Mast cells as immune regulators in atherosclerosis

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Chapter 8

General discussion - Future perspectives

Synopsis

Atherosclerosis is the most prevailing underlying pathology responsible for the increased mortality rates due to acute cardiovascular syndromes[1]. Cardiovascular disease (CVD) was accounted for 1 in 3 deaths in US adults over the year 2014, with approximately 1 death incidence occurring every 40 seconds[2]. In Europe, CVD is the cause of 45% of all deaths, according to the 2017 statistics rates[3]. Despite the misconception that cancer is the leading cause of death nowadays, mortality due to CVD is actually higher than the sum of deaths by cancer and chronic lower respiratory disease[1]. Therefore, research aiming at identifying new therapeutic strategies to battle atherosclerosis pathology from an early stage is mandatory.

Atherosclerotic plaque formation is a chronic process which develops as a result of disturbed cholesterol regulation and immune system dysfunction[4]. Mast cells are immune components that have been described as important contributors in the progression of experimental mouse and human atherosclerosis[5]. The presence of mast cells within the plaques of human carotid arteries, was found to be a predictor of future cardiovascular events and furthermore, was associated with intraplaque hemorrhage levels, a characteristic of end-stage atherosclerosis[6]. Activation of mast cells in the plaque is suggested to lead to plaque destabilization[7], increasing thus the chances for an acute episode. However, the exact mechanism by which these cells get activated inside the plaque tissue has not been studied in detail.

In the work described throughout this thesis, we aimed to decipher the action of mast cells in experimental and human atherosclerosis. In **chapter 3** we determined the therapeutic potential of the $LPA_{1/3}$ antagonist, Ki16425, which was hypothesized to lead to inhibition of a recently described mast cell activator, termed lysophosphatidic acid[8]. **Chapters 4 and 5** examined the novel role of mast cell mediated antigen presentation in atherosclerosis. In **chapter 6**, by applying a translational approach, we determined the abundance and function of mast cells in human atherosclerotic plaques. Finally, in **chapter 7** we explored the influence of mast cells in atherosclerosis regression.

Mast cells act on multiple cardiovascular syndromes

The contribution of mast cells in the pathology of atherosclerosis is a subject that has gained considerable attention over the years. Upon contemplating the adverse effects that mast cell activation can elicit in other autoimmune pathologies, such as allergy[9] or rheumatoid arthritis[10], it is necessary to gain more information regarding their exact function in atherosclerotic disease. Therefore, during this thesis we aimed to unravel their mechanisms of action in atherosclerosis.

Chapter 2 offered a general overview on the implication of mast cells in various cardiovascular diseases (CVD). Even though the field of atherosclerosis has been actively investigating their function for the past 20 years, not much is known regarding other cardiovascular pathologies.

We reviewed the role of mast cells in diet induced obesity. Mast cells have been shown to infiltrate white adipose tissue[11] and contribute to local adipogenesis[12] and probably obesity. However, recently it was revealed that mast cells, through their secretion of leptin, can actually protect against obesity and diabetes, highlighting their dual-face actions[13]. This notion was strengthened by a report stating that mast cells can promote the formation of brown fat cells[14], which comprise the protective kind of adipocytes responsible for body temperature retention[15].

This double-edged nature of mast cells was also discussed in the case of myocardial infarction (MI). We conferred on their detrimental role in end-stage plaque rupture[16]. Their adverse nature in plaque destabilization was suggested in a new paper stating that mast cells are found at increased numbers in patients with stable and unstable coronary plaques, as well as in individuals who suffered from either an acute or chronic MI episode[17]. However, it seems that mast cells do not exert only harmful effects, since they are linked to neo-angiogenesis and healing of the myocardium after an acute MI episode[18,19]. Interestingly, a very recent report stated that following an MI incident, mast cells deriving from white adipose tissue can communicate with cardiomyocytes, increase their contractility and improve heart function[20].

The effect of mast cells on the pace of the myocardium may be particularly important in arrhythmia, as we also discussed in the second chapter. Indeed, the presence of mast cells has also been reported a few years ago in arrhythmic atrial fibrillation[21]. Heart rhythm-related dysfunctions are linked to atherosclerosis[22] and atrial fibrillation in particular shows an increasing predominance at present[1]. In fact, arrhythmia is widely understudied with respect to the contribution of the immune system. Interestingly, mast cells are a population that is fundamentally populating the heart at a normal state[23]. Although information available on the role of mast cells in arrhythmia is scarce, it seems that they act in a negative way[24,25]. Furthermore, a novel paradigm-shifting study revealed that also macrophages can influence the regulation of heart rhythm[26]. Mast cells are reported to interact with macrophages in other cardiovascular diseases such as atherosclerosis[27] and aortic aneurysms[28]. While this does not imply a causative relationship, it would be interesting to investigate in the future a possible interaction between tissue resident macrophages and cardiac mast cells in heart rhythm disorders.

It is therefore apparent that mast cells do not compose a cell type that is clear-

cut pro-inflammatory in cardiovascular syndromes. Examination of their function should be approached in a delicate fashion.

Modulation of immune responses in atherosclerosis through mast cells

Having in mind the above mentioned diverse abilities of mast cells, as well as the fact that atherosclerosis is the underlying pathology for the majority of CVDs, we aimed to experimentally elucidate their effects in atherosclerosis.

In **chapter 3** we examined the downstream effects of the established mast cell activator, lysophosphatidic acid (LPA)[29]. LPA is a bioactive lipid produced inside the atherosclerotic plaques where it has been shown to act in a proatherogenic manner[30,31]. LPA acts *via* its specific receptors $LPA_{1/3}$[32] on an array of immune cells[33] among which the mast cells[29,34]. For that reason, we inhibited the action of LPA by blocking the downstream signaling of receptors $LPA_{1/3}$, with the use of the small molecule Ki16425. $LPA_{1/3}$ inhibition had a significant impact on the systemic immune response, which resulted in a 40% reduction of the plaque burden, caused by a reduced macrophage content. The protective effect of Ki16425 was mainly attributed to the disruption of the CCR2-CCL2 infiltration axis, but we also observed an increase in the non-inflammatory monocyte content and the anti-inflammatory T_{REG} population. These effects were accompanied by a mild reduction in the LDL cholesterol levels. Overall, inhibition of $LPA_{1/3}$ appears to be an appealing therapeutic method to limit the progression of established atherosclerosis. Furthermore, our observations show that a shift of the systemic immune response, from pro-inflammatory to anti-inflammatory, in atherosclerosis can not only halt plaque progression, but can also influence cholesterol regulation. However, in this study we did not observe any significant mast cell contribution. Nonetheless, we still have strong indications that mast cells may also be affected by the LPA-signaling cascade. For example, mast cells have been reported to act also through additional LPA receptors, such as LPA_5, which in our study were still fully-functional[35]. Be that as it may, this did not seem to have any effect on the mast cell response we observed. The previously reported LPA action on the mast cells was introduced at advanced atherosclerosis stages[29], which was not the case in our experimental setup. The beneficial effects that we reported on the adaptive immune response suggest that $LPA_{1/3}$ inhibition may also be therapeutically relevant for a prolonged period. It would therefore be interesting to investigate this agent throughout a longer timeframe. In addition, the induction of T_{REG} cells opens the pathway to investigating this agent in atherosclerosis regression since T_{REG} cells are themselves an attractive therapeutic target[36,37].

The overall importance of the adaptive immune response, and particularly the one evoked by T cells is a known fact[38]. $CD4^+$ T cells, predominantly those of the T_{H1}

phenotype, are the most frequent inside atherosclerotic plaques[39]. In **chapter 4** we discussed a newly found direct interaction between mast cells and $CD4^+$ T cells in atherosclerosis. Knowing that mast cells are in close cross-talk with T cells in other inflammatory diseases[40,41], and based on novel reports on the role of mast cells as inducible antigen presenting cells[42,43], we aimed to study whether they interact with $CD4^+$ T cells in atherosclerosis. We reported that mast cells increase their antigen presentation capacity upon hyperlipidemia, by increasing their MHC-II expression in both experimental atherosclerosis, as well as inside human plaques. Through this crosstalk mast cells can present antigens directly to $CD4^+$ T cells, which, under the influence of a high-fat diet, are skewed towards a pro-atherogenic T_{H1} subtype. This is interesting, considering the fact that mast cells in allergies seem to favor a T_{H2} response instead[44]. It therefore seems that mast cells exert differential effects on T cells according to the local inflammatory milieu. Atherosclerosis, being a T_{H1} mediated disease, is probably favoring presentation of antigenic fragments that are affecting the T_{H1} subset. On the contrary, in allergies, which are mediated by T_{H2} cells, mast cells seem to enhance the T_{H2} response. It would be interesting to see if this mast cell-$CD4^+$ T cell crosstalk results in antibody production by the B cells. Screening for T_{H1}-linked antibody fragments is an attractive concept to explore further. Furthermore, we do not know what is the exact contribution of this pathway in atherosclerosis. It would be intriguing to see if abolishment of MHC-II, specifically from the surface of mast cells, can affect atherosclerosis progression *in vivo*. Also, we do not yet know which antigenic fragments are presented by mast cells to $CD4^+$ T-cells *via* MHC-II, in atherosclerosis. In the future, it may be appealing to scan the MHC-II epitopes of mast cells for lipid-specific antigenic presentation, as well as explore the pathway *via* which mast cells take up antigens from their surroundings. For instance, their uptake capacity could be investigated through differentially charged nanoparticles. Finally, a crucial question arises, on whether mast cells can take up lipids in a "foam-cell" fashion. Although we do not have indications that they possess classical uptake receptors, present on macrophages or dendritic cells, mast cells may take up lipids by other means and store them in forms other than neutral lipid accumulation.

The existence of a direct crosstalk between mast cells and the adaptive immune system was investigated also in **chapter 5,** where we observed a direct interaction with the NKT cell population, through CD1d-mediated lipid-specific presentation. Surprisingly, this pathway showed a protective effect in atherosclerosis development. This was an unexpected finding, considering that NKT cells, which in our study showed reduced activation, are reported to be proatherogenic, particularly upon engagement with CD1d[45]. However, we must remember that NKT cells can function as activators as well as inhibitors of the immune response, depending on the glycolipid that is presented to them[46,47]. In fact, it has been previously reported that NKT cells can negatively regulate

$CD4^+$ cells in atherosclerosis, in a fashion that matches our observations[48]. Furthermore, a protective role of NKT cells was also observed in other autoimmune syndromes where mast cells are known mediators, such as obesity[49] and rheumatoid arthritis[50]. Therefore, it seems that, while classical presentation of lipid antigens through CD1d present on APCs exacerbates atherosclerosis[51,52], antigen presentation through CD1d on the mast cell surface, exerts a protective effect. It is also interesting to observe that, although indirectly, mast cells again affect the $CD4^+$ T_{H1} cell response, as in the case of MHC-II mediated presentation. This interaction seems to be tightly balanced, and of importance, in atherosclerosis development; therefore, it requires further exploration.

The presentation capacity of human mast cells mentioned in the fourth chapter, was accompanied by a general phenotypic characterization of human mast cells as stated in **chapter 6**. While human intraplaque mast cells are the only immune cell type that was found to positively associate with future cardiovascular events[6], it is still not fully understood how these cells become activated within the atherosclerotic plaque. In this study we screened human intraplaque mast cells obtained from 22 carotid and femoral arteries, using for the first time the flow cytometry method. We confirmed immunohistochemical data from previous reports, which stated that the protease secretome of mast cells is highly heterogeneous[53], showing a differential cellular content of tryptase and chymase. Importantly, we detected high levels of activation by human intraplaque mast cells, based on their expression of protein CD63[54]. Of note, the majority of activated mast cells were IgE-sensitized, indicating that this pathway is the main activation mode of the human intraplaque mast cell population. This does not come as a surprise, since mast cells are mostly known for their classical degranulation potency upon antigen-sensitized IgE binding on their Fcε-receptor[55]. However, it may explain why serum IgE levels correlate in a positive manner with coronary artery disease[56]. This observation as mentioned above, has also been attributed to mast cells[6,17]. In addition, we noted that a minor fraction of these cells was activated in a non-IgE specific manner, thus strengthening the case of non-classical Fcε-receptor mediated activation of mast cells. It has been proven that mast cells in cardiovascular diseases can get activated by means other than FcεR, such as through TLRs[57], complement receptors[57] or neuropeptide receptors[58]. However, we did not have an indication on the proportion of these alternative activation pathways within the atherosclerotic plaque. The adverse effects that this seemingly small fraction of mast cells can exert in atherosclerosis progression, like in the case of substance P which is linked to intraplaque hemorrhage levels[59], indicates the magnitude of power that these cells possess in atherosclerosis. The above, therefore, states how important it is to consider therapeutic possibilities that target mast cells.

In **chapter 7**, we explored the therapeutic potential that arises from the

sheer absence of mast cells in atherosclerosis regression conditions. Specifically, we conditionally depleted mast cells, at a systemic level, upon altering the diet content from high-fat to normal chow. In our experimental setup we did not observe any reduction in the atherosclerotic plaque size; which indicates that depletion of mast cells after an ongoing inflammatory cascade, does not have the intensity to reduce the plaque volume. We did detect, however, a reduced neutrophil intraplaque influx, in the absence of mast cells. This effect can be explained by the fact that mast cells induce neutrophil infiltration into the plaque[60]. Interestingly, even though there was no effect detected in overall plaque burden, mast cell depletion affected the collagen content of the plaque in a negative manner. This was unexpected, since mast cells are negatively associated with collagen deposition inside atherosclerotic plaques, in a chymase specific action[61]. Yet, tryptase release has been reported to induce collagen synthesis in a renal fibrosis model[62], indicating again the complex manner by which these cells act.

Overall, the observations stated in this thesis suggest that when it comes to intervention of the mast cell action, we should not aim solely on their abolition as they do not appear to possess only harmful effects, but are rather fine tuners of the overall immune response.

Future perspectives

The research described in this thesis paves the way for exploring the therapeutic potential of targeting mast cells in atherosclerosis. Evidently, this cell type, as most immune cells, shows remarkable plasticity. As such, mast cells require refined targeting of specific pathways that would inhibit their negative actions, while retaining or even enhancing their positive effects.

In the case of atherosclerosis, mast cells are, for the most part damaging, when it comes to disease progression; particularly, through their FcεR mediated activation. This specific response is a promising therapeutic point, mainly due to the already commercially available agents that target its ligand, IgE[63]. Specifically, IgE has been found to circulate at high levels in the serum of patients suffering from an acute cardiovascular syndrome[64]. In addition, IgE levels independently correlate with the severity of coronary syndromes[56]. Here we reported that IgE can penetrate the endothelial barrier, accumulate inside the atherosclerotic plaque and bind on the surface of mast cells, thus sensitizing them. The accumulation of IgE within the atherosclerotic plaque does not necessarily require the participation of an antigen. However, most of the intraplaque mast cells we examined were found to have IgE bound on their surface and to be at an activated state. It therefore seems, that they have undergone at least one cycle of degranulation in the area. A previous study has stated that, IgE is elevated upon myocardial infarction[65]

and the authors detected IgE fragments inside human atherosclerosis specimens. However, this intraplaque IgE was argued to act mainly on the inducible FcεRI of the macrophages, and not on intraplaque mast cells. Here we have provided proof that the intraplaque IgE is also activating mast cells in the plaque area. For this process to take place, the presence of an antigen is also necessary. As mentioned above, we do not know which antigenic fragment may bind on IgE. It may however be speculated that the antigen derives directly from the atherosclerotic plaque environment. Further research is needed on the antigen-mediated activation of mast cells within the plaques. Yet, upon considering the harmful effects linked to intraplaque mast cell activation[7,66], it is obvious that intervention on this pathway may prove beneficial for end-stage CVD related events. The IgE-antibody blocking agent omalizumab[67], which is already on the market and prescribed for allergic asthma cases[68,69], as well as for patients suffering from urticaria[70] and mastocytosis[71], could prove beneficial also in atherosclerosis. Indeed, we are eager to report that we have gained permission to study the effects of omalizumab in atherosclerosis patients.

A more crude method to battle the adverse effects of mast cell activation could be through the use of mast cell stabilizers[72], such as cromolyn[73] or tranilast[74]. These agents act on the mast cell mediated degranulation pathway, mainly by affecting ion exchange between the cell and its microenvironment[75,76]. Mast cell stabilization *via* this method has been proven in the past to be beneficial in experimental atherosclerosis studies[7,77]. Since in our experimental work we showed that human intraplaque mast cells are highly activated, using a mast cell stabilizer to eliminate their degranulation sounds appealing. However, there are two important aspects to keep in mind. The first one is that the mode of action that stabilizers exert is not strictly specific for mast cells. For example, tranilast is known to influence also vascular smooth muscle cells[78], an effect which could risk adverse effects. The second aspect that needs to be noted is that inhibition of the mast cell degranulation machinery exclusively will alter also the release of mediators that are not necessarily pro-inflammatory. For example, mast cells are a source of the anti-inflammatory cytokine IL-10[78], which is known to be athero-protective[79,80]. In fact, mast cells have been recently reported to act in an immunoregulatory manner in bone marrow[81] as well as in solid organ transplantation[82,83]. Therefore, blocking the release of the entire mast cell secretome may interfere with the healing properties that these cells possess, while reducing protection towards pathogen infections. Furthermore, it is important to remember that mast cell activation does not necessarily mean degranulation. Mast cells can secrete pro-inflammatory cytokines like IL-8, in means that do not include release of their pre-stored granules[84]. A similar mode of mast cell activation has been previously reported through IL-1, which results in release of IL-6 but does not involve degranulation[85]. Therefore, while most activation pathways lead to mast cell degranulation, there are means which do not elicit such an effect, but rather lead

to controlled cytokine release. One additional example is the TLR activation pathway which results in increased cytokine production but not mast cell degranulation[86]. As mentioned before, oxLDL activates mast cells through TLR4[57]. This states how different the downstream mast cells action is inside the atherosclerotic plaque. According to our observations, approximately 20% of human intraplaque mast cells get activated *via* pathways other than IgE, which may include cytokine release without degranulation. In addition, it is known that when a cell undergoes activation, the intracellular energy consumption, and therefore the mitochondrial machinery, can alter its metabolic status. In the case of mast cells, recent work has stated that IgE mediated activation induces both oxidative phosphorylation and glycolysis[87]. Considering the fact that oxLDL acts *via* the TLR pathway[57] which does not elicit energy consuming degranulation[86], it would be interesting to see if this atherosclerosis specific pathway evokes any change in the cell metabolism of mast cells. Overall, it is important to exactly refine these differential activation pathways in the future, since distinct activators possibly lead to the release of different mediators, in both quality and quantity. After all it is the prevalence of the net immune effect that shapes disease progression.

As we demonstrated, mast cells possess also immunoregulatory abilities that seem to surpass the classical view of a cell that goes inside the atherosclerotic plaque to get activated, degranulate and inflict damage. The inducible antigenic presentation capacity of mast cells we mentioned, is an interesting target for therapeutic development. In particular, MHC-II expression on foam cells was reported to increase upon oxLDL uptake, which triggered an autophagy mediated pathway[88]. Mast cell degranulation is also reportedly mediated by autophagy signals, in a mechanism that is separate from their cytokine secretion machinery[89]. Interestingly, MHC-II induction on the mast cells is stated to increase in the presence of cytokine IL-33[90], a signal that also activates autophagy pathways[91]. It may therefore be that mast cell autophagy-mediated effects are also taking place in atherosclerosis, and affect the expression of MHC-II on the mast cell surface. Further exploration of such a pathway could be of importance in understanding the mast cell presentation capacity. Additionally, a past report indicated that MHC-II is stored in the intracellular mast cell compartment and fuses with the membrane upon degranulation[92]. Investigating whether disruption of this mechanism could reduce the established crosstalk between mast cells and $CD4^+$ T_{H1} cells could be helpful in a therapeutic context. Here we should also mention that mast cells do not seem to affect the adaptive immune system only *via* a direct action on the $CD4^+$ T cells, but can do so indirectly as well. There are studies that explore the exchange of antigenic content between mast cells and dendritic cells[93]. This can happen both through intracellular bridges[93], but it may also happen *via* the secretion of exosomes[94]. Neither the exosomal release by mast cells, nor the antigen-induced crosstalk between mast cells and DCs has been explored in atherosclerosis. However, in our study, we did see that more mast

cells infiltrate the para-aortic lymph nodes upon hyperlipidemia; particularly in the area where DCs reside. The accumulation of mast cells in the LNs upon atherosclerosis progression is on its own an additional pathway to explore. Are they for instance travelling there through the lymphatics? Are they able to migrate from one tissue to the other, carrying along tissue specific antigens? Or are they newly-maturated mast cells deriving from bone marrow progenitors? Answering these questions may provide evidence towards new therapeutic interventions.

An additional pathway that needs further exploration is the epigenetic pressure exerted on the mast cells, inside an inflammatory tissue. The final mast cell maturation step takes place within the tissue[94] in a multitude of organs where mast cells reside. This suggests that mast cells are a highly diverse population with different properties, depending on their place of residence. A recent study screened the transcriptome of mast cells collected from various tissues and confirmed this exact notion[95]. Mast cells indeed appeared to be highly sensitive depending on their surroundings. This raises a question regarding tissues that are characterized by chronic inflammatory pressure, such as the atherosclerotic plaque. Is the local environmental pressure able to alter the mast cell properties at a genetic level? Very recent pioneering work has in fact stated that mast cell tryptase can elicit epigenetic changes on the mast cell genome, through histone truncation[96]. This epigenetic effect is enhanced through time and affects the cellular identity of mast cells, which begin to show similarities with macrophages. It would be intriguing to examine if such a pathway exists also inside the atherosclerotic plaque. After all, epigenetic changes have gained recent attention in atherosclerosis[96].

In conclusion, the above work states that it is important to target specific pathways involved in differential mast cell actions. This is of particular clinical importance since these cells are not only crucial in inflammatory conditions[97], but are also the first responders in infections[98] and potent immune regulators in cancer[99]. After all it is their balancing nature that makes mast cells a fascinating cell type in atherosclerosis, worthy to be explored in depth.

Lastly, it is necessary to mention the therapeutic potential that is offered in atherosclerosis, by targeting the overall immune response. As shown by our work on the LPA-receptor inhibition, systemic modulation of the immune response can efficiently hamper atherosclerosis progression. At this moment, pre-clinical atherosclerosis is being pharmacologically treated with the use of statins[100]. Originally, the benefits of statin use were appointed to the lowering of non-HDL cholesterol alone. However, it was the combination with lower inflammatory burden that resulted in atherosclerotic plaque stabilization[100]. Yet up to now it has not been possible to reduce the plaque burden of an already established human plaque[101]. A novel approach for the reduction of LDL

has been put to practice very recently. Antibody-mediated inhibition of the expression of the hepatic protein PCSK9 is reported to lower LDL levels by 50%, showing very promising potential[102]. However, long term assessment on this type of medication is not possible at the moment. Atherosclerosis research has been actively focusing on means to achieve plaque regression, with experimental models spanning from stimulation of reverse cholesterol transport by means of micro-RNA antagonism[103], to inhibition of immune pathways, in combination with low-lipid diets[104,105]. Lastly, about two months ago, the large scale CANTOS trial, which introduced the inhibition of the inflammatory cytokine IL-1β, using the antibody canakinumab, met its primary clinical endpoint[106]. The results stated that patients treated with canakinumab showed a significant reduction in the risk of developing secondary cardiac events[107]. This anti-inflammatory therapeutic method is a scientific breakthrough comparable to the discovery of statins.

Ultimately, treating atherosclerosis is the challenge of the near future. A challenge that the scientific field has long accepted, in a duel that will be won. *Et lux in tenebris lucet*.

Reference list:

1. Benjamin, E. J. *et al.* Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* **135,** e146–e603 (2017).
2. Vital statistics data - US Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/nchs/data_access/vitalstatsonline.htm.
3. European Cardiovascular Disease Statistics - 2017. Available at: http://www.ehnheart.org/cvd-statistics.html.
4. Usman, A., Ribatti, D., Sadat, U. & Gillard, J. H. From Lipid Retention to Immune-Mediate Inflammation and Associated Angiogenesis in the Pathogenesis of Atherosclerosis. *J. Atheroscler. Thromb.* **22,** 739–749 (2015).
5. Shi, G.-P., Bot, I. & Kovanen, P. T. Mast cells in human and experimental cardiometabolic diseases. *Nat. Rev. Cardiol.* **12,** 643–658 (2015).
6. Willems, S. *et al.* Mast cells in human carotid atherosclerotic plaques are associated with intraplaque microvessel density and the occurrence of future cardiovascular events. *Eur. Heart J.* **34,** 3699–3706 (2013).
7. Bot, I. *et al.* Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. *Circulation* **115,** 2516–2525 (2007).
8. Bot, M. *et al.* Lysophosphatidic acid triggers mast cell-driven atherosclerotic plaque destabilization by increasing vascular inflammation. *J. Lipid Res.* **54,** 1265–74 (2013).
9. Saluja, R., Khan, M., Church, M. K. & Maurer, M. The role of IL-33 and mast cells in allergy and inflammation. *Clin. Transl. Allergy* **5,** 33 (2015).
10. Maruotti, N., Crivellato, E., Cantatore, F. P., Vacca, A. & Ribatti, D. Mast cells in rheumatoid arthritis. *Clin. Rheumatol.* **26,** 1–4 (2007).
11. Liu, J. *et al.* Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat. Med.* **15,** 940–945 (2009).
12. Tanaka, A., Nomura, Y., Matsuda, A., Ohmori, K. & Matsuda, H. Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-delta-12, 14-prostaglandin J2. *Am. J. Physiol. Cell Physiol.* **301,** C1360-7 (2011).
13. Zhou, Y. *et al.* Leptin Deficiency Shifts Mast Cells toward Anti-Inflammatory Actions and Protects Mice from Obesity and Diabetes by Polarizing M2 Macrophages. *Cell Metab.* **22,** 1045–1058 (2015).
14. Finlin, B. S. *et al.* Mast Cells Promote Seasonal White Adipose Beiging in Humans. *Diabetes* **66,** 1237–1246 (2017).
15. Bartelt, A. & Heeren, J. Adipose tissue browning and metabolic health. *Nat. Rev. Endocrinol.* **10,** 24–36 (2014).
16. Laine, P. *et al.* Association Between Myocardial Infarction and the Mast Cells in the Adventitia of the Infarct-Related Coronary Artery. *Circulation* **99,** 361–369 (1999).
17. Kupreishvili, K. *et al.* Mast cells are increased in the media of coronary lesions in patients with myocardial infarction and may favor atherosclerotic plaque instability. *J. Cardiol.* **69,** 548–554 (2017).
18. Somasundaram, P. *et al.* Mast cell tryptase may modulate endothelial cell phenotype in healing myocardial infarcts. *J. Pathol.* **205,** 102–111 (2005).
19. Kwon, J. S. *et al.* The novel role of mast cells in the microenvironment of acute myocardial infarction. *J. Mol. Cell. Cardiol.* **50,** 814–825 (2011).
20. Ngkelo, A. *et al.* Mast cells regulate myofilament calcium sensitization and heart function after myocardial infarction. *J. Exp. Med.* **213,** 1353–1374 (2016).
21. Liao, C. *et al.* Cardiac mast cells cause atrial fibrillation through PDGF-A-mediated fibrosis in pressure-overloaded mouse hearts. *J. Clin. Invest.* **120,** 242–253 (2010).

22. De Jesus, N. M. *et al.* Atherosclerosis exacerbates arrhythmia following myocardial infarction: Role of myocardial inflammation. *Heart Rhythm* **12,** 169–178 (2015).
23. Rakusan, K., Sarkar, K., Turek, Z. & Wicker, P. Mast cells in the rat heart during normal growth and in cardiac hypertrophy. *Circ. Res.* **66,** 511–516 (1990).
24. Morrey, C. *et al.* Interaction between sensory C-fibers and cardiac mast cells in ischemia/reperfusion: activation of a local renin-angiotensin system culminating in severe arrhythmic dysfunction. *J. Pharmacol. Exp. Ther.* **335,** 76–84 (2010).
25. Koda, K. *et al.* Aldehyde dehydrogenase activation prevents reperfusion arrhythmias by inhibiting local renin release from cardiac mast cells. *Circulation* **122,** 771–781 (2010).
26. Hulsmans, M. *et al.* Macrophages Facilitate Electrical Conduction in the Heart. *Cell* **169,** 510–522.e20 (2017).
27. Yeong, P., Ning, Y., Xu, Y., Li, X. & Yin, L. Tryptase promotes human monocyte-derived macrophage foam cell formation by suppressing LXRalpha activation. *Biochim. Biophys. Acta* **1801,** 567–576 (2010).
28. Wang, J. *et al.* IgE actions on CD4+ T cells, mast cells, and macrophages participate in the pathogenesis of experimental abdominal aortic aneurysms. *EMBO Mol. Med.* **6,** 952–969 (2014).
29. Bot, M. *et al.* Lysophosphatidic acid triggers mast cell-driven atherosclerotic plaque destabilization by increasing vascular inflammation. *J. Lipid Res.* **54,** 1265–74 (2013).
30. Bot, M. *et al.* Atherosclerotic lesion progression changes lysophosphatidic acid homeostasis to favor its accumulation. *Am. J. Pathol.* **176,** 3073–3084 (2010).
31. Schober, A. & Siess, W. Lysophosphatidic acid in atherosclerotic diseases. *Br. J. Pharmacol.* **167,** 465–82 (2012).
32. Fukushima, N. & Chun, J. The LPA receptors. *Prostaglandins Other Lipid Mediat.* **64,** 21–32 (2001).
33. Yung, Y. C., Stoddard, N. C. & Chun, J. *LPA receptor signaling: pharmacology, physiology, and pathophysiology. Journal of lipid research* **55,** (2014).
34. Bagga, S. *et al.* Lysophosphatidic acid accelerates the development of human mast cells. *Blood* **104,** 4080–7 (2004).
35. Lundequist, A. & Boyce, J. A. LPA5 is abundantly expressed by human mast cells and important for lysophosphatidic acid induced MIP-1beta release. *PLoS One* **6,** e18192 (2011).
36. Foks, A. C., Lichtman, A. H. & Kuiper, J. Treating atherosclerosis with regulatory T cells. *Arterioscler. Thromb. Vasc. Biol.* **35,** 280–287 (2015).
37. Foks, A. C. *et al.* Differential effects of regulatory T cells on the initiation and regression of atherosclerosis. *Atherosclerosis* **218,** 53–60 (2011).
38. Tse, K., Tse, H., Sidney, J., Sette, A. & Ley, K. T cells in atherosclerosis. *Int. Immunol.* **25,** 615–622 (2013).
39. Jonasson, L., Holm, J., Skalli, O., Bondjers, G. & Hansson, G. K. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. *Arteriosclerosis* **6,** 131–138 (1986).
40. Gaudenzio, N., Laurent, C., Valitutti, S. & Espinosa, E. Human mast cells drive memory CD4+ T cells toward an inflammatory IL-22+ phenotype. *J. Allergy Clin. Immunol.* **131,** 1400–7.e11 (2013).
41. Sehra, S. *et al.* TH9 cells are required for tissue mast cell accumulation during allergic inflammation. *J. Allergy Clin. Immunol.* **136,** 433–40.e1 (2015).
42. Suurmond, J. *et al.* Communication between human mast cells and CD4(+) T cells through antigen-dependent interactions. *Eur. J. Immunol.* **43,** 1758–1768 (2013).
43. Kambayashi, T. & Laufer, T. M. Atypical MHC class II-expressing antigen-presenting cells:

can anything replace a dendritic cell? *Nat. Rev. Immunol.* **14,** 719–30 (2014).

44. Chai, O. H., Han, E.-H., Lee, H.-K. & Song, C. H. Mast cells play a key role in Th2 cytokine-dependent asthma model through production of adhesion molecules by liberation of TNF-alpha. *Exp. Mol. Med.* **43,** 35–43 (2011).
45. Tupin, E. *et al.* CD1d-dependent activation of NKT cells aggravates atherosclerosis. *J. Exp. Med.* **199,** 417–422 (2004).
46. van Puijvelde, G. H. M. & Kuiper, J. NKT cells in cardiovascular diseases. *Eur. J. Pharmacol.* (2017). doi:10.1016/j.ejphar.2017.03.052
47. Godfrey, D. I. & Kronenberg, M. Going both ways: immune regulation via CD1d-dependent NKT cells. *J. Clin. Invest.* **114,** 1379–1388 (2004).
48. Miyazaki, Y. *et al.* Effect of high fat diet on NKT cell function and NKT cell-mediated regulation of Th1 responses. *Scand. J. Immunol.* **67,** 230–237 (2008).
49. Huh, J. Y. *et al.* Deletion of CD1d in adipocytes aggravates adipose tissue inflammation and insulin resistance in obesity. *Diabetes* **66,** 835–847 (2017).
50. Teige, A. *et al.* CD1d-dependent NKT cells play a protective role in acute and chronic arthritis models by ameliorating antigen-specific Th1 responses. *J. Immunol.* **185,** 345–56 (2010).
51. Nakai, Y. *et al.* Natural killer T cells accelerate atherogenesis in mice. *Blood* **104,** 2051–2059 (2004).
52. Li, Y. *et al.* A CD1d-dependent lipid antagonist to NKT cells ameliorates atherosclerosis in ApoE-/- mice by reducing lesion necrosis and inflammation. *Cardiovasc. Res.* **109,** 305–317 (2016).
53. Kaartinen, M., Penttila, A. & Kovanen, P. T. Mast cells of two types differing in neutral protease composition in the human aortic intima. Demonstration of tryptase- and tryptase/chymase-containing mast cells in normal intimas, fatty streaks, and the shoulder region of atheromas. *Arterioscler. Thromb. a J. Vasc. Biol.* **14,** 966–972 (1994).
54. Kraft, S. *et al.* The tetraspanin CD63 is required for efficient IgE-mediated mast cell degranulation and anaphylaxis. *J. Immunol.* **191,** 2871–2878 (2013).
55. Burd, P. R. *et al.* Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.* **170,** 245–257 (1989).
56. Guo, X. *et al.* Serum IgE levels are associated with coronary artery disease severity. *Atherosclerosis* **251,** 355–360 (2016).
57. Meng, Z. *et al.* Oxidized low-density lipoprotein induces inflammatory responses in cultured human mast cells via Toll-like receptor 4. *Cell. Physiol. Biochem.* **31,** 842–53 (2013).
58. Lagraauw, H. M. *et al.* Vascular neuropeptide Y contributes to atherosclerotic plaque progression and perivascular mast cell activation. *Atherosclerosis* **235,** 196–203 (2014).
59. Bot, I. *et al.* The neuropeptide substance P mediates adventitial mast cell activation and induces intraplaque hemorrhage in advanced atherosclerosis. *Circ. Res.* **106,** 89–92 (2010).
60. Wezel, A. *et al.* Mast cells mediate neutrophil recruitment during atherosclerotic plaque progression. *Atherosclerosis* **241,** 289–296 (2015).
61. Bot, I. *et al.* Mast cell chymase inhibition reduces atherosclerotic plaque progression and improves plaque stability in ApoE-/- mice. *Cardiovasc. Res.* **89,** 244–252 (2011).
62. Kondo, S. *et al.* Role of mast cell tryptase in renal interstitial fibrosis. *J. Am. Soc. Nephrol.* **12,** 1668–1676 (2001).
63. Wright, J. D. *et al.* Structural and Physical Basis for Anti-IgE Therapy. *Sci. Rep.* **5,** 11581 (2015).
64. Erdogan, O., Gul, C., Altun, a. & Ozbay, G. Increased Immunoglobulin E Response in Acute

Coronary Syndromes. *Angiology* **54,** 73–79 (2003).

65. Wang, J. *et al.* IgE stimulates human and mouse arterial cell apoptosis and cytokine expression and promotes atherogenesis in Apoe-/- mice. *J. Clin. Invest.* **121,** 3564–3577 (2011).
66. Leskinen, M. J., Kovanen, P. T. & Lindstedt, K. A. Regulation of smooth muscle cell growth, function and death in vitro by activated mast cells--a potential mechanism for the weakening and rupture of atherosclerotic plaques. *Biochem. Pharmacol.* **66,** 1493–1498 (2003).
67. Kawakami, T. & Blank, U. From IgE to Omalizumab. *J. Immunol.* **197,** 4187–4192 (2016).
68. Pelaia, G. *et al.* Anti-IgE therapy with omalizumab for severe asthma: current concepts and potential developments. *Curr. Drug Targets* **16,** 171–178 (2015).
69. Humbert, M. *et al.* Omalizumab in asthma: an update on recent developments. *J. allergy Clin. Immunol. Pract.* **2,** 525–36.e1 (2014).
70. Sellitto, A. *et al.* Effects of omalizumab treatment on serum cytokine concentrations of atopic patients with chronic spontaneous urticaria: a preliminary report. *Eur. Ann. Allergy Clin. Immunol.* **49,** 171–175 (2017).
71. Sokol, K. C., Ghazi, A., Kelly, B. C. & Grant, J. A. Omalizumab as a desensitizing agent and treatment in mastocytosis: a review of the literature and case report. *J. allergy Clin. Immunol. Pract.* **2,** 266–270 (2014).
72. Finn, D. F. & Walsh, J. J. Twenty-first century mast cell stabilizers. *Br. J. Pharmacol.* **170,** 23–37 (2013).
73. Edwards, A. M. & Norris, A. A. Cromoglycate and asthma. *Lancet (London, England)* **343,** 426 (1994).
74. Baba, A. *et al.* Anti-Allergic Drugs Tranilast and Ketotifen Dose-Dependently Exert Mast Cell-Stabilizing Properties. *Cell. Physiol. Biochem.* **38,** 15–27 (2016).
75. Alton, E. W. & Norris, A. A. Chloride transport and the actions of nedocromil sodium and cromolyn sodium in asthma. *J. Allergy Clin. Immunol.* **98,** S102-5-6 (1996).
76. Garcia Mesa, M. New approach to the mechanism of antiasthmatic action of Tranilast. *Allergol. Immunopathol. (Madr).* **18,** 53–56 (1990).
77. Saiura, A., Sata, M., Hirata, Y., Nagai, R. & Makuuchi, M. Tranilast inhibits transplant-associated coronary arteriosclerosis in a murine model of cardiac transplantation. *Eur. J. Pharmacol.* **433,** 163–168 (2001).
78. Nie, L., Mogami, H., Kanzaki, M., Shibata, H. & Kojima, I. Blockade of DNA synthesis induced by platelet-derived growth factor by tranilast, an inhibitor of calcium entry, in vascular smooth muscle cells. *Mol. Pharmacol.* **50,** 763–769 (1996).
79. Han, X. & Boisvert, W. A. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. *Thromb. Haemost.* **113,** 505–512 (2015).
80. McCarthy, C. *et al.* IL-10 mediates the immunoregulatory response in conjugated linoleic acid-induced regression of atherosclerosis. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **27,** 499–510 (2013).
81. Leveson-Gower, D. B. *et al.* Mast cells suppress murine GVHD in a mechanism independent of CD4+CD25+ regulatory T cells. *Blood* **122,** 3659–3665 (2013).
82. Jungraithmayr, W. The putative role of mast cells in lung transplantation. *Am. J. Transplant* **15,** 594–600 (2015).
83. Nakano, T. *et al.* Immunological and regenerative aspects of hepatic mast cells in liver allograft rejection and tolerance. *PLoS One* **7,** e37202 (2012).
84. Lin, T. J., Issekutz, T. B. & Marshall, J. S. Human mast cells transmigrate through human umbilical vein endothelial monolayers and selectively produce IL-8 in response to stromal cell-derived factor-1 alpha. *J. Immunol.* **165,** 211–220 (2000).

85. Conti, P. *et al.* Mast cells emerge as mediators of atherosclerosis: Special emphasis on IL-37 inhibition. *Tissue Cell* **49,** 393–400 (2017).

86. Suurmond, J., Dorjee, A. L., Knol, E. F., Huizinga, T. W. J. & Toes, R. E. M. Differential TLR-induced cytokine production by human mast cells is amplified by FcvarepsilonRI triggering. *Clin. Exp. Allergy* **45,** 788–796 (2015).

87. Phong, B., Avery, L., Menk, A. V, Delgoffe, G. M. & Kane, L. P. Cutting Edge: Murine Mast Cells Rapidly Modulate Metabolic Pathways Essential for Distinct Effector Functions. *J. Immunol.* **198,** 640–644 (2017).

88. Choi, S.-H. *et al.* SYK regulates macrophage MHC-II expression via activation of autophagy in response to oxidized LDL. *Autophagy* **11,** 785–795 (2015).

89. Nakano, H. & Ushio, H. An unexpected role for autophagy in degranulation of mast cells. *Autophagy* **7,** 657–659 (2011).

90. Ito, T. *et al.* IL-33 promotes MHC class II expression in murine mast cells. *Immunity, Inflamm. Dis.* **3,** 196–208 (2015).

91. Wu, J. *et al.* IL-33 is required for disposal of unnecessary cells during ovarian atresia through regulation of autophagy and macrophage migration. *J. Immunol.* **194,** 2140–2147 (2015).

92. Raposo, G. *et al.* Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. *Mol. Biol. Cell* **8,** 2631–2645 (1997).

93. Carroll-Portillo, A. *et al.* Mast cells and dendritic cells form synapses that facilitate antigen transfer for T cell activation. *J. Cell Biol.* **210,** 851–864 (2015).

94. Skokos, D. *et al.* Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J. Immunol.* **170,** 3037–3045 (2003).

95. Dwyer, D. F., Barrett, N. A. & Austen, K. F. Expression profiling of constitutive mast cells reveals a unique identity within the immune system. *Nat. Immunol.* **17,** 878–887 (2016).

96. Melo, F. R. *et al.* Tryptase-catalyzed core histone truncation: A novel epigenetic regulatory mechanism in mast cells. *J. Allergy Clin. Immunol.* **140,** 474–485 (2017).

97. Frenzel, L. & Hermine, O. Mast cells and inflammation. *Joint. Bone. Spine* **80,** 141–145 (2013).

98. Voehringer, D. Protective and pathological roles of mast cells and basophils. *Nat. Rev. Immunol.* **13,** 362–375 (2013).

99. Khazaie, K. *et al.* The significant role of mast cells in cancer. *Cancer Metastasis Rev.* **30,** 45–60 (2011).

100. Nissen, S. E. Effect of intensive lipid lowering on progression of coronary atherosclerosis: evidence for an early benefit from the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial. *Am. J. Cardiol.* **96,** 61F–68F (2005).

101. Nissen, S. E. *et al.* Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *JAMA* **295,** 1556–1565 (2006).

102. Whayne, T. F. J. PCSK9 inhibitors in the current management of atherosclerosis. *Arch. Cardiol. Mex.* **87,** 43–48 (2017).

103. Rayner, K. J. *et al.* Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J. Clin. Invest.* **121,** 2921–2931 (2011).

104. Foks, A. C. *et al.* Interruption of the OX40 – OX40 Ligand Pathway in LDL Receptor – Deficient Mice Causes Regression of Atherosclerosis. (2013). doi:10.4049/jimmunol.1200708

105. Kita, T. *et al.* Regression of atherosclerosis with anti-CD3 antibody via augmenting a regulatory T-cell response in mice. *Cardiovasc. Res.* **102,** 107–117 (2014).

106. Ridker, P. M., Thuren, T., Zalewski, A. & Libby, P. Interleukin-1beta inhibition and the

prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am. Heart J.* **162,** 597–605 (2011).

107. Ridker, P. M. *et al.* Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N. Engl. J. Med.* (2017). doi:10.1056/NEJMoa1707914