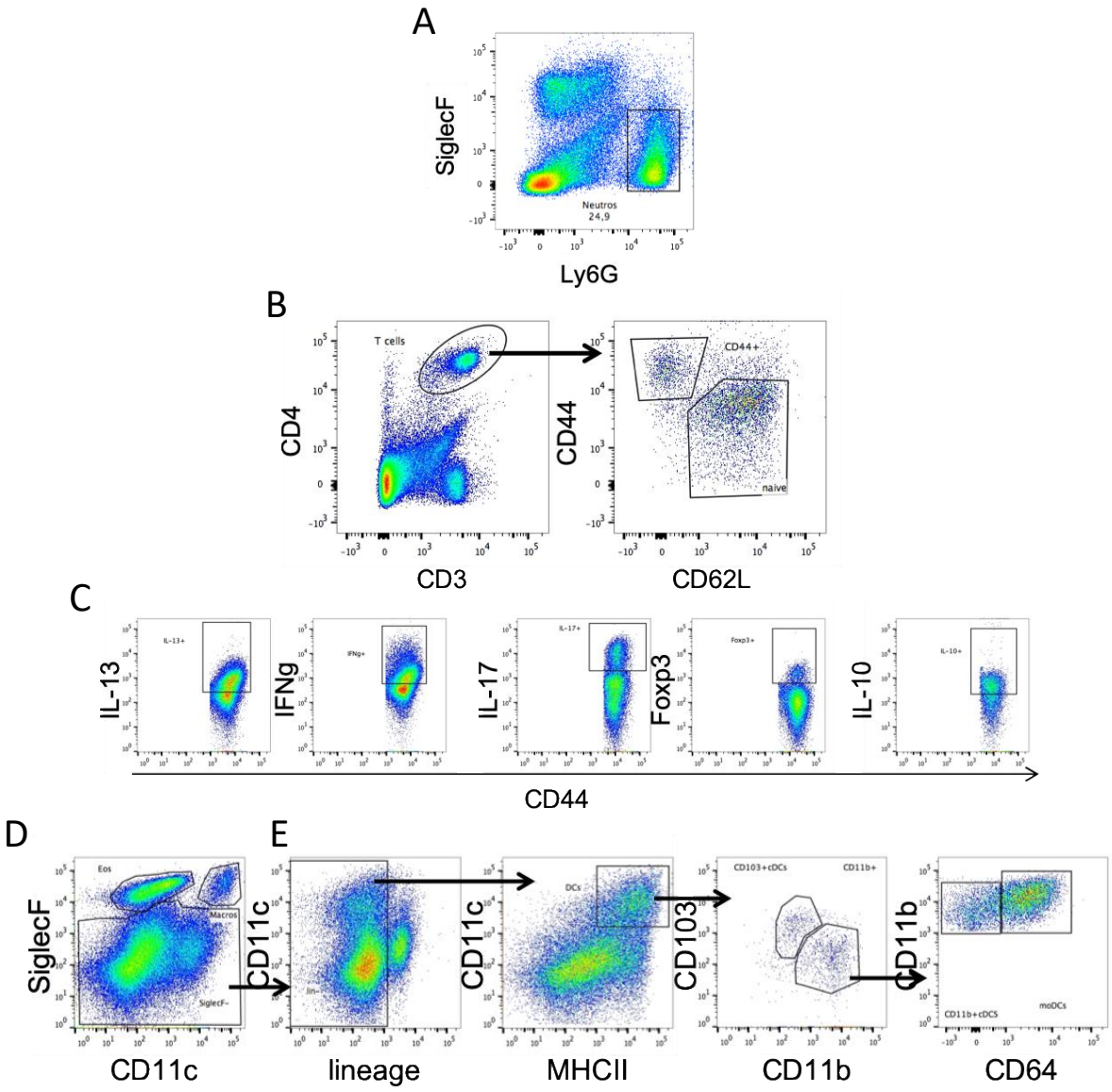

Figure 5: Lung cell gating (neutrophils, T cells, eosinophils, macrophages, DCs). (A) Neutrophils were gated as Siglec $F^-/Ly6G^+$; (B) total T cells as $CD3_+CD4^+$ and distinguished in naïve ($CD62L^+CD44^-$) and effector T cells $CD62L^-CD44^+$, (C) effector T cells were distinguished in IL-13^+, IFNγ$^+$, IL-17^+, Foxp3^+, and IL-10^+ cells using either combinations IL-13/IFNγ, IL-17/Foxp3, or single IL-10 staining, (D) eosinophils were identified as Siglec F+, alveolar macrophages as Siglec F^+CD11c^+) (E) lineage-negative cells (removal of: $CD3e^+$ T cells, $CD19^+$ B cells,

Ly6G$^+$ neutrophils, and CD49b$^+$ NK cells) were used to identify total DCs (CD11c$^+$MHCII$^+$), further subdivided into CD103$^+$ and CD11c$^+$ conventional (c)DCs and in CD64$^+$ monocyte-derived (mo)DCs.

3.7.12 Expression analysis of DC-relevant molecules

To further evaluate the DC functions, the surface expression of chemotactic receptor CCR7, the antigen presenting complex MHCII, and the co-stimulatory molecule CD40 were analyzed together with the antibodies panel used for DC identification (Figure 5 D-E). To perform this experiment, naïve lung DCs from 12 weeks HFD vs. CD feeding were sorted and collected into full medium containing 20 ng/ml CSF-2. After adjustment to 1x10^6 cells/ml and seeding, DCs were stimulated with 1 µM OVA overnight at 37 °C and 5% CO_2. Afterwards cells were prepared for flow cytometric analysis as usually.

3.7.13 Evaluation of DC-T cell crosstalk by co-culture experiment

Living lineage$^-$CD11c$^+$MHCII$^+$ DCs were sorted from whole lung cell isolates from allergic CD and HFD mice (CD/OVA and HFD/OVA). The DCs were collected into full medium containing 20 ng/ml GM-CSF, adjusted to 1x10^5 cells/well (200 µl). DCs were kept in culture medium overnight at 37 °C and 5% CO_2 in presence of Ovalbumin (1:1000, 4%, Sigma grade V). The work was performed under sterile conditions.

On the next day, splenic CD4$^+$ T cells were isolated from spleens of OT-II mice. For this, OT-II mice were sedated by CO_2 and sacrificed. Then, spleens were removed and placed on a 40 µm cell strainer in a 6 wells plate. Single cell suspensions were obtained by mechanical disaggregation of the organ through the 40 µm mesh, using the stamp of a 5 ml syringe and washing repeatedly with PBS. For magnetic cell selection of CD4$^+$ T cells the manufacturer's instructions were followed. Briefly, the cell suspension was stained with a biotinylated antibody cocktail (100 µl/10^8 cells) and then with anti-Biotin MicroBeads (200 µl/10^8 cells). Cells were magnetically separated and collected after appropriate washing steps. The Biotin-antibody cocktail allowed removal of cells positive for CD8a, CD11b, CD11c, CD19, CD45R (B220), CD49b (DX5), CD105, Anti-MHCII, and Ter-119. After separation, CD4$^+$ T cell purity was checked using flow cytometric analysis. When the purity was below 80%, the purification protocol was repeated. Furthermore, purified CD4$^+$ T cells were adjusted to a concentration of 10^7 cells/ml in PBS and labeled with 1 µM carboxyfluorescein diacetate succinimidyl ester (CFSE) at 37 °C for 10 min. Then, the reaction was stopped by addition of 1 ml ice cold PBS and incubation for 1 min on ice. Subsequently, 2.5 x 10^5 purified, CFSE labeled splenic CD4$^+$ T cells from OVA-TCR transgenic OT-II mice (in 50 µl) were added and kept in culture with the DCs. At day 4 and 9, cells were harvested and stained against CD4 in presence of a

live/dead viability dye. Therefore, T cell survival could be estimated by gating on living $CD4^+$ T cells and analyzing the CFSE-signal as a surrogate for T cell proliferation. Indeed, CFSE is a fluorescent dye and binds to intracellular molecules, and upon cell division of CFSE-labelled cells leads to dilution of fluorescence intensity because the cell progeny is received half the number of labeled molecules. Consequently, the decrease in cell fluorescence corresponds with the number of cell divisions. This allows analyzing and comparing cell proliferation in the range of up to eight cell divisions. In addition, co-culture supernatants were collected and used to determine levels of IL-10, IL-13 and IFNγ by ELISA.

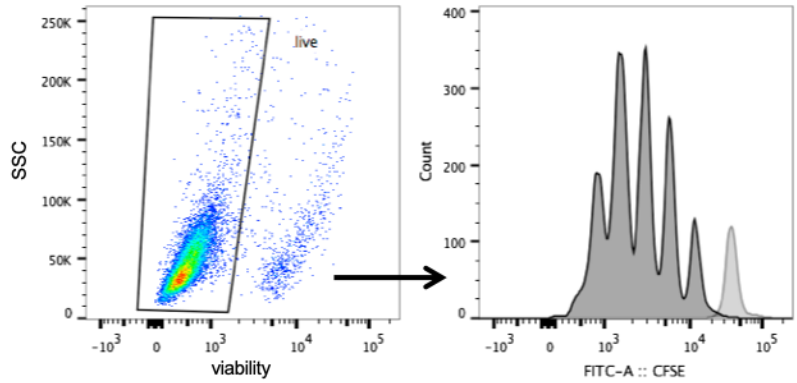

Figure 6: Gating strategy for flow cytometric analysis assessing in vitro splenic T cell survival and proliferation. CD4+ T cells were gated on live/dead-staining negative cells and frequency assessed. Then, CFSE signal intensity, percentage of T cells undergone cell divisions, and number of cell divisions were analyzed.

3.7.14 Cytokine detection in co-culture supernatants

To determine the cytokine levels present in the DC-T cell co-culture supernatants, an enzyme-linked immunosorbent assay (ELISA) was performed using a DuoSet ELISA-kit. The detection limits for IL-13 was 62.5 pg/ml and for IL-10 and IFNγ 31.2 pg/ml according manufacturer's suggestions. Briefly, ELISA plates were firstly coated with the appropriate capture antibody. Following this coating, plates were blocked with PBS/BSA (1%) to prevent unwanted binding of proteins of the sample. Next, the respective samples were given on the coated plate so that the immobilized antibodies would bind to the cytokine. After incubation, removal of the sample, and washing, a biotinylated detection antibody was added, followed by an incubation with an enzyme-linked streptavidin conjugate. After washing and incubation with a substrate, a colorimetric measurements of optical density (OD) at 450 nm using a spectrophotometer, OD which correlates with the content of proteins. The actual concentration of each cytokine measured was determined by comparing that OD to a standard curve measured in parallel and using known concentrations of cytokines.

3.7.15 T cell polarization

The T cell polarization experiments were performed using cells isolated from C57BL/6 wild type mice in Prof. Divanovic' lab at the Division of Immunobiology of Cincinnati Children's Hospital Medical Center in Cincinnati, Ohio, USA. Adult mice were sacrificed, the spleens removed, and single cell suspensions were prepared by smashing cells through a 100 µm filter using a syringe plunger and washing with 10 ml of complete RPMI medium. For magnetic cell selection of $CD4^+$ T cells, cells were handled as aforementioned. After separation, cells were additionally stained with CD62L MicroBeads and the $CD4^+CD62L^+$ cell fraction representing naïve T cells was separated. Then, 3×10^5 naïve T cells per ml medium were seeded into a pre-coated (Goat anti-hamster IgG) 48 wells plate, and naïve T cells were activated with 1 µg/ml anti-mouse CD3 and 0.5 µg/ml anti-mouse CD28. To induce T cell polarization, cells were further incubated with either Th1-, Th2-, or Th17-priming conditions. Thus, Th1 differentiation was achieved using 20 ng/ml recombinant mouse IL-12 and 0.5 µg/ml anti-mouse IL-4; Th2 differentiation using 10 ng/ml recombinant mouse IL-4 and 0.5 µg/ml anti-mouse IFNγ, and Th17 differentiation using 1 ng/ml of recombinant human TGFβ, 10 ng/ml recombinant mouse IL-6, 0.5 µg/ml anti-mouse IFNγ, and 0.5 µg/ml anti-mouse IL-4. Furthermore, leptin was added either in 250 ng/ml or in 500 ng/ml to assess its role in T cell differentiation, while a no leptin control was included for each polarization. Cells were incubated at 37 °C in polarizing conditions for 72 hours, then medium was removed and fresh T cell medium containing the polarization cytokines was added. After 48 hours, T cells were stimulated with 50 ng/ml PMA, 1 µg/ml Ionomycin, in presence of 10 µg/ml Brefeldin A for four hours. Cells were then removed from the wells and fixed and permeabilized using a fixation/permeabilization solution kit following the manufacturer's instructions before staining. T cells were stained with antibodies against TNF-α, IFNγ, IL-4, and IL-17 and then analyzed with a LSR Fortessa 2 flow cytometer.

3.7.16 Semi-quantitative PCR

After isolation, cells were lysed in TRIZOL and stored. Then, total RNA was extracted using the Qiagen RNeasy Mini Kit protocol, and isolated RNA was transcribed into complementary (c)DNA by reverse transcription (reverse transcriptase; Therma Scientific, Schwerte, Germany). Then, real-time semi-quantitative polymerase chain reaction was performed using 10 µL of Maxima SYBR Green qPCR Master Mix (Therma Scientific, Schwerte, Germany), plus 0.5 mM of each primer using a 96-well plate format. After preincubation at 95 °C for 10 minutes for denaturation, 40 cycles of amplification followed with 5 seconds 95 °C, annealing at the appropriate temperature for 10 seconds (see Table 4), and 11 seconds at 72 °C. To

confirm the specificity of the amplification products, their melting curve profiles were determined. Finally, their expression levels were normalized to β actin.

3.7.17 Statistical analysis

Results are usually expressed as mean ± SEM (standard error of the mean) if not stated otherwise. Normal distribution of the data was tested using the Kolmogorov-Smirnov and D'Agostino-Pearson tests, sometime after log transformation. When groups were normally distributed, statistical differences between two groups were analyzed by unpaired t test. Comparisons involving multiple groups were first analyzed by ANOVA followed by Tukey's test. When groups were not normally distributed, they were analyzed using a Mann-Whitney U (two groups), or an ANOVA on ranks followed by a Dunn's multiple comparison test (multiple groups). In case of comparison of two groups, asterisk (*) is used to indicate the p-value. In case of comparison of more than two groups, e.g. the PBS controls and the OVA treated groups within each diet group (e.g. in CD/PBS and HFD/PBS vs. CD/OVA and HFD/OVA), * indicates significant difference between the OVA-group and the respective PBS control (e.g. CD/OVA vs. CD/PBS) and the pound sign (#) indicates significant difference between the OVA-groups of both diets (e.g. CD/OVA vs. HFD/OVA). A p-value < 0.05 was considered statistically significant and marked with * or #, p-value < 0.01 was considered highly statistically significant and marked with ** or ## and in case of p < 0.001 with *** or ###. Correlation and regression analyses were performed using R version 3.2 and the packages ggplot2, dplyr, corrplot, and ggcorrplot.

4 Results

4.1 Metabolic phenotype

Female C57BL/6JRj wild type mice were randomly selected to be either fed with a high fat diet (HFD) or a chow diet (CD) as control. Both dietary regimens were designed for a period of 12 weeks and executed in parallel in the same animal facility. Weekly assessments of bodyweight development and food consumption were performed. The latter revealed no difference between the two diets (data not shown), however, the HFD mice reached a higher bodyweight during the feeding than CD fed mice (Figure 7 A). This weight increase was mirrored by an increase in visceral adipose tissue (VAT) mass as the weight of both perigonadal fat depots was increased in HFD mice as compared to CD mice (Figure 7 B). In addition, the frequency of macrophages, showing a high expression of F4/80 and CD11c, increased in VAT of HFD compared to CD mice (Figure 7 C).

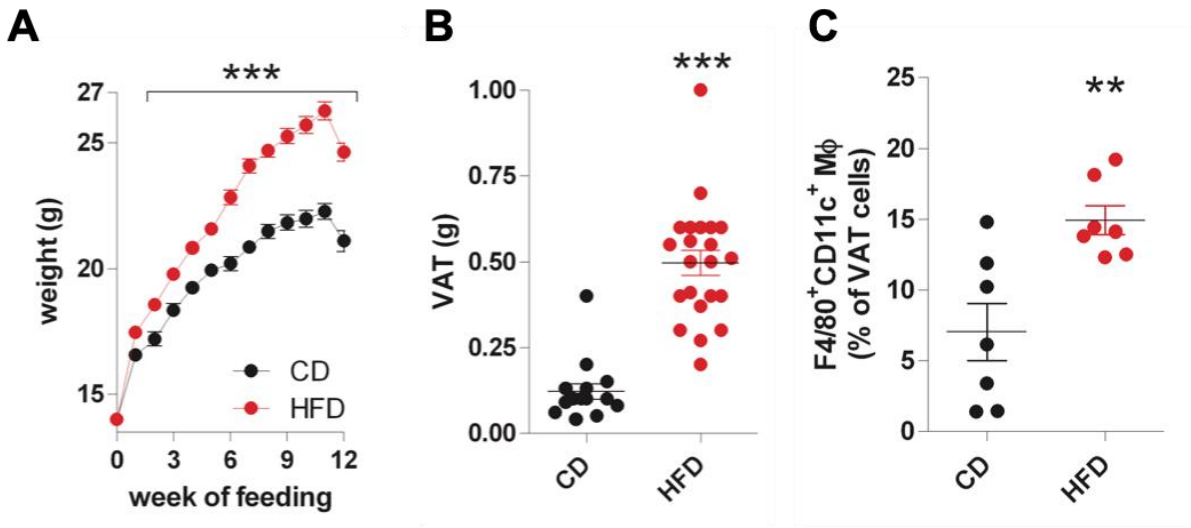

Figure 7: HFD feeding leads to mild bodyweight gain. (A) Bodyweight development in response to 12 weeks feeding with CD- (black circles) or HFD (red circles); n=28-36 out of 5 independent experiments. (B) Weight of perigonadal fat depots representing the visceral adipose tissue (VAT) after 12 weeks of either HFD or CD feeding; n=15-22 out of 5 independent experiments. (C) Frequency of $F4/80^{+}CD11c^{+}$ macrophages in VAT stromal vascular cells 12 weeks after HFD or CD feeding; n=7 out of 2 independent experiments. Values shown are the mean ± SEM. Asterisks show significant differences between HFD- and CD-treatment groups; ** $p < 0.01$; *** $p < 0.001$.

When the bodyweight development was normalized to the bodyweight at beginning of feeding of each mouse (100%), the observations were recapitulated with HFD fed mice

having a higher bodyweight increase compared to CD fed mice irrespective of the starting bodyweight (Figure 8).

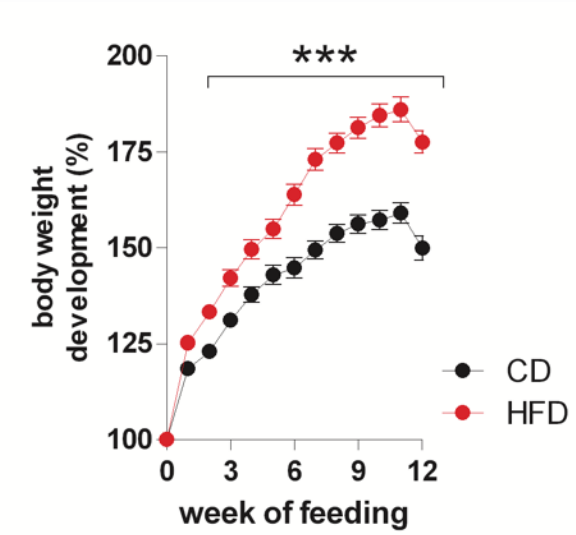

Figure 8: HFD feeding leads to a higher bodyweight development in relation to the beginning bodyweight. Bodyweight development normalized to starting bodyweight of each mouse upon a 12 weeks regimen using CD- (black circles) or HFD (red circles); n=28-36 out of 5 independent experiments. Values shown are the mean ± SEM. Asterisks show significant differences between HFD- and CD-treatment groups; *** $p < 0.001$.

As indicators of systemic metabolic inflammation, the serum leptin and insulin levels were measured in HFD and CD mice. Both parameters were markedly and statistically higher in HFD mice compared to CD controls (Figure 9 A, B). Furthermore, histologic examination of hepatic tissues revealed lipid accumulation in HFD but not CD mice (Figure 9 C).

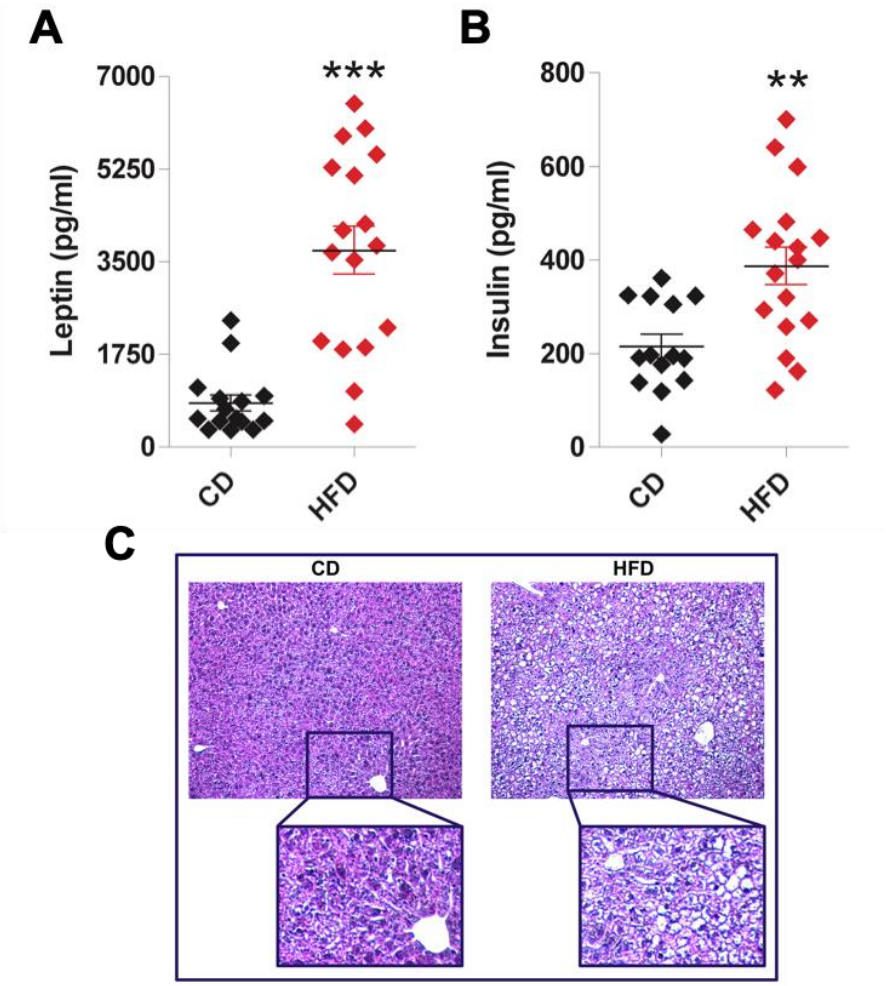

Figure 9: Mild bodyweight gain is associated with signs of systemic and local metabolic alterations. (A) Serum leptin and (B) serum insulin levels (not fasted) in 12 weeks HFD (red rhombus)- and CD fed (black rhombus) mice; n=15-17 out of 3 independent experiments. Values shown are the mean ± SEM. Asterisks show significant differences between HFD- and CD-treatment groups; ** $p < 0.01$; *** $p < 0.001$. (C) Representative pictures of H&E stained liver tissue (amplification 100x) of HFD (right) and CD (left) fed mice with respective digitally zoomed area. HFD mice showed lipid accumulation in contrast to CD mice.

To evaluate the impact on serum lipid profiles, serum cholesterol and triglycerides were measured as additional markers of metabolic disturbances. After 12 weeks of HFD feeding higher values for total cholesterol as well as the subfractions VLDL/LDL and HDL cholesterol compared to the CD controls were detected (Figure 10 A-C). However, values of serum triglycerides were not influenced by the different diet regimens (Figure 10 D).

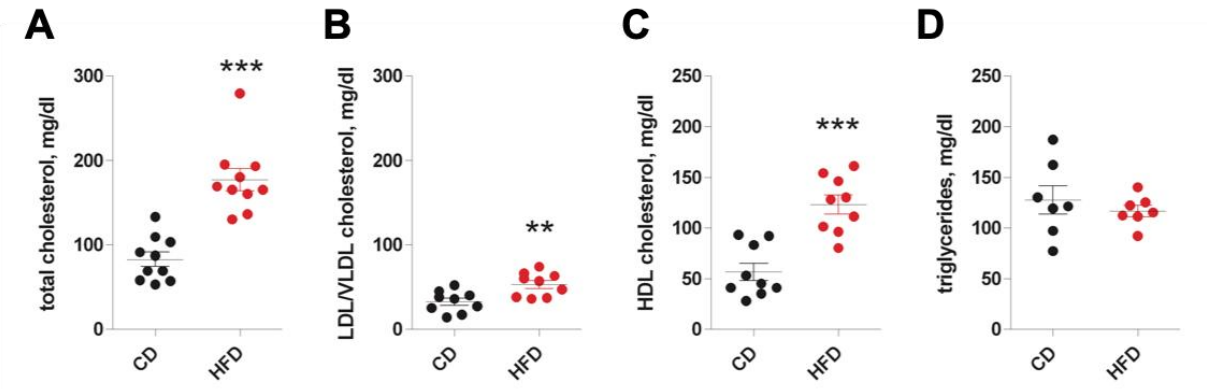

Figure 10: Mild bodyweight gain is associated with changes in serum lipids. (A) Serum total cholesterol, (B) LDL/VLDL-cholesterol, (C) HDL-cholesterol, and (D) serum triglycerides were analyzed after 12 weeks feeding with CD- (black circles) or HFD fed mice (red circles); n=7-10 out of 3 independent experiments. Values shown are the mean ± SEM. Asterisks show significant differences between HFD- and CD-treatment groups; ** $p < 0.01$; *** $p < 0.001$.

Finally, basic stool microbiome analyses were performed prior and after both feeding protocols. After feeding with either HFD or CD, the alpha diversity was different (Chao1, Shannon, Tukey's honesty significance difference of fitted model $p < 0.001$) compared to before the change in diet at age of 4 weeks. Furthermore, the alpha diversity was also different at the end of the feeding between the HFD and the CD (Figure 11 A), showing that HFD and CD feeding led to different microbial richness. Indeed, quantitative analysis of the most abundant gut microbiota phyla showed that HFD mice had increased abundance of Proteobacteria and decreased abundance of Firmicutes (Figure 11 B). In addition, analysis of beta diversity (Bray-Curtis dissimilarity) showed that the diversity of the microbiota tended to be different after HFD feeding (Figure 11 C). However, the comparison of the Firmicutes/Bacteroidetes ratio, a prominent marker for metabolic syndrome associated alterations of the microbiome, did not reveal a significant difference (Figure 11 D).

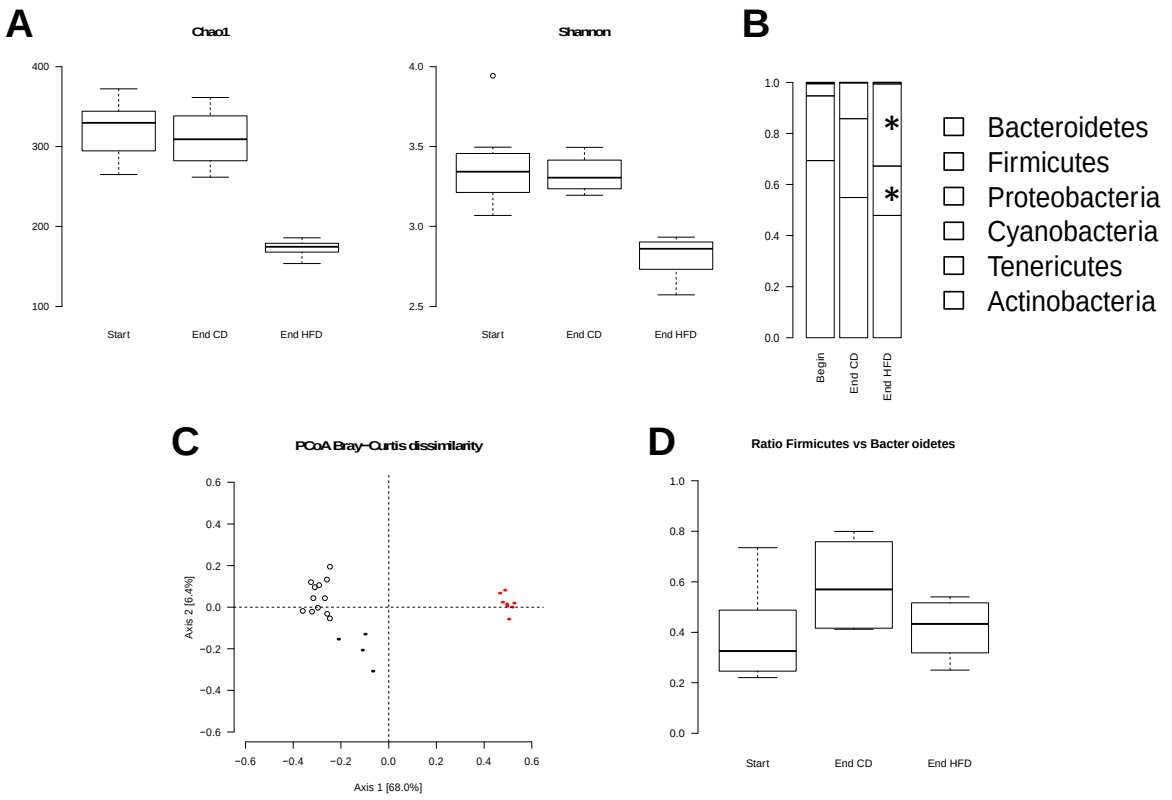

Figure 11: Microbiome analysis after 12 weeks feeding to either HFD or CD. (A) Alpha diversity showing diversity of microbial communities after HFD feeding (End HFD) as compared to after CD feeding (End CD) and to prior of both feedings (Start). (B) Analysis of phyla after HFD feeding (End HFD) as compared to after CD feeding (End CD) and of both feedings (Start). (C) Beta diversity showing species richness after HFD feeding (End HFD) as compared to after CD feeding (End CD) and to prior of feedings (Start). (D) Firmicutes-to-to-Bacteroidetes ratio after HFD feeding (End HFD) as compared to after CD feeding (End CD) and to prior of feedings (Start).* indicates statistical significance $p < 0.05$.

Taken together, my data showed that a 12 weeks HFD feeding induced a higher absolute and relative bodyweight. Moreover, it triggered an expansion of VAT mass, together with an increased numbers of F4/80^{+}CD11c^{+} adipose tissue macrophages, increased serum levels of leptin, insulin, and serum cholesterol, and, lastly, induced alterations of the gut microbiome. All these changes indicated that HFD as compared to CD feeding led to an increased bodyweight and additionally profoundly challenged the metabolic system on a multi-organ level.

4.2 Allergic asthma phenotype

After nine weeks of HFD and CD feeding, allergic asthma was induced using ovalbumin (OVA) as allergen. Thus, on days 0 and 7 mice were injected intra-peritoneally with OVA mixed to aluminum potassium sulfate (as immunological adjuvant), followed by four airway

challenges on days 14, 16, 18, and 20 (CD/OVA and HFD/OVA). After 24 hours, on day 21, pulmonary functions of mice were measured, and organs and body fluids collected to assess the asthma phenotype. The respective diet was continued during the whole sensitization/challenge process. In both diet groups, mice were treated with PBS as controls (CD/PBS and HFD/PBS). The hallmarks of allergic asthma used were airway hyperresponsiveness (AHR), airways inflammation, and mucus production.

For assessment of AHR, mice were intubated, connected to a Flexivent, and lung resistance was determined by measuring the pulmonary functions (elastance and compliance) against increasing dosages of methacholine (Figure 12 A). In both diets, PBS controls did not show an increase of lung resistance. In contrast, CD mice upon OVA treatment (CD/OVA) showed a clear increase in lung resistance, whereas, OVA-treatment in HFD mice (HFD/OVA) resulted in a significantly lower lung resistance compared to CD/OVA (Figure 12 A). To rule out that the different bodyweight of HFD versus CD mice may confound the AHR analysis, lung resistance against increasing dosages of methacholine was analyzed in relation to baseline values for each mouse. Such analysis confirmed the absence of difference in AHR between the HFD and CD PBS controls, and the significantly lower AHR in HFD/OVA compared to CD/OVA (Figure 12 B).

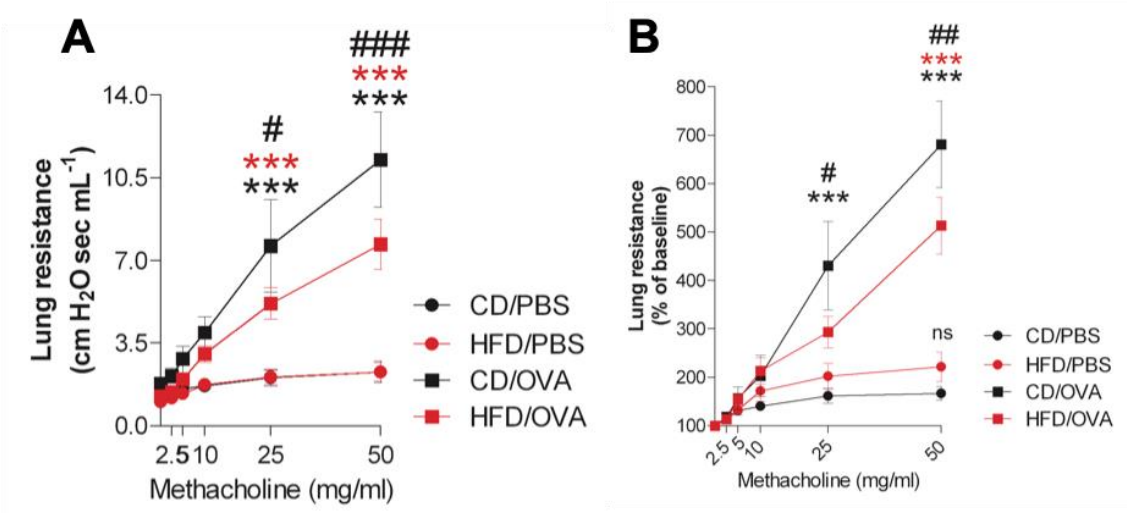

Figure 12: Mild bodyweight gain reduces AHR. (A) AHR in response to increasing dosages of methacholine in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black); (B) Methacholine-induced AHR in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black) in relation to baseline; n=8-16 out of 6 independent experiments. Values shown are the mean ± SEM, * show significant differences between PBS and OVA treatment groups, # indicates significant differences between CD and HFD OVA-treated groups; # or * indicates $p < 0.05$; ## or ** $p < 0.01$; ### or *** $p < 0.001$.

Airway inflammation is characterized by increased numbers of inflammatory cells in the bronchoalveolar space. To determine this infiltration, bronchoalveolar lavage fluid (BAL) was collected after AHR assessment and cell numbers were enumerated manually using a hemocytometer after trypan blue staining. The two PBS control groups, CD/PBS and HFD/PBS did not show increased BAL cell numbers, while both OVA groups, CD/OVA and HFD/OVA, had significantly higher BAL cell numbers than the respective PBS controls. This increase was notably lower in HFD/OVA compared to CD/OVA (Figure 13).

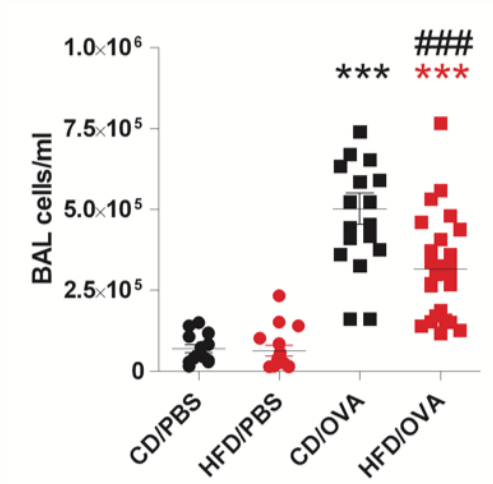

Figure 13: Mild bodyweight gain reduces airway inflammation. Total number of BAL cells in PBS or OVA-treated mice fed with either HFD or CD; n=11-17 out of 3 independent experiments. Values shown are the mean ± SEM. Asterisks indicate significant differences between the PBS and OVA treatment groups; the pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

In addition, mucus production, was assessed by Periodic Acid Schiff (PAS) staining and manual enumeration of PAS-positive airways. While CD- as well as HFD-mice had no PAS-positive airways after PBS treatment, both OVA groups showed around 60% mucus-positive airways in response to OVA challenge with no statistical difference between each other (Figure 14).

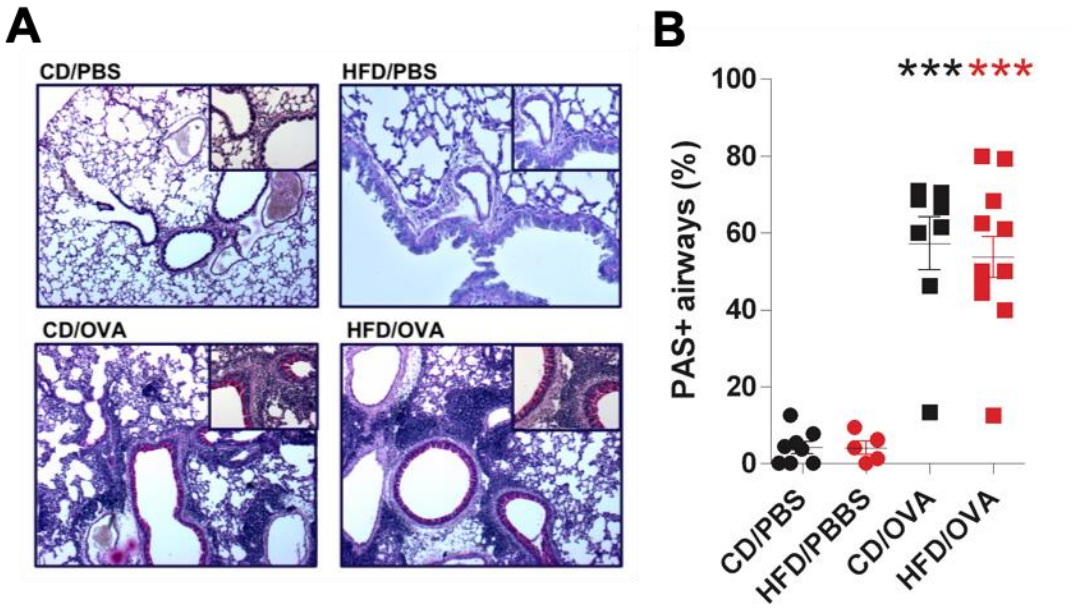

Figure 14: Mild bodyweight gain does not alter mucus production. (A) Representative pictures of histologic examination (PAS staining; 100x) of lung tissue from CD/PBS, CD/OVA, HFD/PBS, and HFD/OVA mice including digitally zoomed areas (upper right). (B) Frequency of PAS-positive airways in the different treatment groups, all airways within a histologic sample were considered; n=5-11 from 3 independent experiments. Values shown are the mean ± SEM, *** indicates $p < 0.001$.

Taken together, my data suggested that the HFD feeding impacts on the development of experimental allergic asthma leading to a reduction in AHR and airway cell number in HFD/OVA mice compared to CD/OVA mice.

4.3 Characterization of airways immune cells

In both OVA groups, CD/OVA and HFD/OVA, BAL cells numbers increased suggesting an influx of immune cells. To further characterize these cells, flow cytometric analysis was performed using antibodies against Siglec F, CD4 and Ly6G and following the gating strategy mentioned in the methods section 3.7.11.3 (Figure 3). Upon OVA treatment, I observed a clear influx of eosinophils, neutrophils, and $CD4^+$ T cells, which were absent in PBS-treated control mice (Figure 15 A, C, D), while the numbers of macrophages were not affected by feeding nor asthma induction (Figure 15 B). Whereas the numbers of eosinophils were not different between CD/OVA and HFD/OVA, the neutrophils and $CD4^+$ T cell numbers were significantly lower in HFD/OVA compared to CD/OVA, suggesting that a lower influx of neutrophils and $CD4^+$ T cells into the airways took place after allergy induction and HFD feeding.

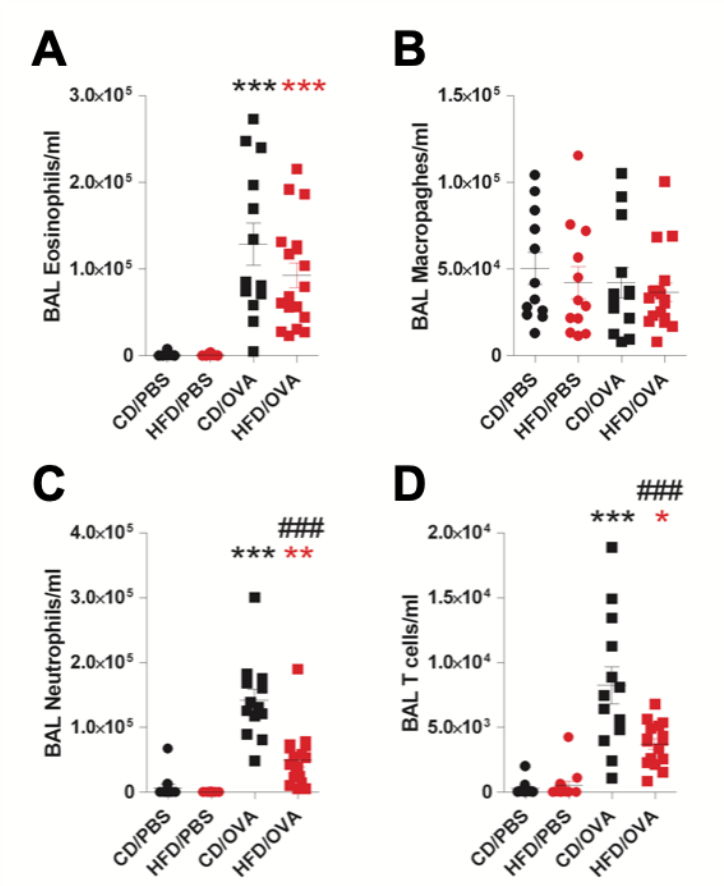

Figure 15: Mild bodyweight gain reduces numbers of neutrophils and CD4+ T cell in the airways. Numbers of (A) eosinophils, (B) macrophages, (C) neutrophils, and (D) $CD4^+$ T cells in BAL of mice fed with either HFD or CD; n=11-17 out of 6 independent experiments. Values shown are the mean ± SEM. * indicate significant differences between the PBS and OVA treatment groups; # indicates significant differences between CD and HFD OVA-treated groups. * or # $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

4.4 Alternative high fat diet feeding

To rule out that the observed impact of HFD feeding on the OVA-induced allergic asthma phenotype was diet-specific, an alternate HFD feeding was used. Therefore, a diet containing 20% more calories derived from fat and 20% less calories from carbohydrates (HFD60) was used and compared to CD fed mice as controls. In other respects, the protocol remains unchanged. Not only HFD60 contained a higher amount of total energy originating from fat, but also the fat type was different with less cholesterol and palmitic acid but more lauric and palmitoleic acids (Table 1). After feeding, HFD60 mice displayed an increased bodyweight gain compared to CD control mice (Figure 16 A). This bodyweight development was similar to the standard HFD40 diet (maximum weight 26.3 in HFD vs. 22.3 in CD, Figure 7 A). Moreover, the mice under HFD60 feeding showed a significantly lower lung resistance and less airway cells after OVA-treatment than the mice fed with CD as controls (Figure 16 B, C), similar to what was observed with the HFD40 feeding (Figure 7).

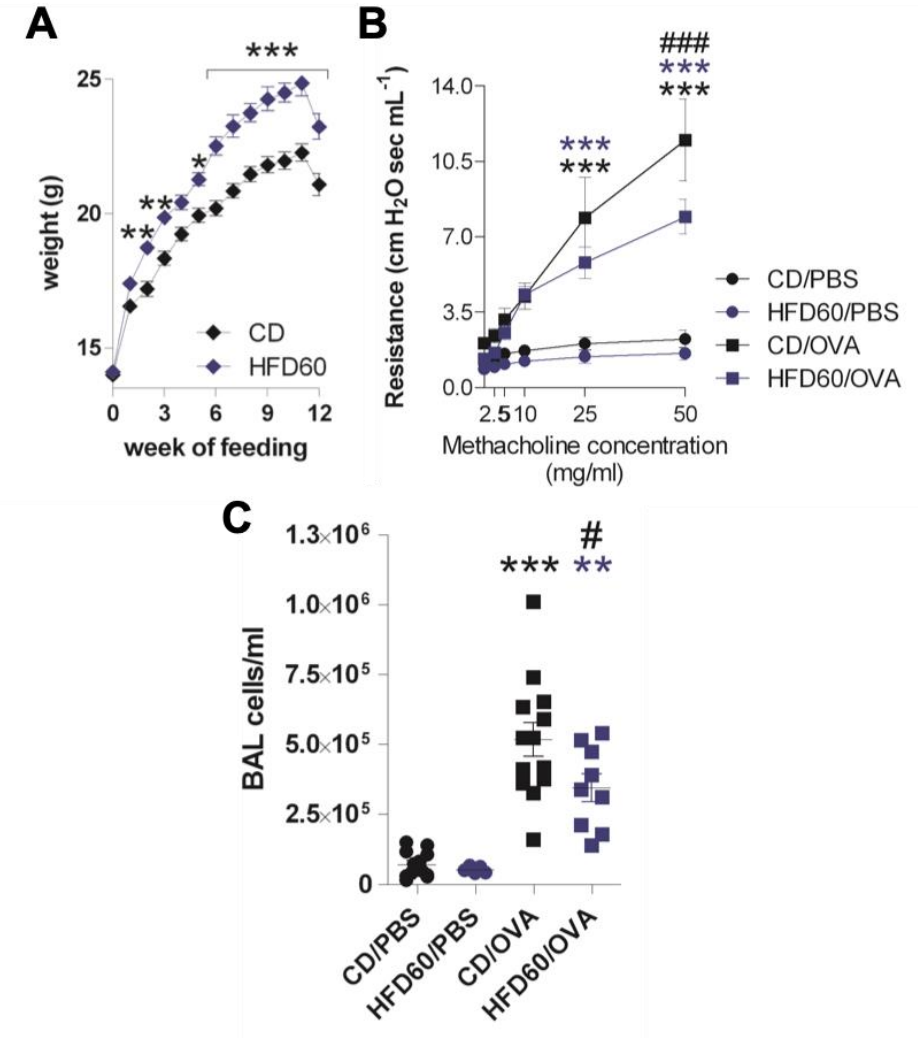

Figure 16: Alternate HFD60 feeding recapitulates mild bodyweight gain and decrease in AHR and airways inflammation. (A) Bodyweight development in response to 12 weeks feeding with CD- (black rhombus) or alternate HFD60 (blue rhombus circles). (B) AHR in response to increasing dosages of methacholine in PBS (circles) or OVA-treated (squares) mice fed with either HFD60 (blue) or CD (black). (C) Total number of BAL cells in PBS or OVA-treated mice fed with either HFD60 or CD. Values shown are the mean ± SEM, n=5-13 out of 2 independent experiments. * shows significant differences between PBS and OVA treatment groups; # indicates significant differences between CD and HFD OVA-treated groups. * or # indicate $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

To further characterize the airway cells in HFD60 as compared to CD, a flow cytometric analysis was performed using the same antibodies against Siglec F, CD4, and Ly6G to identify BAL eosinophils, macrophages, neutrophils, and T cells. Macrophages were equally allocated in all diet and treatment groups, and the numbers of eosinophils, neutrophils, and T cells were increased upon OVA treatment compared to PBS. Furthermore, neutrophils as well as $CD4^+$ T cells were significantly lower in HFD60/OVA compared to CD/OVA (Figure 17).

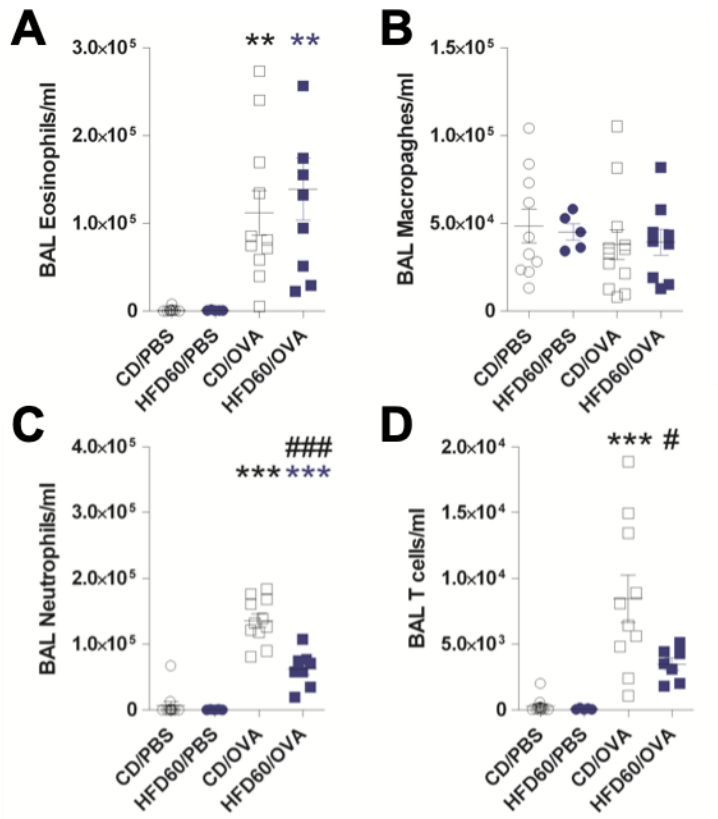

Figure 17: Alternate HFD60 feeding also reduced numbers of neutrophils and CD4+ T cell in the airways. Numbers of (A) eosinophils, (B) macrophages, (C) neutrophils, and (D) $CD4^+$ T cells in BAL of mice fed with either HFD (blue) or CD (white), treated with either PBS (circles) or OVA (squares); n=11-17 out of 6 independent experiments. Values shown are the mean ± SEM. Asterisks indicate significant differences between the PBS and OVA treatment groups; the pound sign indicates significant differences between CD and HFD OVA-treated groups. * or # $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

However, these changes, as a consequence of HFD60 feeding in combination with an OVA-induced allergic asthma, were again in the range of what I observed using the HFD40 diet. Indeed, the average numbers of BAL eosinophils, macrophages, neutrophils, T cells in HFD60/OVA as compared to HFD/OVA did not differ (Table 10). This indicated that the immune cell influx into the airways in HFD60/OVA was similar to HFD/OVA.

	HFD40	HFD60	p-value
Eosinophils	92,758 ± 14,223	139,097 ± 35,272	0.1572
Macrophages	36,620 ± 5,298	39,175 ± 7,359	0.7818
Neutrophils	49,534 ± 10,614	62,546 ± 9.510	0.449
T cells	3.727 ± 408	3.479 ± 475	0.7252

Table 10. BAL cell numbers after HFD and HFD60 feeding. The numbers of eosinophils, macrophages, neutrophils, and T cells were displayed after HFD (HFD40) and HFD60 feeding as cells per ml. Values shown are the mean ± SEM. The right column shows the p-values of the differences of the means are displayed.

Taken together, my observations, using HFD60 as alternate feeding, indicated that the impact of the diet in the context of development of OVA-driven experimental allergic asthma did not depend on the source of the fat and the overall calories.

4.5 Characterization of pulmonary immune cells

Interestingly, histological examination of lung slices revealed a strong inflammatory cell infiltrate into the lung tissue upon OVA-induced allergic asthma in both diet groups (Figure 18 A). However, no difference between the groups could be determined by simple visual examination. Therefore, lung tissue digestion for immune cell isolation was performed, and cells were manually enumerated in a counting chamber. Both, CD- and HFD fed PBS-control mice had around $1x10^7$ cells in lungs, whereas OVA-treated mice of both diet groups had around $3x10^7$ cells per right lung lobe (Figure 18 B). However, no difference was detected between CD/OVA and HFD/OVA.

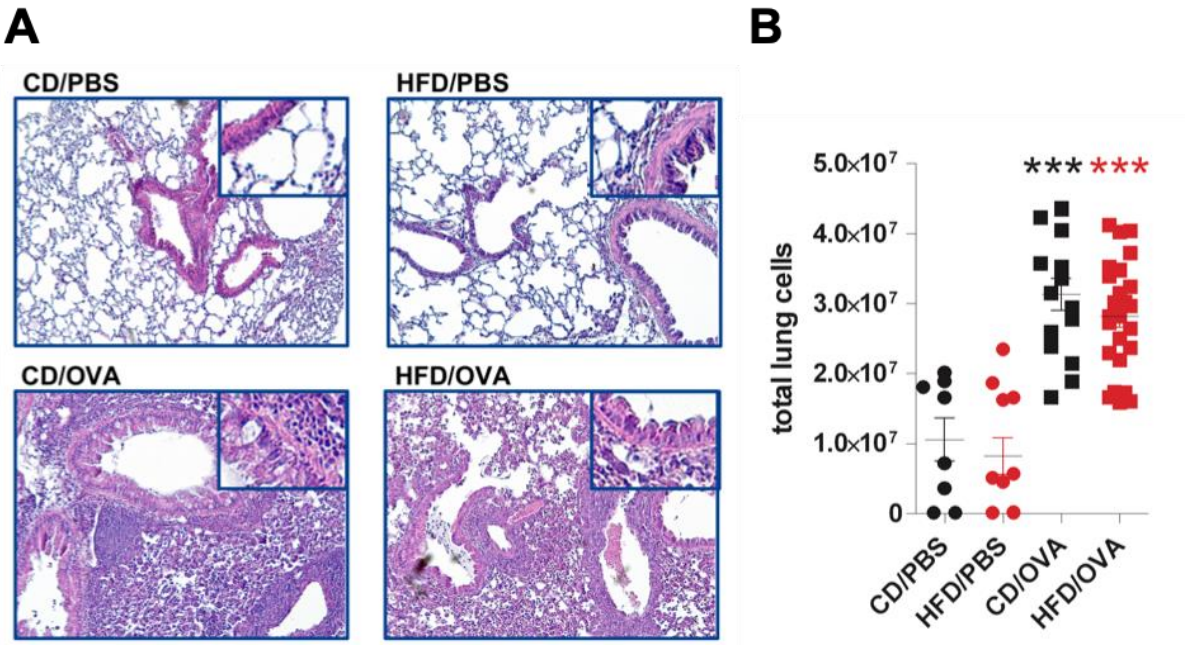

Figure 18: Asthmatic mice of both diet groups exhibit recruitment of inflammatory cell into the lung tissue. (A) Representative pictures of histologic examination (H&E staining; 100x) of lung tissue from CD/PBS, CD/OVA, HFD/PBS, and HFD/OVA mice including digitally zoomed areas (upper right). (B) Counted numbers of isolated lung cells after (right lobe) feeding and asthma induction. Values shown are the mean ± SEM, n=8-16 out of 6 independent experiments. *** indicates significant differences $p < 0.001$.

To further define the cellular composition of the infiltrate, flow cytometry was used. To identify neutrophils, eosinophils, and alveolar macrophages, antibodies against Siglec F, CD11c, and Ly6G were used. Furthermore, to identify dendritic cells (DCs), antibodies against CD11c, MHCII, CD11c, CD103, and CD64 were used in combination with a lineage cell staining. Finally, pulmonary T cell were identified using antibodies against CD3, CD4, CD44, and CD62L. The gating strategies are displayed in the methods section.

A significant increase in lung neutrophils, eosinophils, and alveolar macrophages upon OVA-treatment in both diets was observed compared to the CD controls (Figure 19), although, no diet-related difference could be detected.

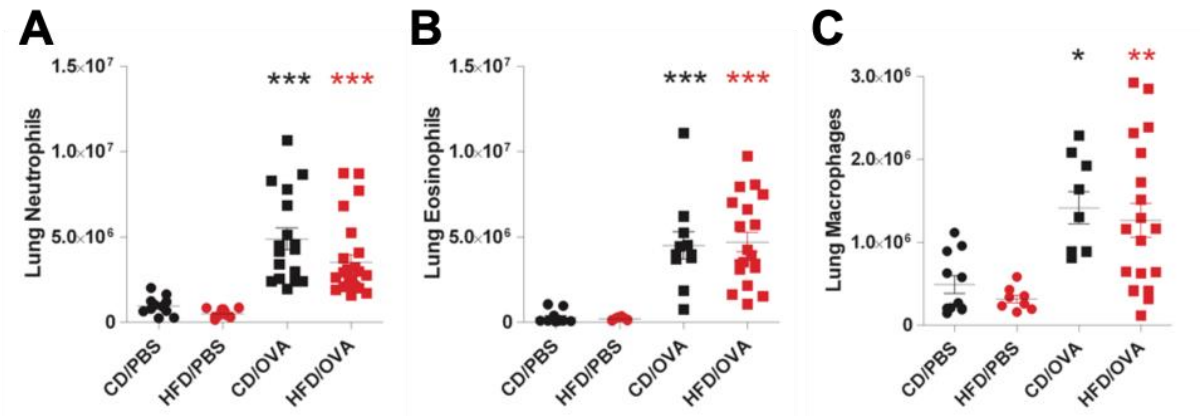

Figure 19: Mild bodyweight gain does not alter the number of lung neutrophils, eosinophils, or alveolar macrophages in experimental asthma. Flow cytometric analysis of digested lung tissue to identify (A) lung neutrophils, (B) lung eosinophils, and (V) lung alveolar macrophages. Values shown are the mean ± SEM, Asterisks show significant differences between PBS and OVA treatment groups; * indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Furthermore, the analysis of lineage$^-$CD11c$^+$MHCII$^+$ lung DCs revealed that DCs were recruited to the lungs upon OVA in both diet groups, CD/OVA and HFD/OVA (Figure 20 A). However, significantly lower numbers of DCs were recruited to the lungs of HFD/OVA mice compared to the CD/OVA. Moreover, using CD11b, CD103 and CD64 as discriminative surface markers to distinguish CD11b$^+$ and CD103$^+$ conventional DCs (cDCs) and CD64$^+$ monocyte-derived DCs (moDCs), I observed that these three lung DC subtypes were increased upon OVA treatment but significantly lower in HFD/OVA as compared to CD/OVA counterparts (Figure 20 B-D).

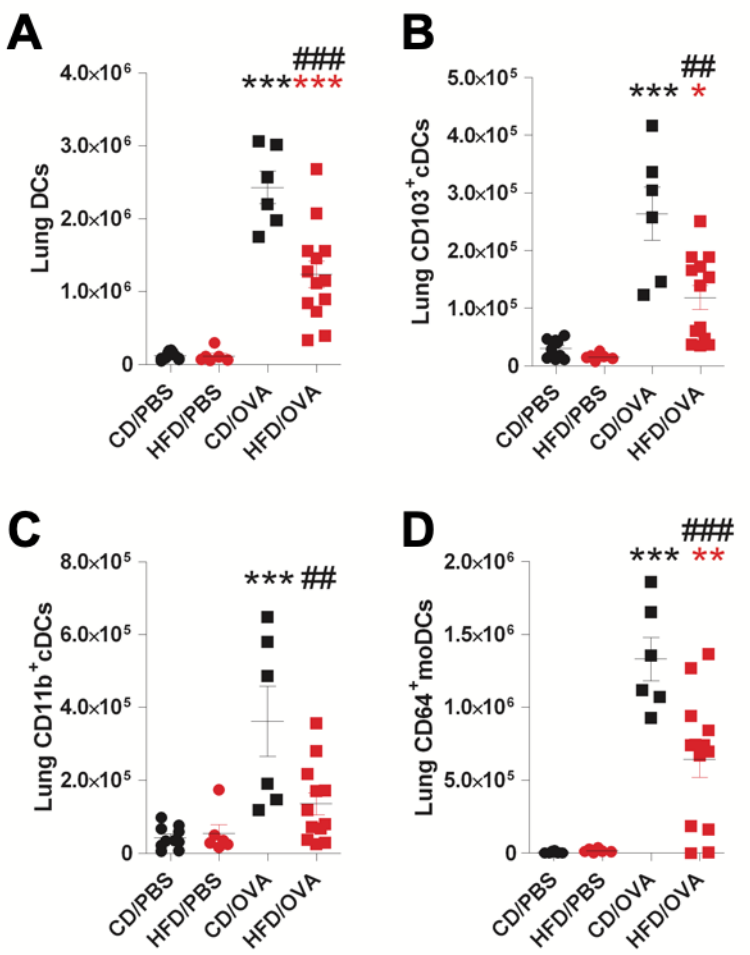

Figure 20: Mild bodyweight gain is associated with decreased numbers of DCs in the lungs of OVA-treated mice. (A) Total numbers of pulmonary CD11c$^+$MHCII$^+$ DCs. Numbers of (B) CD103$^+$ conventional (c)DCs, (C) CD11b$^+$ cDCs, and (D) CD11b+CD64$^+$ monocyte-derived (mo)DCs. Values shown are the mean ± SEM, Asterisks show significant differences between PBS and OVA treatment groups; Pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # indicate $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

Since the type 2 inflammation originates from the release of type 2 cytokines by Th2 T cells, I analyzed the numbers of pulmonary CD4$^+$ T cells, distinguishing naïve and effector CD4$^+$ T cells based on their respective CD62L and CD44 expression. Although in CD and HFD mice CD4$^+$ T cells increased upon OVA treatment, the numbers of T cells were significantly reduced in HFD/OVA as compared to CD/OVA (Figure 21 A). Furthermore, The frequencies of effector and naïve T cells in the CD4$^+$ T cell population revealed that in PBS-control situation the CD4$^+$ T cells were predominantly CD62L$^+$ naïve T cells, while in OVA-driven allergic state predominantly CD44$^+$ effector T cells (Figure 21 B, C). However, the frequencies were not different between CD/PBS and HFD/PBS as well as CD/OVA and HFD/OVA (Figure 21 B, C).

Taken together, my data suggested that such decreased numbers of lungs DCs and $CD4^+$ T cells account for the attenuated allergic asthma phenotype in HFD mice.

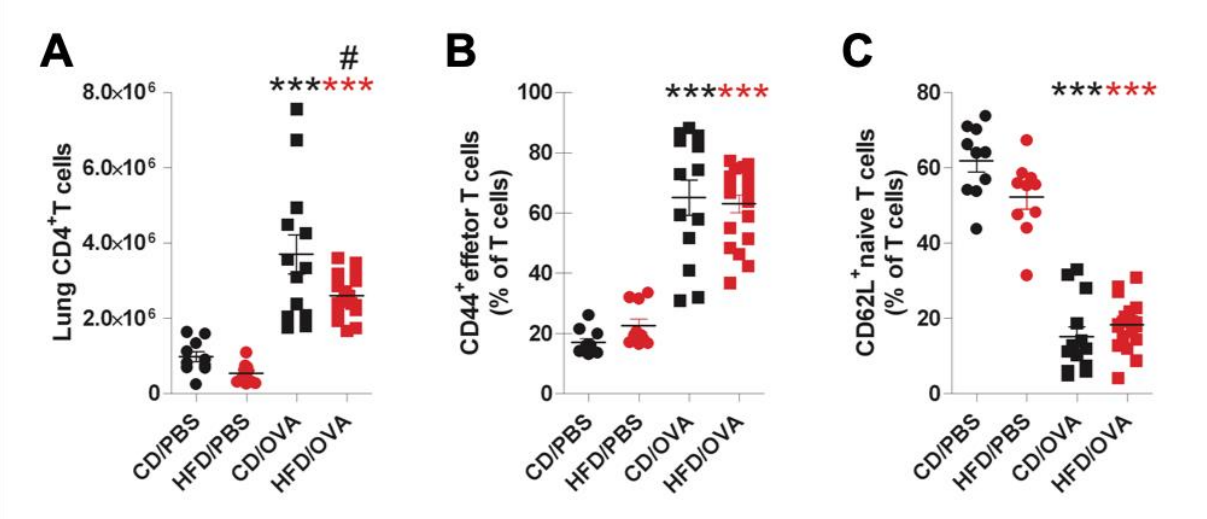

Figure 21: Mild bodyweight gain is associated with decreased numbers of CD4+ T cells in the lungs of OVA-treated mice. Numbers of (A) $CD3^+CD4^+$ T cells and frequency of (B) $CD3^+CD4^+CD44^+$ effector T cells and (C) $CD3^+CD4^+CD44^-CD62L^+$ naïve T cells in PBS or OVA-treated CD/OVA and HFD/OVA mice. Values shown are the mean ± SEM, Asterisks show significant differences between PBS and OVA treatment groups; Pound sign indicates significant differences between CD and HFD OVA-treated groups. * or # indicate $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

4.6 Correlation analysis of most relevant parameters

To follow a broader statistical approach with most relevant parameters of all mice, a statistical description was performed. For this, final bodyweight, VAT weight, BMI, serum leptin, serum insulin, and VAT-macrophage frequency were chosen as metabolic parameters, while BAL cell number, BAL neutrophils and T cells as well as lung DCs and T cells were taken as immunological parameters. The summary of these values is reviewed in Table 11 and plotted against each other in Figure 22.

	body weight (g)	weight VAT (g)	BMI (100g/cm2)	leptin (pg/ml)	insulin (pg/ml)	Macro-phages (% of VAT cells)	AHR (Resis-tance 50 mg/ml MC)	BAL cells/ml	BAL neutro-phils/ml	BAL Tcells/ml	number of Lg-DCs	number of Lg-Tcells
Min.	17,4	0,05	20,12	319,9	27,8	1,4	0,9967	14286	0	0	53760	35964
1st Qu.	20,65	0,115	22,47	533,2	189,5	7,17	2,2357	82309	169,6	113,8	104310	612352
Median	23,65	0,4	24,34	1483,8	320,1	12,4	5,3088	210417	17174,3	1989,4	335580	1089000
Mean	22,96	0,3589	24,86	2272	315,4	10,98	5,9625	290622	39123,7	3113,4	822177	1427727
3rd Qu.	24,9	0,55	27,42	3987,1	426,9	14,32	8,7603	410161	56190,8	4054,3	1365630	2382750
Max.	28,8	1	31,24	6485,3	700,5	19,2	19,3143	1457944	255840	24648	2786400	5751000

Table 11: Summary of most relevant parameters evaluated for correlation. Minimum (Min), maximum (Max), median, mean, first (1st Qu.), and third quartiles (3rd Qu.) are presented for bodyweight, VAT weight, BMI, leptin, insulin, macrophages in VAT, BAL cells, BAL neutrophils, BAL T cells, AHR (lung resistance at 50 mg/ml methacholine), lung DCs, and lung T cells.

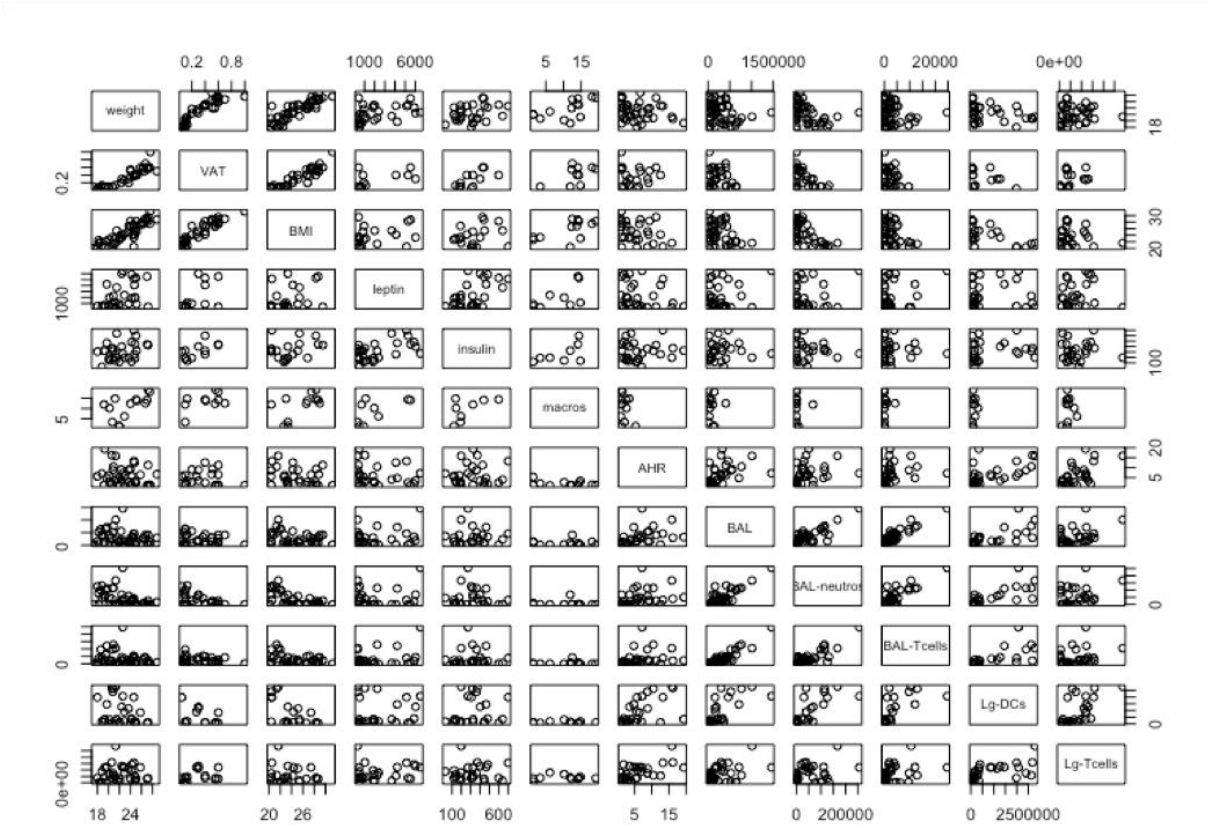

Figure 22: Plots of most relevant factors. As most relevant factors bodyweight (weight, 1st row and column), VAT weight (VAT, 2nd row and column), BMI (3rd row and column), leptin (4th row and column), insulin (5th row and column), macrophages in VAT (macros, 6th tow and column), AHR (lung resistance at 50 mg/ml methacholine, 7th row and column), BAL cells (BAL, 8th row and column), BAL neutrophils (BAL-neutros, 9th row and column), BAL T cells (10th row and column), lung DCs (Lg-DCs, 11th row and column), and lung T cells (Lg-Tcells, 12th row and column) were selected. The single graphs show the plots of all the parameters of the respective row and column against each other. For identification, the parameters are indicated in the diagonal array.

The summary of the metabolic and immunological parameters described the range and distribution of these values. Plotting theses values suggested linear correlation between some of these parameters, e.g. weight and VAT or weight and BMI. To statistically validate a correlation between metabolic and immunological parameters, I used a spearman correlation analysis. The results were plotted as a correlation matrix using color to indicate the correlation coefficient and * to indicate the p-value of this correlation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Figure 23).

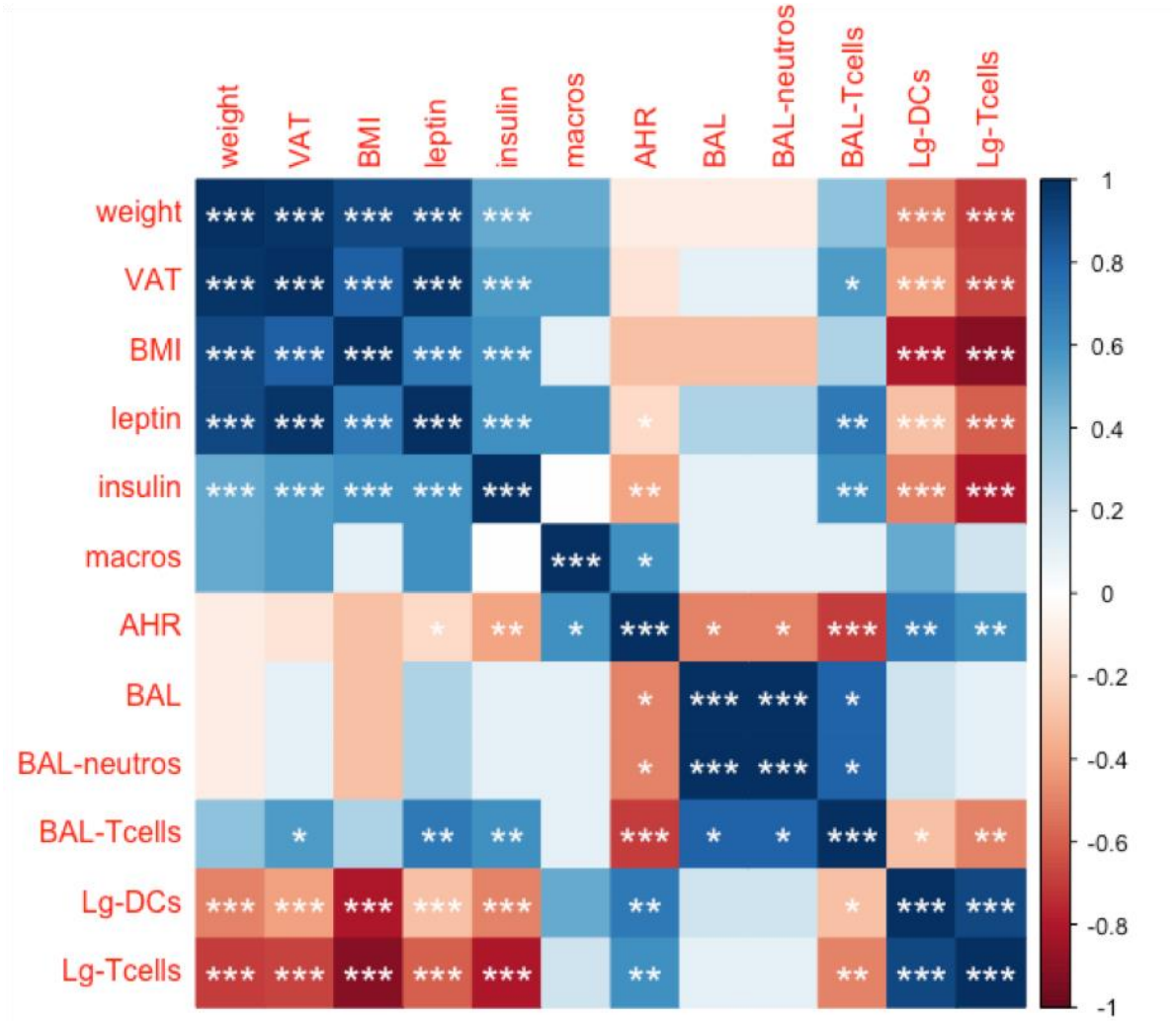

Figure 23: Correlation matrix with most relevant parameters. Most relevant parameters: bodyweight, VAT weight, BMI, leptin, insulin, macrophages in VAT, BAL cells, BAL neutrophils, BAL T cells, AHR (lung resistance at 50 mg/ml methacholine), lung DCs, and lung T cells including p-value (* indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to spearman correlation analysis, the color indicates correlation coefficient from -1 (dark blue) to +1 (dark red).

The correlation matrix revealed that, i) the metabolic parameters bodyweight, VAT weight, BMI, serum leptin, and serum insulin were highly positively correlated to each other and, ii) they negatively correlated to lung DCs and T cell numbers, whereas VAT weight, serum

leptin, and serum insulin were less strongly positively correlated to BAL T cells. In contrast, AHR (lung resistance at 50 mg/ml methacholine) was negatively correlated to leptin and insulin as well as BAL cell number, in particular neutrophils and T cells. Furthermore, AHR was positively correlated to DCs and T cells in lung tissue, while, T cells in BAL and lung tissue were negatively correlated to each other, and lung T cells were negatively associated to lung DCs.

Collectively, the correlation data indicate that changes of metabolic parameters altogether go into the same direction and are predominantly negatively associated with immunological asthma outcome parameters. Furthermore, AHR, a central asthma readout, was more strongly correlated to BAL T cells, which itself was strongly correlating with lung T cells. Lung T cells as well as lung DCs were highly correlated to each other.

To dig deeper into this issue, I plotted the most promising values against AHR and performed a simple linear regression model (Figure 24).

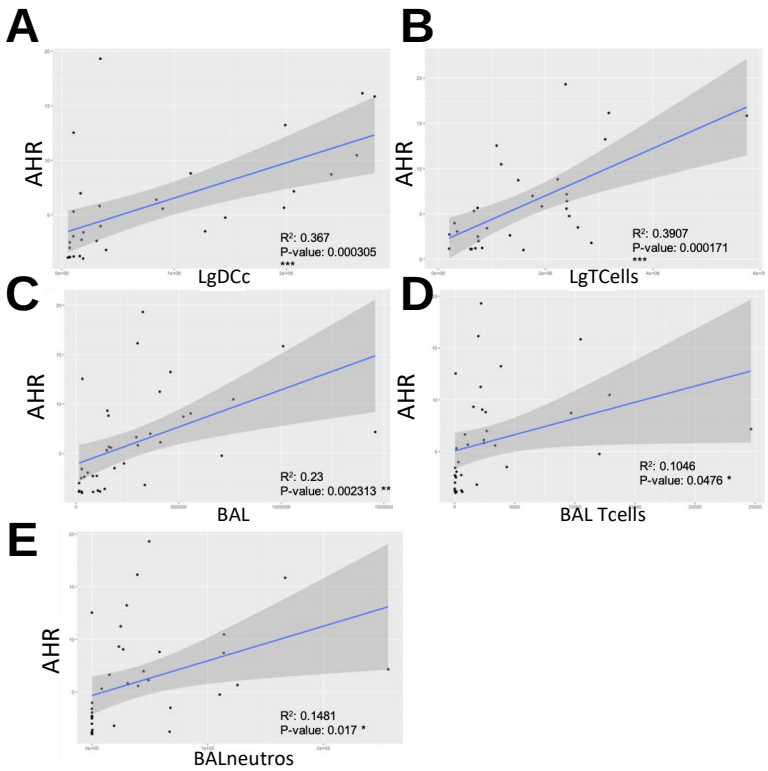

Figure 24: Regression analysis. Linear regression models were performed with AHR (lung resistance at 50 mg/ml methacholine) and the variables (A) lung (Lg) DCs, (B) lung T cells, (C) BAL cell number, (D) BAL T cells, (E) and BAL neutrophils to. The blue line indicates the fitted linear regression, R^2 indicates percentage of the response variable variation that is explained by a linear model. The p-value is indicated in the right corner at the bottom of each graph (* indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

The parameters analyzed by regression analysis recapitulated the pictures of the correlation analysis. The most suitable parameters to predict AHR were lung DCs and T cells and BAL cell number, followed by BAL T cells and neutrophils (Figure 24).

Taken together, these findings suggest that pulmonary DCs and T cells are major drivers of decreasing AHR and airway inflammation in short-term HFD feeding.

4.7 Dendritic cell function

The changes in DC and T cell numbers in HFD/OVA as compared to CD/OVA raised the question whether these changes were involved in the reduction of the allergic asthma severity in HFD/OVA mice. Central DC functions are migration from the site of inflammation to the lymph node, allergen procession and presentation as peptide loaded to MHCII complexes, resulting in proliferation and differentiation of $CD4^+$ T cells toward the Th2 subtype. During the effector phase of the disease, reactivation of Th2 clones occurs mainly in the lung tissue rather than in local mediastinal lymph nodes (mLN). It has been reported that during the sensitization phase, $CD11b^+$ cDCs are the main DC population to home to the mLN (Plantinga et al., 2013), therefore the presence of $CD11b^+$ cDCs in mLN was analyzed. Indeed, I observed the presence of $CD11b^+$ cDCs in the mLN of both OVA groups, although, no difference between the CD/OVA and HFD/OVA could be observed (Figure 25).

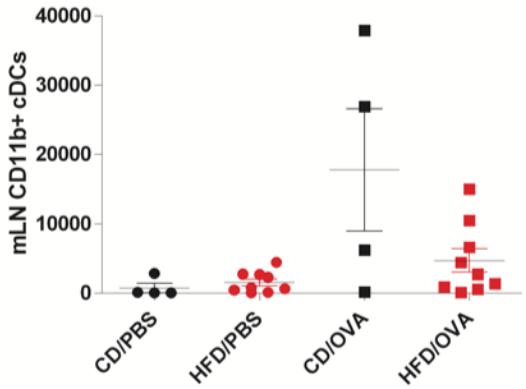

Figure 25: Local mediastinal lymph nodes. CD11b+ cDCs in local mediastinal lymph nodes (mLN) in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black); n=4-9 out of 2 independent experiments. Values shown are the mean ± SEM.

Next, to evaluate which basal DC function might be altered in my model, I screened several DC functions, starting with an analysis of DC-relevant molecules expression, in particular the mLN homing receptor CCR7 and CD40 and MHCII, both molecules relevant for antigen presentation. Analysis of chemokine receptor CCR7 expression in cDCs and moDCs

revealed no difference between PBS and OVA in both diet groups (Figure 26), indicating that DC migration capacity was not affected by HFD feeding or OVA treatment.

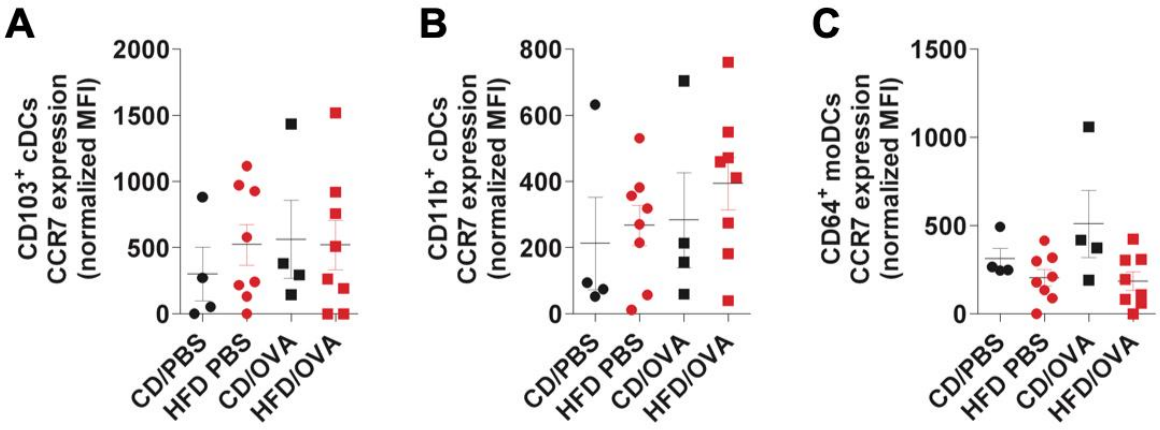

Figure 26: CCR7 expression. Mild bodyweight gain does not lead to changes in CCR7 expression in lung DC subtypes. (A-C) CCR7 surface expression presented as mean fluorescence intensity (MFI) in (A) $CD103^+$ cDCs, (B) $CD11b^+$ cDCs, and (C) $CD64^+$ moDCs. Values shown are the mean ± SEM, n=4-8 out of 2 independent experiments.

To further screen DCs' ability to present allergens, the expression of MHCII (Figure 27 A-C) and CD40 (Figure 27 D-F) was assessed in all DC subtypes by flow cytometry. This analysis revealed that MHCII surface expression was similar between all groups in $CD103^+$ cDCs and $CD64^+$ moDCs (Figure 27 A, C). In contrast, the expression of MHCII in $CD11b^+$ cDCs was significantly reduced in HFD/OVA compared to CD/OVA mice (Figure 27 B).

Besides of MHCII, allergen-driven activation of T cells by DCs depends on the expression of co-stimulatory molecules, such as CD40 (Ma and Clark, 2010). The expression analysis of CD40 showed that in all three DC subtypes CD40 expression increased upon OVA treatment, irrespective of the diet (Figure 27 D-F), but again, the CD40 expression at the surface of $CD11b^+$ cDCs was significantly lower in HFD/OVA compared to CD/OVA (Figure 27 E).

These findings suggested that in HFD/OVA mice as compared to CD/OVA mice, the $CD11b^+$ cDCs' abilities to present OVA peptide was altered and impacted the development of the Th2 immune response.

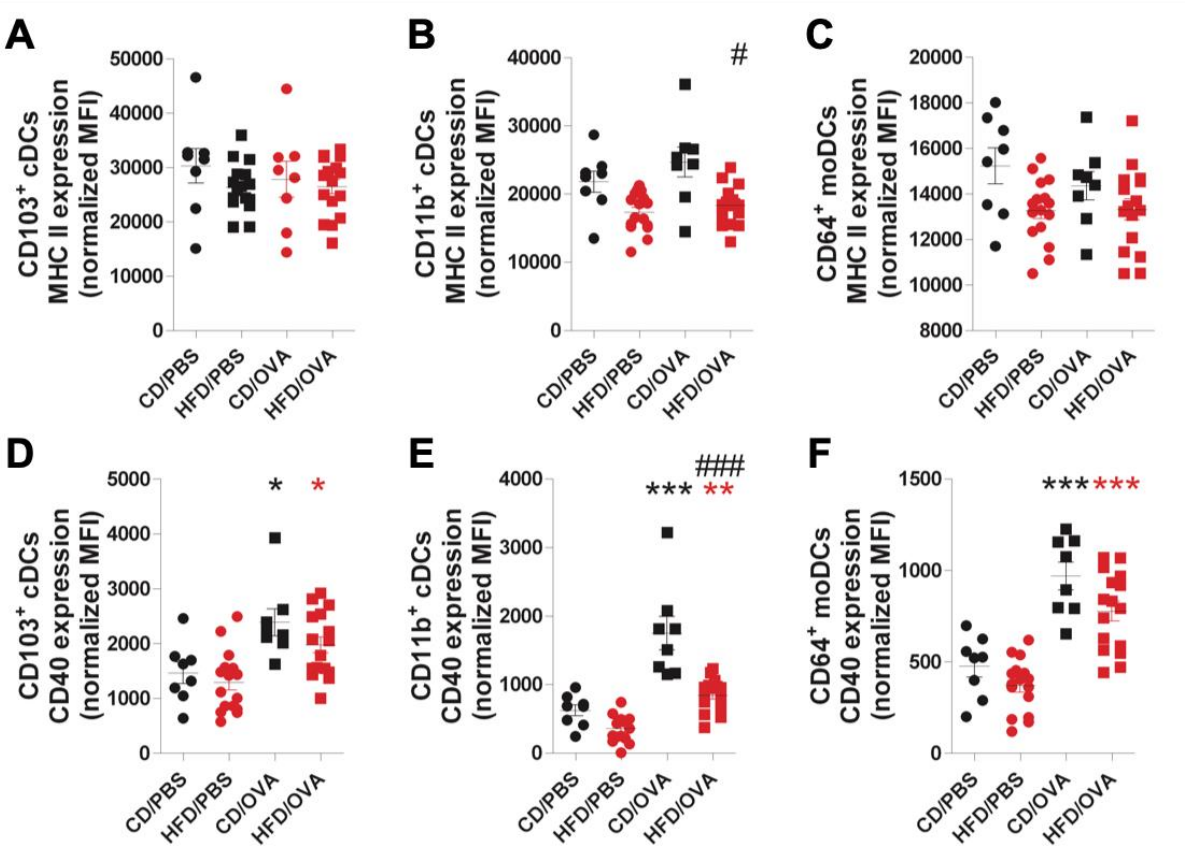

Figure 27: MHCII and CD40 expression. Mild bodyweight gain induces changes in MHCII and CD40 expression in lung DC subtypes. MHCII (A-C) and CD40 (D-F) surface expression presented as mean fluorescence intensity (MFI) in (A,D) CD103$^+$ cDCs, (B,E) CD11b$^+$ cDCs, and (C,F) CD64$^+$ moDCs. Values shown are the mean ± SEM, n=8-16 out of 4 independent experiments. * show significant differences between PBS and OVA treatment groups; # indicates significant differences between CD and HFD OVA-treated groups. * or # indicate $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

4.8 DC-T cell crosstalk

To follow the hypothesis that CD4$^+$ T cell activation was affected in HFD/OVA mice as a consequence of altered DC function, CD4$^+$ T cell proliferation and survival was determined. Briefly, a CD4$^+$ T cell proliferation assay was performed using co-cultures of OVA-pulsed sorted lung DCs isolated from allergic CD- or HFD-mice, with CFSE-labeled CD4$^+$ T cells from transgenic OVA-specific T cell receptor mice (OT-II mice). After co-culturing the cells for four days, CD4$^+$ T cell proliferation was evaluated by measuring the dilution CFSE among daughter cells. The frequency of proliferating CD4$^+$ T cells in co-culture with DCs from OVA-treated allergic mice was unaffected by the diet feeding (Figure 28 A). After prolonging the co-culture up to nine days, CD4$^+$ T cell survival was assessed by live-dead-staining. Around 95% of CD4$^+$ T cells in culture were alive after nine days. However, no differences were detected between co-culture with DCs of CD/OVA or HFD/OVA mice (Figure 28 B).

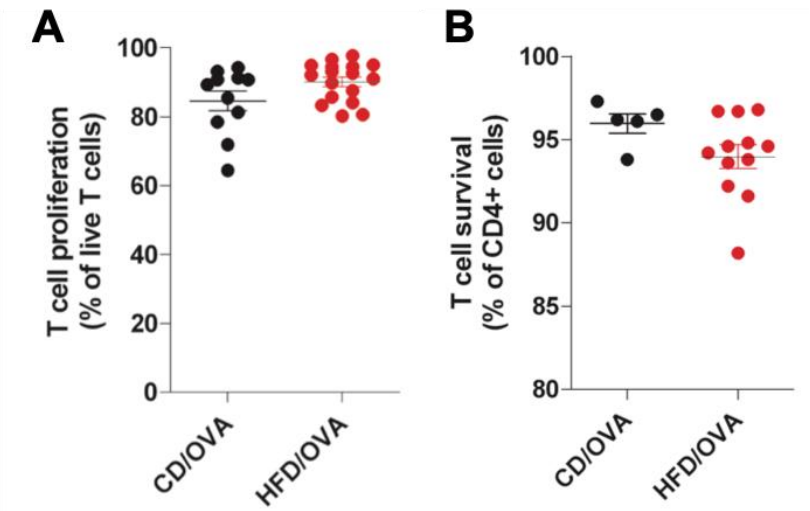

Figure 28: HFD feeding did not alter OVA-associated DC-dependent T cell proliferation and survival. (A) T cell proliferation and (B) T cell differentiation after 4 days co-culture of *in vivo* OVA-primed DCs of CD- and HFD fed mice with splenic OTII T cells. Values shown are the mean ± SEM, n=5-17 from 3 independent experiments.

As the experiments indicated no effect of the diet on $CD4^+$ T cell proliferation and survival when co-cultured with DCs from OVA-treated allergic mice, I measured the T cell cytokines concentrations in co-culture supernatants. Interestingly, screening of IFNγ, IL-10, and IL-13 concentration revealed lower IFNγ and higher IL-10 concentration (Figure 29 A, B). Of note, IL-13 concentration was unchanged (Figure 29 C).

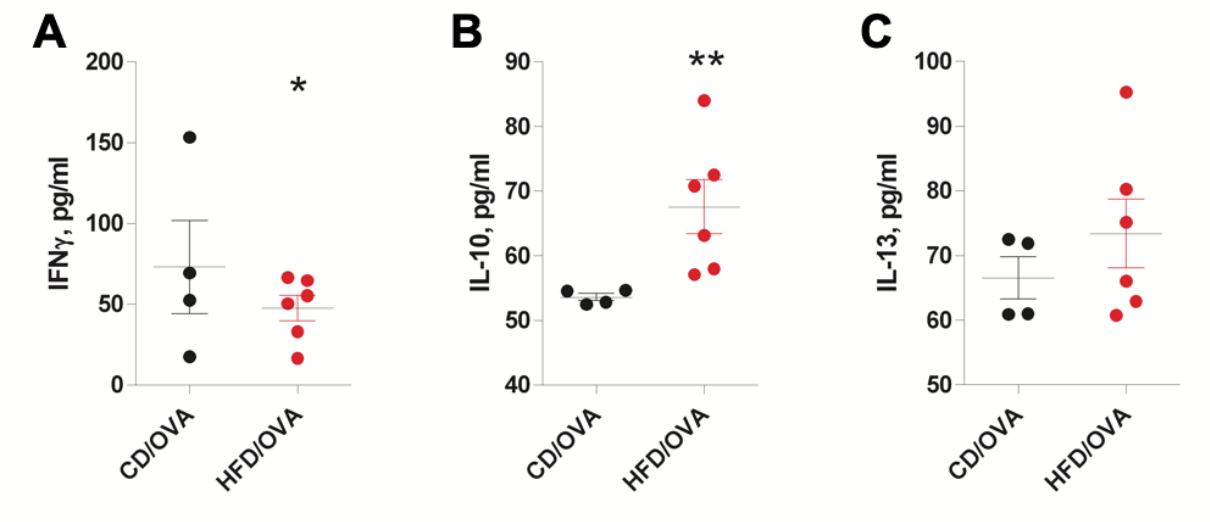

Figure 29: HFD feeding changes the concentration of IFNγ, IL-10, and IL-13 in DC-T cell co-culture supernatant. Concentration of (A) IFNγ, (B) IL-10, and (C) IL-13 after 9 days co-culture of *in vivo* OVA-primed DCs of CD- and HFD fed mice with splenic OTII T cells. Values shown are the mean ± SEM, n=4-6 from 2 independent experiments.

Since the experiments with co-cultures of DCs from OVA-treated mice and OVA receptor transgenic T cells indicated unchanged T cell proliferation and survival but alterations of T cell differentiation, I examined the relevance of these findings in the *in vivo* model of HFD feeding and OVA-driven experimental allergic asthma. Therefore, I characterized the *in vivo* polarization of T cells by stimulating for four hours lung cell isolates *in vitro*. Then, intracellular cytokine staining and flow cytometric analysis was performed to assess T cell polarization. The frequency of IL-13$^+$ T cells was unchanged in CD/OVA and HFD/OVA recapitulating the *in vitro* indications of an unchanged Th2 polarization (Figure 30 A). In contrast, frequencies of IFNγ$^+$ and IL-17$^+$ T cells were lower in HFD/OVA than CD/OVA pointing towards altered Th1 and Th17 polarization in HFD fed and OVA-treated mice (Figure 30 B, C). In addition, the frequency of Foxp3$^+$ T cell was lower in HFD/OVA than CD/OVA; however, no difference was observed in IL-10$^+$ T cells (Figure 30 D, E).

Altogether, these findings suggested that HFD/OVA mice displayed an attenuated allergic asthma phenotype as a consequence of an altered DC-T cell crosstalk leading to alterations in T cell polarization.

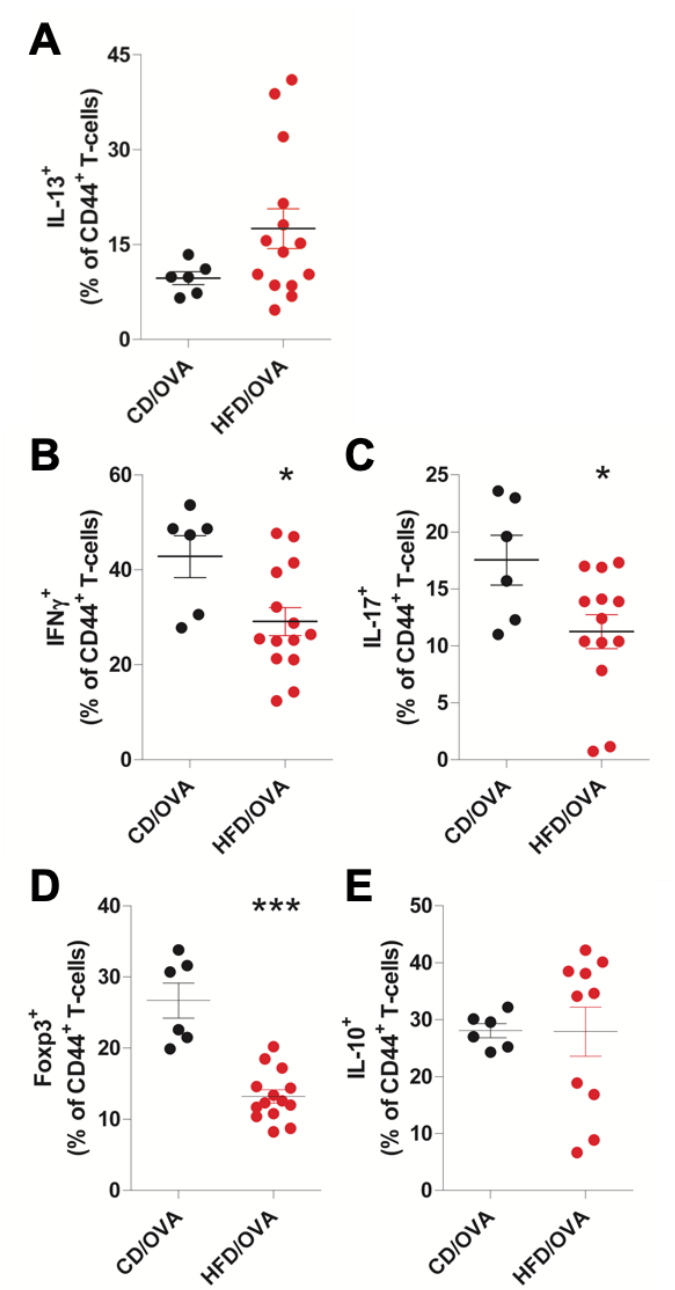

Figure 30: Mild bodyweight gain suppresses the differentiation of Th1, Th17, and Treg but not of Th2 cells. Frequency of (A) IL-13$^+$, (B) IFNγ$^+$, (C) IL-17$^+$, (D) Foxp3$^+$, or (E) IL-10$^+$ CD3$^+$CD4$^+$CD44$^+$ effector T cells in CD/OVA and HFD/OVA mice; n=6-14 out of 3 independent experiments. Values shown are the mean ± SEM, asterisks indicate significant differences, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4.9 Role of leptin and its receptor

Since leptin, as a classical adipokine increasingly secreted along with increased adipose tissue mass, has been linked to the pathogenesis of allergic asthma, and is increased in my HFD feeding model (Figure 7**,** Figure 9), I evaluated whether leptin signaling could have an impact in this model. Therefore, I assessed the expression of leptin receptor (LepR) by qPCR using mRNA from DCs and T cells of mice fed either using CD or HFD for 12 weeks. Indeed,

I could not evidence a difference in expression of the leptin receptor (LepR) in DCs and T cell between CD and HFD mice (Figure 31).

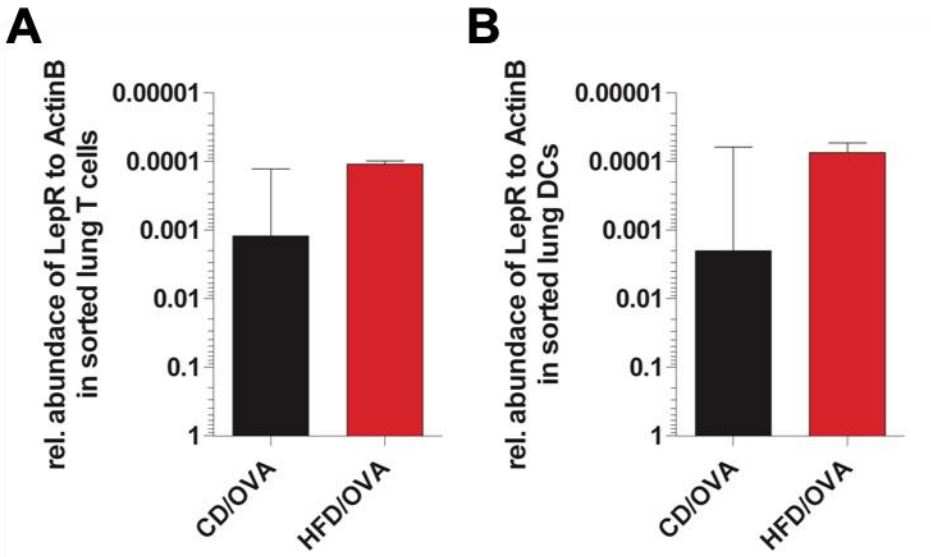

Figure 31: Leptin receptor expression. Leptin receptor expression in lung DCs and T cells of CD/OVA and HFD/OVA. Relative (LepR) and adiponectin (AdipoR1/2) receptor expression in sorted (A) lung T cells and (B) total lung DCs after OVA treatment in CD and HFD fed mice. Values shown are the mean ± SEM, log scale, n=3-4 from 2 independent experiments.

However, increased serum levels of leptin in HFD mice suggested that, despite unchanged receptor expression, leptin might play a role in T cell functions, since my data indicated that HFD feeding altered Th1 and Th17 polarization in my model. To analyze whether leptin mediates such effects on T cell polarization, an *in vitro* T cell polarization assay, performed in presence or absence of leptin was done. There, splenic naïve $CD4^+$ T cells were isolated from C57BL/6J wild type mice, and either cultured in Th1, Th2, or Th17 priming culture conditions with or without leptin, at the concentrations of 250 or 500 ng/ml. More precisely, IL-12 and IL-4 were used to prime for Th1 differentiation, IL-4 and anti-mouse IFNγ for Th2 differentiation, and TGFβ, and anti-mouse IFNγ for Th17 differentiation. Then, T cell survival was determined by manual enumeration of T cell numbers as well as by flow cytometry live/dead staining. In such conditions, I could not observe any difference in live/dead staining (Figure 32 B). However, T cell number enumeration revealed a tendency for increased cell numbers after incubation with 250 and 500 ng/ml leptin in Th1 and Th17 priming conditions and reduced numbers in Th2 priming conditions compared to untreated controls (Figure 32 A). In line, the frequency of $IL\text{-}4^+$ T cells tended to be reduced upon incubation of 250 ng/ml leptin (Figure 32 D). In contrast, cell numbers in Th1 and Th17 polarizing conditions as well

as frequencies of IFNγ$^+$ and IL-17$^+$ T cell were not changed in this experiment (Figure 32 A, C, E).

Taken together, these experiments did not provide evidence for functional involvement of leptin signaling in the differentiation of the subsets of Th cells.

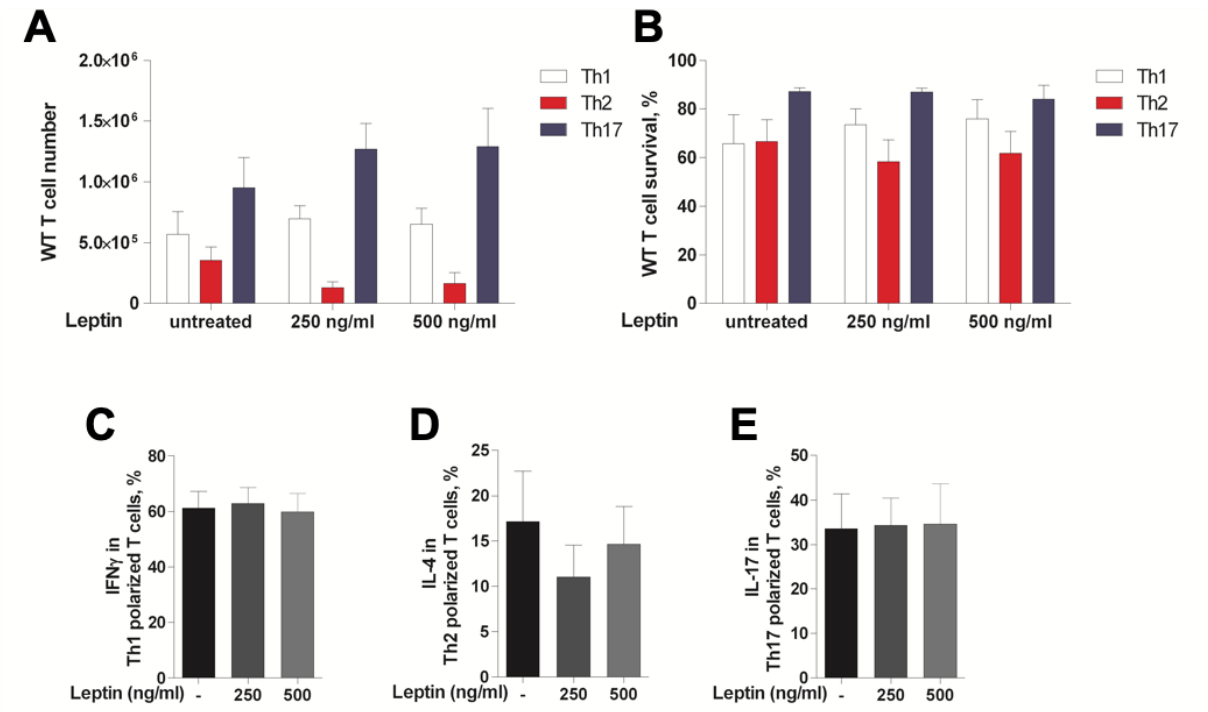

Figure 32: Effect of leptin on in vitro T cell polarization. Effect of leptin on (A) T cell number and (B) survival in either Th1-, Th2-, or Th17 priming culture conditions. Effect of leptin on the frequency of (C) IFNγ$^+$, (D) IL-4$^+$, and (E) IL-17$^+$ CD4$^+$ T cells. Values shown are the mean ± SEM, n=4-8 from 3 independent experiments.

4.10 Role of anaphylatoxin receptor C5aR1

Altogether, the assembled data suggest that DCs and T cells are predominantly involved in reducing allergic asthma severity in this model. However, another observation was that although neutrophils were increased in BAL and lung tissue upon allergic asthma induction, their numbers decreased in BAL but not lung tissue of HFD/OVA mice compared to CD/OVA mice (Figure 15, Figure 19). Interestingly, the anaphylatoxin receptor C5aR1 is known to participate in sensitization and severity of allergic asthma induction and plays a critical role in neutrophil functions on the one hand (Engelke et al., 2014; Karsten et al., 2015; Köhl et al., 2006), while HFD feeding is reported to be involved in neutrophil trafficking on the other hand (Manicone et al., 2016). To unravel a possible involvement of C5aR1 in this model of HFD feeding followed by experimental OVA-driven allergic asthma induction, further experiments were performed.

First, neutrophils from BAL and lung tissue of HFD/OVA and CD/OVA mice were identified by flow cytometry and additionally stained for C5aR1 receptor expression. Interestingly, C5aR1 receptor expression was slightly increased in neutrophils from HFD/OVA mice as compared to CD/OVA in both, BAL and lung tissue (Figure 33).

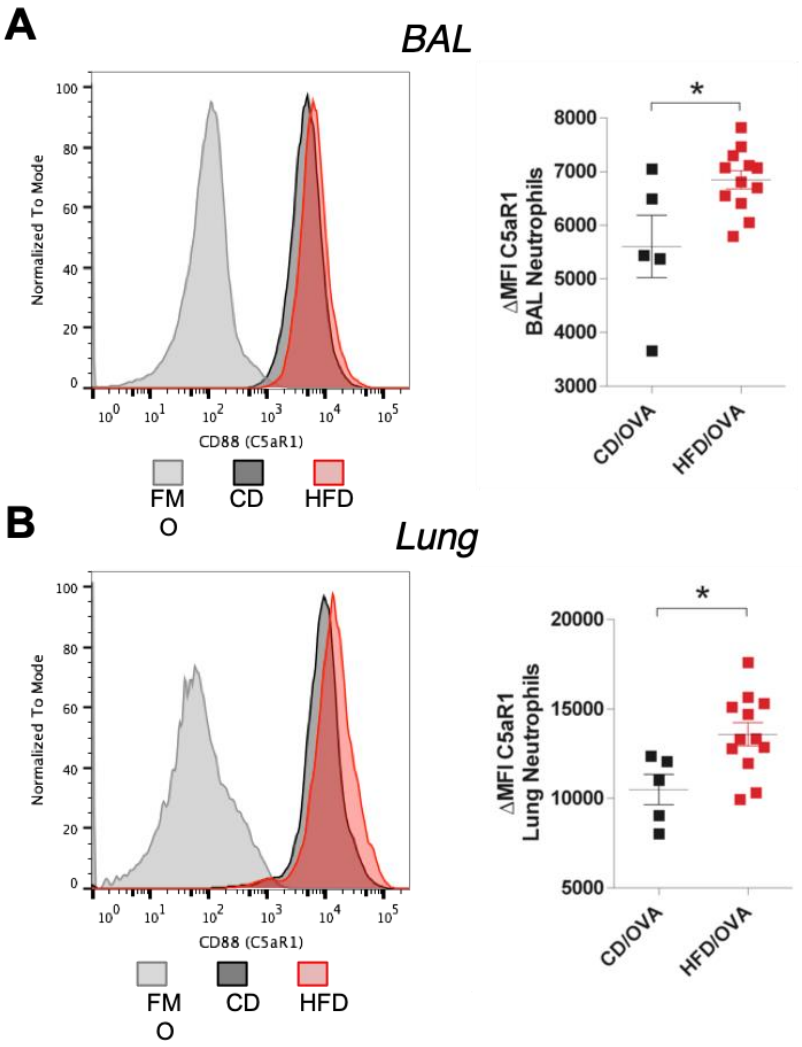

Figure 33: C5aR1 surface expression. HFD feeding results in increases C5aR1 surface expression in airway and lung neutrophils. Relative C5aR1 surface expression presented as MFI in (A, left) BAL and (B, left) lung neutrophils of HFD/OVA vs. CD/OVA. FMO displays negative control. Quantification of C5aR1 surface expression in (A, right) BAL and (B, right) lung neutrophils of HFD/OVA vs. CD/OVA in comparison to FMO control. Values shown are the mean ± SEM, n=5-12 from 2 independent experiments, asterisks indicate significant differences, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

An additional set of experiments was performed aiming to identify a functional involvement of C5aR1 in HFD induced mild bodyweight increase and subsequent experimental allergic asthma. For this, female C5aR1 deficient mice (*C5ar1*$^{-/-}$) were fed to either HFD or CD and

treated with OVA to induce allergic asthma following the same protocol like in above mentioned experiments. C5aR1 knockout mice reached a final bodyweight of 29.5 g (± 0.66 g SEM) with HFD as compared to 22.9 g (± 0.63 g SEM) with CD. In addition, C5aR1 knockout presented with an increase in AHR upon OVA in CD and HFD feeding (Figure 34). However, no difference could be detected between HFD/OVA and CD/OVA. Thus, this experiment rather suggests that systemic C5aR1 signaling is not involved in the dampening effect of mild bodyweight increase on OVA-driven allergic asthma.

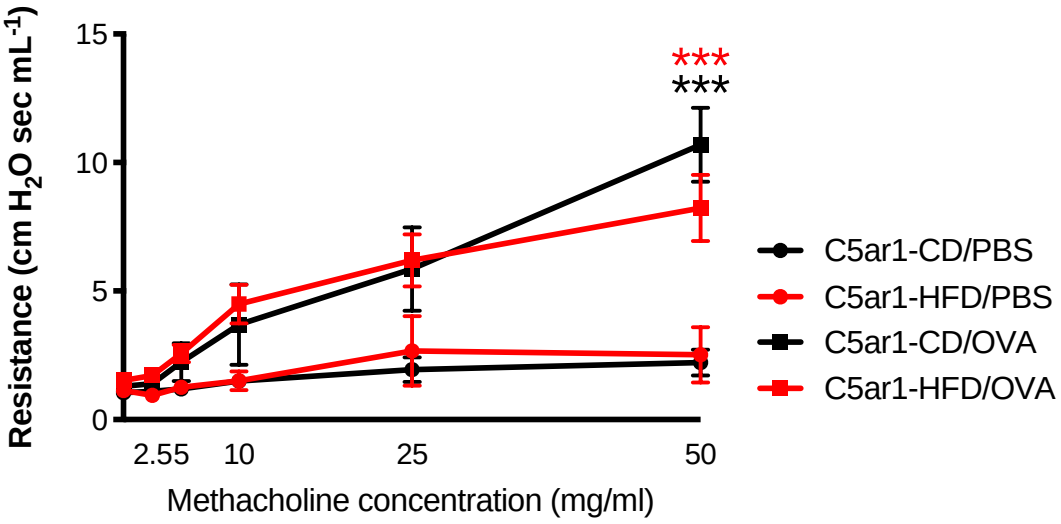

Figure 34: HFD feeding of C5aR1 deficient mice did not lead to a reduction in AHR. AHR in response to increasing dosages of methacholine in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black). Values shown are the mean ± SEM, n=4-8 out of 2 independent experiments, *** show significant differences between PBS and OVA treatment groups ($p < 0.001$, red HFD, black CD).

5 Discussion

5.1 Evaluation of the experimental model

5.1.1 Decision on the mouse model

Experimental data on asthma pathogenesis and obesity are most frequently derived from mouse models. Indeed, mice breed quickly and easily and can be well controlled and manipulated. However, the choice of the best mouse model to use may be challenging. For instance, in obesity research, the genetic-gender-diet-interaction is of high importance, and as such, the C57BL/6 genetic background is well predisposed to store fat and to develop an increased bodyweight accompanied by hyperglycemia and hyperinsulinemia on a high fat diet (HFD) (Surwit et al., 1998). Similarly, the Balb/c genetic background shows a very high sensitivity to develop strong allergen-induced airway obstruction and remodeling (Chen et al., 2015; Silva et al., 2017; Takeda et al., 2013).

Since the main goal of my study was to evaluate a potential impact of a mild bodyweight increase associated with early metabolic affections, due to HFD feeding, on the development of allergic asthma, I had to develop an appropriate mouse model. C57BL/6 wildtype mice are used for diet-induced-obesity models because of their relatively robust susceptibility to diet-induced metabolic challenges (Buettner et al., 2007; Hariri and Thibault, 2010; Heydemann, 2016; Lutz and Woods, 2012). Furthermore, several sub-strains of C57BL/6 wildtype mice exist and show different responses to dietary challenges (Fontaine and Davis, 2016). In particular, it has been demonstrated that in response to different short-term HFD feedings, C57BL/6JRj presents lower bodyweight than C57BL/6NTac mice, suggesting a milder metabolic phenotype (Heiker et al., 2014; Kern et al., 2012). Nonetheless, it should be noted that the use of a mouse model like C57BL/6 mice for obesity studies often inadequately replicate the human condition. In particular, C57BL/6J female mice do not exhibit the same magnitude or rapidity of weight gain and develop lower levels of obesity and obesity-associated sequelae as opposed to male mice (Nickelson et al., 2012; Pettersson et al., 2012). Such prominent sex difference in development of obesity is not observed in humans (Finucane et al., 2011; NCD Risk Factor Collaboration (NCD-RisC), 2017).

While C57BL/6 are considered as prototypic model for metabolic research, Balb/c mice are frequently used for allergic asthma research (Aun et al., 2017; Gueders et al., 2009). Furthermore, Balb/c mice also show a gender skewing in the development of asthma, with female Balb/c mice displaying higher AHR, airway inflammation, and airway remodeling in various models of allergic asthma than their male counterparts (Blacquière et al., 2010; Chen et al., 2015; Melgert et al., 2005; Silva et al., 2017; Takeda et al., 2013). However, Balb/c

mice are reported to be relatively protected from metabolic alterations under a HFD-induced metabolic challenge following a short phase of feeding (Montgomery et al., 2013).

Another prominent mouse model in the field of asthma research is A/J mice (Cozzi et al., 2011; Hadeiba et al., 2000; De Vooght et al., 2010). This mouse model was not taken into consideration because A/J mice are reported to be obesity-resistant with very low bodyweight differences between HFD and chow diet fed animals (Bullen et al., 2004; Surwit et al., 1995). On the contrary, AKR mice were demonstrated to develop AHR in experimental models (De Vooght et al., 2010) and are susceptible to diet-induced obesity (Meng et al., 2017).

However, as in several studies induction of asthma has been achieved in C57BL/6 mice using OVA as allergen (Calixto et al., 2010; Jung et al., 2013; Kim et al., 2015), and C57BL/6JRj mice would develop HFD-induced mild bodyweight increase, characterized by early metabolic alterations (Heiker et al., 2014; Kern et al., 2012), and female mice could be expected to be more prone to develop an allergen-induced allergic asthma (Blacquière et al., 2010; Chen et al., 2015; Melgert et al., 2005; Silva et al., 2017; Takeda et al., 2013), finally, female C57BL/6JRj mice were chosen as experimental mouse model for this study.

5.1.2 Metabolic phenotype

Female C57BL/6JRj mice were fed 12 weeks with HFD, in a way that mice reach metabolic alterations below what is reported in obesity and obesity-related metabolic disturbance in mice. Indeed, the HFD fed mice developed a mild bodyweight increase mainly due to an expansion of visceral adipose tissue (VAT) mass. Alongside, I observed the typical accumulation of pro-inflammatory adipose tissue macrophages, accounting for a beginning systemic pro-inflammatory state, leading to mild insulinemia and leptinemia as well as hepatic lipid storage (Boutens et al., 2018; McNelis and Olefsky, 2014). In line, serum cholesterol, i.e. total cholesterol as well as VLDL/LDL and HDL cholesterol subfractions, were higher in HFD compared to CD animals, a finding consistent with previously reported models of diet-induced overweight as well as typical obesity-associated metabolic alterations (Buettner et al., 2007; Dietze et al., 2012; Heydemann, 2016; Jung et al., 2013; Lutz and Woods, 2012; De Vries et al., 2009). Furthermore, the microbiota changed towards an increased abundance of Proteobacteria and decreased abundance of Bacteroidetes, in line with previously reported microbiota changes in the context of HFD-associated systemic inflammation (Hildebrandt et al., 2009; Ley et al., 2005). Altogether, these metabolic changes in HFD mice compared to CD mice support the idea that the used feeding model indeed induced metabolically challenged mice.

Of note, all mice, either fed with CD, HFD, or HFD60, showed a comparable decline of bodyweight at the end of the feeding regimens. This decline started with the repeated airway allergen airway challenges and can be explained by the stress induced by the deep anesthesia required (Dholakia et al., 2017).

5.1.3 Defining “mild increase” in bodyweight

In order to more precisely define the metabolic aspects of my model, I compared several parameters to commonly reported values from mouse models of obesity present in the literature, in particular genetically induced obese mice (ob/ob mice) (Arteaga-Solis et al., 2013; Favero et al., 2013), genetically induced atherosclerosis mice (ApoE-/- mice) (Giles et al., 2016), and HFD fed C56BL/6 female wildtype mice housed under thermoneutral conditions reaching morbid obesity (Giles et al., 2017). A summary of these comparisons is included in Table 12.

Such comparison reveals that, although the weight gain during the 12 weeks of feeding was 10.6 g (± 2.04) higher than in CD mice, the usual weight gain found in the literature was ranging from 18 to 40 g in comparable feeding regimens using leptin-receptor-deficient mice (ob/ob mice) (Giles et al., 2017; The Jackson Laboratory) (Table 12). Similarly, my HFD mice exhibited higher increase in perigonadal fat depots, clearly suggesting that an increase in VAT accounted for the increase in total bodyweight. Although such increase in VAT was twice as much for HFD mice compared to CD mice (0.5 ± 0.04 vs. 0.12 ± 0.01 g VAT), it remained below what has been reported in ob/ob mice (0.7 to 1.5 g) (O’Brien et al., 2017; Picard et al., 1998) (Table 12).

Although the body mass index (BMI) is clearly linked to the diagnosis of overweight or obesity in humans, there is no clear definition of overweight and obesity in mice, nor is the BMI widely used for mice. However, in some examples an adjusted BMI applied to rodents have been used (Gargiulo et al., 2014; Laaksonen et al., 2013). Interestingly, a mean adjusted BMI calculated for the CD and HFD mice revealed that the former showed a value of 22.4 (± 0.39) while the latter reached a mean adjusted BMI of 26.4 (± 0.48) (Table 12). In comparison to human, where a BMI ≥ 25 defines overweight and a BMI ≥ 30 defines obesity (WHO), the mean adjusted BMI value indicates that HFD fed mice reached a metabolic state in the range of overweight rather than obesity. In addition, as liver fat deposition is a sign of visceral adiposity (Kawada, 2015) and a key measure to objectify histological signs of fatty liver (Brunt et al., 2011), I used histology section and staining, and observed that HFD fed mice show a mild liver lipid accumulation, whereas CD mice did not show lipid accumulation in the liver at all. However, a comparison of this feature to reported mouse models of diet- or genetically-induced obesity, confirmed again that the extent of metabolic changes I observed

were below obesity-related alterations (Arisqueta et al., 2018; Giles et al., 2017; Schröder et al., 2016) (Table 12). Finally, serum lipids such as cholesterol and triglycerides were also measured to assess the severity of the metabolic disturbances. Interestingly, the HFD feeding resulted in higher values for cholesterol but not for triglycerides compared to the CD mice. However, comparing these values to data obtained in morbid obese female mice housed under thermoneutral conditions as well as genetically-induced lipidemia in the context of atherosclerosis (Giles et al., 2016, 2017; Madenspacher et al., 2010; Nishina et al., 1994), the levels observed in my HFD fed mice can again be considered mild (Table 12). Furthermore, mean serum leptin level was at 3710 pg/ml (± 452.4 pg/ml) in HFD mice, approximately 4.5 times higher than in CD mice (827.5 ± 156.3 pg/ml), and insulin was at 387.5 pg/ml (± 40.0 pg/ml), nearly twice as high as in CD mice. Once more, these two parameters were lower than those reported in obesity models in mice and report about obese humans (Boi et al., 2016; Gilbert et al., 2011; Maffei et al., 1995; O'Brien et al., 2017; Sood and Shore, 2013) (Table 12).

Of note, insulin was measured not fasted and glucose values were missing because an overnight fast may have caused further stress in addition to the burden mice experienced from the repeated OVA airway challenges and narcosis every other day at the end of the protocol.

Table 12: Comparison of cellular and metabolic parameters between chow diet (CD), short-term high fat diet (HFD) feeding and literature reports of obesity.

Mouse model	Body-weight[$]	Body-weight gain[$] (g)	Body-weight gain[$] (%)	VAT (g)	Adjusted BMI (100g/cm^2)	VAT Mϕ (% of VAT cells)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL/VLDL (mg/dl)	Triglyceride (mg/dl)	serum leptin (pg/ml)	serum insulin (pg/ml)	liver lipid accumulation
CD fed mice	20.4 ± 0.39	7.3 ± 1.65	52.6 ± 12.8	0.12 ± 0.02	22.4 ± 0.39	7 ± 2.0	82.9 ± 8.3	56.8 ± 8.5	32.7 ± 4.3	127.6 ± 14.1	827.5 ± 156.3	215.3 ± 26.1	none
HFD fed mice	24.6 ± 0.37	10.6 ± 2.04	76.5 ± 17.3	0.5 ± 0.04	26.4 ± 0.48	15 ± 1 (2x increase vs. CD)	177.2 ± 13.1	123.0 ± 9.3	53.1 ± 4.7	116.7 ± 5.6	3710 ± 452.4 (4.5x increase)	387.5 ± 40.0 (1.8x increase vs. CD)	mild
obese mice[*]	NA	18 ± 0,5 (Giles et al., 2017)	200 vs. 125 (Kim et al., 2014)	2x increase vs. lean mice (Montgomery et al., 2013)	> 30 [human] (WHO)	28 ± 2.5 (Giles et al., 2017); 5-6x increase vs. lean mice (Montgomery et al., 2013)	200 (Giles et al., 2017)	50 (Madenspacher et al., 2010)	60-100 (Madenspacher et al., 2010)	140 (Giles et al., 2017)	4-6x higher [human] (Maffei et al., 1995; Sood and Shore, 2013)	1.5 to 4x versus lean controls (Gilbert et al., 2011); 2000 pg/ml (Boi et al., 2016)	Strong (Giles et al., 2017)
ob/ob mice[#]	45 ± 3,6 (Arteaga-Solis et al., 2013) to 53 (Favero et al., 2013)	39,8 (The Jackson Laboratory)	175 (The Jackson Laboratory)	0.7-1.5 (O'Brien et al., 2017; Picard et al., 1998)	NA	8-10x increase (F4/80 mRNA expression) vs. lep^{ob}-heterozygous mice (O'Brien et al., 2017)	134 ± 10 (Nishina et al., 1994)	107 ± 11 (Nishina et al., 1994)	NA	57.3 ± 3 (Nishina et al., 1994)	NA	5000 (O'Brien et al., 2017)	Strong (Arisqueta et al., 2018)
ApoE$^{-/-}$ mice[§]							600 (Giles et al., 2016)	NA	NA	150 (Giles et al., 2016)			

If applicable data are shown as mean ± SEM; * due to data from literature according to indicated reference, only diet-induced obesity was taken in account; # genetically induced obesity model due to complete deficiency of leptin signaling; $ equivalent to 12 weeks feeding; § used for positive control for serum lipids only.

Altogether, in particular considering the bodyweight development, the mouse adjusted body mass index (BMI), the epididymal VAT mass, the pro-inflammatory macrophage accumulation in adipose tissue as well as changes in hepatic lipid accumulation, the serum cholesterol, and the leptin and insulin concentrations, HFD fed mice used in this thesis experimental model exhibit consistently changes in-between those observed in CD fed mice and mice reported to develop an obese phenotype. Therefore, this level of physiological alterations was referred as a “mild increase”.

Conclusively, the HFD feeding regimen I used induces a stage of physiological change, defined as a “mild increase” in bodyweight and is accompanied by the initiation of metabolic changes on a systemic level. This mimics an early phase of metabolic challenge without reaching a morbid obesity phenotype. Thus, this model rather reflects changes described in overweight individuals (Flegal et al., 2013; Wang et al., 2016).

5.1.4 Experimental asthma induction

In this study, ovalbumin (OVA) was used as allergen to induce allergic asthma. Although OVA is not a classic aeroallergen accounting for pulmonary allergy in humans, it has been widely used to induce asthma in mice, in particular in mice with a C57BL/6 background (Calixto et al., 2010; Diaz et al., 2015; Jung et al., 2013; Kim et al., 2014, 2015; Mito et al., 2002). Intraperitoneal injections of OVA, followed by repeated forced inhalations of OVA typically induce an eosinophil-dominant asthma including AHR in response to methacholine, airway inflammation, airway remodeling with increase mucus production, and Th2-biased immune regulation (Yu and Chen, 2018). As these are classical features of allergic asthma (Schatz and Rosenwasser, 2014), such allergen-induced allergic asthma was used as primary model for this thesis.

CD fed mice were treated either with OVA or PBS as control, the latter served as baseline control of the asthma model itself. Although CD/PBS as well as CD/OVA mice were intraperitoneally injected with Alum as immunological adjuvant. Although immunological changes triggered by Alum itself could not be ruled out, the CD/PBS mice readouts were comparable with earlier reports about pulmonary immunology under baseline conditions and experiments with PBS without an adjuvant in CD fed mice (e.g. in a house dust mite model) (Zhang et al., 2010), therefore I considered it unlikely that Alum plays a role in asthma relevant immunological changes.

When analyzing asthma key features in CD/PBS, they reflect the physiological state I found in healthy lungs, i.e. low immune cell numbers in both, lung tissue and airways, few PAS-positive airways, indicating no excessive mucus production, as well as a low lung airway resistance was revealed. In contrast, OVA treatment in CD fed mice (CD/OVA) led to

increases in immune cell numbers in lung tissue and airways, increases in lung resistance as well as PAS-positivity indicating mucus production, showing that OVA is an appropriate allergen. More specifically, the immune cell recruitment to the airways was characterized by higher numbers of eosinophils, neutrophils, and T cells, all major players in allergic asthma. In addition, eosinophils, neutrophils, alveolar macrophages, DCs, and T cells, also characterizing an asthma-typical and -relevant immune cells composition, dominated the lung tissue immune cell infiltrate (Hoffmann et al., 2016; Locksley, 2010). Finally, the final values of lung resistance also reflected what was to be expected due to earlier reports about AHR in asthmatic mice (Ender et al., 2017; Gueders et al., 2009). In addition, mucus production was in a range comparable to previous OVA-induced allergic asthma experiments with C57BL/6 or Balb/c animals performed in our group (direct OVA induction or adoptive transfer of OVA-pulsed bone marrow derived DCs) (Ender et al., 2017; Schmudde et al., 2013; Zhang et al., 2010).

Taken together, the usage of OVA as allergen, led to an allergic asthma phenotype in CD/OVA mice compared to CD/PBS. This allergic asthma phenotype was characterized by a eosinophilic-dominated inflammation, AHR, and mucus production. Accordingly, the experimental set-up was sufficiently marked to be used to analyze the impact of a metabolic challenge by a short period of HFD feeding on asthma relevant read-outs.

5.2 Impact of HFD feeding on the allergic asthma phenotype

5.2.1 HFD impact on airway resistance and mucus production

While CD/PBS mice demonstrated low immune cell numbers, low PAS-positive airways, and low AHR, which collectively reflect the physiological state of healthy non-asthmatic lungs, the HFD/PBS presented similar values for AHR, PAS positivity, and immune cell numbers like the CD/PBS mice. This clearly indicates that HFD feeding alone does not lead to an asthma-like phenotype. In contrast, another study reported that short-term HFD feeding alone is already sufficient to induce a non-allergic asthma phenotype in C57BL/6 mice, characterized by a marked increase in AHR (Kim et al., 2014). However, that study used male C57BL/6 mice, reaching a final bodyweight of 45 g or 200% bodyweight increase, respectively. Since the final bodyweight of my HFD fed mice was much lower - ~25 g or ~77% bodyweight increase -, therefore, the difference may be explained by the final bodyweight for which HFD mice may not have reached a threshold required to trigger a non-allergic form of asthma. However, in other studies where HFD-fed mice also reached a final bodyweight of 40 to 45 g, they did not necessarily show an increase in AHR (Kim et al., 2015; Park et al., 2016). In fact, several mouse studies were performed and predominantly dealt with the impact of genetically or dietary induced obesity on asthma (Calixto et al., 2010; Chen et al., 2015; Diaz

et al., 2015; Dietze et al., 2012; Johnston et al., 2007; Jung et al., 2013; Kim et al., 2015; Mito et al., 2002; Silva et al., 2017; De Vries et al., 2009). Nonetheless, these studies could not reach a clear consensus on the effects of obesity on allergic asthma. This is in particular with respect to AHR, which is a pathological key feature of asthma and an objective and reproducible parameter reflecting typical asthma symptoms, such as wheezing and shortness of breath (Brannan and Lougheed, 2012; Dixon et al., 2011; Walker et al., 2013). Importantly, these discrepancies between studies could not be explained by differences in experimental set-up, such as mouse strain, gender of mice, mode of feeding, or used allergen for experimental induction of allergic asthma. However, the studies reporting an increase in AHR also report a HFD induced bodyweight increase in the range of 160 to 250% as compared to control diet fed mice (Johnston et al., 2007; Kim et al., 2014, 2015), whereas the studies reporting no change of AHR report only a bodyweight increase in the range of 120% (Dietze et al., 2012; Jung et al., 2013), which is the range of bodyweight increase of my HFD mice. Nonetheless, the correlation analysis performed with various parameters assessed in this thesis indicates that the mild bodyweight gain of HFD mice rather modulate the DC and T cell numbers and that this leads to a decrease of allergen induced AHR as compared to CD mice.

HFD fed mice treated with and sensitized against OVA developed an allergic asthma, which was characterized by an increased AHR, mucus production, and higher numbers of eosinophils, neutrophils, and T cells infiltrating the airways and lung tissue, as well as higher numbers of alveolar macrophages and DCs in the lung tissue compared to their respective PBS controls, showing that HFD did not alter the capabilities of mice to develop allergic asthma. However, the increases in AHR and several immune cells, such as neutrophils, DCs, and T cells, were significantly lower in HFD/OVA compared to the CD/OVA control mice, indicating that the diet impacted on the severity of the asthma disease; thus, interfering with the effector phase rather than the sensitization phase. Intriguingly, this reduction of the AHR in HFD/OVA mice was characterized by a lower maximal response to methacholine rather than a shift of the slop in the methacholine dose-response curve suggesting that changes in smooth muscle cell contractility, wall thickness or elastic loads may have occurred (Berend et al., 2008). However, my allergic asthma model is an acute model, which typically do not feature structural airway remodeling. In addition, it is unlikely that diet-induced changes in bodyweight have exerted mechanical effects because the baseline measurement without methacholine did not show higher resistance values for mice with a higher bodyweight.

Altogether, this clearly indicates that in terms of HFD and allergic asthma, a mild increase of bodyweight has a different impact on asthma development than obesity and that it is not the bodyweight *per se* which impacts on the asthma development. In addition, environmental

factors other than the diet may be involved in the development of allergic asthma in pre-existing obesogenic diet and accompanied pro-inflammatory conditions.

Interestingly, obesity-associated hyperinsulinemia (Schenk et al., 2008; Suratt, 2016) is frequently associated with increased mucus production (Kwak et al., 2018) and insulin has been shown to have an impact on airway reactivity potentially leading to increases in AHR (Berend et al., 2008; Leiria et al., 2015b). Therefore, one would have expected an increased severity of allergic asthma in the HFD/OVA compared to the CD/OVA mice. However, although HFD mice had higher insulin levels than CD mice, it was below what is usually reported for obesity models (Boi et al., 2016; Gilbert et al., 2011) and might not be sufficient to trigger a change in mucus production or that insulin signaling is either not involved in reducing the maximal lung resistance in HFD/OVA mice or that insulin may play a bimodal role in establishing AHR in dependence on its blood concentration.

Similarly, although mucus secretion is linked to Th2-associated cytokines, such as IL-4, IL-5, and IL-13 (Hall et al., 2016; Vercelli, 2008; Wills-Karp et al., 1998), my co-culture experiments using DCs from allergic CD and HFD fed mice and OVA-specific T cells as well as intracellular cytokine analysis in effector T cells from allergic mice indicated that IL-13 production and secretion was not altered by HFD feeding in this model. This probably explain the unchanged mucus production in HFD/OVA as compared to CD/OVA.

5.2.2 HFD impact on inflammatory cell recruitment

The recruitment of immune cells into the airways and lung tissue is the third hallmark of allergic asthma and occurred in both, CD/OVA and HFD/OVA groups, compared to their respective diet PBS controls. However, to be more specific, only neutrophils and T cells were lower in the airways, and DCs and T cells were lower in the lungs of HFD/OVA than CD/OVA mice. In contrast other immune cells, such as eosinophils, remained unchanged when adding the HFD diet to the OVA asthma model.

5.2.2.1 Eosinophils

This unaltered eosinophilia in my HFD/OVA mice is in contrast to other reports about HFD feeding and classical experimental models, using house dust mite (HDM) or OVA as allergen or diesel particles to drive asthma. Such studies have either reported a decrease in eosinophilia (Diaz et al., 2015; Johnston et al., 2007; De Vries et al., 2009; Yanagisawa et al., 2014), or a higher pulmonary recruitment of eosinophils (Dietze et al., 2012).

Such discrepancy between the lack of impact of HFD on eosinophil numbers in my model and other studies reporting increase (Dietze et al., 2012) or decrease numbers (Diaz et al., 2015; Johnston et al., 2007; De Vries et al., 2009; Yanagisawa et al., 2014) may originate in differences of the terminal weight of animals (varying from 22 to 55 g) achieved in these studies. Indeed, heavy weight gain (> 35 g weight gain in the course of feeding) has been frequently associated with lower airway eosinophilia in experimental allergic asthma models (Calixto et al., 2010; Diaz et al., 2015; Johnston et al., 2007), whereas my HFD-fed and OVA-treated asthmatic mice presented with a moderate weight gain of 10.6 g (± 2.04 g) and no difference in eosinophilic infiltration as compared to CD counterparts. Additionally, I could demonstrate that this correlation is independent of macronutrient composition and energy source of the applied HFD. Indeed, a change in the HFD diet towards more calories from fat and different fat sources by using the HFD60 diet did not result in a changed impact on eosinophils numbers in the airways.

Alternatively, while increases in leptin concentration have reported to impair eosinophil recruitment into the airways (Silva et al., 2017). Although the leptin levels in my HFD mice were in the range of reported immune-modulating effects of leptin, in particular with respect to the recruitment of eosinophils into the airways (Leiria et al., 2015a; Locksley, 2010), neither airway nor lung tissue eosinophil numbers were altered after HFD feeding, indicating that changes in leptin concentration in my HFD/OVA mice were not sufficient to modulate pulmonary and airway eosinophilia. In line with my data, a chronic OVA-induced asthma model revealed that high leptin levels are not always associated with changes in eosinophilic recruitment to the airways (Jung et al., 2013).

5.2.2.2 Neutrophils

Noticeably in HFD/OVA mice as compared to CD/OVA mice, airway neutrophils were decreased but pulmonary neutrophil abundance were unchanged, which suggests that HFD feeding has an impact specifically on the recruitment of neutrophils into the airways of allergic asthma. The mechanism involved in the translocation of neutrophils from the lung tissue into the airways is incompletely understood. Molecules like anaphylatoxins have been for long considered as a major driver of neutrophil migration to the airways, but a recent study conditionally invalidating the expression of the C5a receptor C5aR1 in LysM expressing cells (mostly pulmonary neutrophils, macrophages and DCs) showed lower airway but unchanged lung neutrophils in OVA-asthma (Wiese et al., 2017).

Additionally, leptin has also been demonstrated to be involved in neutrophil migration with a potential dual effect. On the one hand, leptin acts directly as a neutrophil chemoattractant or through the release of TNF-α and CXCL1 (Montecucco et al., 2006; Souza-Almeida et al.,

2018). On the other hand, leptin interferes with the neutrophil chemotaxis to classical chemoattractants in end stage renal disease or acute lung injury (Kordonowy et al., 2012; Ottonello et al., 2004). Therefore, it can be speculated that the slight increased leptin in HFD/OVA compared to CD/OVA mice may play a role in modulating neutrophil trafficking from the lungs to the airways.

Finally, IL-17 producing T cells have previously implicated in determining the severity of allergic asthma through a synergism of IL-17 and IL-13 (Hall et al., 2016; Lajoie et al., 2010). Furthermore, Th17 signaling is involved in recruiting neutrophils to the site of inflammation in OVA-induced allergic asthma (Aujla and Alcorn, 2011; McKinley et al., 2008; Newcomb and Peebles, 2013), and may also be relevant for airway neutrophilia in humans (Laan et al., 1999). Accordingly, it is likely, that the decreased frequency of Th17 cells in HFD fed allergic mice compared to CD mice is, at least partly, responsible for the decrease in airway neutrophils in allergic HFD and CD mice.

5.2.3 Role of DCs and T cells

In allergic mice of both diet groups high numbers of pulmonary DCs were observed, but numbers were significantly lower in HFD compared to CD mice. In line with the decrease in total DCs, all DC subtypes, i.e. $CD11b^+$ and $CD103^+$ cDCs as well as $CD64^+$ moDCs, were decreased. Despite lower numbers of cDCs in the lungs, the numbers of $CD11b^+$ cDCs in draining mediastinal lymph nodes was not changed, indicating that HFD did not modulate the homing of DCs toward the lymph nodes but rather the effector phase of asthma. Interestingly, $CD11b^+$ cDCs and $CD64^+$ moDCs, besides being key regulators of allergic asthma by driving the polarization of effector T cells (Herbert et al., 1995; Roche et al., 2000; Salazar and Ghaemmaghami, 2013; Sung et al., 2006; Wan et al., 2000), are exerting local pro-inflammatory effects amplifying the Th2 immunity (Hoffmann et al., 2016; Lambrecht and Hammad, 2014b; Martínez-López et al., 2015; Plantinga et al., 2013). Consequently, it can be speculated that HFD feeding primarily interferes with DC proliferation and function in the lungs and that T cell recruitment to the lungs and local downstream consequences in allergic asthma, such as AHR and further immune cell recruitment, are secondarily diminished.

A deeper look at the T cell compartment revealed that the frequencies of naïve and effector T cells were not altered by the HFD feeding, however, the overall number of T cells was lower in HFD/OVA than in CD/OVA. This suggests that a general decrease in T cell accumulation in HFD mice was at play, rather than a specific reduction in effector or naïve T cells. While DCs drive T cell survival and general proliferation, *in vitro* HFD-primed DCs acted in this respect similarly to CD-primed. However, since DCs also impact T cells polarization, I broadly screen the pattern of secreted cytokines after co-culturing with *in vivo* OVA-primed

DCs with OVA-specific T cells. As a consequence, I disclosed lower IFNγ and higher IL-10 concentration suggesting that a change in the development of effector T cells occurred. For a better reflection of the local *in vivo* situation, intracellular cytokines profile of T cells of allergic CD and HFD fed mice was characterized. This analysis revealed that levels of IFNγ and IL-17 cytokines together with the transcription factor Foxp3 were diminished in HFD/OVA as compared to CD/OVA effector T cells. In contrast, the frequencies of IL-13 and IL-10 positive cells were not altered. This together clearly indicates that HFD altered the Th1 and Th17 differentiation but not Th2 differentiation. Furthermore, a decrease of Foxp3 but not IL-10 positive cells indicate that HFD feeding acts differently on regulatory T cell subtypes (Vieira et al., 2004). Of note, such unchanged development of a pulmonary Th2 response *in vivo* is in line with the similar mucus production and the similar secretion of IL-13 *in vitro* I observed, suggesting that in both, CD and HFD mice, a Th2-driven, eosinophilic allergic asthma was achieved (Locksley, 2010). Thus, HFD does not interfere with the development of classical immunological aspects of corticosteroid sensitive allergic asthma, i.e. airway eosinophilia and Th2-dominance ,(Holgate and Polosa, 2006). These data again point toward an impact of HFD on the severity rather the development of the allergic asthma phenotype by interfering with the effector phase but not the sensitization phase.

Interestingly, while the specific changes of DCs and T cells in HFD and CD mice suggest that mild increase in bodyweight impact on the severity of allergic asthma via alterations of the DC-T cell crosstalk, I observed that MHCII expression was significantly lower in $CD11b^+$ cDCs of HFD/OVA mice compared to CD/OVA. Similarly, CD40 expression, higher in all OVA-treated groups irrespective of the feeding, was also significantly lower in $CD11b^+$ cDCs of HFD/OVA compared to CD/OVA mice. Indeed, CD40 is necessary for the initiation of antigen-specific immune responses and has been proven to be crucial for T cell activation and polarization towards Th1, Th17, and Tregs (Haase et al., 2004; Kowal et al., 2006). Moreover, increases of CD40 ligand has been linked to allergic asthma in humans and correlates with obesity-associated inflammation (Kowal et al., 2006; Lam et al., 2007; Steven et al., 2017).

Collectively, the finding that MHCII and CD40 expression were reduced in $CD11b^+$ cDCs suggests that the reduced allergic phenotype upon HFD feeding resulted from an impaired potency of cDCs to (re)activate $CD4^+$ T cells leading to a decreased Th1/Th17 immune response.

5.2.4 Role of leptin and its receptor

In my model, the HFD-driven increase in leptin levels was below what is frequently observed in obesity models (Maffei et al., 1995; Sood and Shore, 2013); however, since leptin levels

were reported to be increased in the lungs of obese mice (Silva et al., 2017), the mild increase of serum leptin in my HFD mice could be associated with mild increases in the lungs and exert immune-modulatory effects accounting for the diminished asthma phenotype in HFD mice. Interestingly, a possible involvement of leptin signaling in asthma development, especially in the context of obesity, has been described but is debated (Jung et al., 2013; Kordonowy et al., 2012; Lam et al., 2007; Leiria et al., 2015a; Montecucco et al., 2006; Moraes-Vieira et al., 2014; Ottonello et al., 2004; Ramirez and Garza, 2014; Silva et al., 2017; Souza-Almeida et al., 2018). In particular, leptin has been involved in changed neutrophil migration from the lungs to the airways in my HFD/OVA mice. (Kordonowy et al., 2012; Montecucco et al., 2006; Ottonello et al., 2004; Souza-Almeida et al., 2018). In addition, leptin has been shown to play an anti-apoptotic and differentiation enhancing role on DCs isolated from bone marrow or human periphery blood (Lam et al., 2006; Mattioli et al., 2005, 2009). Furthermore, leptin in high dosages has previously been shown to induce CD40 expression in bone marrow-derived DCs *in vitro* and up-regulate T cell proliferation (Lam et al., 2007), and leptin deficiency was associated with decreased CD40 expression (Moraes-Vieira et al., 2014). On the contrary, a study with splenic DCs from leptin deficient mice showed an increased ability to drive a DC-dependent T cell activation, what rather suggests that increased leptin levels reduce T cell activation via DCs (Ramirez and Garza, 2014). The role of leptin in DC functions specifically in allergic asthma remain elusive, however, the evidence of decreased DC function in my HFD model implies that either mild increases in leptin lead to lower CD40 expression in cDCs in asthma and that this contributes to an overall attenuation of asthma severity or that mild increases in leptin do not exert effects on DCs in my model.

With respect to T cells, leptin has been shown to modulate T cell activation and differentiation of all $CD4^+$ effector and regulatory T cells, in particular, it has been reported that leptin promote Th1 and Th17 differentiation while reducing Th2 differentiation (Batra et al., 2010; Moraes-Vieira et al., 2013; Procaccini et al., 2012b; De Rosa et al., 2007). I performed a deeper functional analysis of *in vitro* T cell polarization with and without leptin but found no evidence that leptin modulate Th1, Th2, and Th17 immune response. However, as these experiments were performed *in vitro*, it is possible that the experimental conditions do not sufficiently recapitulate the *in vivo* situation.

Collectively, an impact of mild increase of leptin in my model remain uncertain. However, an involvement in modulating DC function and subsequent DC-T cell interaction in terms of allergic asthma may be missed and should be addressed in future projects.

5.2.5 Role of the complement receptor C5aR1

As aforementioned, neutrophil numbers were lower in BAL but not lung tissue upon allergic asthma induction in HFD/OVA mice as compared to CD/OVA controls suggesting that the trafficking of neutrophils from the lungs to the airways is altered. Interestingly, the anaphylatoxin C5a receptor C5aR1 is known to be, on the one hand, involved in allergic asthma induction and on the other hand to play a major functions of neutrophils (Engelke et al., 2014; Karsten et al., 2015; Köhl, 2006). Furthermore, HFD feeding has been reported to be involved in neutrophil trafficking (Manicone et al., 2016). Moreover, a conditional deletion of C5aR1 in LysMCre-expressing cells, in particular neutrophils, resulted in reduced numbers of airway but not of pulmonary neutrophils in the course of OVA-driven experimental allergic asthma (Wiese et al., 2017). Altogether, these data indicates that neutrophils require C5aR1 to infiltrate the airways, although the relevance for the overall asthma disease severity remains uncertain as the conditional knock-out mice presented an asthma phenotype like the C5aR1 sufficient control mice (Wiese et al., 2017). However, I performed further experiments to delineate a possible modulatory role of HFD in the role of C5aR1 in allergic asthma. Interestingly, a quantitative analysis of both, airway and pulmonary neutrophils, showed a slight but significant increase of C5aR1 receptor surface expression in asthmatic HFD mice when compared to CD controls (Figure 33), although, this finding was not in line with the impact of the conditional deletion of C5aR1 on neutrophil numbers in allergic asthma (Wiese et al., 2017), and, thus, the biological relevance remains uncertain. Therefore, to assess a possible *in vivo* relevance of C5aR1 in the reduced allergic asthma severity observed in HFD mice, I choose to feed either with HFD or CD, a mouse strain completely deficient for C5aR1 and to drive an OVA-triggered allergic asthma. After 12 weeks of CD feeding, female *C5ar1*$^{-/-}$ mice reached a final bodyweight of 22.9 ± 0.63 g, which was around 10% higher than in C57BL/6JRj mice, who reached a bodyweight of 20.4 ± 0.39 g. After HFD feeding female *C5ar1*$^{-/-}$ mice reached a final bodyweight of 29.5 ± 0.66 g, around 20% higher than in HFD fed C57BL/6JRj mice (final bodyweight: 24.6 ± 0.37 g). This is in line with reports showing a higher susceptibility of *C5ar1*$^{-/-}$ mice to HFD-induced bodyweight increase (Phieler et al., 2013b). However, analyzing the impact of the absence of C5aR1 on the course of allergic asthma in regular CD mice revealed that OVA treatment increased the AHR to a range similar to the one observed in wildtype mice. In addition, HFD feeding appeared to have no impact on this shape of response curve to methacholine, while the absolute values of lung resistance were similar between HFD and CD mice. Taken together, despite slightly decreased C5aR1 expression on neutrophils in HFD mice could be seen, the feeding experiment in *C5ar1*$^{-/-}$ mice do not highlight a functional involvement in the dampening effect of mild bodyweight increase on OVA-driven allergic asthma. However, the screening experiments do not allow for final conclusions and this issue should be part of future projects.

5.3 Conclusions and outlook

Mice, which are mildly metabolically challenged by a short-term high-fat and high-caloric diet, exhibit a milder form of experimental allergic asthma. This was shown by a lower airway hyperresponsiveness and reduced airway as well as pulmonary immune cell recruitment in the effector phase of allergic asthma. Particularly, DC and T cell numbers were decreased, and the data hint at an alleviation of Th1 and Th17 immunity as a consequence of impaired DC-T cell crosstalk via CD40. Of interest, Th2 immunity was excluded from this link suggesting that not classical allergy-promoting pathways are involved.

While the general concept of obese asthma implies that AHR develop as a result of adipokine-induced dysregulation of airway contractility and fibrosis as well as changes in collagen and elastin expression with little impact of a Th2 inflammation (Dinger et al., 2016; Jain et al., 2011; Mathews et al., 2014; Schaafsma et al., 2007; Silva et al., 2017), my model of short-term HFD feeding induced metabolic alterations in OVA-driven experimental allergic asthma rather suggests that a mild metabolic challenge protects from a complete asthma development and rather reduce the disease severity. This clearly indicates a differential impact of metabolic challenges in allergic and non-allergic asthma.

As an additional conclusion, this project brought new evidence to the field of obesity-associated asthma and underlines that multiple phenotypes with numerous immunological pathways are involved. In particular, a major outcome of this thesis is that there may be no linear relationship between increasing bodyweight and asthma-typical features. Alternatively, overweight might be a distinct state exerting a distinct impact on allergic asthma and may not “just” be a mild version of obesity, exerting milder, intermediate modulatory effects on allergic asthma.

However, future studies should aim at directly comparing lean, overweight and obese conditions on the development of allergic asthma. A recently demonstrated experimental model allows for induction of severe obesity in female mice without genetic modification (Giles et al., 2016, 2017), thus, would be suitable for such experiment. Further research with overweight asthmatics is highly needed for a deeper understanding of the involved immune mechanisms. Overweight as well as asthma are tremendously increasing all over the world and affecting children particularly, so, we face a clear medical need to elucidate the impact of overweight on the early development of allergic asthma. The presented evidence that dietary-associated metabolic stress associated with mild bodyweight increase primarily alters the function of lung DCs leading to altered T cell function may be prototypic as functional link between metabolism and the inflammatory system in terms of chronic disorders. In addition,

environmental factors other than the diet may be involved in the development of allergic asthma in pre-existing obesogenic diet and accompanied pro-inflammatory conditions.

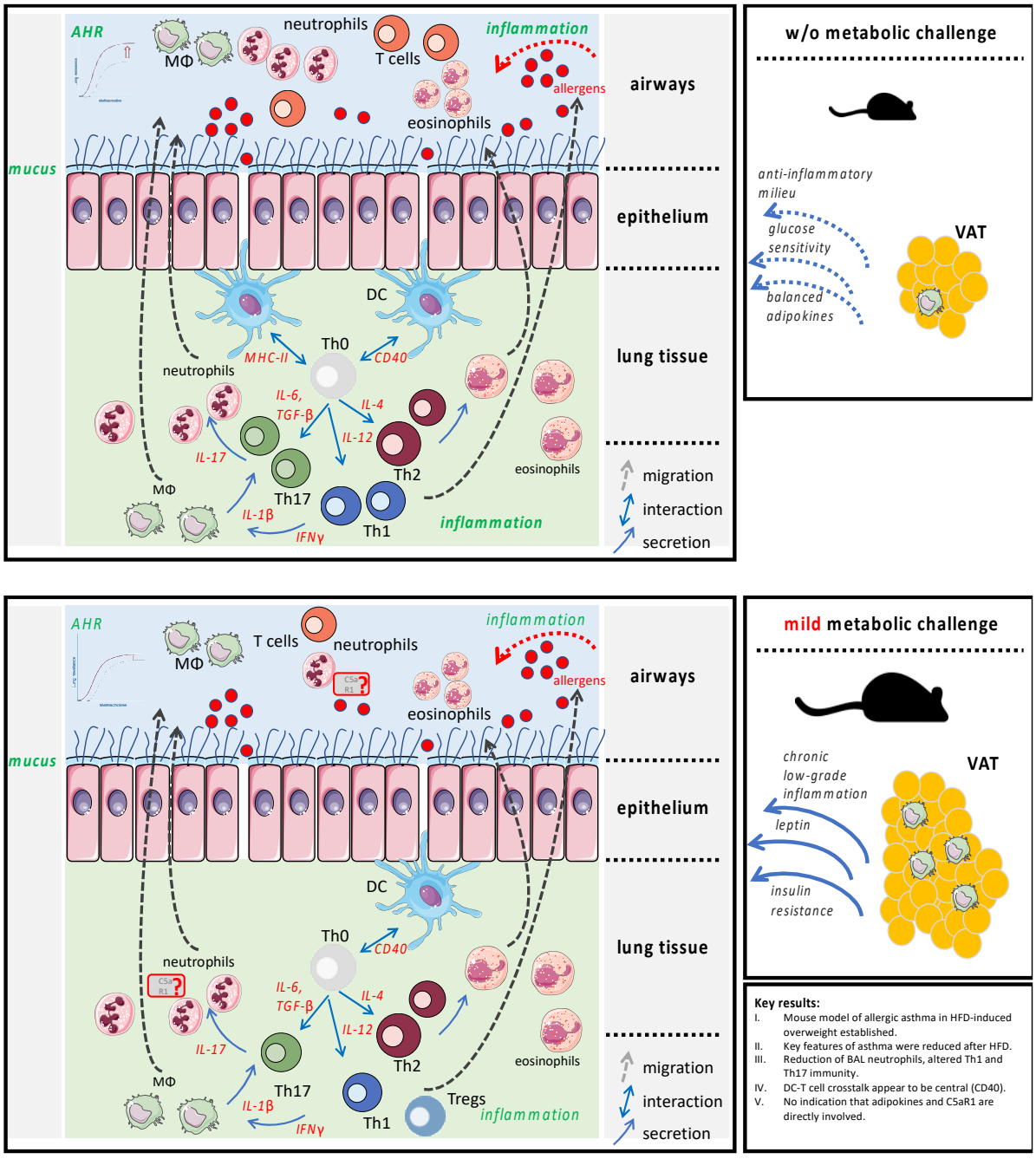

Figure 35: Graphical abstract of findings. Upon high fat diet (HFD), mice developed a mild increase in bodyweight with significant metabolic alterations but were still sensitive to allergic asthma development (I). However, metabolically challenged mice developed a milder form of experimental allergic asthma compared to lean mice (II). This included a lower airway hyperresponsiveness (AHR), less allergen-induced airway recruitment of T cells and neutrophils as well as lower pulmonary T cells and DC numbers. Mechanistically, Th1 and Th17 immunity were altered as a consequence of impaired DC-T cell crosstalk at the level of MHCII/CD40 (IV). However, although changes in expression could be observed, no indication that adipokines were directly involved could be found. Similarly, C5aR1 did not seem to play an important role in these processes (V).

6 References

Abdel Fattah, M., El Baz, M., Sherif, A., and Adel, A. (2010). Complement components (C3, C4) as inflammatory markers in asthma. Indian J. Pediatr. *77*, 771–773.

Akerman, M.J.H., Calacanis, C.M., and Madsen, M.K. (2004). Relationship between asthma severity and obesity. J. Asthma *41*, 521–526.

Al-Ramli, W., Préfontaine, D., Chouiali, F., Martin, J.G., Olivenstein, R., Lemière, C., and Hamid, Q. (2009). T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. J. Allergy Clin. Immunol. *123*, 1185–1187.

Ali, Z., and Ulrik, C.S. (2013). Obesity and asthma: a coincidence or a causal relationship? A systematic review. Respir. Med. *107*, 1287–1300.

Alphonse, M.P., Saffar, A.S., Shan, L., HayGlass, K.T., Simons, F.E.R., and Gounni, A.S. (2008). Regulation of the high affinity IgE receptor (Fc epsilonRI) in human neutrophils: role of seasonal allergen exposure and Th-2 cytokines. PLoS One *3*, e1921.

Alvarez, D., Vollmann, E.H., and von Andrian, U.H. (2008). Mechanisms and consequences of dendritic cell migration. Immunity *29*, 325–342.

Amelink, M., de Nijs, S.B., de Groot, J.C., van Tilburg, P.M.B., van Spiegel, P.I., Krouwels, F.H., Lutter, R., Zwinderman, a H., Weersink, E.J.M., ten Brinke, a, et al. (2013). Three phenotypes of adult-onset asthma. Allergy *68*, 674–680.

Amin, K., Janson, C., and Bystrom, J. (2016). Role of Eosinophil Granulocytes in Allergic Airway Inflammation Endotypes. Scand. J. Immunol. *84*, 75–85.

Anderson, G.P. (2008). Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet (London, England) *372*, 1107–1119.

Antonopoulos, A.S., and Tousoulis, D. (2017). The molecular mechanisms of obesity paradox. Cardiovasc. Res. *113*, 1074–1086.

Arango Duque, G., and Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. Front. Immunol. *5*, 491.

Arbore, G., Kemper, C., and Kolev, M. (2017). Intracellular complement - the complosome - in immune cell regulation. Mol. Immunol. *89*, 2–9.

Ardura-Garcia, C., Garner, P., and Cooper, P.J. (2018). Is childhood wheeze and asthma in Latin America associated with poor hygiene and infection? A systematic review. BMJ Open Respir. Res. *5*, e000249.

Arisqueta, L., Navarro-Imaz, H., Labiano, I., Rueda, Y., and Fresnedo, O. (2018). High fat

diet overfeeding promotes non-detrimental liver steatosis in female mice. Am. J. Physiol. Gastrointest. Liver Physiol.

Arteaga-Solis, E., Zee, T., Emala, C.W., Vinson, C., Wess, J., and Karsenty, G. (2013). Inhibition of leptin regulation of parasympathetic signaling as a cause of extreme body weight-associated asthma. Cell Metab. *17*, 35–48.

Atanes, P., Ruz-Maldonado, I., Pingitore, A., Hawkes, R., Liu, B., Zhao, M., Huang, G.C., Persaud, S.J., and Amisten, S. (2018). C3aR and C5aR1 act as key regulators of human and mouse β-cell function. Cell. Mol. Life Sci. *75*, 715–726.

Aujla, S.J., and Alcorn, J.F. (2011). T H17 cells in asthma and inflammation. Biochim. Biophys. Acta - Gen. Subj. *1810*, 1066–1079.

Aun, M.V., Bonamichi-Santos, R., Arantes-Costa, F.M., Kalil, J., and Giavina-Bianchi, P. (2017). Animal models of asthma: utility and limitations. J. Asthma Allergy *10*, 293–301.

Bach, J. (2002). The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. *347*, 911–920.

Bamberg, C.E., Mackay, C.R., Lee, H., Zahra, D., Jackson, J., Lim, Y.S., Whitfeld, P.L., Craig, S., Corsini, E., Lu, B., et al. (2010). The C5a receptor (C5aR) C5L2 is a modulator of C5aR-mediated signal transduction. J. Biol. Chem. *285*, 7633–7644.

Barlow, S.E., and Expert Committee (2007). Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics *120 Suppl*, S164-92.

Batra, A., Okur, B., Glauben, R., Erben, U., Ihbe, J., Stroh, T., Fedke, I., Chang, H.-D., Zeitz, M., and Siegmund, B. (2010). Leptin: a critical regulator of CD4+ T-cell polarization in vitro and in vivo. Endocrinology *151*, 56–62.

Bayly-Jones, C., Bubeck, D., and Dunstone, M.A. (2017). The mystery behind membrane insertion: a review of the complement membrane attack complex. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *372*.

Bedoret, D., Wallemacq, H., Marichal, T., Desmet, C., Quesada Calvo, F., Henry, E., Closset, R., Dewals, B., Thielen, C., Gustin, P., et al. (2009). Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice. J. Clin. Invest. *119*, 3723–3738.

Belamarich, P.F., Luder, E., Kattan, M., Mitchell, H., Islam, S., Lynn, H., and Crain, E.F. (2000). Do obese inner-city children with asthma have more symptoms than nonobese children with asthma? Pediatrics *106*, 1436–1441.

Bello, M.G.D., Knight, R., Gilbert, J.A., and Blaser, M.J. (2018). Preserving microbial

diversity. Science *362*, 33–34.

Bénard, M., Gonzalez, B.J., Schouft, M.-T., Falluel-Morel, A., Vaudry, D., Chan, P., Vaudry, H., and Fontaine, M. (2004). Characterization of C3a and C5a receptors in rat cerebellar granule neurons during maturation. Neuroprotective effect of C5a against apoptotic cell death. J. Biol. Chem. *279*, 43487–43496.

Berend, N., Salome, C.M., and King, G.G. (2008). Mechanisms of airway hyperresponsiveness in asthma. Respirology *13*, 624–631.

Beuther, D.A., and Sutherland, E.R. (2007). Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am. J. Respir. Crit. Care Med. *175*, 661–666.

Biswas, S.K., and Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat. Immunol. *11*, 889–896.

Blacquière, M.J., Hylkema, M.N., Postma, D.S., Geerlings, M., Timens, W., and Melgert, B.N. (2010). Airway inflammation and remodeling in two mouse models of asthma: comparison of males and females. Int. Arch. Allergy Immunol. *153*, 173–181.

Bloch, E.F., Knight, E.M., Carmon, T., McDonald-Pinkett, S., Carter, J., Boomer, A., Ogunfusika, M., Petersen, M., Famakin, B., Aniagolu, J., et al. (1997). C5b-7 and C5b-8 precursors of the membrane attack complex (C5b-9) are effective killers of E. coli J5 during serum incubation. Immunol. Invest. *26*, 409–419.

Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. Nat. Rev. Endocrinol. *15*, 288–298.

Boi, S.K., Buchta, C.M., Pearson, N.A., Francis, M.B., Meyerholz, D.K., Grobe, J.L., and Norian, L.A. (2016). Obesity alters immune and metabolic profiles: New insight from obese-resistant mice on high-fat diet. Obesity (Silver Spring). *24*, 2140–2149.

Börjeson, M. (1976). The aetiology of obesity in children. A study of 101 twin pairs. Acta Paediatr. Scand. *65*, 279–287.

Bosmann, M., Haggadone, M.D., Hemmila, M.R., Zetoune, F.S., Sarma, J.V., and Ward, P.A. (2012). Complement activation product C5a is a selective suppressor of TLR4-induced, but not TLR3-induced, production of IL-27(p28) from macrophages. J. Immunol. *188*, 5086–5093.

Botelho, S.C., Saghafian, M., Pavlova, S., Hassan, M., DePierre, J.W., and Abedi-Valugerdi, M. (2014). Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid. Chemosphere.

Boutens, L., Hooiveld, G.J., Dhingra, S., Cramer, R.A., Netea, M.G., and Stienstra, R.

(2018). Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia *61*, 942–953.

Brannan, J.D., and Lougheed, M.D. (2012). Airway hyperresponsiveness in asthma: Mechanisms, clinical significance, and treatment. Front. Physiol. *3 DEC*, 1–11.

Braun, L., Christophe, T., and Boulay, F. (2003). Phosphorylation of key serine residues is required for internalization of the complement 5a (C5a) anaphylatoxin receptor via a beta-arrestin, dynamin, and clathrin-dependent pathway. J. Biol. Chem. *278*, 4277–4285.

Bruno, A., Pace, E., Chanez, P., Gras, D., Vachier, I., Chiappara, G., La Guardia, M., Gerbino, S., Profita, M., and Gjomarkaj, M. (2009). Leptin and leptin receptor expression in asthma. J. Allergy Clin. Immunol. *124*, 230-237.e4.

Brunt, E.M., Kleiner, D.E., Wilson, L.A., Belt, P., Neuschwander-Tetri, B.A., and NASH Clinical Research Network (CRN) (2011). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology *53*, 810–820.

Buettner, R., Schölmerich, J., and Bollheimer, L.C. (2007). High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity (Silver Spring). *15*, 798–808.

Bullen, J.W.J., Ziotopoulou, M., Ungsunan, L., Misra, J., Alevizos, I., Kokkotou, E., Maratos-Flier, E., Stephanopoulos, G., and Mantzoros, C.S. (2004). Short-term resistance to diet-induced obesity in A/J mice is not associated with regulation of hypothalamic neuropeptides. Am. J. Physiol. Endocrinol. Metab. *287*, E662-70.

Bundesärztekammer (BÄK), Kassenärztliche Bundesvereinigung (KBV), and Arbeitsgemeinschaft der Wissenschaftli- chen Medizinischen Fachgesellschaften (AWMF) (2018). Nationale VersorgungsLeitlinie Asthma – Langfassung, 3. Auf- lage. Version 1. 2018. AWMF-Register-Nr.: Nvl-002.

Busse, W., Corren, J., Lanier, B.Q., McAlary, M., Fowler-Taylor, A., Cioppa, G.D., van As, A., and Gupta, N. (2001). Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J. Allergy Clin. Immunol. *108*, 184–190.

Bustos, P., Amigo, H., Oyarzún, M., and Rona, R.J. (2005). Is there a causal relation between obesity and asthma? Evidence from Chile. Int. J. Obes. *29*, 804–809.

Cai, Y., Sugimoto, C., Arainga, M., Alvarez, X., Didier, E.S., and Kuroda, M.J. (2014). In vivo characterization of alveolar and interstitial lung macrophages in rhesus macaques: implications for understanding lung disease in humans. J. Immunol. *192*, 2821–2829.

Calixto, M.C., Lintomen, L., Schenka, a, Saad, M.J., Zanesco, a, and Antunes, E. (2010). Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. Br. J.

Pharmacol. *159*, 617–625.

Camargo, C.A., Weiss, S.T., Zhang, S., Willett, W.C., and Speizer, F.E. (1999). Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch. Intern. Med. *159*, 2582–2588.

Capelo, A.V., de Fonseca, V.M., Peixoto, M.V.M., de Carvalho, S.R., and Guerino, L.G. (2015). Central obesity and other factors associated with uncontrolled asthma in women. Allergy, Asthma Clin. Immunol. *11*, 12.

Carden, T.J., and Carr, T.P. (2013). Food availability of glucose and fat, but not fructose, increased in the U.S. between 1970 and 2009: analysis of the USDA food availability data system. Nutr. J. *12*, 130.

Chawla, A., Nguyen, K.D., and Goh, Y.P.S. (2011). Macrophage-mediated inflammation in metabolic disease. Nat. Rev. Immunol. *11*, 738–749.

Chen, J.H., Qin, L., Shi, Y.Y., Feng, J.T., Zheng, Y.L., Wan, Y.F., Xu, C.Q., Yang, X.M., and Hu, C.P. (2016). IL-17 protein levels in both induced sputum and plasma are increased in stable but not acute asthma individuals with obesity. Respir. Med. *121*, 48–58.

Chen, N.-J., Mirtsos, C., Suh, D., Lu, Y.-C., Lin, W.-J., McKerlie, C., Lee, T., Baribault, H., Tian, H., and Yeh, W.-C. (2007). C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. Nature *446*, 203–207.

Chen, Y.-P., Zhang, J.-H., Li, C.-Q., Sun, Q.-X., and Jiang, X.-H. (2015). Obesity enhances Th2 inflammatory response via natural killer T cells in a murine model of allergic asthma. Int. J. Clin. Exp. Med. *8*, 15403–15412.

Chen, Y., Dales, R., Krewski, D., and Breithaupt, K. (1999). Increased effects of smoking and obesity on asthma among female Canadians: the National Population Health Survey, 1994-1995. Am. J. Epidemiol. *150*, 255–262.

Chen, Y., Dales, R., and Jiang, Y. (2006). The Association Between Obesity and Asthma Is Stronger in Nonallergic Than Allergic Adults. Chest *130*, 890–895.

Chen, Y., Tian, J., Tian, X., Tang, X., Rui, K., Tong, J., Lu, L., Xu, H., and Wang, S. (2014). Adipose tissue dendritic cells enhances inflammation by prompting the generation of Th17 cells. PLoS One *9*, 1–8.

Cheng, O.Z., and Palaniyar, N. (2013). NET balancing: a problem in inflammatory lung diseases. Front. Immunol. *4*, 1.

Chiou, W.-F., Tsai, H.-R., Yang, L.-M., and Tsai, W.-J. (2004). C5a differentially stimulates the ERK1/2 and p38 MAPK phosphorylation through independent signaling pathways to induced chemotactic migration in RAW264.7 macrophages. Int. Immunopharmacol. *4*, 1329–

1341.

Di Cicco, M., D'Elios, S., Peroni, D.G., and Comberiati, P. (2020). The role of atopy in asthma development and persistence. Curr. Opin. Allergy Clin. Immunol. *20*, 131–137.

Ciepiela, O., Ostafin, M., and Demkow, U. (2015). Neutrophils in asthma-A review. Respir. Physiol. Neurobiol. *209*, 13–16.

Cozzi, E., Ackerman, K.G., Lundequist, A., Drazen, J.M., Boyce, J.A., and Beier, D.R. (2011). The naive airway hyperresponsiveness of the A/J mouse is Kit-mediated. Proc. Natl. Acad. Sci. U. S. A. *108*, 12787–12792.

D, P.B.M., D, J.D.M., D, J.S.M., Vega, J.M., D, M.J.P.M., D, R.B.M., García-río, F., D, A.P.M., and D, S.Q.M. (2009). Obesity is not Associated with Mild Asthma Diagnosis in a Population of Spanish Adults of Spanish Adults †. *0903*.

DeKruyff, R.H., Yu, S., Kim, H.Y., and Umetsu, D.T. (2014). Innate immunity in the lung regulates the development of asthma. Immunol. Rev. *260*, 235–248.

Dembitzer, F.R., Kinoshita, Y., Burstein, D., Phelps, R.G., Beasley, M.B., Garcia, R., Harpaz, N., Jaffer, S., Thung, S.N., Unger, P.D., et al. (2012). gC1qR Expression in Normal and Pathologic Human Tissues: Differential Expression in Tissues of Epithelial and Mesenchymal Origin. J. Histochem. Cytochem. *60*, 467–474.

Dholakia, U., Clark-Price, S.C., Keating, S.C.J., and Stern, A.W. (2017). Anesthetic effects and body weight changes associated with ketamine-xylazine-lidocaine administered to CD-1 mice. PLoS One *12*, e0184911.

Diaz, J., Warren, L., Helfner, L., Xue, X., Chatterjee, P.K., Gupta, M., Solanki, M.H., Esposito, M., Bonagura, V., and Metz, C.N. (2015). Obesity shifts house dust mite-induced airway cellular infiltration from eosinophils to macrophages: effects of glucocorticoid treatment. Immunol. Res. *63*, 197–208.

Dietze, J., Böcking, C., Heverhagen, J.T., Voelker, M.N., and Renz, H. (2012). Obesity lowers the threshold of allergic sensitization and augments airway eosinophilia in a mouse model of asthma. Allergy *67*, 1519–1529.

Dinger, K., Kasper, P., Hucklenbruch-Rother, E., Vohlen, C., Jobst, E., Janoschek, R., Bae-Gartz, I., van Koningsbruggen-Rietschel, S., Plank, C., Dötsch, J., et al. (2016). Early-onset obesity dysregulates pulmonary adipocytokine/insulin signaling and induces asthma-like disease in mice. Sci. Rep. *6*, 24168.

Dixon, A.E., and Peters, U. (2018). The effect of obesity on lung function. Expert Rev. Respir. Med. *12*, 755–767.

Dixon, A.E., Pratley, R.E., Forgione, P.M., Kaminsky, D.A., Whittaker-Leclair, L.A., Griffes,

L.A., Garudathri, J., Raymond, D., Poynter, M.E., Bunn, J.Y., et al. (2011). Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. J. Allergy Clin. Immunol. *128*, 508–515.

Dobson, R., Burgess, M.I., Sprung, V.S., Irwin, A., Hamer, M., Jones, J., Daousi, C., Adams, V., Kemp, G.J., Shojaee-Moradie, F., et al. (2016). Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity. Int. J. Obes. (Lond). *40*, 153–161.

Doerner, S.K., Reis, E.S., Leung, E.S., Ko, J.S., Heaney, J.D., Berger, N.A., Lambris, J.D., and Nadeau, J.H. (2016). High-Fat Diet-Induced Complement Activation Mediates Intestinal Inflammation and Neoplasia, Independent of Obesity. Mol. Cancer Res. *14*, 953–965.

Dombrowicz, D., and Capron, M. (2001). Eosinophils, allergy and parasites. Curr. Opin. Immunol. *13*, 716–720.

Douros, K., Moustaki, M., Tsabouri, S., Papadopoulou, A., Papadopoulos, M., and Priftis, K.N. (2017). Prenatal Maternal Stress and the Risk of Asthma in Children. Front. Pediatr. *5*, 202.

Draijer, C., and Peters-Golden, M. (2017). Alveolar Macrophages in Allergic Asthma: the Forgotten Cell Awakes. Curr. Allergy Asthma Rep. *17*, 12.

Drouin, S.M., Sinha, M., Sfyroera, G., Lambris, J.D., and Wetsel, R.A. (2006). A protective role for the fifth complement component (c5) in allergic airway disease. Am. J. Respir. Crit. Care Med. *173*, 852–857.

Eckel, N., Meidtner, K., Kalle-Uhlmann, T., Stefan, N., and Schulze, M.B. (2016). Metabolically healthy obesity and cardiovascular events: A systematic review and meta-analysis. Eur. J. Prev. Cardiol. *23*, 956–966.

Eder, W., and von Mutius, E. (2004). Hygiene hypothesis and endotoxin: what is the evidence? Curr. Opin. Allergy Clin. Immunol. *4*, 113–117.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics *27*, 2194–2200.

Ekström, S., Hallberg, J., Kull, I., Protudjer, J.L.P., Thunqvist, P., Bottai, M., Gustafsson, P.M., Bergström, A., and Melén, E. (2018). Body mass index status and peripheral airway obstruction in school-age children: a population-based cohort study. Thorax thoraxjnl-2017-210716.

Ender, F., Wiese, A. V., Schmudde, I., Sun, J., Vollbrandt, T., König, P., Laumonnier, Y., and Köhl, J. (2017). Differential regulation of C5a receptor 1 in innate immune cells during the allergic asthma effector phase. PLoS One *12*, 1–21.

Engelke, C., Wiese, A. V, Schmudde, I., Ender, F., Strover, H.A., Vollbrandt, T., Konig, P., Laumonnier, Y., and Kohl, J. (2014). Distinct Roles of the Anaphylatoxins C3a and C5a in Dendritic Cell-Mediated Allergic Asthma. J. Immunol. *193*, 5387–5401.

Engin, A. (2017). The Definition and Prevalence of Obesity and Metabolic Syndrome. Adv. Exp. Med. Biol. *960*, 1–17.

European Academy of Allergy and Clinical Immunology (2013). Global Atlas of Asthma.

Evans, C.M., Fryer, A.D., Jacoby, D.B., Gleich, G.J., and Costello, R.W. (1997). Pretreatment with antibody to eosinophil major basic protein prevents hyperresponsiveness by protecting neuronal M2 muscarinic receptors in antigen-challenged guinea pigs. J. Clin. Invest. *100*, 2254–2262.

Fahy, J. V (2015). Type 2 inflammation in asthma--present in most, absent in many. Nat. Rev. Immunol. *15*, 57–65.

Farooqi, I.S., Jebb, S.A., Langmack, G., Lawrence, E., Cheetham, C.H., Prentice, A.M., Hughes, I.A., McCamish, M.A., and O'Rahilly, S. (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N. Engl. J. Med. *341*, 879–884.

Fasshauer, M., and Blüher, M. (2015). Adipokines in health and disease. Trends Pharmacol. Sci. *36*, 461–470.

Favero, G., Lonati, C., Giugno, L., Castrezzati, S., Rodella, L.F., and Rezzani, R. (2013). Obesity-related dysfunction of the aorta and prevention by melatonin treatment in ob/ob mice. Acta Histochem. *115*, 783–788.

Figueroa-Muñoz, J.I., Chinn, S., and Rona, R.J. (2001). Association between obesity and asthma in 4-11 year old children in the UK. Thorax *56*, 133–137.

Finkelstein, E.A., Khavjou, O.A., Thompson, H., Trogdon, J.G., Pan, L., Sherry, B., and Dietz, W. (2012). Obesity and severe obesity forecasts through 2030. Am. J. Prev. Med. *42*, 563–570.

Finucane, M.M., Stevens, G.A., Cowan, M.J., Danaei, G., Lin, J.K., Paciorek, C.J., Singh, G.M., Gutierrez, H.R., Lu, Y., Bahalim, A.N., et al. (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. Lancet (London, England) *377*, 557–567.

Fischer, W.H., and Hugli, T.E. (1997). Regulation of B cell functions by C3a and C3a(desArg): suppression of TNF-alpha, IL-6, and the polyclonal immune response. J. Immunol. *159*, 4279–4286.

Fischer, W.H., Jagels, M.A., and Hugli, T.E. (1999). Regulation of IL-6 synthesis in human

peripheral blood mononuclear cells by C3a and C3a(desArg). J. Immunol. *162*, 453–459.

Flaherman, V., and Rutherford, G.W. (2006). A meta-analysis of the effect of high weight on asthma. Arch. Dis. Child. *91*, 334–339.

Flegal, K.M., Kit, B.K., Orpana, H., and Graubard, B.I. (2013). Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA *309*, 71–82.

Fontaine, D.A., and Davis, D.B. (2016). Attention to Background Strain Is Essential for Metabolic Research: C57BL/6 and the International Knockout Mouse Consortium. Diabetes *65*, 25–33.

Fontaine, K.R., Redden, D.T., Wang, C., Westfall, A.O., and Allison, D.B. (2003). Years of life lost due to obesity. JAMA *289*, 187–193.

Ford, E.S. (2005). The epidemiology of obesity and asthma. J. Allergy Clin. Immunol. *115*, 897–910.

Fricker, M., and Gibson, P.G. (2017). Macrophage dysfunction in the pathogenesis and treatment of asthma. Eur. Respir. J. *50*.

Fukuoka, Y., and Hugli, T.E. (1988). Demonstration of a specific C3a receptor on guinea pig platelets. J. Immunol. *140*, 3496–3501.

Fulkerson, P.C., Fischetti, C.A., McBride, M.L., Hassman, L.M., Hogan, S.P., and Rothenberg, M.E. (2006). A central regulatory role for eosinophils and the eotaxin/CCR3 axis in chronic experimental allergic airway inflammation. Proc. Natl. Acad. Sci. U. S. A. *103*, 16418–16423.

Galesanu, R.G., Bernard, S., Marquis, K., Lacasse, Y., Poirier, P., Bourbeau, J., and Maltais, F. (2014). Obesity and chronic obstructive pulmonary disease: Is fatter really better? Can. Respir. J. *21*, 297–301.

Galli, S.J., Tsai, M., and Piliponsky, A.M. (2008). The development of allergic inflammation. Nature *454*, 445–454.

Gao, H., Neff, T.A., Guo, R.-F., Speyer, C.L., Sarma, J.V., Tomlins, S., Man, Y., Riedemann, N.C., Hoesel, L.M., Younkin, E., et al. (2005). Evidence for a functional role of the second C5a receptor C5L2. FASEB J. *19*, 1003–1005.

Gargiulo, S., Gramanzini, M., Megna, R., Greco, A., Albanese, S., Manfredi, C., and Brunetti, A. (2014). Evaluation of growth patterns and body composition in C57Bl/6J mice using dual energy X-ray absorptiometry. Biomed Res. Int. *2014*, 253067.

Gibbings, S.L., Thomas, S.M., Atif, S.M., McCubbrey, A.L., Desch, A.N., Danhorn, T., Leach,

S.M., Bratton, D.L., Henson, P.M., Janssen, W.J., et al. (2017). Three Unique Interstitial Macrophages in the Murine Lung at Steady State. Am. J. Respir. Cell Mol. Biol. *57*, 66–76.

Gilardini, L., Zambon, A., Soranna, D., Croci, M., and Invitti, C. (2018). Predictors of the transition from metabolically healthy obesity to unhealthy obesity. Eat. Weight Disord. *23*, 739–744.

Gilbert, E.R., Fu, Z., and Liu, D. (2011). Development of a nongenetic mouse model of type 2 diabetes. Exp. Diabetes Res. *2011*, 416254.

Giles, D.A., Ramkhelawon, B., Donelan, E.M., Stankiewicz, T.E., Hutchison, S.B., Mukherjee, R., Cappelletti, M., Karns, R., Karp, C.L., Moore, K.J., et al. (2016). Modulation of ambient temperature promotes inflammation and initiates atherosclerosis in wild type C57BL/6 mice. Mol. Metab. *5*, 1121–1130.

Giles, D.A., Moreno-Fernandez, M.E., Stankiewicz, T.E., Graspeuntner, S., Cappelletti, M., Wu, D., Mukherjee, R., Chan, C.C., Lawson, M.J., Klarquist, J., et al. (2017). Thermoneutral housing exacerbates nonalcoholic fatty liver disease in mice and allows for sex-independent disease modeling. Nat. Med. *23*, 829–838.

Gold, M.J., Antignano, F., Halim, T.Y.F., Hirota, J.A., Blanchet, M.-R., Zaph, C., Takei, F., and McNagny, K.M. (2014). Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. J. Allergy Clin. Immunol. *133*, 1142–1148.

Gordon, S. (2003). Alternative activation of macrophages. Nat. Rev. Immunol. *3*, 23–35.

Gosselin, D., Link, V.M., Romanoski, C.E., Fonseca, G.J., Eichenfield, D.Z., Spann, N.J., Stender, J.D., Chun, H.B., Garner, H., Geissmann, F., et al. (2014). Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell *159*, 1327–1340.

van Greevenbroek, M.M.J., Ghosh, S., van der Kallen, C.J.H., Brouwers, M.C.G.J., Schalkwijk, C.G., and Stehouwer, C.D.A. (2012). Up-regulation of the complement system in subcutaneous adipocytes from nonobese, hypertriglyceridemic subjects is associated with adipocyte insulin resistance. J. Clin. Endocrinol. Metab. *97*, 4742–4752.

Gueders, M.M., Paulissen, G., Crahay, C., Quesada-Calvo, F., Hacha, J., Van Hove, C., Tournoy, K., Louis, R., Foidart, J.-M., Noël, A., et al. (2009). Mouse models of asthma: a comparison between C57BL/6 and BALB/c strains regarding bronchial responsiveness, inflammation, and cytokine production. Inflamm. Res. *58*, 845–854.

Gundel, R.H., Letts, L.G., and Gleich, G.J. (1991). Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. J. Clin. Invest. *87*, 1470–1473.

Haase, C., Michelsen, B.K., and Jørgensen, T.N. (2004). CD40 is necessary for activation of naïve T cells by a dendritic cell line in vivo but not in vitro. Scand. J. Immunol. *59*, 237–245.

Hadeiba, H., Corry, D.B., and Locksley, R.M. (2000). Baseline airway hyperreactivity in A/J mice is not mediated by cells of the adaptive immune system. J. Immunol. *164*, 4933–4940.

Hajishengallis, G., Reis, E.S., Mastellos, D.C., Ricklin, D., and Lambris, J.D. (2017). Novel mechanisms and functions of complement. Nat. Immunol. *18*, 1288–1298.

Haldar, P., Pavord, I., and Shaw, D. (2008). Cluster Analysis and Clinical Asthma Phenotypes. Am J Respir Crit Care Med. *178*, 218–224.

Hall, S.L., Baker, T., Lajoie, S., Richgels, P.K., Yang, Y., Mcalees, J.W., Lier, A. Van, Wills-karp, M., Sivaprasad, U., Kovacic, M.B., et al. (2016). IL-17A enhances IL-13 activity by enhancing IL-13 – induced signal transducer and activator of transcription 6 activation. J. Allergy Clin. Immunol.

Hammad, H., and Lambrecht, B.N. (2008). Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. Nat. Rev. Immunol. *8*, 193–204.

Hariri, N., and Thibault, L. (2010). High-fat diet-induced obesity in animal models. Nutr. Res. Rev. *23*, 270–299.

Hauner, H., Bramlage, P., Lösch, C., Jöckel, K.-H., Moebus, S., Schunkert, H., and Wasem, J. (2008). Overweight, obesity and high waist circumference: regional differences in prevalence in primary medical care. Dtsch. Arztebl. Int. *105*, 827–833.

Hayashi, D., Li, D., Hayashi, C., Shatos, M., Hodges, R.R., and Dartt, D.A. (2012). Role of histamine and its receptor subtypes in stimulation of conjunctival goblet cell secretion. Invest. Ophthalmol. Vis. Sci. *53*, 2993–3003.

de Heer, H.J., Hammad, H., Soullié, T., Hijdra, D., Vos, N., Willart, M.A.M., Hoogsteden, H.C., and Lambrecht, B.N. (2004). Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. J. Exp. Med. *200*, 89–98.

Heiker, J.T., Kunath, A., Kosacka, J., Flehmig, G., Knigge, A., Kern, M., Stumvoll, M., Kovacs, P., Blüher, M., and Klöting, N. (2014). Identification of genetic loci associated with different responses to high-fat diet-induced obesity in C57BL/6N and C57BL/6J substrains. Physiol. Genomics *46*, 377–384.

Herbert, C.A., King, C.M., Ring, P.C., Holgate, S.T., Stewart, G.A., Thompson, P.J., and Robinson, C. (1995). Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1. Am. J. Respir. Cell Mol. Biol. *12*, 369–378.

Heydemann, A. (2016). An Overview of Murine High Fat Diet as a Model for Type 2 Diabetes Mellitus. J. Diabetes Res. *2016*, 2902351.

Hildebrandt, M. a, Hoffmann, C., Sherrill-Mix, S.A., Keilbaugh, S. a, Hamady, M., Chen, Y., Knight, R., Ahima, R.S., Bushman, F., and Wu, G.D. (2009). High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology *137*, 1716-24.e1-2.

Hill, J.O., Wyatt, H.R., and Peters, J.C. (2012). Energy balance and obesity. Circulation *126*, 126–132.

Hillebrandt, S., Wasmuth, H.E., Weiskirchen, R., Hellerbrand, C., Keppeler, H., Werth, A., Schirin-Sokhan, R., Wilkens, G., Geier, A., Lorenzen, J., et al. (2005). Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. Nat. Genet. *37*, 835–843.

Hillian, A.D., McMullen, M.R., Sebastian, B.M., Roychowdhury, S., Rowchowdhury, S., Kashyap, S.R., Schauer, P.R., Kirwan, J.P., Feldstein, A.E., and Nagy, L.E. (2013). Mice lacking C1q are protected from high fat diet-induced hepatic insulin resistance and impaired glucose homeostasis. J Biol Chem *288*, 22565–22575.

Hoffmann, F., Ender, F., Schmudde, I., Lewkowich, I.P., Köhl, J., König, P., and Laumonnier, Y. (2016). Origin, Localization, and Immunoregulatory Properties of Pulmonary Phagocytes in Allergic Asthma. Front. Immunol. *7*, 1–16.

Holgate, S.T., and Polosa, R. (2006). The mechanisms, diagnosis, and management of severe asthma in adults. Lancet (London, England) *368*, 780–793.

Holguin, F., Bleecker, E.R., Busse, W.W., Calhoun, W.J., Castro, M., Erzurum, S.C., Fitzpatrick, A.M., Gaston, B., Israel, E., Jarjour, N.N., et al. (2011). Obesity and asthma: an association modified by age of asthma onset. J. Allergy Clin. Immunol. *127*, 1486-93.e2.

Hosoki, K., Itazawa, T., Boldogh, I., and Sur, S. (2016). Neutrophil recruitment by allergens contribute to allergic sensitization and allergic inflammation. Curr. Opin. Allergy Clin. Immunol. *16*, 45–50.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. Nature *444*, 860–867.

Howard, R., Rattray, M., Prosperi, M., and Custovic, A. (2015). Distinguishing Asthma Phenotypes Using Machine Learning Approaches. Curr. Allergy Asthma Rep. *15*, 38.

Hsueh, W.-C., Bennett, P.H., Esparza-Romero, J., Urquidez-Romero, R., Valencia, M.E., Ravussin, E., Williams, R.C., Knowler, W.C., Baier, L.J., Schulz, L.O., et al. (2018). Analysis of type 2 diabetes and obesity genetic variants in Mexican Pima Indians: Marked allelic differentiation among Amerindians at HLA. Ann. Hum. Genet. *82*, 287–299.

Jacobsen, E.A., Zellner, K.R., Colbert, D., Lee, N.A., and Lee, J.J. (2011). Eosinophils regulate dendritic cells and Th2 pulmonary immune responses following allergen

provocation. J. Immunol. *187*, 6059–6068.

Jain, M., Budinger, G.R.S., Lo, A., Urich, D., Rivera, S.E., Ghosh, A.K., Gonzalez, A., Chiarella, S.E., Marks, K., Donnelly, H.K., et al. (2011). Leptin promotes fibroproliferative acute respiratory distress syndrome by inhibiting peroxisome proliferator-activated receptor-γ. Am. J. Respir. Crit. Care Med. *183*, 1490–1498.

Jiang, H., Kuang, Y., Wu, Y., Smrcka, A., Simon, M.I., and Wu, D. (1996). Pertussis toxin-sensitive activation of phospholipase C by the C5a and fMet-Leu-Phe receptors. J. Biol. Chem. *271*, 13430–13434.

Johari, V., and Loke, C. (2012). Brief overview of the coagulation cascade. Dis. Mon. *58*, 421–423.

Johnston, R.A., Zhu, M., Rivera-Sanchez, Y.M., Lu, F.L., Theman, T. a, Flynt, L., and Shore, S. a (2007). Allergic airway responses in obese mice. Am. J. Respir. Crit. Care Med. *176*, 650–658.

Julia, V., Macia, L., and Dombrowicz, D. (2015). The impact of diet on asthma and allergic diseases. Nat. Rev. Immunol. *15*, 308–322.

Jung, S.H., Kwon, J.M., Shim, J.W., Kim, D.S., Jung, H.L., Park, M.S., Park, S.H., Lee, J., Lee, W.Y., and Shim, J.Y. (2013). Effects of diet-induced mild obesity on airway hyperreactivity and lung inflammation in mice. Yonsei Med. J. *54*, 1430–1437.

Kabesch, M., Tzotcheva, I., Carr, D., Höfler, C., Weiland, S.K., Fritzsch, C., von Mutius, E., and Martinez, F.D. (2003). A complete screening of the IL4 gene: novel polymorphisms and their association with asthma and IgE in childhood. J. Allergy Clin. Immunol. *112*, 893–898.

Kanda, A., Driss, V., Hornez, N., Abdallah, M., Roumier, T., Abboud, G., Legrand, F., Staumont-Sallé, D., Quéant, S., Bertout, J., et al. (2009). Eosinophil-derived IFN-gamma induces airway hyperresponsiveness and lung inflammation in the absence of lymphocytes. J. Allergy Clin. Immunol. *124*, 573–582, 582.e1-9.

Karsten, C.M., Laumonnier, Y., Eurich, B., Ender, F., Bröker, K., Roy, S., Czabanska, A., Vollbrandt, T., Figge, J., and Köhl, J. (2015). Monitoring and cell-specific deletion of C5aR1 using a novel floxed GFP-C5aR1 reporter knock-in mouse. J. Immunol. *194*, 1841–1855.

Karsten, C.M., Wiese, A. V, Mey, F., Figge, J., Woodruff, T.M., Reuter, T., Scurtu, O., Kordowski, A., Almeida, L.N., Briukhovetska, D., et al. (2017). Monitoring C5aR2 Expression Using a Floxed tdTomato-C5aR2 Knock-In Mouse. J. Immunol. *199*, 3234–3248.

Kawada, T. (2015). Liver fat, visceral fat and metabolic syndrome in patients with severe obesity. Int. J. Surg. *22*, 153.

Kawano, H., Kayama, H., Nakama, T., Hashimoto, T., Umemoto, E., and Takeda, K. (2016).

IL-10-producing lung interstitial macrophages prevent neutrophilic asthma. Int. Immunol. *28*, 489–501.

Kelley, C.F., Mannino, D.M., Homa, D.M., Savage-Brown, A., and Holguin, F. (2005). Asthma phenotypes, risk factors, and measures of severity in a national sample of US children. Pediatrics *115*, 726–731.

Kern, M., Knigge, A., Heiker, J.T., Kosacka, J., Stumvoll, M., Kovacs, P., Blüher, M., and Klöting, N. (2012). C57BL/6JRj mice are protected against diet induced obesity (DIO). Biochem. Biophys. Res. Commun. *417*, 717–720.

Kim, H., Chang, Y., and Subramanian, S. (2012). Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. J. Allergy … *129*, 216–227.

Kim, H.Y., Lee, H.J., Chang, Y.-J., Pichavant, M., Shore, S. a, Fitzgerald, K. a, Iwakura, Y., Israel, E., Bolger, K., Faul, J., et al. (2014). Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. Nat. Med. *20*, 54–61.

Kim, H.Y., Umetsu, D.T., and Dekruyff, R.H. (2016). Innate lymphoid cells in asthma: Will they take your breath away? Eur. J. Immunol. *46*, 795–806.

Kim, J.Y., Sohn, J., Lee, J., and Park, J. (2015). Obesity increases airway hyperresponsiveness via the TNF-α pathway and treating obesity induces recovery. PLoS One *10*, e0116540.

Kirchhoff, K., Weinmann, O., Zwirner, J., Begemann, G., Götze, O., Kapp, A., and Werfel, T. (2001). Detection of anaphylatoxin receptors on CD83+ dendritic cells derived from human skin. Immunology *103*, 210–217.

Kita, H., Ohnishi, T., Okubo, Y., Weiler, D., Abrams, J.S., and Gleich, G.J. (1991). Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. J. Exp. Med. *174*, 745–748.

Klos, A., Tenner, A.J., Johswich, K.-O., Ager, R.R., Reis, E.S., and Köhl, J. (2009). The role of the anaphylatoxins in health and disease. Mol. Immunol. *46*, 2753–2766.

von Köckritz-Blickwede, M., and Nizet, V. (2009). Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. J. Mol. Med. (Berl). *87*, 775–783.

Köhl, J. (2006). The role of complement in danger sensing and transmission. Immunol. Res. *34*, 157–176.

Köhl, J., Baelder, R., Lewkowich, I.P., Pandey, M.K., Hawlisch, H., Wang, L., Best, J., Herman, N.S., Sproles, A.A., Zwirner, J., et al. (2006). A regulatory role for the C5a

anaphylatoxin in type 2 immunity in asthma. J. Clin. Invest. *116*, 783–796.

Kolev, M., Friec, G. Le, and Kemper, C. (2014). Complement — tapping into new sites and effector systems. Nat. Rev. Immunol. *14*, 811–820.

Kordonowy, L.L., Burg, E., Lenox, C.C., Gauthier, L.M., Petty, J.M., Antkowiak, M., Palvinskaya, T., Ubags, N., Rincón, M., Dixon, A.E., et al. (2012). Obesity is associated with neutrophil dysfunction and attenuation of murine acute lung injury. Am. J. Respir. Cell Mol. Biol. *47*, 120–127.

Korem, T., Zeevi, D., Zmora, N., Weissbrod, O., Bar, N., Lotan-Pompan, M., Avnit-Sagi, T., Kosower, N., Malka, G., Rein, M., et al. (2017). Bread Affects Clinical Parameters and Induces Gut Microbiome-Associated Personal Glycemic Responses. Cell Metab. *25*, 1243-1253.e5.

Kouzaki, H., O'Grady, S.M., Lawrence, C.B., and Kita, H. (2009). Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. J. Immunol. *183*, 1427–1434.

Kowal, K., Pampuch, A., Kowal-Bielecka, O., Iacoviello, L., and Bodzenta-Lukaszyk, A. (2006). Soluble CD40 ligand in asthma patients during allergen challenge. J. Thromb. Haemost. *4*, 2718–2720.

Koyuncuoğlu Güngör, N. (2014). Overweight and Obesity in Children and Adolescents. J. Clin. Res. Pediatr. Endocrinol. *6*, 129–143.

Krug, N., Tschernig, T., Erpenbeck, V.J., Hohlfeld, J.M., and Köhl, J. (2001). Complement factors C3a and C5a are increased in bronchoalveolar lavage fluid after segmental allergen provocation in subjects with asthma. Am. J. Respir. Crit. Care Med. *164*, 1841–1843.

Kuperman, D.A., Huang, X., Koth, L.L., Chang, G.H., Dolganov, G.M., Zhu, Z., Elias, J.A., Sheppard, D., and Erle, D.J. (2002). Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat. Med. *8*, 885–889.

Kurukulaaratchy, R.J., Matthews, S., and Arshad, S.H. (2006). Relationship between childhood atopy and wheeze: what mediates wheezing in atopic phenotypes? Ann. Allergy. Asthma Immunol. *97*, 84–91.

Kuruvilla, M.E., Lee, F.E.-H., and Lee, G.B. (2019). Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clin. Rev. Allergy Immunol. *56*, 219–233.

Kwak, S., Kim, Y.-D., Na, H.G., Bae, C.H., Song, S.-Y., and Choi, Y.S. (2018). Resistin upregulates MUC5AC/B mucin gene expression in human airway epithelial cells. Biochem. Biophys. Res. Commun. *499*, 655–661.

Kwon, J., Kim, S.-H., Kim, T.-B., Kim, S., Park, H.-W., Chang, Y.-S., Jang, A.-S., Cho, Y.S.,

Nahm, D.-H., Park, J.-W., et al. (2012). Airway hyperresponsiveness is negatively associated with obesity or overweight status in patients with asthma. Int. Arch. Allergy Immunol. *159*, 187–193.

Laaksonen, K.S., Nevalainen, T.O., Haasio, K., Kasanen, I.H.E., Nieminen, P.A., and Voipio, H.-M. (2013). Food and water intake, growth, and adiposity of Sprague-Dawley rats with diet board for 24 months. Lab. Anim. *47*, 245–256.

Laan, M., Cui, Z.H., Hoshino, H., Lötvall, J., Sjöstrand, M., Gruenert, D.C., Skoogh, B.E., and Lindén, A. (1999). Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. J. Immunol. *162*, 2347–2352.

Lajoie, S., Lewkowich, I.P., Suzuki, Y., Clark, J.R., Sproles, A.A., Dienger, K., Budelsky, A.L., and Wills-Karp, M. (2010). Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. Nat. Immunol. *11*, 928–935.

Lalli, P.N., Strainic, M.G., Yang, M., Lin, F., Medof, M.E., and Heeger, P.S. (2008). Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. Blood *112*, 1759–1766.

Lam, Q.L.K., Liu, S., Cao, X., and Lu, L. (2006). Involvement of leptin signaling in the survival and maturation of bone marrow-derived dendritic cells. Eur. J. Immunol. *36*, 3118–3130.

Lam, Q.L.K., Zheng, B.J., Jin, D.Y., Cao, X., and Lu, L. (2007). Leptin induces CD40 expression through the activation of Akt in murine dendritic cells. J. Biol. Chem. *282*, 27587–27597.

Lambrecht, B.N., and Hammad, H. (2012). The airway epithelium in asthma. Nat. Med. *18*, 684–692.

Lambrecht, B.N., and Hammad, H. (2014a). Allergens and the airway epithelium response: gateway to allergic sensitization. J. Allergy Clin. Immunol. *134*, 499–507.

Lambrecht, B.N., and Hammad, H. (2014b). Dendritic cell and epithelial cell interactions at the origin of murine asthma. Ann. Am. Thorac. Soc. *11 Suppl 5*, S236-43.

Lambrecht, B.N., and Hammad, H. (2015). The immunology of asthma. Nat. Immunol. *16*, 45–56.

Lapin, B., Piorkowski, J., Ownby, D., Freels, S., Chavez, N., Hernandez, E., Wagner-Cassanova, C., Pelzel, D., Vergara, C., and Persky, V. (2015). Relationship between prenatal antibiotic use and asthma in at-risk children. Ann. Allergy. Asthma Immunol. *114*, 203–207.

Larsson, L. (1995). Incidence of asthma in Swedish teenagers: relation to sex and smoking

habits. Thorax *50*, 260–264.

Laumonnier, Y., Karsten, C.M., and Köhl, J. (2017). Novel insights into the expression pattern of anaphylatoxin receptors in mice and men. Mol. Immunol. *89*, 44–58.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., and Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell *159*, 1312–1326.

Leckie, M.J., ten Brinke, A., Khan, J., Diamant, Z., O'Connor, B.J., Walls, C.M., Mathur, A.K., Cowley, H.C., Chung, K.F., Djukanovic, R., et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet (London, England) *356*, 2144–2148.

Leiria, L.O.S., Martins, M.A., and Saad, M.J.A. (2015a). Obesity and asthma: beyond T(H)2 inflammation. Metabolism. *64*, 172–181.

Leiria, L.O.S., Arantes-Costa, F.M., Calixto, M.C., Alexandre, E.C., Moura, R.F., Folli, F., Prado, C.M., Prado, M.A., Prado, V.F., Velloso, L.A., et al. (2015b). Increased airway reactivity and hyperinsulinemia in obese mice are linked by ERK signaling in brain stem cholinergic neurons. Cell Rep. *11*, 934–943.

Levy, H., Raby, B.A., Lake, S., Tantisira, K.G., Kwiatkowski, D., Lazarus, R., Silverman, E.K., Richter, B., Klimecki, W.T., Vercelli, D., et al. (2005). Association of defensin beta-1 gene polymorphisms with asthma. J. Allergy Clin. Immunol. *115*, 252–258.

Lewkowich, I.P., Lajoie, S., Clark, J.R., Herman, N.S., Sproles, A.A., and Wills-Karp, M. (2008). Allergen uptake, activation, and IL-23 production by pulmonary myeloid DCs drives airway hyperresponsiveness in asthma-susceptible mice. PLoS One *3*, e3879.

Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C. a, Knight, R.D., and Gordon, J.I. (2005). Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U. S. A. *102*, 11070–11075.

León, B. (2017). T Cells in Allergic Asthma: Key Players Beyond the Th2 Pathway. Curr. Allergy Asthma Rep. *17*, 43.

Li, J.-G., DU, Y.-M., Yan, Z.-D., Yan, J., Zhuansun, Y.-X., Chen, R., Zhang, W., Feng, S.-L., and Ran, P.-X. (2016). CD80 and CD86 knockdown in dendritic cells regulates Th1/Th2 cytokine production in asthmatic mice. Exp. Ther. Med. *11*, 878–884.

Lim, J., Iyer, A., Suen, J.Y., Seow, V., Reid, R.C., Brown, L., and Fairlie, D.P. (2013). C5aR and C3aR antagonists each inhibit diet-induced obesity, metabolic dysfunction, and adipocyte and macrophage signaling. FASEB J. *27*, 822–831.

Liszewski, M.K., Kolev, M., Le Friec, G., Leung, M., Bertram, P.G., Fara, A.F., Subias, M.,

Pickering, M.C., Drouet, C., Meri, S., et al. (2013). Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. Immunity *39*, 1143–1157.

Lloyd, C.M., and Saglani, S. (2010). Asthma and allergy: the emerging epithelium. Nat. Med. *16*, 273–274.

Lloyd, C.M., and Saglani, S. (2013). Eosinophils in the spotlight: Finding the link between obesity and asthma. Nat. Med. *19*, 976–977.

Locksley, R.M. (2010). Asthma and Allergic Inflammation. Cell *140*, 777–783.

Loewen, K., Monchka, B., Mahmud, S.M., 't Jong, G., and Azad, M.B. (2018). Prenatal antibiotic exposure and childhood asthma: a population-based study. Eur. Respir. J. *52*.

Loffreda, S., Yang, S.Q., Lin, H.Z., Karp, C.L., Brengman, M.L., Wang, D.J., Klein, A.S., Bulkley, G.B., Bao, C., Noble, P.W., et al. (1998). Leptin regulates proinflammatory immune responses. FASEB J. *12*, 57–65.

Lötvall, J., Akdis, C.A., Bacharier, L.B., Bjermer, L., Casale, T.B., Custovic, A., Lemanske, R.F., Wardlaw, A.J., Wenzel, S.E., and Greenberger, P.A. (2011). Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J. Allergy Clin. Immunol. *127*, 355–360.

Louis, R., Lau, L.C., Bron, A.O., Roldaan, A.C., Radermecker, M., and Djukanović, R. (2000). The relationship between airways inflammation and asthma severity. Am. J. Respir. Crit. Care Med. *161*, 9–16.

Luthe, S.K., Hirayama, A., Goto, T., Faridi, M.K., Camargo, C.A., and Hasegawa, K. (2018). Association Between Obesity and Acute Severity Among Patients Hospitalized for Asthma Exacerbation. J. Allergy Clin. Immunol. Pract.

Lutz, T.A., and Woods, S.C. (2012). Overview of animal models of obesity. Curr. Protoc. Pharmacol. *Chapter 5*, Unit5.61.

Ma, D.Y., and Clark, E. a (2010). The role of CD40 and CD40L in Dendritic Cells. Semin Immunol. 2009 *21*, 265–272.

Madenspacher, J.H., Draper, D.W., Smoak, K.A., Li, H., Griffiths, G.L., Suratt, B.T., Wilson, M.D., Rudel, L.L., and Fessler, M.B. (2010). Dyslipidemia induces opposing effects on intrapulmonary and extrapulmonary host defense through divergent TLR response phenotypes. J. Immunol. *185*, 1660–1669.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., and Ranganathan, S. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. *1*, 1155–1161.

Malik, V.S., Willett, W.C., and Hu, F.B. (2013). Global obesity: trends, risk factors and policy implications. Nat. Rev. Endocrinol. *9*, 13–27.

Mamane, Y., Chung Chan, C., Lavallee, G., Morin, N., Xu, L.-J., Huang, J., Gordon, R., Thomas, W., Lamb, J., Schadt, E.E., et al. (2009). The C3a anaphylatoxin receptor is a key mediator of insulin resistance and functions by modulating adipose tissue macrophage infiltration and activation. Diabetes *58*, 2006–2017.

Manicone, A.M., Gong, K., Johnston, L.K., and Giannandrea, M. (2016). Diet-induced obesity alters myeloid cell populations in naïve and injured lung. Respir. Res. *17*, 24.

Martín-Romero, C., Santos-Alvarez, J., Goberna, R., and Sánchez-Margalet, V. (2000). Human leptin enhances activation and proliferation of human circulating T lymphocytes. Cell. Immunol. *199*, 15–24.

Martínez-López, M., Iborra, S., Conde-Garrosa, R., and Sancho, D. (2015). Batf3-dependent CD103+ dendritic cells are major producers of IL-12 that drive local Th1 immunity against Leishmania major infection in mice. Eur. J. Immunol. *45*, 119–129.

Martinez, F.O., and Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. *6*, 13.

Maruo, K., Akaike, T., Ono, T., Okamoto, T., and Maeda, H. (1997). Generation of anaphylatoxins through proteolytic processing of C3 and C5 by house dust mite protease. J. Allergy Clin. Immunol. *100*, 253–260.

Masoli, M., Fabian, D., Holt, S., Beasley, R., and Global Initiative for Asthma (GINA) Program (2004). The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy *59*, 469–478.

Mastellos, D., Papadimitriou, J.C., Franchini, S., Tsonis, P.A., and Lambris, J.D. (2001). A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. J. Immunol. *166*, 2479–2486.

Mathews, J.A., Wurmbrand, A.P., Ribeiro, L., Neto, F.L., and Shore, S.A. (2014). Induction of IL-17A Precedes Development of Airway Hyperresponsiveness during Diet-Induced Obesity and Correlates with Complement Factor D. Front. Immunol. *5*, 440.

Mathis, D. (2013). Immunological goings-on in visceral adipose tissue. Cell Metab. *17*, 851–859.

Matsui, E.C. (2014). Environmental exposures and asthma morbidity in children living in urban neighborhoods. Allergy *69*, 553–558.

Mattioli, B., Straface, E., Quaranta, M.G., Giordani, L., and Viora, M. (2005). Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming. J.

Immunol. *174*, 6820–6828.

Mattioli, B., Giordani, L., Quaranta, M.G., and Viora, M. (2009). Leptin exerts an anti-apoptotic effect on human dendritic cells via the PI3K-Akt signaling pathway. FEBS Lett. *583*, 1102–1106.

McCullough, R.L., McMullen, M.R., Poulsen, K.L., Kim, A., Medof, M.E., and Nagy, L.E. (2018). Anaphylatoxin Receptors C3aR and C5aR1 Are Important Factors That Influence the Impact of Ethanol on the Adipose Secretome. Front. Immunol. *9*, 2133.

McGinnis, J.M., and Nestle, M. (1989). The Surgeon General's Report on Nutrition and Health: policy implications and implementation strategies. Am. J. Clin. Nutr. *49*, 23–28.

McKinley, L., Kim, J., Bolgos, G.L., Siddiqui, J., and Remick, D.G. (2006). Allergens induce enhanced bronchoconstriction and leukotriene production in C5 deficient mice. Respir. Res. *7*, 129.

McKinley, L., Alcorn, J.F., Peterson, A., DuPont, R.B., Kapadia, S., Logar, A., Henry, A., Irvin, C.G., Piganelli, J.D., Ray, A., et al. (2008). TH17 Cells Mediate Steroid-Resistant Airway Inflammation and Airway Hyperresponsiveness in Mice. J. Immunol. *181*, 4089–4097.

McNelis, J.C., and Olefsky, J.M. (2014). Macrophages, immunity, and metabolic disease. Immunity *41*, 36–48.

Medoff, B.D., Okamoto, Y., Leyton, P., Weng, M., Sandall, B.P., Raher, M.J., Kihara, S., Bloch, K.D., Libby, P., and Luster, A.D. (2009). Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. Am. J. Respir. Cell Mol. Biol. *41*, 397–406.

Melgert, B.N., Postma, D.S., Kuipers, I., Geerlings, M., Luinge, M.A., van der Strate, B.W.A., Kerstjens, H.A.M., Timens, W., and Hylkema, M.N. (2005). Female mice are more susceptible to the development of allergic airway inflammation than male mice. Clin. Exp. Allergy *35*, 1496–1503.

Melgert, B.N., ten Hacken, N.H., Rutgers, B., Timens, W., Postma, D.S., and Hylkema, M.N. (2011). More alternative activation of macrophages in lungs of asthmatic patients. J. Allergy Clin. Immunol. *127*, 831–833.

Mendes-Soares, H., Raveh-Sadka, T., Azulay, S., Edens, K., Ben-Shlomo, Y., Cohen, Y., Ofek, T., Bachrach, D., Stevens, J., Colibaseanu, D., et al. (2019). Assessment of a Personalized Approach to Predicting Postprandial Glycemic Responses to Food Among Individuals Without Diabetes. JAMA Netw. Open *2*, e188102.

Meng, Q., Lai, Y.-C., Kelly, N.J., Bueno, M., Baust, J.J., Bachman, T.N., Goncharov, D., Vanderpool, R.R., Radder, J.E., Hu, J., et al. (2017). Development of a Mouse Model of

Metabolic Syndrome, Pulmonary Hypertension, and Heart Failure with Preserved Ejection Fraction. Am. J. Respir. Cell Mol. Biol. *56*, 497–505.

Miethe, S., Guarino, M., Alhamdan, F., Simon, H.-U., Renz, H., Dufour, J.-F., Potaczek, D.P., and Garn, H. (2018). The effects of obesity on asthma: immunometabolic links. Polish Arch. Intern. Med.

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Delgado Palacio, S., Arboleya Montes, S., Mancabelli, L., et al. (2017). The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol. Mol. Biol. Rev. *81*.

Miller, R.L. (2008). Prenatal maternal diet affects asthma risk in offspring. J. Clin. Invest. *118*, 3265–3268.

Mito, N., Kitada, C., Hosoda, T., and Sato, K. (2002). Effect of diet-induced obesity on ovalbumin-specific immune response in a murine asthma model. Metabolism *51*, 1241–1246.

Moffatt, M.F., and Cookson, W.O. (1997). Tumour necrosis factor haplotypes and asthma. Hum. Mol. Genet. *6*, 551–554.

Montecucco, F., Bianchi, G., Gnerre, P., Bertolotto, M., Dallegri, F., and Ottonello, L. (2006). Induction of neutrophil chemotaxis by leptin: crucial role for p38 and Src kinases. Ann. N. Y. Acad. Sci. *1069*, 463–471.

Montgomery, M.K., Hallahan, N.L., Brown, S.H., Liu, M., Mitchell, T.W., Cooney, G.J., and Turner, N. (2013). Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. Diabetologia *56*, 1129–1139.

Moore, K.J., Sheedy, F.J., and Fisher, E.A. (2013). Macrophages in atherosclerosis: a dynamic balance. Nat. Rev. Immunol. *13*, 709–721.

Moraes-Vieira, P.M.M., Bassi, E.J., Larocca, R.A., Castoldi, A., Burghos, M., Lepique, A.P., Quintana, F.J., Araujo, R.C., Basso, A.S., Strom, T.B., et al. (2013). Leptin deficiency modulates allograft survival by favoring a Th2 and a regulatory immune profile. [corrected]. Am. J. Transplant *13*, 36–44.

Moraes-Vieira, P.M.M., Larocca, R.A., Bassi, E.J., Peron, J.P.S., Andrade-Oliveira, V., Wasinski, F., Araujo, R., Thornley, T., Quintana, F.J., Basso, A.S., et al. (2014). Leptin deficiency impairs maturation of dendritic cells and enhances induction of regulatory T and Th17 cells. Eur J Immunol *44*, 794–806.

Moreira, A.P., and Hogaboam, C.M. (2011). Macrophages in allergic asthma: fine-tuning their pro- and anti-inflammatory actions for disease resolution. J. Interferon Cytokine Res. *31*, 485–491.

Morgan, B.P., and Gasque, P. (1997). Extrahepatic complement biosynthesis: where, when and why? Clin. Exp. Immunol. *107*, 1–7.

Mullmann, T.J., Siegel, M.I., Egan, R.W., and Billah, M.M. (1990). Complement C5a activation of phospholipase D in human neutrophils. A major route to the production of phosphatidates and diglycerides. J. Immunol. *144*, 1901–1908.

Murphy, K., Travers, P., Walport, M., and Janeway, C. (2008). Janeway's immunobiology. (New York: Garland Science).

Nagel, G., Koenig, W., Rapp, K., Wabitsch, M., Zoellner, I., and Weiland, S.K. (2009). Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren. Pediatr. Allergy Immunol. *20*, 81–88.

Natal, C., Restituto, P., Iñigo, C., Colina, I., Díez, J., and Varo, N. (2008). The proinflammatory mediator CD40 ligand is increased in the metabolic syndrome and modulated by adiponectin. J. Clin. Endocrinol. Metab. *93*, 2319–2327.

NCD Risk Factor Collaboration (NCD-RisC) (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet (London, England) *387*, 1377–1396.

NCD Risk Factor Collaboration (NCD-RisC) (2017). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet (London, England) *390*, 2627–2642.

Newcomb, D.C., and Peebles, R.S. (2013). Th17-mediated inflammation in asthma. Curr. Opin. Immunol. *25*, 755–760.

Nickelson, K.J., Stromsdorfer, K.L., Pickering, R.T., Liu, T.-W., Ortinau, L.C., Keating, A.F., and Perfield, J.W. (2012). A comparison of inflammatory and oxidative stress markers in adipose tissue from weight-matched obese male and female mice. Exp. Diabetes Res. *2012*, 859395.

Nilsson, G., Johnell, M., Hammer, C.H., Tiffany, H.L., Nilsson, K., Metcalfe, D.D., Siegbahn, A., and Murphy, P.M. (1996). C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. J. Immunol. *157*, 1693–1698.

Nishina, P.M., Lowe, S., Wang, J., and Paigen, B. (1994). Characterization of plasma lipids in genetically obese mice: the mutants obese, diabetes, fat, tubby, and lethal yellow. Metabolism. *43*, 549–553.

Nunes, C., Pereira, A.M., and Morais-Almeida, M. (2017). Asthma costs and social impact.

Asthma Res. Pract. *3*, 1.

Nurmagambetov, T., Kuwahara, R., and Garbe, P. (2018). The Economic Burden of Asthma in the United States, 2008-2013. Ann. Am. Thorac. Soc. *15*, 348–356.

O'Brien, P.D., Hinder, L.M., Parlee, S.D., Hayes, J.M., Backus, C., Zhang, H., Ma, L., Sakowski, S.A., Brosius, F.C., and Feldman, E.L. (2017). Dual CCR2/CCR5 antagonist treatment attenuates adipose inflammation, but not microvascular complications in ob/ob mice. Diabetes. Obes. Metab. *19*, 1468–1472.

OECD (2017). Obesity Update 2017.

Osborn, O., and Olefsky, J.M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. Nat. Med. *18*, 363–374.

Ottonello, L., Gnerre, P., Bertolotto, M., Mancini, M., Dapino, P., Russo, R., Garibotto, G., Barreca, T., and Dallegri, F. (2004). Leptin as a uremic toxin interferes with neutrophil chemotaxis. J. Am. Soc. Nephrol. *15*, 2366–2372.

Ozdemir, C., Kucuksezer, U.C., Akdis, M., and Akdis, C.A. (2018). The concepts of asthma endotypes and phenotypes to guide current and novel treatment strategies. Expert Rev. Respir. Med. *12*, 733–743.

Pai, S.-Y., Truitt, M.L., and Ho, I.-C. (2004). GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc. Natl. Acad. Sci. U. S. A. *101*, 1993–1998.

Pancer, Z., and Cooper, M.D. (2006). The evolution of adaptive immunity. Annu. Rev. Immunol. *24*, 497–518.

Panda, S., and Ding, J.L. (2015). Natural antibodies bridge innate and adaptive immunity. J. Immunol. *194*, 13–20.

Papoutsakis, C., Priftis, K.N., Drakouli, M., Prifti, S., Konstantaki, E., Antonogeorgos, G., Chondronikola, M., and Matziou, V. (2013). Childhood Overweight/Obesity and Asthma: Is There a Link? A Systematic Review of Recent Epidemiologic Evidence. J. Acad. Nutr. Diet. *113*, 77–105.

Park, H.J., Lee, J.-H., Park, Y.H., Han, H., Sim, D.W., Park, K.H., and Park, J.-W. (2016). Roflumilast Ameliorates Airway Hyperresponsiveness Caused by Diet-Induced Obesity in a Murine Model. Am. J. Respir. Cell Mol. Biol. *55*, 82–91.

Passalacqua, G., and Ciprandi, G. (2008). Allergy and the lung. Clin. Exp. Immunol. *153 Suppl*, 12–16.

Pelaia, G., Vatrella, A., and Maselli, R. (2012). The potential of biologics for the treatment of asthma. Nat. Rev. Drug Discov. *11*, 958–972.

Pennock, N.D., White, J.T., Cross, E.W., Cheney, E.E., Tamburini, B.A., and Kedl, R.M. (2013). T cell responses: naive to memory and everything in between. Adv. Physiol. Educ. *37*, 273–283.

Perianayagam, M.C., Balakrishnan, V.S., King, A.J., Pereira, B.J.G., and Jaber, B.L. (2002). C5a delays apoptosis of human neutrophils by a phosphatidylinositol 3-kinase-signaling pathway. Kidney Int. *61*, 456–463.

Perros, F., Hoogsteden, H.C., Coyle, A.J., Lambrecht, B.N., and Hammad, H. (2009). Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation. Allergy *64*, 995–1002.

Pettersson, U.S., Waldén, T.B., Carlsson, P.-O., Jansson, L., and Phillipson, M. (2012). Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLoS One *7*, e46057.

Phieler, J., Garcia-Martin, R., Lambris, J.D., and Chavakis, T. (2013a). The role of the complement system in metabolic organs and metabolic diseases. Semin. Immunol. *25*, 47–53.

Phieler, J., Chung, K.-J., Chatzigeorgiou, A., Klotzsche-von Ameln, A., Garcia-Martin, R., Sprott, D., Moisidou, M., Tzanavari, T., Ludwig, B., Baraban, E., et al. (2013b). The complement anaphylatoxin C5a receptor contributes to obese adipose tissue inflammation and insulin resistance. J. Immunol. *191*, 4367–4374.

Picard, F., Richard, D., Huang, Q., and Deshaies, Y. (1998). Effects of leptin adipose tissue lipoprotein lipase in the obese ob/ob mouse. Int. J. Obes. Relat. Metab. Disord. *22*, 1088–1095.

Pinedo, C., Cano, M.P., and Martín, C. (2009). Body mass index and asthma severity in the National Asthma Survey. Rev. Patol. Respir. *12*, 50–51.

Plantinga, M., Guilliams, M., Vanheerswynghels, M., Deswarte, K., Branco-Madeira, F., Toussaint, W., Vanhoutte, L., Neyt, K., Killeen, N., Malissen, B., et al. (2013). Conventional and Monocyte-Derived CD11b+ Dendritic Cells Initiate and Maintain T Helper 2 Cell-Mediated Immunity to House Dust Mite Allergen. Immunity *38*, 322–335.

Possa, S.S., Leick, E.A., Prado, C.M., Martins, M.A., and Tibério, I.F.L.C. (2013). Eosinophilic inflammation in allergic asthma. Front. Pharmacol. *4*, 46.

Poursharifi, P., Lapointe, M., Fisette, A., Lu, H., Roy, C., Munkonda, M.N., Fairlie, D.P., and Cianflone, K. (2014a). C5aR and C5L2 act in concert to balance immunometabolism in adipose tissue. Mol. Cell. Endocrinol. *382*, 325–333.

Poursharifi, P., Rezvani, R., Gupta, A., Lapointe, M., Marceau, P., Tchernof, A., and Cianflone, K. (2014b). Association of immune and metabolic receptors C5aR and C5L2 with adiposity in women. Mediators Inflamm. *2014*, 413921.

Procaccini, C., Jirillo, E., and Matarese, G. (2012a). Leptin as an immunomodulator. Mol. Aspects Med. *33*, 35–45.

Procaccini, C., De Rosa, V., Galgani, M., Carbone, F., Cassano, S., Greco, D., Qian, K., Auvinen, P., Calì, G., Stallone, G., et al. (2012b). Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. J. Immunol. *189*, 2941–2953.

Prospective Studies Collaboration, Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., Qizilbash, N., Collins, R., and Peto, R. (2009). Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet (London, England) *373*, 1083–1096.

Pykäläinen, M., Kinos, R., Valkonen, S., Rydman, P., Kilpeläinen, M., Laitinen, L.A., Karjalainen, J., Nieminen, M., Hurme, M., Kere, J., et al. (2005). Association analysis of common variants of STAT6, GATA3, and STAT4 to asthma and high serum IgE phenotypes. J. Allergy Clin. Immunol. *115*, 80–87.

Qin, X., and Gao, B. (2006). The complement system in liver diseases. Cell. Mol. Immunol. *3*, 333–340.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. *41*, D590-6.

Ramirez, O., and Garza, K.M. (2014). Leptin deficiency in vivo enhances the ability of splenic dendritic cells to activate T cells. Int. Immunol. *26*, 627–636.

Reilly, J.J., Armstrong, J., Dorosty, A.R., Emmett, P.M., Ness, A., Rogers, I., Steer, C., Sherriff, A., and Avon Longitudinal Study of Parents and Children Study Team (2005). Early life risk factors for obesity in childhood: cohort study. BMJ *330*, 1357.

Reis, E.S., Mastellos, D.C., Hajishengallis, G., and Lambris, J.D. (2019). New insights into the immune functions of complement. Nat. Rev. Immunol. *19*, 503–516.

Rensen, S.S., Slaats, Y., Driessen, A., Peutz-Kootstra, C.J., Nijhuis, J., Steffensen, R., Greve, J.W., and Buurman, W.A. (2009). Activation of the complement system in human nonalcoholic fatty liver disease. Hepatology *50*, 1809–1817.

Rettig, T.A., Harbin, J.N., Harrington, A., Dohmen, L., and Fleming, S.D. (2015). Evasion and interactions of the humoral innate immune response in pathogen invasion, autoimmune

disease, and cancer. Clin. Immunol. *160*, 244–254.

Rezvani, R., Smith, J., Lapointe, M., Marceau, P., Tchernof, A., and Cianflone, K. (2014). Complement Receptors C5aR and C5L2 Are Associated with Metabolic Profile, Sex Hormones, and Liver Enzymes in Obese Women Pre- and Postbariatric Surgery. J. Obes. *2014*, 1–12.

Ricklin, D., Hajishengallis, G., Yang, K., and Lambris, J.D. (2010). Complement: a key system for immune surveillance and homeostasis. Nat. Immunol. *11*, 785–797.

Roberto, C.A., Swinburn, B., Hawkes, C., Huang, T.T.-K., Costa, S.A., Ashe, M., Zwicker, L., Cawley, J.H., and Brownell, K.D. (2015). Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking. Lancet (London, England) *385*, 2400–2409.

Roche, N., Chinet, T.C., Belouchi, N.E., Julié, C., and Huchon, G.J. (2000). Dermatophagoides pteronyssinus and bioelectric properties of airway epithelium: role of cysteine proteases. Eur. Respir. J. *16*, 309–315.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ *4*, e2584.

Rokholm, B., Baker, J.L., and Sørensen, T.I.A. (2010). The levelling off of the obesity epidemic since the year 1999--a review of evidence and perspectives. Obes. Rev. *11*, 835–846.

Romagnani, S. (2006). Regulation of the T cell response. Clin. Exp. Allergy *36*, 1357–1366.

De Rosa, V., Procaccini, C., Calì, G., Pirozzi, G., Fontana, S., Zappacosta, S., La Cava, A., and Matarese, G. (2007). A key role of leptin in the control of regulatory T cell proliferation. Immunity *26*, 241–255.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P.I., Godneva, A., Kalka, I.N., Bar, N., et al. (2018). Environment dominates over host genetics in shaping human gut microbiota. Nature *555*, 210–215.

Rouaud, T., Siami, N., Dupas, T., Gervier, P., Gardahaut, M.-F., Auda-Boucher, G., and Thiriet, C. (2017). Complement C3 of the innate immune system secreted by muscle adipogenic cells promotes myogenic differentiation. Sci. Rep. *7*, 171.

Roy, C., Gupta, A., Fisette, A., Lapointe, M., Poursharifi, P., Richard, D., Lu, H.L., Lu, B., Gerard, N., Gerard, C., et al. (2013). C5a Receptor Deficiency Alters Energy Utilization and Fat Storage. PLoS One *8*.

Sabatel, C., Radermecker, C., Fievez, L., Paulissen, G., Chakarov, S., Fernandes, C., Olivier, S., Toussaint, M., Pirottin, D., Xiao, X., et al. (2017). Exposure to Bacterial CpG DNA

Protects from Airway Allergic Inflammation by Expanding Regulatory Lung Interstitial Macrophages. Immunity *46*, 457–473.

Saglani, S., Payne, D.N., Zhu, J., Wang, Z., Nicholson, A.G., Bush, A., and Jeffery, P.K. (2007). Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am. J. Respir. Crit. Care Med. *176*, 858–864.

Saklayen, M.G. (2018). The Global Epidemic of the Metabolic Syndrome. Curr. Hypertens. Rep. *20*, 12.

Salazar, F., and Ghaemmaghami, A.M. (2013). Allergen recognition by innate immune cells: critical role of dendritic and epithelial cells. Front. Immunol. *4*, 356.

Saradna, A., Do, D.C., Kumar, S., Fu, Q.-L., and Gao, P. (2018). Macrophage polarization and allergic asthma. Transl. Res. *191*, 1–14.

Sargent, M. (2009). Why inequality is fatal. Nature *458*, 1109–1110.

Saxon, A., and Diaz-Sanchez, D. (2005). Air pollution and allergy: you are what you breathe. Nat. Immunol. *6*, 223–226.

Schaafsma, D., McNeill, K.D., Stelmack, G.L., Gosens, R., Baarsma, H.A., Dekkers, B.G.J., Frohwerk, E., Penninks, J.-M., Sharma, P., Ens, K.M., et al. (2007). Insulin increases the expression of contractile phenotypic markers in airway smooth muscle. Am. J. Physiol. Cell Physiol. *293*, C429-39.

Schachter, L.M., Salome, C.M., Peat, J.K., and Woolcock, A.J. (2001). Obesity is a risk for asthma and wheeze but not airway hyperresponsiveness. Thorax *56*, 4–8.

Schachter, L.M., Peat, J.K., and Salome, C.M. (2003). Asthma and atopy in overweight children. *1*, 1031–1036.

Schäffler, A., Schölmerich, J., and Salzberger, B. (2007). Adipose tissue as an immunological organ: Toll-like receptors, C1q/TNFs and CTRPs. Trends Immunol. *28*, 393–399.

Schatz, M., and Rosenwasser, L. (2014). The Allergic Asthma Phenotype. J. Allergy Clin. Immunol. Pract. *2*, 645–648.

Schenk, S., Saberi, M., and Olefsky, J.M. (2008). Insulin sensitivity: modulation by nutrients and inflammation. J. Clin. Invest. *118*, 2992–3002.

Schleich, F., Brusselle, G., Louis, R., Vandenplas, O., Michils, A., Pilette, C., Peche, R., Manise, M., and Joos, G. (2014). Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). Respir. Med. *108*, 1723–1732.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,

Lesniewski, R. a., Oakley, B.B., Parks, D.H., Robinson, C.J., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. *75*, 7537–7541.

Schmudde, I., Ströver, H. a, Vollbrandt, T., König, P., Karsten, C.M., Laumonnier, Y., and Köhl, J. (2013). C5a receptor signalling in dendritic cells controls the development of maladaptive Th2 and Th17 immunity in experimental allergic asthma. Mucosal Immunol. *6*, 807–825.

Schröder, T., Kucharczyk, D., Bär, F., Pagel, R., Derer, S., Jendrek, S.T., Sünderhauf, A., Brethack, A.-K., Hirose, M., Möller, S., et al. (2016). Mitochondrial gene polymorphisms alter hepatic cellular energy metabolism and aggravate diet-induced non-alcoholic steatohepatitis. Mol. Metab. *5*.

Schulz, L.O., Bennett, P.H., Ravussin, E., Kidd, J.R., Kidd, K.K., Esparza, J., and Valencia, M.E. (2006). Effects of traditional and western environments on prevalence of type 2 diabetes in Pima Indians in Mexico and the U.S. Diabetes Care *29*, 1866–1871.

Scudellari, M. (2017). News Feature: Cleaning up the hygiene hypothesis. Proc. Natl. Acad. Sci. U. S. A. *114*, 1433–1436.

Sears, M.R. (2014). Trends in the prevalence of asthma. Chest *145*, 219–225.

Segata, N. (2015). Gut Microbiome: Westernization and the Disappearance of Intestinal Diversity. Curr. Biol. *25*, R611-3.

Shi, H.Z., Humbles, A., Gerard, C., Jin, Z., and Weller, P.F. (2000). Lymph node trafficking and antigen presentation by endobronchial eosinophils. J. Clin. Invest. *105*, 945–953.

Shishido, S.N., Varahan, S., Yuan, K., Li, X., and Fleming, S.D. (2012). Humoral innate immune response and disease. Clin. Immunol. *144*, 142–158.

Shore, S. a., Terry, R.D., Flynt, L., Xu, A., and Hug, C. (2006). Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. J. Allergy Clin. Immunol. *118*, 389–395.

Shore, S. a, Schwartzman, I.N., Mellema, M.S., Flynt, L., Imrich, A., and Johnston, R. a (2005). Effect of leptin on allergic airway responses in mice. J. Allergy Clin. Immunol. *115*, 103–109.

Sibille, Y., and Marchandise, F.X. (1993). Pulmonary immune cells in health and disease: polymorphonuclear neutrophils. Eur. Respir. J. *6*, 1529–1543.

Sica, A., and Mantovani, A. (2012). Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. *122*, 787–795.

Sideleva, O., Black, K., and Dixon, A.E. (2013). Effects of obesity and weight loss on airway physiology and inflammation in asthma. Pulm. Pharmacol. Ther. *26*, 455–458.

Silva, F.M.C., Oliveira, E.E., Gouveia, A.C.C., Brugiolo, A.S.S., Alves, C.C., Correa, J.O.A., Gameiro, J., Mattes, J., Teixeira, H.C., and Ferreira, A.P. (2017). Obesity promotes prolonged ovalbumin-induced airway inflammation modulating T helper type 1 (Th1), Th2 and Th17 immune responses in BALB/c mice. Clin. Exp. Immunol. *189*, 47–59.

Silverman, E.S., Palmer, L.J., Subramaniam, V., Hallock, A., Mathew, S., Vallone, J., Faffe, D.S., Shikanai, T., Raby, B.A., Weiss, S.T., et al. (2004). Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. Am. J. Respir. Crit. Care Med. *169*, 214–219.

Simpson, A., Tan, V.Y.F., Winn, J., Svensén, M., Bishop, C.M., Heckerman, D.E., Buchan, I., and Custovic, A. (2010). Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am. J. Respir. Crit. Care Med. *181*, 1200–1206.

Sinisgalli, S., Collins, M.S., and Schramm, C.M. (2012). Clinical features cannot distinguish allergic from non-allergic asthma in children. J. Asthma *49*, 51–56.

Solèr, M., Matz, J., Townley, R., Buhl, R., O'Brien, J., Fox, H., Thirlwell, J., Gupta, N., and Della Cioppa, G. (2001). The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. Eur. Respir. J. *18*, 254–261.

Solomkin, J.S., Jenkins, M.K., Nelson, R.D., Chenoweth, D., and Simmons, R.L. (1981). Neutrophil dysfunction in sepsis. II. Evidence for the role of complement activation products in cellular deactivation. Surgery *90*, 319–327.

Sood, A., and Shore, S. a (2013). Adiponectin, Leptin, and Resistin in Asthma: Basic Mechanisms through Population Studies. J. Allergy *2013*, 785835.

Soroosh, P., Doherty, T.A., Duan, W., Mehta, A.K., Choi, H., Adams, Y.F., Mikulski, Z., Khorram, N., Rosenthal, P., Broide, D.H., et al. (2013). Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance. J. Exp. Med. *210*, 775–788.

Souza-Almeida, G., D'Avila, H., Almeida, P.E., Luna-Gomes, T., Liechocki, S., Walzog, B., Hepper, I., Castro-Faria-Neto, H.C., Bozza, P.T., Bandeira-Melo, C., et al. (2018). Leptin MediatesIn VivoNeutrophil Migration: Involvement of Tumor Necrosis Factor-Alpha and CXCL1. Front. Immunol. *9*, 111.

Steven, S., Dib, M., Hausding, M., Kashani, F., Oelze, M., Kröller-Schön, S., Hanf, A., Daub, S., Roohani, S., Gramlich, Y., et al. (2017). CD40L controls obesity-associated vascular inflammation, oxidative stress, and endothelial dysfunction in high fat diet-treated and db/db

mice. Cardiovasc. Res. 312–323.

Stockinger, B., Barthlott, T., and Kassiotis, G. (2004). The concept of space and competition in immune regulation. Immunology *111*, 241–247.

Strachan, D.P. (1989). Hay fever, hygiene, and household size. BMJ *299*, 1259–1260.

Strainic, M.G., Liu, J., Huang, D., An, F., Lalli, P.N., Muqim, N., Shapiro, V.S., Dubyak, G.R., Heeger, P.S., and Medof, M.E. (2008). Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. Immunity *28*, 425–435.

Strey, C.W., Markiewski, M., Mastellos, D., Tudoran, R., Spruce, L.A., Greenbaum, L.E., and Lambris, J.D. (2003). The proinflammatory mediators C3a and C5a are essential for liver regeneration. J. Exp. Med. *198*, 913–923.

Stunkard, A.J., Harris, J.R., Pedersen, N.L., and McClearn, G.E. (1990). The body-mass index of twins who have been reared apart. N. Engl. J. Med. *322*, 1483–1487.

Sung, S.-S.J., Fu, S.M., Rose, C.E., Gaskin, F., Ju, S.-T., and Beaty, S.R. (2006). A major lung CD103 (alphaE)-beta7 integrin-positive epithelial dendritic cell population expressing Langerin and tight junction proteins. J. Immunol. *176*, 2161–2172.

Suratt, B.T. (2016). Mouse Modeling of Obese Lung Disease. Insights and Caveats. Am. J. Respir. Cell Mol. Biol. *55*, 153–158.

Surwit, R.S., Feinglos, M.N., Rodin, J., Sutherland, A., Petro, A.E., Opara, E.C., Kuhn, C.M., and Rebuffé-Scrive, M. (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism. *44*, 645–651.

Surwit, R.S., Feinglos, M.N., Rodin, J., Sutherland, A., Petro, A.E., Opara, E.C., Kuhn, C.M., and Rebuffe-Scribe, M. (1998). Differential effects of fat and sucrose on body composition in C57BL/6 and A/J mice. Metabolism. *47*, 1354–1359.

Sutherland, E.R., Lehman, E.B., Teodorescu, M., Wechsler, M.E., and National Heart, Lung, and B.I.A.C.R.N. (2009). Body mass index and phenotype in subjects with mild-to-moderate persistent asthma. J. Allergy Clin. Immunol. *123*, 1328-34.e1.

Sutherland, T.J.T., Cowan, J.O., Young, S., Goulding, A., Grant, A.M., Williamson, A., Brassett, K., Herbison, G.P., and Taylor, D.R. (2008). The association between obesity and asthma: Interactions between systemic and airway inflammation. Am. J. Respir. Crit. Care Med. *178*, 469–475.

Swain, S.L. (1999). Helper T cell differentiation. Curr. Opin. Immunol. *11*, 180–185.

Swain, S.L., Weinberg, A.D., English, M., and Huston, G. (1990). IL-4 directs the

development of Th2-like helper effectors. J. Immunol. *145*, 3796–3806.

Swinburn, B.A., Sacks, G., Hall, K.D., McPherson, K., Finegood, D.T., Moodie, M.L., and Gortmaker, S.L. (2011). The global obesity pandemic: shaped by global drivers and local environments. Lancet (London, England) *378*, 804–814.

Sybilski, A.J., Raciborski, F., Lipiec, A., Tomaszewska, A., Lusawa, A., Furmańczyk, K., Krzych-Fałta, E., Komorowski, J., and Samoliński, B. (2015). Obesity-a risk factor for asthma, but not for atopic dermatitis, allergic rhinitis and sensitization. Public Health Nutr. *18*, 530–536.

Takafuji, S., Tadokoro, K., Ito, K., and Dahinden, C.A. (1994). Degranulation from human eosinophils stimulated with C3a and C5a. Int. Arch. Allergy Immunol. *104 Suppl*, 27–29.

Takeda, M., Tanabe, M., Ito, W., Ueki, S., Konnno, Y., Chihara, M., Itoga, M., Kobayashi, Y., Moritoki, Y., Kayaba, H., et al. (2013). Gender difference in allergic airway remodelling and immunoglobulin production in mouse model of asthma. Respirology *18*, 797–806.

Telenga, E.D., Tideman, S.W., Kerstjens, H. a M., Hacken, N.H.T. Ten, Timens, W., Postma, D.S., and Van Den Berge, M. (2012). Obesity in asthma: More neutrophilic inflammation as a possible explanation for a reduced treatment response. Allergy Eur. J. Allergy Clin. Immunol. *67*, 1060–1068.

Thavagnanam, S., Fleming, J., Bromley, A., Shields, M.D., and Cardwell, C.R. (2008). A meta-analysis of the association between Caesarean section and childhood asthma. Clin. Exp. Allergy *38*, 629–633.

The Global Asthma Network (2018). The Global Asthma Report 2018.

The Jackson Laboratory BODY WEIGHT INFORMATION FOR B6.CG-LEPOB/J (000632).

Toennesen, L.L., Bjerregaard, A., Porsbjerg, C., Ulrik, C.S., Harmsen, L., and Backer, V. (2018). Overweight in childhood and adolescence: Does it lead to airway hyperresponsiveness in adulthood? J. Asthma *55*, 137–144.

Tomlinson, S., Taylor, P.W., Morgan, B.P., and Luzio, J.P. (1989). Killing of gram-negative bacteria by complement. Fractionation of cell membranes after complement C5b-9 deposition on to the surface of Salmonella minnesota Re595. Biochem. J. *263*, 505–511.

Turvey, S.E., and Broide, D.H. (2010). Innate immunity. J. Allergy Clin. Immunol. *125*, S24-32.

Valdes, A.M., Walter, J., Segal, E., and Spector, T.D. (2018). Role of the gut microbiota in nutrition and health. BMJ *361*, k2179.

Vannella, K.M., and Wynn, T.A. (2017). Mechanisms of Organ Injury and Repair by

Macrophages. Annu. Rev. Physiol. *79*, 593–617.

Van Veen, I.H., Ten Brinke, A., Sterk, P.J., Rabe, K.F., and Bel, E.H. (2008). Airway inflammation in obese and nonobese patients with difficult-to-treat asthma. Allergy Eur. J. Allergy Clin. Immunol. *63*, 570–574.

Vercelli, D. (2008). Discovering susceptibility genes for asthma and allergy. Nat. Rev. Immunol. *8*, 169–182.

Vieira, P.L., Christensen, J.R., Minaee, S., O'Neill, E.J., Barrat, F.J., Boonstra, A., Barthlott, T., Stockinger, B., Wraith, D.C., and O'Garra, A. (2004). IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. J. Immunol. *172*, 5986–5993.

Vijayakanthi, N., Greally, J.M., and Rastogi, D. (2016). Pediatric Obesity-Related Asthma: The Role of Metabolic Dysregulation. Pediatrics *137*, e20150812–e20150812.

Visness, C.M., London, S.J., Daniels, J.L., Kaufman, J.S., Yeatts, K.B., Siega-Riz, A.-M., Calatroni, A., and Zeldin, D.C. (2010). Association of childhood obesity with atopic and nonatopic asthma: results from the National Health and Nutrition Examination Survey 1999-2006. J. Asthma *47*, 822–829.

Vladich, F.D., Brazille, S.M., Stern, D., Peck, M.L., Ghittoni, R., and Vercelli, D. (2005). IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. J. Clin. Invest. *115*, 747–754.

Vlaicu, S.I., Tatomir, A., Boodhoo, D., Vesa, S., Mircea, P.A., and Rus, H. (2016). The role of complement system in adipose tissue-related inflammation. Immunol. Res. *64*, 653–664.

De Vooght, V., Vanoirbeek, J.A.J., Luyts, K., Haenen, S., Nemery, B., and Hoet, P.H.M. (2010). Choice of mouse strain influences the outcome in a mouse model of chemical-induced asthma. PLoS One *5*, e12581–e12581.

De Vries, A., Hazlewood, L., Fitch, P.M., Seckl, J.R., Foster, P., and Howie, S.E.M. (2009). High-fat feeding redirects cytokine responses and decreases allergic airway eosinophilia. Clin. Exp. Allergy *39*, 731–739.

Walker, J.K.L., Kraft, M., and Fisher, J.T. (2013). Assessment of murine lung mechanics outcome measures: alignment with those made in asthmatics. Front. Physiol. *3*, 1–14.

Walsh, E.R., Sahu, N., Kearley, J., Benjamin, E., Kang, B.H., Humbles, A., and August, A. (2008). Strain-specific requirement for eosinophils in the recruitment of T cells to the lung during the development of allergic asthma. J. Exp. Med. *205*, 1285–1292.

Wan, H., Winton, H.L., Soeller, C., Gruenert, D.C., Thompson, P.J., Cannell, M.B., Stewart, G.A., Garrod, D.R., and Robinson, C. (2000). Quantitative structural and biochemical

analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1. Clin. Exp. Allergy *30*, 685–698.

Wang, H.-B., Ghiran, I., Matthaei, K., and Weller, P.F. (2007). Airway eosinophils: allergic inflammation recruited professional antigen-presenting cells. J. Immunol. *179*, 7585–7592.

Wang, Y., Zhang, H., and He, Y.-W. (2019). The Complement Receptors C3aR and C5aR Are a New Class of Immune Checkpoint Receptor in Cancer Immunotherapy. Front. Immunol. *10*, 1574.

Wang, Z., Liu, M., Pan, T., and Tong, S. (2016). Lower Mortality Associated With Overweight in the U.S. National Health Interview Survey: Is Overweight Protective? Medicine (Baltimore). *95*, e2424–e2424.

Weissbrod, O., Rothschild, D., Barkan, E., and Segal, E. (2018). Host genetics and microbiome associations through the lens of genome wide association studies. Curr. Opin. Microbiol. *44*, 9–19.

Wensveen, F.M., Valentić, S., Šestan, M., Turk Wensveen, T., and Polić, B. (2015). The "Big Bang" in obese fat: Events initiating obesity-induced adipose tissue inflammation. Eur. J. Immunol. *45*, 2446–2456.

Wenzel, S.E. (2012). Asthma phenotypes: the evolution from clinical to molecular approaches. Nat. Med. *18*, 716–725.

Werfel, T., Oppermann, M., Schulze, M., Krieger, G., Weber, M., and Götze, O. (1992). Binding of fluorescein-labeled anaphylatoxin C5a to human peripheral blood, spleen, and bone marrow leukocytes. Blood *79*, 152–160.

Werner, M., Topp, R., Wimmer, K., Richter, K., Bischof, W., Wjst, M., and Heinrich, J. (2003). TLR4 gene variants modify endotoxin effects on asthma. J. Allergy Clin. Immunol. *112*, 323–330.

WHO Body mass index - BMI - http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.

WHO (2017). Noncommunicable Diseases Progress Monitor.

WHO (2018). Obesity and overweight.

Wiese, A. V, Ender, F., Quell, K.M., Antoniou, K., Vollbrandt, T., König, P., Köhl, J., and Laumonnier, Y. (2017). The C5a/C5aR1 axis controls the development of experimental allergic asthma independent of LysM-expressing pulmonary immune cells. PLoS One *12*, e0184956.

Williams, C.M., and Galli, S.J. (2000). Mast cells can amplify airway reactivity and features of

chronic inflammation in an asthma model in mice. J. Exp. Med. *192*, 455–462.

Wills-Karp, M., and Koehl, J. (2005). New insights into the role of the complement pathway in allergy and asthma. Curr. Allergy Asthma Rep. *5*, 362–369.

Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L., and Donaldson, D.D. (1998). Interleukin-13: central mediator of allergic asthma. Science *282*, 2258–2261.

Wills-Karp, M., Santeliz, J., and Karp, C.L. (2001). The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat. Rev. Immunol. *1*, 69–75.

Withers, N.J., Low, L., Holgate, S.T., and Clough, J.B. (1998). The natural history of respiratory symptoms in a cohort of adolescents. Am. J. Respir. Crit. Care Med. *158*, 352–357.

Wlazlo, N., van Greevenbroek, M.M.J., Ferreira, I., Jansen, E.H.J.M., Feskens, E.J.M., van der Kallen, C.J.H., Schalkwijk, C.G., Bravenboer, B., and Stehouwer, C.D. a (2013). Activated complement factor 3 is associated with liver fat and liver enzymes: the CODAM study. Eur. J. Clin. Invest. *43*, 679–688.

Wong, C.K., Cheung, P.F.Y., and Lam, C.W.K. (2007). Leptin-mediated cytokine release and migration of eosinophils: Implications for immunopathophysiology of allergic inflammation. Eur. J. Immunol. *37*, 2337–2348.

Würzner, R. (2003). Deficiencies of the complement MAC II gene cluster (C6, C7, C9): is subtotal C6 deficiency of particular evolutionary benefit? Clin. Exp. Immunol. *133*, 156–159.

Xu, C., Chen, Y., Xu, L., Miao, M., Li, Y., and Yu, C. (2016). Serum complement C3 levels are associated with nonalcoholic fatty liver disease independently of metabolic features in Chinese population. Sci. Rep. *6*, 23279.

Yamamoto, R., Ueki, S., Moritoki, Y., Kobayashi, Y., Oyamada, H., Konno, Y., Tamaki, M., Itoga, M., Takeda, M., Ito, W., et al. (2013). Adiponectin attenuates human eosinophil adhesion and chemotaxis: implications in allergic inflammation. J. Asthma *50*, 828–835.

Yan, C., and Gao, H. (2012). New insights for C5a and C5a receptors in sepsis. Front. Immunol. *3*, 368.

Yanagisawa, R., Koike, E., Ichinose, T., and Takano, H. (2014). Obese mice are resistant to eosinophilic airway inflammation induced by diesel exhaust particles. J. Appl. Toxicol. *34*, 688–694.

Yanovski, J.A. (2018). Obesity: Trends in underweight and obesity - scale of the problem. Nat. Rev. Endocrinol. *14*, 5–6.

Yoshihara, S., Yamada, Y., Abe, T., Lindén, A., and Arisaka, O. (2006). Association of

epithelial damage and signs of neutrophil mobilization in the airways during acute exacerbations of paediatric asthma. Clin. Exp. Immunol. *144*, 212–216.

Yu, Q.-L., and Chen, Z. (2018). Establishment of different experimental asthma models in mice. Exp. Ther. Med. *15*, 2492–2498.

Yu, S., Kim, H.Y., Chang, Y.-J., DeKruyff, R.H., and Umetsu, D.T. (2014). Innate lymphoid cells and asthma. J. Allergy Clin. Immunol. *133*, 943–950; quiz 51.

Zaal, A., Dieker, M., Oudenampsen, M., Turksma, A.W., Lissenberg-Thunnissen, S.N., Wouters, D., van Ham, S.M., and Ten Brinke, A. (2017). Anaphylatoxin C5a Regulates 6-Sulfo-LacNAc Dendritic Cell Function in Human through Crosstalk with Toll-Like Receptor-Induced CREB Signaling. Front. Immunol. *8*, 818.

Zaal, A., van Ham, S.M., and Ten Brinke, A. (2019). Differential effects of anaphylatoxin C5a on antigen presenting cells, roles for C5aR1 and C5aR2. Immunol. Lett. *209*, 45–52.

Zacharasiewicz, A. (2016). Maternal smoking in pregnancy and its influence on childhood asthma. ERJ Open Res. *2*.

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., et al. (2015). Personalized Nutrition by Prediction of Glycemic Responses. Cell *163*, 1079–1094.

Zhang, X., Schmudde, I., Laumonnier, Y., Pandey, M.K., Clark, J.R., König, P., Gerard, N.P., Gerard, C., Wills-Karp, M., and Köhl, J. (2010). A critical role for C5L2 in the pathogenesis of experimental allergic asthma. J. Immunol. *185*, 6741–6752.

Zhou, Z.-H., Zhang, Y., Hu, Y.-F., Wahl, L.M., Cisar, J.O., and Notkins, A.L. (2007). The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies. Cell Host Microbe *1*, 51–61.

Zipfel, P.F., and Skerka, C. (2009). Complement regulators and inhibitory proteins. Nat. Rev. Immunol. *9*, 729–740.

7 Abbreviations and Symbols

-/-	knock out
°C	degree Celsius
%	percent
*	indication of p-value
	in case of comparison of more than two groups, * indicates significant difference between OVA-group and PBS control
	* p < 0.05, ** p < 0.01; *** p < 0.001
#	indication of p-value in case of comparison of > 2 groups, indicates significant difference between OVA-groups of both diets (e.g. CD/OVA vs. HFD/OVA)
	# p < 0.05, ## p < 0.01; ### p < 0.001
AHR	airway hyperresponsiveness
Alum	aluminum potassium sulfate
APC	allophycocyanin
BAL	bronchoalveolar lavage
BMI	body mass index
BSA	bovine serum albumin
BV	Brilliant Violet (e.g. BV421)
C5aR1	complement component 5a receptor 1, also: CD88
CCL	Chemokine (C-C motif) ligand
CCR	C-C chemokine receptor
CD	cluster of differentiation (followed by respective digit)
CD	chow diet
cDC	conventional DC
CFSE	carboxyfluorescein diacetate succinimidyl ester
CSF-2	colony stimulating factor 2 (a.k.a. GM-CSF)
CXCL	Chemokine (C-X-C motif) ligand

DMSO	Dimethyl sulfoxide
DC(s)	dendritic cell(s)
EDTA	Ethylenediaminetetraacetic acid
eF	EFluor
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence activated cell sorting
FBS	fetal bovine serum
FCS	Fetal calf serum
FITC	fluorescein isothiocyanate
Foxp3	forkhead-box-protein P3
FSC	forward scatter light
g	gram (unit)
g	gravity (in centrifugation steps)
GM-CSF	granulocyte-macrophage colony stimulating factor
H/hrs	hour(s)
HDL	high-density lipoprotein
HFD	high fat diet
HFD60	high fat diet with 60 energy% fat (alternate feeding experiment)
H&E	hematoxylin-eosin
IFN	interferon
IgE	immunglobuline E
IL	interleukin
i.p.	intra-peritoneal
i.t.	intra-tracheal
kcal	kilocalorie(s)
kJ	kilojoule
LDL	low-density lipoprotein
LepR	leptin receptor
Lg	lung

Ly6G	lymphocyte antigen 6 complex locus G6D
Mϕ	macrophage
MACS	magnetic cell separation
methacholine	Acetyl-β-Methyl-Choline
MFI	mean fluorescence intensity
mg	milligram(s)
MHC	major histocompatibility complex
min	minute(s)
MJ	megajoule
mLN	mediastinal lymph node(s)
NA	not available, not assessed
ns	not significant
µl	microliter(s)
ml	milliliter(s)
moDC	monocyte-derived DC
OT-II	transgenic OVA-specific T cell receptor mice
OTU	operational taxonomic unit
OVA	ovalbumin
PAS	periodic acid-Schiff
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	phycoerythrin
PECy	PE-cyanine
Per-CP-Cy	Peridinin chlorophyl protein-cyanine
PMA	phorbol 12-myristate 13-acetate
RBC	red blood cell lysis
VAT	visceral adipose tissue
RT	room temperature
sec	second(s)

SEM	standard error of the mean
Siglec	sialic acid-binding immunoglobulin-type lectin
SSC	side scattered light
STAT	signal transducer and activator of transcription
TGF	transforming growth factor
Th	T helper cell
TM	trademark
TNF	tumor necrosis factor
VAT	visceral adipose tissue
VLDL	very low-density lipoprotein
WT	wildtype

8 Figure list

*The schematics in figures 1 and 35, in part, are composed by available artwork (http://www.servier.com/Powerpoint-image-bank), per licensing agreement (https://creativecommons.org/licenses/by/3.0/legalcode).

9 Table list

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