

Monocytes and macrophages in abdominal aortic aneurysm

Juliette Raffort^{1,2,3,4}, Fabien Lareyre^{1,4,5}, Marc Clément¹, Réda Hassen-Khodja^{4,5}, Giulia Chinetti^{3,4}, Ziad Mallat^{1,2}

¹ Division of Cardiovascular Medicine, Department of Medicine, University of Cambridge, Cambridge, UK, CB20 SZ.

² Institut National de la Santé et de la Recherche Médicale (Inserm), Unit 970, Paris Cardiovascular Research Center, 75015 Paris, France

³ Clinical Chemistry Laboratory, University Hospital of Nice, France

⁴ Université Côte d'Azur, CHU, CNRS, Inserm, IRCAN, France

⁵ Department of Vascular Surgery, University Hospital of Nice, France

Correspondence should be addressed to: Ziad Mallat, MD, PhD, at Division of Cardiovascular Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK. E-Mail: zm255@medchl.cam.ac.uk.

Abstract word count: 184

Text word count: 7927

Figures: 4

References: 168

Abstract

Abdominal aortic aneurysm (AAA) is a life threatening disease associated with high morbidity and high rates of mortality in case of aortic rupture. Major advances in surgical treatment have been achieved during the past few years. However, drug-based therapies are still lacking, highlighting a real need for better understanding of the cellular and molecular mechanisms involved in AAA formation and progression. Main pathological features of AAA include extracellular matrix remodeling associated with degeneration and loss of smooth muscle cells, as well as inflammatory cell accumulation and activation. The inflammatory process plays a crucial role in the disease and substantially impacts many determinants of aortic wall remodeling. Here, we focus on the implication of monocytes and macrophages, and summarize the current knowledge regarding their roles, origins and functions in AAA development and complications. We show and propose that distinct monocyte/macrophage subsets play critical differential roles in the initiation, propagation, and healing of the aneurysmal process. Based on experimental and clinical studies, we review potential translational applications to detect, assess and image the various subsets of macrophages in AAA, and discuss their relevance for clinical practice.

Key points

- Most of the macrophages that accumulate in the aneurysmal aortic wall originate from circulating monocytes, mobilized from the bone marrow and the spleen. Some AAA macrophages may originate from aortic tissue resident macrophages.
- Main factors involved in macrophage accumulation in AAA include chemokines/cytokines produced in response to tissue injury, products of extracellular matrix degradation, and microenvironmental conditions (blood flow, thrombus, periaortic fat, etc.).

- Monocytes and macrophages display distinct phenotypes during the development and progression of AAA, with major implications for their activation and biological functions.
- Macrophages exert both pathogenic and reparative roles in AAA through their involvement in extracellular matrix remodeling, the promotion and resolution of inflammation, and various aspects of the tissue healing response.
- State-of-the-art translational applications are available, and can be improved and harnessed for the use of monocytes and macrophages as diagnostic and prognostic biomarkers, but also as therapeutic targets in AAA.

Abbreviations:

AAA: Abdominal Aortic Aneurysm

ANGPTL2: Angiopoietin-Like Protein 2

ApoE^{-/-}: apolipoprotein E-deficient mice

ASC: Adaptor molecule apoptosis-associated speck like protein containing a caspase recruitment domain

CaCl₂: Calcium Chloride

Casp1: Caspase 1

CCL: CC-chemokine Ligand

CCR: CC-chemokine Receptor

CXCR: CXC chemokine Receptor

CXCL: CXC chemokine Ligand

CD: Cluster of Differentiation

CLIO: Cross-Linked Iron Oxide

CSF1: Colony Stimulating Factor 1

C/EBP β : CCAAT/enhancer binding protein

DOTATATE: [1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid]-d-Phe1,Tyr3-octreotate

ECM: Extracellular Matrix

GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor

GTP: Guanosine Triphosphate

IL: Interleukin

IL1RN: gene encoding interleukin-1 receptor antagonist

KLF6: Krüppel-Like Factor 6

Ldlr^{-/-}: low density lipoprotein receptor deficient mice

LFA-1: Lymphocyte Function-Associated Antigen 1

M-CSF: Macrophage-Colony Stimulating Factor

MerTK: Myeloid-epithelial-reproductive Tyrosine Kinase

mRNA: messenger RNA

MMP: Metalloproteinase

NADPH oxidase: Nicotinamide Adenine Dinucleotide Phosphate

NLRP3: NOD-like receptor family, pyrin domain containing 3

NOS2: Nitric Oxide Synthase 2

PET/CT: Positron Emission Tomography/ Computed Tomography

PET/MRI: Positron Emission Tomography/ Magnetic Resonance Imaging

PPAR: Peroxisome Proliferator-Activated Receptors

PRDX-1: Peroxiredoxin-1

RANKL: Receptor Activator of Nuclear Factor KappaB Ligand

RGS: Regulators of G-Protein Signaling

SatM: segregated-nucleus-containing atypical monocytes

TERT: Telomerase Reverse Transcriptase

TGF: Transforming Growth Factor

TLR4: Toll Like Receptor 4

TIMP: Tissue Inhibitor of Metalloproteinase

TNF: Tumor Necrosis Factor

USPIO: Ultrasmall Superparamagnetic Iron Oxide

VEGF-C: Vascular Endothelial Growth Factor-C

¹⁸F-FDG: 18F-Fluorodeoxyglucose

⁶⁴Cu: Copper-64

⁶⁸Ga: Gallium-68

Introduction

Aortic aneurysm corresponds to a focal dilatation of the aorta with substantial morbidity and mortality, as a result of complications such as aortic rupture ¹. Although a remarkable progress has been achieved in both open and endovascular surgery, curative pharmacological treatments are still lacking ², underlining a real need to improve our knowledge of the mechanisms involved in disease development and progression. While vessel dilatation can occur in the abdominal or thoracic aorta, the location of the most common form of aneurysm in humans is the infra-renal region of the abdominal aorta, and is the subject of the present review.

Detailed pathological and imaging studies of human abdominal aortic aneurysm (AAA), associated with the development of several animal models of the disease have led to the characterization of the main mechanisms underlying disease development and complications ^{3,4}. Pathological features include extracellular matrix (ECM) degradation and loss of vascular smooth muscle cells (VSMCs), associated with adventitial and medial inflammatory cell infiltration, contributing to vascular remodeling and weakening of the aortic wall. Innate and adaptive immune cells including neutrophils, macrophages, mast cells, natural killer cells, dendritic cells, B and T cells have been identified in the aortic wall during aneurysm, and shown to contribute to its development ⁵. In this review we focus on the characterization of monocytes and macrophages in aortic aneurysm given their critical and distinct roles in several steps of the vascular response to injury, and we summarize the current knowledge on their origins and roles in disease development and complications. In the limelight of human and animal studies, we discuss potential translational applications.

Circulating monocytes and AAA

Circulating blood monocytes originate from the bone marrow and correspond to a heterogeneous leukocyte population with various subsets differing in terms of morphology, cell surface markers, gene expression and functions.

In humans, 3 main subsets can be distinguished based on the expression of the cell surface markers CD14 and CD16. Classical monocytes, defined by high CD14 expression but no CD16 (CD14⁺⁺ CD16⁻), usually represent up to 90% of the blood monocytes^{6,7}. They express high levels of chemokine receptor 2 (CCR2) and CD62L but low levels of CX3CR1 and are characterized by a high expression of genes coding for antimicrobial and phagocytosis related proteins, underlining their major role in innate immune responses^{7,8}. Besides, they express genes involved in angiogenesis, wound healing and coagulation, suggesting a potential role in tissue repair⁹. In contrast, non-classical monocytes are characterized by low level of CD14 but high CD16 expression (CD14⁺ CD16⁺⁺), and display low levels of CCR2 with high levels of CX3CR1⁶⁻⁸. Due to their vascular patrolling properties, they are involved in innate immune surveillance of tissues^{7,8}. An intermediate phenotype characterized by high level of CD14 with low CD16 (CD14⁺⁺ CD16⁺) has also been described and display pro-inflammatory and phagocytic properties⁶⁻⁸.

In mice, monocyte subsets express CD11b and CD115 but low levels of F4/80, and are classified according to the expression of Ly6C into 2 major subsets that are close to the major human subsets in terms of chemokine receptor expression and function^{10,11}. Ly6C high (Ly6C^{hi}) monocytes are considered to be the counterpart of the classical human monocytes and are thought to promote inflammatory responses. In contrast, Ly6C low (Ly6C^{lo}) monocytes display similar functions as non-classical human monocytes and are involved in patrolling, immune surveillance and tissue repair^{8,10,11}.

Monocytosis (increased number of circulating monocytes) has been associated with a number of cardiovascular diseases including atherosclerosis and myocardial infarction ^{7,8,12,13}. In aortic aneurysm, clinical studies revealed significant modifications of peripheral blood monocytes, suggesting their involvement in the disease. A higher proportion of circulating intermediate CD14⁺⁺CD16⁺ monocytes, concomitantly with a decrease of classical CD14⁺⁺CD16⁻ monocytes levels was observed in patients with AAA compared to healthy controls ^{14,15}. Less consistent results were found for CD14⁺CD16⁺ monocytes; one study identified an increase of this subset in AAA patients compared to controls, whereas another study could not find any significant difference ^{14,15}. Other investigators performed proteomic and transcriptomic analyses on macrophages derived from human peripheral blood monocytes, and revealed differences in protein and gene expression profiles between monocyte-derived macrophages of patients with AAA and patients with peripheral arterial disease ¹⁶. Among the differentially expressed mRNAs and proteins, some were gene products involved in pathophysiological mechanisms relevant to AAA such as ECM remodeling or inflammation, suggesting a specific role of blood monocyte-derived macrophages in AAA pathogenesis.

To gain further insight into the pathological significance of circulating monocytes in AAA, investigators studied their variation in animal models of AAA.

Several experimental models have been developed, each of them reproduces some of the main features of human AAA ¹⁷. Schematically, non-dissecting AAA can be created mainly through local perfusion/application of porcine pancreatic elastase or calcium chloride, and lead to aortic dilation in association with ECM degradation and induction of an inflammatory response ¹⁷⁻¹⁹. In contrast, dissecting AAA models, as those induced by angiotensin II infusion, are characterized by the induction of medial dissection and are also associated with ECM degradation and vascular inflammation. The angiotensin II infusion model has been

developed in different strains including apolipoprotein E-deficient (*ApoE*^{-/-}), low density lipoprotein receptor deficient (*Ldlr*^{-/-}) or in some cases *C57BL/6* mice, and usually induces a dissecting AAA located to the suprarenal aorta, but can also lead to thoracic aortic rupture ^{17,20-23}.

An increase of circulating monocytes expressing CCR2 was observed in *ApoE*^{-/-} mice infused with angiotensin II for 14 days ²⁴. Given the ability of CCR2⁺ monocytes to migrate to sites of injury and inflamed tissues ²⁵, their increase in the blood after angiotensin II infusion suggests that they could contribute to the vascular inflammatory response. Another study reported higher levels of circulating Ly6C^{hi} monocytes and Ly6C^{lo} monocytes, at respectively day 3 and day 7, in mice that later developed AAA (after 28 days of angiotensin II infusion) compared to mice that did not develop AAA ²⁶. Remarkably, monocyte levels did not increase and remained stable over time in mice that did not develop AAA after angiotensin II ²⁶. Moreover, the efficacy of some treatment strategies (such as administration of recombinant angiopoietin-2) in attenuating angiotensin II-induced aortic dilatation and rupture in *ApoE*^{-/-} mice was associated with a reduction of the circulating levels of Ly6C^{hi} inflammatory monocytes ²⁷. Interestingly, mice deficient in myeloid differentiation factor 88 (MyD88), a critical adaptor protein required for signaling downstream of several toll-like receptors (TLR) as well as receptors of the interleukin-1 (IL-1) family of cytokines, showed reduced redistribution of monocytes from Ly6C^{lo} to Ly6C^{hi} in both the peripheral blood and spleens after angiotensin II infusion, which was associated with a significant attenuation of AAA formation ²⁸. The data suggested a pathogenic role of Ly6C^{hi} but a potentially less critical role for Ly6C^{lo} monocytes in the development of AAA. However, *Nr4a1*^{-/-} mice, which are deficient in Ly6C^{lo} monocytes ²⁹, displayed increased aortic dilatation and elastin disruption in response to angiotensin II ³⁰, potentially suggesting a direct role for Ly6C^{lo} monocytes. Nevertheless, the phenotype of *Nr4a1* deficient mice could also be due to a role of *Nr4a1* in

vascular smooth muscle cells³⁰, or to functional alteration of the remaining Ly6C^{hi} monocytes and bone marrow-derived macrophages in the absence of *Nr4a1*^{31,32}. The latter hypothesis can potentially be addressed by use of mice deleted for an *Nr4a1* super-enhancer subdomain that differentially controls and decouples Ly6C^{lo} monocyte development from the regulation of gene expression in macrophages³³.

Taken together, these results point to a potentially interesting role of circulating monocytes in regulating vascular inflammation and remodeling during the development of AAA, and suggest that determination or modulation of their numbers in the blood may be of predictive or therapeutic value (see below). Interestingly, a recent study reported the identification of a novel atypical monocyte subset (CD11b⁺ Ly6C⁻ Ceacam1⁺ Msr1⁺), called segregated-nucleus-containing atypical monocytes (SatM), derived from a Ly6C⁻ FcεRI⁺ SatM progenitor downstream of granulocyte/macrophage progenitors. SatM share granulocyte characteristics, require CCAAT/enhancer binding protein (C/EBPβ) for their differentiation, and play a critical role in fibrosis³⁴. We suspect that they have a human equivalent and may play interesting roles in tissue repair during AAA. Hence, monocytes are composed of multiple subsets whose various and distinct functions would be worth of detailed investigation in the context of AAA.

Macrophage characterization in AAA

Over the past decades, several phenotypes of macrophages have been described in inflamed tissues, with a particular focus on the dichotomy between “classically activated” M1 and “alternatively activated” M2 macrophages³⁵⁻³⁷. M1 macrophages are considered to be pro-inflammatory and characterized by the production of proteolytic enzymes and pro-inflammatory cytokines such as TNF, IL6, IL12, IL1β, NOS2 or CCL2^{5,35}. In contrast, M2

macrophages play anti-inflammatory roles through the secretion of factors such as IL10 or TGF β , and are involved in matrix remodeling and tissue repair³⁵. Recently, a specific pathway has been described for the generation of NOS2⁺ macrophages from a subset of Ly6C^{hi} monocytes, indicating that specific subsets of monocytes may be endowed with the ability to generate functionally distinct macrophage subsets³⁸. However, while the classification between macrophage subsets is well defined *in vitro*, their distinction *in vivo* is less obvious and more problematic given that selective M1 and M2 stimuli do not occur separately *in vivo*, and markers used to differentiate between the various macrophage subsets lack specificity^{35,39}. Hence, macrophage subsets *in vivo* rather correspond to a spectrum of activation and are characterized by a combination of markers³⁵.

Several studies reported the presence of macrophages in the aneurysmal aortic wall both in human aortic sections⁴⁰ and in animal models of AAA^{20,22,41-44}. Macrophages accumulate in the 3 layers of the aneurysmal aorta but display predominant accumulation in the adventitia and the intraluminal thrombus^{45,46}. To gain further insight into the accumulation of macrophages in different aortic layers, Dutertre et al. analyzed the composition of human aneurysmal tissues by flow cytometry after separation of the media from the adventitia⁴⁵. In that study, M2 macrophages were defined as CD15⁻ CD14^{hi} cells which strongly expressed the M2 markers CD163 (a high affinity scavenger receptor for the hemoglobin-haptoglobin complex), CD206 (mannose receptor) and MerTK (tyrosine kinase receptor involved in efferocytosis of apoptotic cells); whereas M1 macrophages corresponded to CD15⁺ CD14^{lo} cells and did not express CD163, CD206, or MerTK. The authors revealed a decrease of M1 in favor of M2 macrophages in the adventitia of aneurysmal tissue as compared to controls⁴⁵. Another study on human aortic aneurysmal sections identified M1 macrophages as CD68⁺ CD206⁻ cells and M2 macrophages as CD68⁺ CD206⁺ cells⁴⁷. The authors also found a higher cellular density for M2 in the aortic wall. However, they reported a preferential

localization of the M1 subset in the adventitia, while M2 macrophages were predominant in the intraluminal thrombus. Isolation of each macrophage subset by laser capture microdissection further revealed that gene and protein expression of peroxiredoxin-1 (PRDX-1), involved in redox status, was higher in M1 compared to M2 macrophages. These results are discordant with the findings of Dutertre et al. and could be explained by differences in the methods and markers used to identify M1 and M2 macrophages. Besides, it is possible that the samples represented different stages of the disease, and that macrophage infiltration and polarization evolve during the various steps of aneurysm development and complications. Both studies however suggest potentially distinct roles of macrophage subsets in disease development and aortic remodeling.

In that context, macrophage polarization was also studied in animal models of AAA. Increased accumulation of total macrophages with a higher M1/M2 ratio was observed in the suprarenal aorta of *ApoE*^{-/-} mice after 7 to 10 days of angiotensin II infusion, and was maintained over the 28 days of the experiment⁴⁸. The reasons for this M1 shift are not fully understood. However, a few pathways were suggested to play a role, including upregulation of TLR4 by angiotensin II⁴⁸, and a role for ECM remodeling and elastin degradation products, particularly VGVAPG repeat sequence⁴⁹. Other investigators addressed the status and implications of macrophage polarization after prolonged angiotensin II infusion in *ApoE*^{-/-} mice⁵⁰. Compared to mice infused with angiotensin II for 28 days, prolonged infusion for additional 56 days led to increased aneurysm expansion and propensity to rupture with an increase of CD68 positive cells. Immunostaining for CD206 (a marker of M2) was more common than NOS2 (a marker of M1-like) in these tissues suggesting that end stage aneurysm could be associated with a shift toward M2 polarization. **Given their role in tissue repair and wound healing, we can hypothesize that the shift toward M2 macrophages may result from a compensatory effect, aiming to prevent AAA expansion and rupture. The**

mechanisms behind such a polarization are not well understood. Intriguingly, although angiotensin II promotes the infiltration of Ly6C^{hi} monocytes in the aortic wall, it may also directly limit the development of M1 type macrophages, and promote an M2 phenotype ⁵¹. Besides angiotensin II, factor XIIIa transglutaminase has been identified to play an important role in mediating gene expression during M2 polarization ⁵² and is induced in adventitial CXCR3⁺ macrophages in response to impairment of blood flow ^{53,54}. Macrophages with the latter phenotype accumulate in the aneurysmal aortic wall ⁵⁴. It is tempting to hypothesize that reduction of shear stress in advanced aortic aneurysms due to profound alterations of aortic wall geometry and hemodynamics may promote that pathway in adventitial macrophages in an attempt to limit expansive wall remodeling. Other mechanisms of M2-like polarization may involve pathways related to apoptotic cell efferocytosis ⁵⁵, or clearance of red blood cells after hemorrhage. Indeed, hemorrhage associated macrophages including HA-mac, M(Hb), and Mhem have been identified in areas of intraplaque hemorrhage and play roles in hemoglobin clearance and tissue remodeling ^{37,56}. These macrophages are characterized by the expression of CD163, which is involved in hemoglobin-heme uptake and endocytosis of haemoglobin-haptoglobin complexes, and their activation leads to the induction of an anti-inflammatory macrophage phenotype ^{57,58}. CD163 expression is increased in human aneurysmal walls as compared with healthy aorta and is mainly found in areas rich in hemosiderin adjacent to neoangiogenic microvessels in the adventitia ¹⁵. Thus, similarly to M2 macrophages, anti-inflammatory HA-mac macrophage subsets characterized by high levels of CD163 and low levels of HLA-DR also accumulate in the adventitial layer of AAA ^{15,45}.

In summary, based on the results of current studies in mice and humans, we can hypothesize that early stages of AAA are associated with a predominant infiltration of macrophages whose characteristics are close to the M1 phenotype and probably enhance vascular inflammation.

We suspect that some, like NOS2⁺ macrophages, may directly derive from a selective subset of Ly6C^{hi} monocytes³⁸. This could contribute to increased aortic dilatation and adverse vascular remodeling (see below). When the disease evolves, several changes in the artery wall such as altered hemodynamics, distinct chemokine/cytokine expression profile, intra-luminal thrombus formation, or the occurrence of micro-dissections may lead to the recruitment of distinct subsets of monocytes with reparative potential, like Ly6C^{lo} or SatM monocytes, or may impact macrophage polarization and promote anti-inflammatory and pro-resolving macrophage phenotypes in an attempt to heal the aortic wall and maintain vascular integrity.

Origin of macrophages in the AAA wall

For decades, it has been assumed that tissue macrophages originate from bone marrow-derived circulating blood monocytes that extravasate into tissues^{59,60}. However, recent advances in macrophage ontogeny have substantially revisited and refined that concept. At steady state, the majority of tissue resident macrophages is self-maintained during adulthood, independently of bone marrow progenitors, and originate from embryonic progenitor cells that have migrated into these tissues during the embryonic and fetal life^{59,60}. This is also the case for cardiac⁶¹ and vascular-associated resident macrophages⁶². Local proliferation of tissue macrophages is involved in several cardiovascular processes^{63,64}, and a greater proliferation index has been reported in macrophages of aneurysmal aortas compared to controls⁴⁵. However, proliferation can occur in both tissue-resident macrophages and those derived from circulating monocytes after their infiltration into injured vessels⁶⁵. Thus, whether vascular-resident macrophages contribute any significant role to aortic remodeling during aneurysm development is still unclear (Figure 1).

Most of the available evidence, derived in part from bone marrow transplantation experiments, points to an important contribution of monocyte-derived macrophages to macrophage accumulation in AAA. The use of green fluorescent protein positive bone marrow showed that a proportion of macrophages that infiltrated the adventitial layers originated from bone marrow monocytes recruited in response to increased expression of CXCL12 in the aorta during aneurysm formation after local application of calcium chloride (CaCl₂)⁶⁶. Experiments in *Apoe*^{-/-} mice infused with angiotensin II also indicate that most of the aneurysmal wall macrophages derive from circulating monocytes²⁶. Interestingly, angiotensin II infusion leads to an increase of blood Ly6C^{hi} monocytes at day 3, in parallel to their decrease in the spleen with no significant change in the bone marrow, suggesting that monocytes/macrophages that accumulate in the abdominal aorta may be mobilized from the spleen by angiotensin II. This hypothesis is corroborated by the fact that splenectomy in *Apoe*^{-/-} mice infused with angiotensin II induces a decrease of aortic macrophage accumulation^{26,67}. Investigation of the mechanisms involved in monocyte mobilization from the spleen revealed that the process did not result from a direct mechanical contraction of the spleen in response to angiotensin II, and that splenic B lymphocytes were required for monocyte mobilization²⁶. These results show that monocyte/macrophage mobilization from peripheral reservoirs is an important and complex mechanism that contributes to aortic inflammation, and merits further investigations into its molecular mechanisms.

Consistent with the importance of circulating monocytes in the pathogenesis of AAA, their depletion in mice using clodronate containing liposomes substantially limits macrophage accumulation in the aortic wall and reduces the development and severity of AAA^{26,68,69}. Intriguingly, genetic ablation of monocytes/macrophages in *Csf1*^{-/-} mice did not prevent AAA formation in *Apoe*^{-/-} mice, and unexpectedly induced intra-mural hemorrhage in the thoracic aortas under both *Apoe*^{-/-} and *Apoe*^{+/+} backgrounds⁷⁰. Those results should be interpreted with

caution given the low number of *Csf1*^{-/-} mice used in that study and the unusually low percentage of angiotensin II-induced AAA in the *Csf1*^{+/+} controls⁷⁰. However, an important difference between clodronate depletion and *Csf1*^{-/-} mice is that clodronate only depletes circulating monocytes without affecting vascular-associated resident macrophages of the adventitia, whereas *Csf1*^{-/-} are also deficient in arterial resident macrophages⁶². It is thus tempting to hypothesize that the latter play essential roles in preserving vascular integrity.

Monocyte recruitment to the AAA wall

The importance of monocyte mobilization from peripheral organs led to the study of the mechanisms responsible for their mobilization and recruitment into the injured aortic wall. Given that 3 major chemokine/chemokine receptor pathways (CCL2/CCR2, CX3CL1/CX3CR1 and CCL5/CCR5) control the recruitment of circulating Ly6C^{hi} and Ly6C^{lo} monocyte subsets in atherosclerosis⁷¹, those pathways were the most studied in the context of AAA.

CCL2 expression and secretion from aortic explants is increased in response to angiotensin II infusion compared to control mice^{40,72}. In fact, an amplification loop between CCL2 and IL6 has been described in the aortic wall following angiotensin II infusion, where IL-6 and CCL2 production by aortic adventitial fibroblasts initiate monocyte recruitment, which in turn, differentiate into macrophages and stimulate the proliferation of aortic adventitial fibroblast, leading to additional cytokine/chemokine production in a vicious circle⁷³. CCR2 global knockout or CCR2 specific deficiency in leukocytes decreased aortic dissection and aneurysm formation, which was associated with a decreased number of aortic macrophages and an inhibitory effect on inflammatory cytokine expression in angiotensin II infused mice^{40,72}. Similarly, CCL2 global knockout as well as CCL2 deficiency in bone marrow derived cells

showed protective effects in the model of elastase-induced aneurysm, and was associated with a drastic reduction of aortic Mac-2⁺ (galectin-3) macrophages⁷⁴. These results confirm the role of Ly6C^{hi} monocytes and of CCL2/CCR2 axis in macrophage accumulation and the promotion of vascular inflammation during the development of aortic aneurysm.

The role of the other chemokine pathways received less attention. Deletion of CX3CR1 led to partial protection against fatal aortic dissection in a model of angiotensin II and anti-TGFβ infusion²². Interestingly, CX3CR1 and CCR2 were sequentially and differently expressed in the aortic wall during the early stages of AAA development, and this was associated with early predominance of Ly6C^{hi} over Ly6C^{lo} monocyte recruitment in the abdominal aorta²⁶.

Inhibition of CXCL4/CCL5 heterodimer formation was associated with a reduction of monocyte/macrophage infiltration in an elastase-induced aneurysm⁷⁵. However, deletion of CCR5 did not alter aortic disease in a model of CaCl₂-induced AAA⁷⁶.

In addition to monocyte recruitment, chemokines may also modulate macrophage retention within the tissue. Regulators of G-Protein Signaling (RGS) proteins target GTPase activity of chemokine receptors that are coupled to G_{oi} subunits, and desensitize receptor signaling⁷⁷. Interestingly, RGS1 is highly expressed in human AAA tissue compared to non-aneurysmal vascular tissue⁷⁸. *Rgs1* deficient mice showed reduced leukocyte trafficking, increased macrophage chemotaxis and lower macrophage retention into the aortic wall, which was associated with a protection against aneurysm formation and progression⁷⁸.

In addition to chemokines and cytokines, the selectin family is known for its role in immune cell interaction with the vessel wall⁷⁹. L-selectin deficiency impaired both neutrophil and monocyte/macrophage recruitment and suppressed elastase-induced AAA formation⁸⁰. As neutrophils were recruited to the aortic wall before macrophages, impaired macrophage accumulation could be attributed at least in part to diminished neutrophil recruitment and

activation. Indeed, neutrophils are able to promote monocyte recruitment through cytokine secretion, neutrophil extracellular trap formation or secretion of granule proteins, thereby inducing adhesion and migration of monocytes to the site of inflammation ⁸¹.

What induces chemokines and chemotactic products in the vascular wall during the development of AAA? Several risk factors of AAA, including smoking and hypertension, have been associated with inflammatory activation of the vascular wall ^{82,83}. Moreover, ECM remodeling, changes in hemodynamics and circumferential stress, or the occurrence of microdissections and intra-mural hemorrhage during the progression of AAA may further impact inflammatory cell recruitment and activation. Those mechanisms have been addressed in several experimental studies.

Macrophages were observed to accumulate predominantly in regions of medial disruption on the adventitial side as indicated by elastin fragmentation, suggesting that ECM components could act as chemotactic stimuli ⁵⁰. *In vitro* experiments further revealed that soluble proteins extracted from human AAA tissue enhanced human U937 monocyte migration in a concentration dependent manner ⁸⁴. The authors attributed the monocyte chemotactic activity in the AAA extracts to the presence of elastin degradation products and intact cell surface receptors for elastin related ligands ⁸⁴. Hence, elastin degradation products may promote monocyte recruitment in the aneurysmal wall, which in turn will secrete proteolytic enzymes that further degrade the tissue. A variety of other molecules associated with ECM remodeling including osteopontin, thrombospondin-1, and plasminogen, have also been shown to impact monocyte/macrophage recruitment, either directly or indirectly ⁸⁵⁻⁸⁷. ECM degradation may lead to microdissections and intra-mural hemorrhage, which could further recruit more monocytes/macrophages into the aortic wall. Indeed, increased migration of human monocytes was reported in response to hemoglobin and conditioned medium obtained from adventitial human AAA ¹⁵.

Macrophage infiltration can also be modulated by the intraluminal microenvironment, and more particularly, by changes in hemodynamic conditions. Macrophage accumulation in the elastase-induced aneurysm model was inversely correlated to luminal flow and wall shear stress⁸⁸. High aortic flow and wall shear stress were associated with increased medial macrophage apoptosis. Interestingly, CCL2 mRNA expression in the aorta was inversely correlated with the level of blood flow and could partly explain the reduction of macrophage accumulation in media of the high flow group. The abluminal microenvironment, such as the peri-aortic adipose tissue, may also impact macrophage infiltration.

Obesity is associated with AAA⁸⁹ and periaortic fat volume correlated with the dimensions of both thoracic and abdominal aortas, suggesting that local fat accumulation may contribute to aortic remodeling⁹⁰. In fact, periaortic adipose explants from abdominal aortas of high fat-fed mice showed greater CCL2 release and higher capacity to induce migration of peritoneal macrophages, compared to mice fed a low fat diet⁹¹.

Risk factors and changes in the AAA microenvironment may also directly impact the activation, adhesive and migratory properties of circulating monocytes. For example, thrombomodulin and CD14 expression in monocytes significantly modulate monocyte production of inflammatory mediators, adhesion to endothelial cells and monocyte chemotaxis, resulting in significant attenuation of macrophage accumulation in the aortic wall and significant prevention of AAA development in mice with either total CD14 or LysM-Cre restricted thrombomodulin deficiency^{92,93}. Human studies confirm that circulating monocytes of patients with AAA display higher levels of circulating monocytes exhibiting LFA-1 and Mac-1, as compared to controls⁹⁴. Moreover, blood monocytes from AAA patients exhibit higher monocyte adhesion to the endothelial monolayer and higher transmigration ability through the endothelium *in vitro* compared to controls⁹⁴.

Roles of macrophages in AAA

ECM degradation

Macrophage accumulation in the aneurysmal aortic wall plays crucial roles in the response to tissue injury. Macrophages may profoundly alter the balance between destructive tissue remodeling and reparative tissue healing through their control of extracellular matrix remodeling and their roles in both the promotion and resolution of the inflammatory response⁹⁵.

ECM remodeling results from a balance between the activities of proteases and their inhibitors. Increased expression of proteases such as cathepsins or metalloproteinases (MMPs) is observed during AAA development, and deficiency in these enzymes has revealed protective effects^{42,96-98}. Conversely, enhanced AAA formation is observed in mice deficient in tissue inhibitors of metalloproteinases (TIMPs)⁹⁹. The expression of cathepsins, MMPs and TIMPs is altered in blood monocyte-derived macrophages from patients with AAA compared with patients with peripheral arterial disease, and increased expression of MMPs or cathepsins colocalize with macrophages in the aneurysmal wall, suggesting a role for monocyte/macrophage-derived proteases in ECM remodeling during aneurysm^{16,100,101}. This is supported by mechanistic studies in animal models of AAA. *Mmp9* knockout mice were protected against aneurysm formation, and reconstitution with MMP9-competent macrophages through bone marrow transplantation or reinfusion of wild type peritoneal macrophages, re-established increased susceptibility to aneurysm formation^{42,96}. Some investigators further revealed a differential mRNA and protein expression of MMP9 between M1 and M2 macrophages within the aneurysmal wall, suggesting that macrophages may differentially regulate ECM degradation depending on their phenotype and activation profile

⁴⁷. Another study identified macrophages positive for osteoclast markers in human aneurysmal aortas, and revealed higher protease activity in these cells compared to undifferentiated macrophages ¹⁰². Inhibition of osteoclastogenic differentiation by bisphosphonate treatment in an animal model inhibited aneurysm development, supporting the contribution of osteoclast-like macrophages to AAA pathogenesis. Others identified previously unsuspected roles for telomerase reverse transcriptase (TERT) in the regulation of macrophage proteolytic activity during AAA formation, through the modulation of MMP2 expression ¹⁰³.

Another process related to ECM degradation is macrophage invasiveness and migration within the tissue. A recent study showed increased formation of podosomes by macrophages infiltrating the aneurysmal wall ¹⁰⁴. KLF5 expression in macrophages was increased in the context of murine and human AAA, promoted podosome formation and macrophage migration through upregulation of mammalian myosin Myo9b and modulation of RhoA signaling, and increased the susceptibility to experimental AAA ¹⁰⁴. However, whether podosome formation per se is required for KLF5-dependent pro-aneurysmal effects in macrophages is still unclear.

Inflammatory response

In addition to ECM remodeling, inflammation is one of the main features of aneurysm pathophysiology ¹⁰⁵. Macrophages are both producers of and respond to inflammatory mediators ¹⁰⁶⁻¹⁰⁸. Macrophages infiltrated in the aneurysmal wall have been identified to be a major source of the chemokine CXCL1, which attracts and recruits neutrophils that produce IL-6 ¹⁰⁹. As mentioned above, increased IL6 levels during aneurysm formation promote monocyte differentiation into activated macrophages that secrete CCL2, which in turn

promotes more monocyte recruitment into the aorta ⁴⁰. Cytokines are key mediators of inflammation and important regulators of various immune and non-immune cells in the aortic wall. Most of the knowledge on the direct roles of cytokines in AAA comes from studies using animals with global rather than monocyte/macrophage selective deficiency in the cytokine of interest or in the pathways that leads to their production. This is the case for total deletion of each of the components of the canonical inflammasome pathway *Nlrp3*, *Asc*, *Casp1*, or *Il1b* ^{110,111}, or for total deletion of *Tnf* ¹¹² or *Il6* ¹¹³, which significantly limit AAA development. Conversely, total deletion of *Il10*, an immunosuppressive cytokine that reduces MMP activation and preserves matrix integrity, substantially aggravates AAA formation ¹¹⁴. Interestingly, a negative correlation was found between IL6 and IL10 expression in human AAA explant cultures, and the balance between these cytokines appears to modulate the immune response in the aortic wall ¹¹⁵.

A better proof of the direct contribution of leukocytes, and more particularly macrophages, to the inflammatory process in AAA is exemplified in an animal model with myeloid specific deficiency of Krüppel-like factor 6 (KLF6). KLF6 is a transcription factor robustly expressed in macrophages ¹¹⁶. *Klf6* heterozygous mice as well as mice with myeloid-specific deletion of *Klf6* manifest a phenotype of exacerbated AAA, with increased infiltration of macrophages and increased IL6 expression in the aortic wall ¹¹⁷. The investigators revealed that granulocyte macrophage colony-stimulating factor (GM-CSF) is a direct target of KLF6, and its increased production by macrophages contributed to AAA development.

Regulation of macrophage inflammatory activity by a variety of factors other than chemokines and cytokines may also alter the development of AAA. Angiopoietin-like protein 2 (ANGPTL2) is abundantly expressed in both murine and human macrophages infiltrating the aneurysmal aortic wall ¹¹⁸. Treatment of macrophages with ANGPTL2 increases the expression of pro-inflammatory cytokines and MMP9, suggesting that Angptl2 may regulate

macrophage activity in AAA in an autocrine manner. This is supported by decreased inflammation and MMP9 expression in macrophages of *Angptl2* deficient mice, along with reduced AAA development¹¹⁸. Other mediators of macrophage activity may include Peroxisome Proliferator-Activated Receptor (PPAR)- δ , whose activation in macrophages attenuated the incidence and severity of AAA in experimental models, concomitantly with a decrease of inflammatory mediators such as CCL2 or IL1 β ¹¹⁹.

A recent study identified a major role for NOS2 expression in VSMCs in mediating the development of thoracic aortic aneurysms¹²⁰. The authors suggested an impact of high levels of NOS2 on elastin degradation and potentially VSMC contraction, although the exact pathogenic mechanisms are still unclear. NOS2 is highly expressed by a subset of macrophages³⁸ and is found in AAA disease¹²¹. NOS2 inhibition did not affect aortic dilatation in a mouse model of elastase infusion¹²² but significantly limited AAA formation in a more severe elastase-induced model in rats^{123,124}. Moreover, angiotensin II markedly stimulates the accumulation of NOS2⁺ macrophages in the aorta¹²⁵, particularly under conditions of reduced TGF- β signaling⁶⁹, and NOS2 production by those macrophages directly contribute to angiotensin II-induced aortic AAA and dissection, in part through enhanced MMP activation and ECM degradation⁶⁹.

Tissue healing and repair

Beside their roles in promoting inflammation and matrix degradation, macrophages are important for tissue remodeling during the healing response. Tissue debris and toxic products like extracellular hemoglobin¹²⁶ accumulate in the aortic wall during AAA and can be engulfed by macrophages⁴⁷. Macrophage differentiation toward the HA-mac phenotype is associated with enhanced hemoglobin uptake and increased anti-inflammatory IL-10 secretion

¹⁵, pointing to a protective role of macrophages through hemoglobin clearance, regulation of the oxidative stress and resolution of the inflammatory response during AAA.

It is noteworthy that despite their roles in the clearance of toxic products, macrophages are capable themselves of generating large amounts of reactive oxygen species. However, the impact of oxidative stress on macrophage activity during AAA appears to be more complex than initially anticipated. For example, protection of thrombomodulin deficient mice against AAA was partly attributed to decreased generation of reactive oxygen species by macrophages ⁹³. A major source of reactive oxygen species in macrophages is the NADPH oxidase, which is composed of transmembrane and cytosolic subunits including p47phox and gp91 (also known as NOX2) ¹²⁷. *p47phox* global deficiency decreased incidence of angiotensin II-induced AAA and was associated with decreased generation of reactive oxygen species as well as reduced aortic macrophage infiltration ¹²⁸. In contrast, both *gp91* global deficiency and *gp91* specific deletion in bone marrow-derived cells reduced the level of reactive oxygen species in AAA lesions but unexpectedly aggravated AAA and promoted M1 phenotype with enhanced expression of IL1 β and MMP9 ¹²⁹. However, a recent study suggested a role for gp91 in promoting HMGB1 production by macrophages, which was associated with an overactivation of the adaptive Th17 immune response, and aggravation of AAA in the experimental mouse model of elastase perfusion ¹³⁰. Thus, the role of macrophage-dependent oxidative stress in AAA will need further investigation.

To summarize, macrophages display both pathogenic and protective functions in AAA pathophysiology through their involvement in ECM remodeling, inflammation and oxidative stress (Figure 2). The regulation of these functions is complex and may evolve during the different stages of disease development and progression, and is influenced by the local microenvironment. In addition to the roles described above, we believe that other interesting functions of macrophages would be worth of investigation in relation to AAA pathogenesis.

For instance, macrophages are involved in the clearance of apoptotic debris ¹³¹. Impaired efferocytosis profoundly alters the development and progression of atherosclerosis ^{132,133} and ischemic cardiovascular diseases ^{134,135}, and is probably highly relevant to tissue remodeling and repair in the context of AAA. CXCL10-dependent CXCR3⁺ macrophages that produce FXIIIa accumulate in the adventitia of human AAAs ⁵⁴. We believe that they may contribute to matrix cross-linking and limitation of aneurysmal expansion of the aortic wall. CXCR3⁺ macrophages may also co-express CD163 ⁵⁴, suggesting additional roles in hemorrhage clearance and induction of an anti-inflammatory environment. Recent findings also revealed the contribution of perivascular macrophages to vessel permeability in non-pathological conditions ¹³⁶. Wall thickening occurring during AAA is often associated with the occurrence of microdissections, and it is tempting to hypothesize a role for adventitial macrophages in regulating vascular permeability during AAA. Finally, a role has been identified for skin macrophages in regulating salt-dependent volume retention through VEGF-C-dependent regulation of the lymphcapillary network ¹³⁷. Whether aortic wall macrophages may also play such a role is still unknown. We believe that this merits further exploration given the recently proposed role of interstitial fluid pressure regulation in the pathogenesis of aortic aneurysm and the susceptibility to aortic dissection ¹³⁸.

Potential translational applications

Monocytes and macrophages as diagnostic and prognostic biomarkers of AAA

The mechanistic role of monocytes/macrophages in the development and complications of AAA suggested interesting perspectives for translational applications (Figure 3).

Variations in circulating monocyte subsets during AAA, as well as changes in monocyte activation status, ^{14,16,94} suggest that such parameters could possibly be useful as non-invasive

biomarkers. Nevertheless, further studies are required to address the correlation between those variations and the characteristics of the aneurysm, such as its morphology, size or risk of rupture. Moreover, the specificity of those variations should be explored as the phenotype and activation status of circulating blood monocytes may be altered in a wide range of diseases including cardiovascular and inflammatory pathologies^{7,8,12}.

In addition to their potential use as circulating biomarkers, other experimental studies indicate that macrophages could be used as imaging targets. Macrophage activity may be tracked by magnetic resonance imaging (MRI) used to detect the phagocytosis of ultrasmall superparamagnetic iron oxide (USPIO) contrast agent. The use of this technique in experimental models of AAA showed co-localization of these particles with phagocytic cells in the adventitia⁴¹, suggesting a potential utility in assessing the impact of macrophage-dependent processes on the development and progression of AAA *in vivo*. Nevertheless, surrounding tissues, such as the liver, are also able to uptake USPIO and can represent a challenge to acquire correct and reliable imaging of the aorta *in vivo*. Besides, the turnover of USPIO in aortic macrophages remains to be determined before a potential use of these particles for multiple serial imaging of the inflammatory response during the progression of AAA.

Another targeted approach is PET-CT imaging with fluorine-18-labeled cross-linked iron oxide (CLIO) nanoparticles⁶⁷. The technique was used in *ApoE*^{-/-} mice infused with angiotensin II, and *ex vivo* analysis of the aortas by flow cytometry confirmed that 90% of the PET signal was due to uptake of nanoparticles by monocytes/macrophages, supporting the reliability of the method for macrophage quantification in the aortic wall *in vivo*. Early PET-CT imaging revealed that the signal was increased in aneurysms that grew over time, compared with aneurysm that did not increase in diameter. As the doses used in experimental

models are lower than the approved clinical doses, the results are highly encouraging for translational applications.

^{18}F -fluorodeoxyglucose (^{18}F -FDG) is a marker that accumulates in cells with high metabolic activity, which led to consider ^{18}F -FDG as a potential marker of vessel wall inflammation ¹³⁹. However, only a few small clinical studies addressed the usefulness of PET-CT imaging in the context of AAA. A review comparing the results of six clinical studies revealed heterogeneous results when determining the association between ^{18}F -FDG uptake and AAA growth ¹⁴⁰. One study investigating USPIO-MRI showed that AAA growth was higher in patients with focal USPIO uptake in the aneurysmal wall compared to patients with diffuse or no USPIO uptake ¹⁴¹. Another study compared ^{18}F -FDG PET-CT imaging with USPIO-enhanced MRI in the same cohort of patients ¹⁴². While both ^{18}F -FDG PET-CT and USPIO-MRI uptake detected vascular inflammation in association with AAA, their distribution was distinct, ^{18}F -FDG activity being mainly detected in the shoulder region, whereas USPIO uptake was more apparent in the main body of the aneurysm. These results suggest that the two techniques may be complementary in the characterization of AAAs, ^{18}F -FDG uptake reflecting glycolytic activity and potentially M1-like inflammatory pathogenic properties, whereas USPIO detection more probably reflects phagocytic activity and may potentially be a better indicator of the reparative function of macrophages. Given the complexity of macrophage roles in the pathogenesis of AAA, further investigations will be useful to validate the use of those techniques to predict AAA progression or risk of rupture before their potential use in medical practice.

In the near future, other imaging tools that better identify macrophages will be available. For example, PET tracers such as DOTATATE, which can be labeled using either ^{68}Ga or ^{64}Cu , have shown macrophage binding properties thanks to a specific binding affinity for somatostatin receptors. Studies in animal models demonstrated the uptake of ^{68}Ga -

DOTATATE in atherosclerotic plaques¹⁴³, and a PET/CT clinical study found an association between increased ⁶⁸Ga-DOTATATE uptake in large arteries and the presence of cardiovascular risk factors¹⁴⁴. Interestingly, a PET/MRI study in patients with carotid artery plaques revealed correlation between CD163 gene expression and ⁶⁴Cu-DOTATATE uptake, suggesting more selective detection of alternatively activated macrophages by DOTATATE¹⁴⁵. More recently, PET imaging using ¹⁸F-Macroflor, a small-size renally excreted polyglucose nanoparticle that displays high affinity for macrophages, was used successfully to detect tissue-resident or newly infiltrated macrophages in several experimental models of atherosclerosis and myocardial infarction¹⁴⁶. There is currently no published study on the use of ⁶⁴Cu-DOTATATE or ¹⁸F-Macroflor to image AAA. We believe that PET/MRI using ⁶⁴Cu-DOTATATE or ¹⁸F-Macroflor deserves further investigation in this context.

Monocytes and macrophages as therapeutic targets in AAA

There are several potential possibilities to target monocyte/macrophage recruitment, accumulation and activation during the progression of AAA, although most of the approaches would suffer a lack of selectivity for the monocyte/macrophage lineage.

Inhibition of the chemokine axis has shown efficacy in limiting monocyte recruitment in animal models of AAA. This was the case after blockade of either CXCR4/CXCL12 or CCL2/CCR2 signaling pathways^{66,68}. With regard to CCL2/CCR2, further studies revealed that its specific inhibition in leukocytes or in bone marrow-derived cells was protective against aneurysm formation^{74,147}. In contrast, global CCL2 deficiency or systemic CCL2 blockade, were respectively less protective or even worsened aneurysm formation^{74,147}. The reasons for those unexpected findings are still unclear. Nevertheless, the data suggest that future research should be oriented to test drugs that inhibit CCL2/CCR2 pathway in specific

cell subsets in order to optimize therapeutic efficiency and limit adverse effects. For example, targeting CCR2 degradation in circulating monocytes using siRNA nanoparticles prevented monocyte accumulation at sites of inflammation¹⁴⁸. This treatment has been tested in several cardiovascular disease settings including atherosclerosis, myocardial infarction or myocarditis, and revealed beneficial effects^{148,149}. It will be interesting to test the same therapeutic strategy in the context of AAA. The protective effect of other drugs on AAA has also been linked to modulation of the CCR2 pathway. For example, administration of everolimus, an immunosuppressive drug mostly used in drug-eluting stents, protected mice against angiotensin II-induced aortic dilatation through a decrease of both bone marrow and circulating CCR2 monocytes²⁴.

Besides CCL2/CCR2, treatments targeting CCL5/CCR5 may also be useful. Treatment of mice with MKEY, an inhibitor of CXCL4/CCL5 heterodimer formation, limited AAA enlargement and was associated with a reduction of mural macrophage infiltration and elastin degradation⁷⁵. MKEY treatment suppressed aortic leukocyte migration without affecting their migration into other lymphoid organs such as lymph nodes and spleen. Further investigations are required to substantiate the feasibility and safety of the approach. However, these results are encouraging for translational applications in AAA. Indeed, some CCR5 antagonists are drugs already approved and currently used in medical practice in the setting of HIV infection, and several clinical studies using CCL5/CCR5 antagonists have been completed or are still ongoing in other disease settings¹⁵⁰. It is noteworthy that the chemokine axis is highly redundant. The development of evasins (chemokine-binding proteins) with broad C, CC and CXC-binding properties¹⁵¹ may allow a better and more efficient targeting of monocyte/macrophage recruitment in AAA.

Targeting of cytokines would be less selective for monocytes/macrophages but is worth of consideration in translational approaches. Tocilizumab is a humanized monoclonal antibody

specific for the IL6 receptor, and has proven efficacy in the treatment of rheumatoid arthritis¹⁵². IL6 is an important regulator of inflammation and macrophage activation during AAA⁴⁰, and Mendelian randomization approaches provided strong evidence for the involvement of the IL6 receptor pathway in human AAA¹⁵³ and human coronary artery disease^{154,155}. Thus, we believe it will be worth testing Tocilizumab or other inhibitors of the IL6 pathway (including IL6 transsignaling) in the context of AAA. Blockade of IL1 β has shown efficacy in experimental models of AAA¹¹¹. Intriguingly however, a recent randomized, double-blind, placebo-controlled clinical trial using the monoclonal anti-IL1 β antibody ACZ885 (canakinumab) showed lack of efficacy on aneurysmal growth rate in AAA patients (31 ACZ885 and 33 placebo) with infrarenal AAAs of 40 mm to 50 mm¹⁵⁰. **Although the results do not exclude the involvement of the IL1 pathway in AAA (IL1 receptor can be activated by other ligands than IL1 β), and the lack of efficiency of canakinumab** should prompt us to reconsider our understanding of IL1 β in the pathogenesis of AAA. It is interesting to note that Mendelian randomization studies did not confirm the causality between the IL1 pathway and the occurrence of cardiovascular diseases, including AAA¹⁵⁶. However, the latter study addressed the relationship between genetic variants of IL1RN (IL1 receptor antagonist) and cardiovascular diseases, and does not allow to draw valid conclusions about the causality of the individual IL1 α or IL1 β cytokines in disease development. Another potential explanation of the disappointing clinical results is that anti-IL1 β therapy may be useful only in a subset of AAA with a high inflammatory burden. Combining state-of-the-art diagnostic approaches to detect AAA that harbor an active inflammatory component (see above) with more targeted anti-inflammatory therapy may prove useful in improving the management of patients with AAA. Another example of such a combinatory diagnostic and therapeutic approach is the detection of increased uptake of ¹⁸FDG in AAA, indicative of high glycolytic activity, followed by the administration of a glycolysis inhibitor to limit macrophage activation¹⁵⁷.

This treatment has proven to be successful in animal models of AAA ¹⁵⁷, and may deserve consideration for future translation to the human setting.

Modulation of macrophage phenotype and activity could represent another therapeutic option. Experimentally, intravenous injection of M2 macrophages exhibited protective effect on CaCl₂-induced aneurysm, as demonstrated by improved survival, decreased aortic dilatation and elastin preservation ⁴⁹. Thus, therapeutic strategies or drugs with known impact on macrophage polarization may be of interest. For example, D-series resolvins increase M2 and decrease M1 macrophages, and their administration either before or after induction of experimental AAA halted disease development and progression ¹⁵⁸. Treatment of experimental AAA with mesenchymal stem cells reduced pathogenic NOX2 activation in macrophages, although the mechanisms remained unclear ¹³⁰. T regulatory cells control the inflammatory response in AAA ¹¹⁴. Several studies revealed that administration of T regulatory cells or their endogenous induction by IL2 had protective effects on aneurysm development, which were associated with reduced macrophage infiltration in the aorta ¹⁵⁹⁻¹⁶¹. *In vitro* experiments further revealed that T regulatory cells decreased macrophage CCL2 secretion as well as protease expression and activity, in part through the induction of IL10 ¹⁶⁰. Besides, T regulatory cells downregulated M1- and upregulated M2-associated genes ¹⁶¹, making them attractive candidates for the immunotherapy of AAA.

Finally, while systemic treatments targeting macrophage polarization have provided encouraging results, recent advances in the understanding of the impact of biomaterials on macrophage phenotype should offer new perspectives ¹⁶². Several studies revealed a previously unappreciated impact of roughness, size, shape and chemical composition of biomaterials on macrophage phenotype. These recent findings could offer new opportunities to locally modulate M1/ M2 polarization in order to limit aneurysm expansion.

Perspectives for future research

In the limelight of experimental and clinical studies, future directions for fundamental and translational research can be suggested. Even if different subsets of monocytes and macrophages have been identified, their distinct origins, roles and functions appear more complex than previously anticipated and several points remain to be elucidated. Are specific monocyte subsets wired to differentiate into specific macrophage subsets or are they able to indifferently differentiate depending on the local micro-environment? What is the precise role in AAA of recently identified subsets such as SatM or tissue resident macrophages? Are the various subsets directly representative of the stages of disease development and progression? Do they closely correlate with clinical outcomes and could they be used in daily practice as diagnostic or prognostic tools, or as therapeutic targets?

Epidemiological studies have highlighted the association of AAA with risk factors including age, male sex, smoking, hypertension or low HDL-cholesterol levels¹. Interestingly, these factors may impact on monocyte/ macrophage activity and function^{37,163-168}. Additional research will be required to explore the molecular pathways that could link those risk factors with the contribution of monocytes/macrophages to AAA. The development of experimental models of AAA over the past decade has offered great opportunities to study the role of monocytes/ macrophages in AAA pathogenesis. However, none of those models perfectly reproduces the human features of AAA, highlighting a real need to develop new and more relevant models¹⁷.

Conclusion

Several experimental and clinical studies implicate monocytes/macrophages in all stages of AAA formation, from initial development to the occurrence of microdissections and rupture (Figure 4). In the last decade, we witnessed an impressive advance in our understanding of the cellular and molecular mechanisms involved in monocyte mobilization and recruitment to sites of vascular injury, and the mechanisms that control macrophage differentiation, proliferation, activation and phenotype switch. This knowledge is now backed by the development of state-of-the-art imaging techniques to detect and track monocyte/macrophage accumulation and activation *in vivo*, the development of new anti-inflammatory therapies as well as additional technological improvements that will allow selective manipulation of various macrophage functions *in vivo*. We truly believe that the combination of such innovative approaches will improve the stratification and management of patients with AAA.

Funding

The authors are supported by British Heart Foundation, European Research Council. We apologise for being unable to discuss all the relevant papers due to space limitations.

Acknowledgements: none

Conflict of interest: none

Contribution statements

All authors confirmed that they have contributed to the intellectual content of this article and have approved the final version.

Author biographies

Juliette Raffort is **Assistant Professor at the University Hospital of Nice, France**. She is a medical biologist specialised in cardiovascular and metabolic related diseases. Her research focuses on the identification and investigation of the relevance of new biomarkers for applications in clinical practice.

Fabien Lareyre is a **Registrar in Vascular Surgery** who develops clinical trials and experimental surgical models of abdominal aortic aneurysm for a better understanding of the inflammatory process underlying the disease.

Marc Clément is a research associate **at the University of Cambridge, UK, and** works on the molecular and cellular mechanisms involved in the development of cardiovascular diseases, with a focus on inflammation in atherosclerosis and abdominal aortic aneurysm.

Réda Hassen-Khodja is a **Professor of Vascular Surgery at the University Hospital of Nice, France**. He is specialised in endovascular and open vascular repair. His work focuses on translational research on aortic aneurysm and atherosclerosis related diseases.

Giulia Chinetti is Professor at University Côte d'Azur, Nice, France. She is an expert in macrophage functions and biology. Her current research focuses on the roles of different macrophage sub-populations in human cardiovascular diseases.

Ziad Mallat is a BHF Professor of Cardiovascular Medicine at the University of Cambridge, UK. He is expert in the immune mechanisms of cardiovascular diseases. Besides his basic and experimental research, Ziad Mallat is leading several phase II clinical trials testing the immuno-inflammatory hypothesis of cardiovascular diseases.

Figure legends

Figure 1: Origins of macrophages in the aneurysmal aortic wall.

Most macrophages accumulating in the aneurysmal aortic wall probably derive from circulating monocytes, which are produced in the bone marrow and can be mobilized from peripheral reservoirs such as the spleen. A contingent of macrophages may also originate from tissue resident macrophages developed from embryonic precursors and early post-natal circulating monocytes. The diverse origin of macrophages lead to the development of macrophage subsets with distinct phenotypes, with major implications for their activation and biological functions in AAA development and progression.

Figure 2: Role of macrophages in AAA pathogenesis and the main factors that modulate their activities.

Macrophages accumulating in the aneurysmal aortic wall play crucial roles in AAA pathogenesis and complications through their implication in extracellular matrix remodeling, the promotion and resolution of inflammation, and various aspects of the tissue healing response. In turn, their recruitment, accumulation, proliferation and activation are modulated by chemokines/cytokines produced in response to tissue injury, products of extracellular matrix degradation, and microenvironmental conditions (blood flow, circumferential stress, thrombus, peri-aortic fat, etc.).

Figure 3: Translational approaches.

Circulating monocytes and infiltrated macrophages can be used as biomarkers and imaging targets, with potential diagnostic and/or prognostic value in medical practice. Various potential applications can be envisaged to treat and/or limit AAA growth and complications based on modulation of cytokine and chemokine pathways, or modulation of macrophage phenotype and activity.

Figure 4: **Hypothesized contribution** of monocyte and macrophage subsets to AAA pathogenesis.

AAA lesions are characterized by ECM degradation, loss of VSMCs, and immune cell infiltration (A). Classical monocytes infiltrate all layers of the vascular wall. Their differentiation into several types of macrophages, including M1 macrophages, NOS2⁺ macrophages and osteoclast-like macrophages is associated with enhanced production of pro-inflammatory mediators and matrix degrading proteases, which weaken the aortic wall and promote expansive aortic remodelling. Eventually, this favours microdissections and intramural haemorrhage, neo-angiogenesis and intraluminal thrombus formation, and may further enhance immune cell infiltration and activation. If those processes are not contained, they may ultimately promote aortic wall rupture.

(B) The development and progression of AAA may be delayed through compensatory mechanisms that limit AAA expansion and maintain vascular integrity. Thanks to their ability to secrete anti-inflammatory mediators and pro-fibrotic factors, M2 macrophages may contribute to the resolution of inflammation and may promote tissue healing. HA-mac macrophages are involved in haemoglobin clearance, exert anti-inflammatory properties and regulate oxidative stress, and may contribute to aortic tissue repair. Other macrophage subsets such as CXCR3⁺ macrophages may limit expansive aortic wall remodelling through their

production of transglutaminase factor XIIIa. Non-classical monocytes may give rise to M2, HA-mac or CXCR3⁺ macrophages, but all those tissue healing-associated macrophages may also arise from other monocyte subsets or from locally differentiated tissue resident adventitial macrophages. Other monocyte subsets such as SatM may be involved in tissue healing and repair through their pro-fibrotic properties.

References

1. Nordon, I.M., Hinchliffe, R.J., Loftus, I.M. & Thompson, M.M. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nat Rev Cardiol* **8**, 92-102 (2011).
2. Golledge, J., Muller, J., Daugherty, A. & Norman, P. Abdominal aortic aneurysm: pathogenesis and implications for management. *Arterioscler Thromb Vasc Biol* **26**, 2605-2613 (2006).
3. Hong, H., Yang, Y., Liu, B. & Cai, W. Imaging of Abdominal Aortic Aneurysm: the present and the future. *Curr Vasc Pharmacol* **8**, 808-819 (2010).
4. Trollope, A., Moxon, J.V., Moran, C.S. & Golledge, J. Animal models of abdominal aortic aneurysm and their role in furthering management of human disease. *Cardiovasc Pathol* **20**, 114-123 (2011).
5. Dale, M.A., Ruhlman, M.K. & Baxter, B.T. Inflammatory cell phenotypes in AAAs: their role and potential as targets for therapy. *Arterioscler Thromb Vasc Biol* **35**, 1746-1755 (2015).
6. Ziegler-Heitbrock, L., *et al.* Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74-80 (2010).
7. Idzkowska, E., *et al.* The Role of Different Monocyte Subsets in the Pathogenesis of Atherosclerosis and Acute Coronary Syndromes. *Scand J Immunol* **82**, 163-173 (2015).
8. Yang, J., Zhang, L., Yu, C., Yang, X.F. & Wang, H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark Res* **2**, 1 (2014).
9. Wong, K.L., *et al.* Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* **118**, e16-31 (2011).
10. Randolph, G.J. The fate of monocytes in atherosclerosis. *J Thromb Haemost* **7 Suppl 1**, 28-30 (2009).
11. Auffray, C., Sieweke, M.H. & Geissmann, F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol* **27**, 669-692 (2009).
12. Dutta, P. & Nahrendorf, M. Regulation and consequences of monocytoysis. *Immunol Rev* **262**, 167-178 (2014).
13. Drechsler, M., Duchene, J. & Soehnlein, O. Chemokines control mobilization, recruitment, and fate of monocytes in atherosclerosis. *Arterioscler Thromb Vasc Biol* **35**, 1050-1055 (2015).
14. Ghigliotti, G., *et al.* CD16(+) monocyte subsets are increased in large abdominal aortic aneurysms and are differentially related with circulating and cell-associated biochemical and inflammatory biomarkers. *Dis Markers* **34**, 131-142 (2013).
15. Rubio-Navarro, A., *et al.* Hemoglobin induces monocyte recruitment and CD163-macrophage polarization in abdominal aortic aneurysm. *Int J Cardiol* **201**, 66-78 (2015).
16. Lamblin, N., *et al.* Profile of macrophages in human abdominal aortic aneurysms: a transcriptomic, proteomic, and antibody protein array study. *J Proteome Res* **9**, 3720-3729 (2010).
17. Senemaud, J., *et al.* Translational Relevance and Recent Advances of Animal Models of Abdominal Aortic Aneurysm. *Arterioscler Thromb Vasc Biol* **37**, 401-410 (2017).
18. Azuma, J., Asagami, T., Dalman, R. & Tsao, P.S. Creation of murine experimental abdominal aortic aneurysms with elastase. *J Vis Exp* (2009).
19. Wang, Y., Krishna, S. & Golledge, J. The calcium chloride-induced rodent model of abdominal aortic aneurysm. *Atherosclerosis* **226**, 29-39 (2013).
20. Daugherty, A., Manning, M.W. & Cassis, L.A. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest* **105**, 1605-1612 (2000).
21. Daugherty, A. & Cassis, L. Angiotensin II and abdominal aortic aneurysms. *Curr Hypertens Rep* **6**, 442-446 (2004).
22. Wang, Y., *et al.* TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *J Clin Invest* **120**, 422-432 (2010).

23. Trachet, B., *et al.* Ascending Aortic Aneurysm in Angiotensin II-Infused Mice: Formation, Progression, and the Role of Focal Dissections. *Arterioscler Thromb Vasc Biol* **36**, 673-681 (2016).
24. Moran, C.S., *et al.* Everolimus limits aortic aneurysm in the apolipoprotein E-deficient mouse by downregulating C-C chemokine receptor 2 positive monocytes. *Arterioscler Thromb Vasc Biol* **33**, 814-821 (2013).
25. Tsou, C.L., *et al.* Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest* **117**, 902-909 (2007).
26. Mellak, S., *et al.* Angiotensin II mobilizes spleen monocytes to promote the development of abdominal aortic aneurysm in Apoe^{-/-} mice. *Arterioscler Thromb Vasc Biol* **35**, 378-388 (2015).
27. Yu, H., *et al.* Angiopietin-2 attenuates angiotensin II-induced aortic aneurysm and atherosclerosis in apolipoprotein E-deficient mice. *Sci Rep* **6**, 35190 (2016).
28. Owens, A.P., 3rd, *et al.* MyD88 deficiency attenuates angiotensin II-induced abdominal aortic aneurysm formation independent of signaling through Toll-like receptors 2 and 4. *Arterioscler Thromb Vasc Biol* **31**, 2813-2819 (2011).
29. Hanna, R.N., *et al.* The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C⁺ monocytes. *Nat Immunol* **12**, 778-785 (2011).
30. Cui, M., *et al.* Orphan Nuclear Receptor Nur77 Inhibits Angiotensin II-Induced Vascular Remodeling via Downregulation of beta-Catenin. *Hypertension* **67**, 153-162 (2016).
31. Hanna, R.N., *et al.* NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis. *Circ Res* **110**, 416-427 (2012).
32. Hamers, A.A., *et al.* Bone marrow-specific deficiency of nuclear receptor Nur77 enhances atherosclerosis. *Circ Res* **110**, 428-438 (2012).
33. Thomas, G.D., *et al.* Deleting an Nr4a1 Super-Enhancer Subdomain Ablates Ly6C⁺ Monocytes while Preserving Macrophage Gene Function. *Immunity* **45**, 975-987 (2016).
34. Satoh, T., *et al.* Identification of an atypical monocyte and committed progenitor involved in fibrosis. *Nature* **541**, 96-101 (2017).
35. Murray, P.J., *et al.* Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41**, 14-20 (2014).
36. Tabas, I. & Bornfeldt, K.E. Macrophage Phenotype and Function in Different Stages of Atherosclerosis. *Circ Res* **118**, 653-667 (2016).
37. Chinetti-Gbaguidi, G., Colin, S. & Staels, B. Macrophage subsets in atherosclerosis. *Nat Rev Cardiol* **12**, 10-17 (2015).
38. Menezes, S., *et al.* The Heterogeneity of Ly6C⁺ Monocytes Controls Their Differentiation into iNOS⁺ Macrophages or Monocyte-Derived Dendritic Cells. *Immunity* **45**, 1205-1218 (2016).
39. Martinez, F.O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* **6**, 13 (2014).
40. Tieu, B.C., *et al.* An adventitial IL-6/MCP1 amplification loop accelerates macrophage-mediated vascular inflammation leading to aortic dissection in mice. *J Clin Invest* **119**, 3637-3651 (2009).
41. Turner, G.H., *et al.* Assessment of macrophage infiltration in a murine model of abdominal aortic aneurysm. *J Magn Reson Imaging* **30**, 455-460 (2009).
42. Pyo, R., *et al.* Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest* **105**, 1641-1649 (2000).
43. Wang, Y.X., *et al.* Angiotensin II increases urokinase-type plasminogen activator expression and induces aneurysm in the abdominal aorta of apolipoprotein E-deficient mice. *Am J Pathol* **159**, 1455-1464 (2001).
44. Saraff, K., Babamusta, F., Cassis, L.A. & Daugherty, A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin II-infused, apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* **23**, 1621-1626 (2003).

45. Dutertre, C.A., *et al.* Deciphering the stromal and hematopoietic cell network of the adventitia from non-aneurysmal and aneurysmal human aorta. *PLoS One* **9**, e89983 (2014).
46. Rao, J., *et al.* Distinct macrophage phenotype and collagen organization within the intraluminal thrombus of abdominal aortic aneurysm. *J Vasc Surg* **62**, 585-593 (2015).
47. Boytard, L., *et al.* Role of proinflammatory CD68(+) mannose receptor(-) macrophages in peroxiredoxin-1 expression and in abdominal aortic aneurysms in humans. *Arterioscler Thromb Vasc Biol* **33**, 431-438 (2013).
48. Qin, Z., *et al.* Angiotensin II-induced TLR4 mediated abdominal aortic aneurysm in apolipoprotein E knockout mice is dependent on STAT3. *J Mol Cell Cardiol* **87**, 160-170 (2015).
49. Dale, M.A., *et al.* Elastin-Derived Peptides Promote Abdominal Aortic Aneurysm Formation by Modulating M1/M2 Macrophage Polarization. *J Immunol* **196**, 4536-4543 (2016).
50. Rateri, D.L., *et al.* Prolonged infusion of angiotensin II in apoE(-/-) mice promotes macrophage recruitment with continued expansion of abdominal aortic aneurysm. *Am J Pathol* **179**, 1542-1548 (2011).
51. Moore, J.P., *et al.* M2 macrophage accumulation in the aortic wall during angiotensin II infusion in mice is associated with fibrosis, elastin loss, and elevated blood pressure. *Am J Physiol Heart Circ Physiol* **309**, H906-917 (2015).
52. Torocsik, D., *et al.* Factor XIII-A is involved in the regulation of gene expression in alternatively activated human macrophages. *Thromb Haemost* **104**, 709-717 (2010).
53. Bakker, E.N., *et al.* Flow-dependent remodeling of small arteries in mice deficient for tissue-type transglutaminase: possible compensation by macrophage-derived factor XIII. *Circ Res* **99**, 86-92 (2006).
54. Zhou, J., *et al.* CXCR3-dependent accumulation and activation of perivascular macrophages is necessary for homeostatic arterial remodeling to hemodynamic stresses. *J Exp Med* **207**, 1951-1966 (2010).
55. Zizzo, G., Hilliard, B.A., Monestier, M. & Cohen, P.L. Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction. *J Immunol* **189**, 3508-3520 (2012).
56. Haskard, D.O., Boyle, J.J., Evans, P.C., Mason, J.C. & Randi, A.M. Cytoprotective signaling and gene expression in endothelial cells and macrophages-lessons for atherosclerosis. *Microcirculation* **20**, 203-216 (2013).
57. Finn, A.V., *et al.* Hemoglobin directs macrophage differentiation and prevents foam cell formation in human atherosclerotic plaques. *J Am Coll Cardiol* **59**, 166-177 (2012).
58. Boyle, J.J., *et al.* Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection. *Circ Res* **110**, 20-33 (2012).
59. Perdiguero, E.G. & Geissmann, F. The development and maintenance of resident macrophages. *Nat Immunol* **17**, 2-8 (2016).
60. Ginhoux, F. & Guilliams, M. Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity* **44**, 439-449 (2016).
61. Epelman, S., *et al.* Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* **40**, 91-104 (2014).
62. Ensan, S., *et al.* Self-renewing resident arterial macrophages arise from embryonic CX3CR1(+) precursors and circulating monocytes immediately after birth. *Nat Immunol* **17**, 159-168 (2016).
63. Lhotak, S., *et al.* Characterization of Proliferating Lesion-Resident Cells During All Stages of Atherosclerotic Growth. *J Am Heart Assoc* **5**(2016).
64. Lavine, K.J., *et al.* Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc Natl Acad Sci U S A* **111**, 16029-16034 (2014).

65. Woollard, K.J. & Geissmann, F. Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol* **7**, 77-86 (2010).
66. Michineau, S., *et al.* Chemokine (C-X-C motif) receptor 4 blockade by AMD3100 inhibits experimental abdominal aortic aneurysm expansion through anti-inflammatory effects. *Arterioscler Thromb Vasc Biol* **34**, 1747-1755 (2014).
67. Nahrendorf, M., *et al.* Detection of macrophages in aortic aneurysms by nanoparticle positron emission tomography-computed tomography. *Arterioscler Thromb Vasc Biol* **31**, 750-757 (2011).
68. Zhang, P., *et al.* Smad4 Deficiency in Smooth Muscle Cells Initiates the Formation of Aortic Aneurysm. *Circ Res* **118**, 388-399 (2016).
69. Tan, C.K., *et al.* SMAD3 deficiency promotes inflammatory aortic aneurysms in angiotensin II-infused mice via activation of iNOS. *J Am Heart Assoc* **2**, e000269 (2013).
70. Babamusta, F., *et al.* Angiotensin II infusion induces site-specific intra-laminar hemorrhage in macrophage colony-stimulating factor-deficient mice. *Atherosclerosis* **186**, 282-290 (2006).
71. Combadiere, C., *et al.* Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation* **117**, 1649-1657 (2008).
72. Ishibashi, M., *et al.* Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arterioscler Thromb Vasc Biol* **24**, e174-178 (2004).
73. Tieu, B.C., *et al.* Aortic adventitial fibroblasts participate in angiotensin-induced vascular wall inflammation and remodeling. *J Vasc Res* **48**, 261-272 (2011).
74. Moehle, C.W., *et al.* Bone marrow-derived MCP1 required for experimental aortic aneurysm formation and smooth muscle phenotypic modulation. *J Thorac Cardiovasc Surg* **142**, 1567-1574 (2011).
75. Iida, Y., *et al.* Peptide inhibitor of CXCL4-CCL5 heterodimer formation, MKEY, inhibits experimental aortic aneurysm initiation and progression. *Arterioscler Thromb Vasc Biol* **33**, 718-726 (2013).
76. MacTaggart, J.N., Xiong, W., Knispel, R. & Baxter, B.T. Deletion of CCR2 but not CCR5 or CXCR3 inhibits aortic aneurysm formation. *Surgery* **142**, 284-288 (2007).
77. Kehrl, J.H. Heterotrimeric G protein signaling: roles in immune function and fine-tuning by RGS proteins. *Immunity* **8**, 1-10 (1998).
78. Patel, J., *et al.* RGS1 regulates myeloid cell accumulation in atherosclerosis and aortic aneurysm rupture through altered chemokine signalling. *Nat Commun* **6**, 6614 (2015).
79. Ley, K. The role of selectins in inflammation and disease. *Trends Mol Med* **9**, 263-268 (2003).
80. Hannawa, K.K., *et al.* L-selectin-mediated neutrophil recruitment in experimental rodent aneurysm formation. *Circulation* **112**, 241-247 (2005).
81. Soehnlein, O., Lindbom, L. & Weber, C. Mechanisms underlying neutrophil-mediated monocyte recruitment. *Blood* **114**, 4613-4623 (2009).
82. Norman, P.E. & Curci, J.A. Understanding the effects of tobacco smoke on the pathogenesis of aortic aneurysm. *Arterioscler Thromb Vasc Biol* **33**, 1473-1477 (2013).
83. Harrison, D.G., *et al.* Inflammation, immunity, and hypertension. *Hypertension* **57**, 132-140 (2011).
84. Hance, K.A., Tataria, M., Ziporin, S.J., Lee, J.K. & Thompson, R.W. Monocyte chemotactic activity in human abdominal aortic aneurysms: role of elastin degradation peptides and the 67-kD cell surface elastin receptor. *J Vasc Surg* **35**, 254-261 (2002).
85. Bruemmer, D., *et al.* Angiotensin II-accelerated atherosclerosis and aneurysm formation is attenuated in osteopontin-deficient mice. *J Clin Invest* **112**, 1318-1331 (2003).
86. Liu, Z., *et al.* Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm. *Circ Res* **117**, 129-141 (2015).

87. Gong, Y., Hart, E., Shchurin, A. & Hoover-Plow, J. Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice. *J Clin Invest* **118**, 3012-3024 (2008).
88. Sho, E., *et al.* Hemodynamic forces regulate mural macrophage infiltration in experimental aortic aneurysms. *Exp Mol Pathol* **76**, 108-116 (2004).
89. Golledge, J., Clancy, P., Jamrozik, K. & Norman, P.E. Obesity, adipokines, and abdominal aortic aneurysm: Health in Men study. *Circulation* **116**, 2275-2279 (2007).
90. Thanassoulis, G., *et al.* Periaortic adipose tissue and aortic dimensions in the Framingham Heart Study. *J Am Heart Assoc* **1**, e000885 (2012).
91. Police, S.B., Thatcher, S.E., Charnigo, R., Daugherty, A. & Cassis, L.A. Obesity promotes inflammation in periaortic adipose tissue and angiotensin II-induced abdominal aortic aneurysm formation. *Arterioscler Thromb Vasc Biol* **29**, 1458-1464 (2009).
92. Blomkalns, A.L., *et al.* CD14 directs adventitial macrophage precursor recruitment: role in early abdominal aortic aneurysm formation. *J Am Heart Assoc* **2**, e000065 (2013).
93. Wang, K.C., *et al.* Membrane-Bound Thrombomodulin Regulates Macrophage Inflammation in Abdominal Aortic Aneurysm. *Arterioscler Thromb Vasc Biol* **35**, 2412-2422 (2015).
94. Samadzadeh, K.M., *et al.* Monocyte activity is linked with abdominal aortic aneurysm diameter. *J Surg Res* **190**, 328-334 (2014).
95. Murray, P.J. & Wynn, T.A. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* **11**, 723-737 (2011).
96. Longo, G.M., *et al.* Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J Clin Invest* **110**, 625-632 (2002).
97. Longo, G.M., *et al.* MMP-12 has a role in abdominal aortic aneurysms in mice. *Surgery* **137**, 457-462 (2005).
98. Qin, Y., *et al.* Deficiency of cathepsin S attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. *Cardiovasc Res* **96**, 401-410 (2012).
99. Eskandari, M.K., *et al.* Enhanced abdominal aortic aneurysm in TIMP-1-deficient mice. *J Surg Res* **123**, 289-293 (2005).
100. Curci, J.A., Liao, S., Huffman, M.D., Shapiro, S.D. & Thompson, R.W. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. *J Clin Invest* **102**, 1900-1910 (1998).
101. Liu, J., *et al.* Cathepsin L expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. *Atherosclerosis* **184**, 302-311 (2006).
102. Takei, Y., Tanaka, T., Kent, K.C. & Yamanouchi, D. Osteoclastogenic Differentiation of Macrophages in the Development of Abdominal Aortic Aneurysms. *Arterioscler Thromb Vasc Biol* **36**, 1962-1971 (2016).
103. Findeisen, H.M., *et al.* Telomerase deficiency in bone marrow-derived cells attenuates angiotensin II-induced abdominal aortic aneurysm formation. *Arterioscler Thromb Vasc Biol* **31**, 253-260 (2011).
104. Ma, D., *et al.* Inhibition of KLF5-Myo9b-RhoA pathway-mediated podosome formation in macrophages ameliorates abdominal aortic aneurysm. *Circ Res* (2017).
105. Shimizu, K., Mitchell, R.N. & Libby, P. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* **26**, 987-994 (2006).
106. Mallat, Z. Macrophages. *Arterioscler Thromb Vasc Biol* **34**, 2509-2519 (2014).
107. Arango Duque, G. & Descoteaux, A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* **5**, 491 (2014).
108. Mosser, D.M. & Edwards, J.P. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* **8**, 958-969 (2008).
109. Anzai, A., *et al.* Adventitial CXCL1/G-CSF expression in response to acute aortic dissection triggers local neutrophil recruitment and activation leading to aortic rupture. *Circ Res* **116**, 612-623 (2015).

110. Usui, F., *et al.* Inflammasome activation by mitochondrial oxidative stress in macrophages leads to the development of angiotensin II-induced aortic aneurysm. *Arterioscler Thromb Vasc Biol* **35**, 127-136 (2015).
111. Johnston, W.F., *et al.* Genetic and pharmacologic disruption of interleukin-1beta signaling inhibits experimental aortic aneurysm formation. *Arterioscler Thromb Vasc Biol* **33**, 294-304 (2013).
112. Xiong, W., *et al.* Blocking TNF-alpha attenuates aneurysm formation in a murine model. *J Immunol* **183**, 2741-2746 (2009).
113. Ju, X., *et al.* Interleukin-6-signal transducer and activator of transcription-3 signaling mediates aortic dissections induced by angiotensin II via the T-helper lymphocyte 17-interleukin 17 axis in C57BL/6 mice. *Arterioscler Thromb Vasc Biol* **33**, 1612-1621 (2013).
114. Ait-Oufella, H., *et al.* Natural regulatory T cells limit angiotensin II-induced aneurysm formation and rupture in mice. *Arterioscler Thromb Vasc Biol* **33**, 2374-2379 (2013).
115. Vucevic, D., *et al.* Inverse production of IL-6 and IL-10 by abdominal aortic aneurysm explant tissues in culture. *Cardiovasc Pathol* **21**, 482-489 (2012).
116. Date, D., *et al.* Kruppel-like transcription factor 6 regulates inflammatory macrophage polarization. *J Biol Chem* **289**, 10318-10329 (2014).
117. Son, B.K., *et al.* Granulocyte macrophage colony-stimulating factor is required for aortic dissection/intramural haematoma. *Nat Commun* **6**, 6994 (2015).
118. Tazume, H., *et al.* Macrophage-derived angiopoietin-like protein 2 accelerates development of abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* **32**, 1400-1409 (2012).
119. Hwang, J.S., *et al.* PPARdelta reduces abdominal aortic aneurysm formation in angiotensin II-infused apolipoprotein E-deficient mice by regulating extracellular matrix homeostasis and inflammatory responses. *Int J Cardiol* **174**, 43-50 (2014).
120. Oller, J., *et al.* Nitric oxide mediates aortic disease in mice deficient in the metalloprotease Adamts1 and in a mouse model of Marfan syndrome. *Nat Med* (2017).
121. Zhang, J., *et al.* Inducible nitric oxide synthase is present in human abdominal aortic aneurysm and promotes oxidative vascular injury. *J Vasc Surg* **38**, 360-367 (2003).
122. Lee, J.K., Borhani, M., Ennis, T.L., Upchurch, G.R., Jr. & Thompson, R.W. Experimental abdominal aortic aneurysms in mice lacking expression of inducible nitric oxide synthase. *Arterioscler Thromb Vasc Biol* **21**, 1393-1401 (2001).
123. Johanning, J.M., Franklin, D.P., Han, D.C., Carey, D.J. & Elmore, J.R. Inhibition of inducible nitric oxide synthase limits nitric oxide production and experimental aneurysm expansion. *J Vasc Surg* **33**, 579-586 (2001).
124. Armstrong, P.J., Franklin, D.P., Carey, D.J. & Elmore, J.R. Suppression of experimental aortic aneurysms: comparison of inducible nitric oxide synthase and cyclooxygenase inhibitors. *Ann Vasc Surg* **19**, 248-257 (2005).
125. Kossmann, S., *et al.* Inflammatory monocytes determine endothelial nitric-oxide synthase uncoupling and nitro-oxidative stress induced by angiotensin II. *J Biol Chem* **289**, 27540-27550 (2014).
126. Rifkind, J.M., Mohanty, J.G. & Nagababu, E. The pathophysiology of extracellular hemoglobin associated with enhanced oxidative reactions. *Front Physiol* **5**, 500 (2014).
127. Keane, J.F., Jr. Oxidative stress and the vascular wall: NADPH oxidases take center stage. *Circulation* **112**, 2585-2588 (2005).
128. Thomas, M., *et al.* Deletion of p47phox attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. *Circulation* **114**, 404-413 (2006).
129. Kigawa, Y., *et al.* NADPH oxidase deficiency exacerbates angiotensin II-induced abdominal aortic aneurysms in mice. *Arterioscler Thromb Vasc Biol* **34**, 2413-2420 (2014).
130. Sharma, A.K., *et al.* Mesenchymal Stem Cells Attenuate NADPH Oxidase-Dependent High Mobility Group Box 1 Production and Inhibit Abdominal Aortic Aneurysms. *Arterioscler Thromb Vasc Biol* **36**, 908-918 (2016).

131. Henson, P.M., Bratton, D.L. & Fadok, V.A. Apoptotic cell removal. *Curr Biol* **11**, R795-805 (2001).
132. Kojima, Y., *et al.* Cyclin-dependent kinase inhibitor 2B regulates efferocytosis and atherosclerosis. *J Clin Invest* **124**, 1083-1097 (2014).
133. Kojima, Y., *et al.* CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis. *Nature* **536**, 86-90 (2016).
134. Wan, E., *et al.* Enhanced efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction. *Circ Res* **113**, 1004-1012 (2013).
135. Howangyin, K.Y., *et al.* Myeloid-Epithelial-Reproductive Receptor Tyrosine Kinase and Milk Fat Globule Epidermal Growth Factor 8 Coordinately Improve Remodeling After Myocardial Infarction via Local Delivery of Vascular Endothelial Growth Factor. *Circulation* **133**, 826-839 (2016).
136. He, H., *et al.* Perivascular Macrophages Limit Permeability. *Arterioscler Thromb Vasc Biol* **36**, 2203-2212 (2016).
137. Machnik, A., *et al.* Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* **15**, 545-552 (2009).
138. Mallat, Z., Tedgui, A. & Henrion, D. Role of Microvascular Tone and Extracellular Matrix Contraction in the Regulation of Interstitial Fluid: Implications for Aortic Dissection. *Arterioscler Thromb Vasc Biol* **36**, 1742-1747 (2016).
139. Golestani, R. & Sadeghi, M.M. Emergence of molecular imaging of aortic aneurysm: implications for risk stratification and management. *J Nucl Cardiol* **21**, 251-267; quiz 268-270 (2014).
140. Jalalzadeh, H., *et al.* Inflammation as a Predictor of Abdominal Aortic Aneurysm Growth and Rupture: A Systematic Review of Imaging Biomarkers. *Eur J Vasc Endovasc Surg* **52**, 333-342 (2016).
141. Richards, J.M., *et al.* Abdominal aortic aneurysm growth predicted by uptake of ultrasmall superparamagnetic particles of iron oxide: a pilot study. *Circ Cardiovasc Imaging* **4**, 274-281 (2011).
142. McBride, O.M., *et al.* Positron Emission Tomography and Magnetic Resonance Imaging of Cellular Inflammation in Patients with Abdominal Aortic Aneurysms. *Eur J Vasc Endovasc Surg* **51**, 518-526 (2016).
143. Rinne, P., *et al.* Comparison of Somatostatin Receptor 2-Targeting PET Tracers in the Detection of Mouse Atherosclerotic Plaques. *Mol Imaging Biol* **18**, 99-108 (2016).
144. Li, X., *et al.* 68Ga-DOTATATE PET/CT for the detection of inflammation of large arteries: correlation with 18F-FDG, calcium burden and risk factors. *EJNMMI Res* **2**, 52 (2012).
145. Pedersen, S.F., *et al.* 64Cu-DOTATATE PET/MRI for Detection of Activated Macrophages in Carotid Atherosclerotic Plaques: Studies in Patients Undergoing Endarterectomy. *Arterioscler Thromb Vasc Biol* **35**, 1696-1703 (2015).
146. Keliher, E.J., *et al.* Polyglucose nanoparticles with renal elimination and macrophage avidity facilitate PET imaging in ischaemic heart disease. *Nat Commun* **8**, 14064 (2017).
147. de Waard, V., *et al.* Systemic MCP1/CCR2 blockade and leukocyte specific MCP1/CCR2 inhibition affect aortic aneurysm formation differently. *Atherosclerosis* **211**, 84-89 (2010).
148. Leuschner, F., *et al.* Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* **29**, 1005-1010 (2011).
149. Leuschner, F., *et al.* Silencing of CCR2 in myocarditis. *Eur Heart J* **36**, 1478-1488 (2015).
150. US National Library of Medicine. ClinicalTrials.gov.
151. Bonvin, P., Power, C.A. & Proudfoot, A.E. Evasins: Therapeutic Potential of a New Family of Chemokine-Binding Proteins from Ticks. *Front Immunol* **7**, 208 (2016).
152. Hennigan, S. & Kavanaugh, A. Interleukin-6 inhibitors in the treatment of rheumatoid arthritis. *Ther Clin Risk Manag* **4**, 767-775 (2008).

153. Harrison, S.C., *et al.* Interleukin-6 receptor pathways in abdominal aortic aneurysm. *Eur Heart J* **34**, 3707-3716 (2013).
154. Interleukin-6 Receptor Mendelian Randomisation Analysis, C., *et al.* The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* **379**, 1214-1224 (2012).
155. Collaboration, I.R.G.C.E.R.F., *et al.* Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet* **379**, 1205-1213 (2012).
156. Interleukin 1 Genetics, C. Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis. *Lancet Diabetes Endocrinol* **3**, 243-253 (2015).
157. Tsuruda, T., *et al.* Inhibition of development of abdominal aortic aneurysm by glycolysis restriction. *Arterioscler Thromb Vasc Biol* **32**, 1410-1417 (2012).
158. Pope, N.H., *et al.* D-series resolvins inhibit murine abdominal aortic aneurysm formation and increase M2 macrophage polarization. *FASEB J* **30**, 4192-4201 (2016).
159. Yodoi, K., *et al.* Foxp3+ regulatory T cells play a protective role in angiotensin II-induced aortic aneurysm formation in mice. *Hypertension* **65**, 889-895 (2015).
160. Zhou, Y., *et al.* Regulatory T cells in human and angiotensin II-induced mouse abdominal aortic aneurysms. *Cardiovasc Res* **107**, 98-107 (2015).
161. Meng, X., *et al.* Regulatory T cells prevent angiotensin II-induced abdominal aortic aneurysm in apolipoprotein E knockout mice. *Hypertension* **64**, 875-882 (2014).
162. Alvarez, M.M., *et al.* Delivery strategies to control inflammatory response: Modulating M1-M2 polarization in tissue engineering applications. *J Control Release* **240**, 349-363 (2016).
163. Fulop, T., *et al.* From inflamm-aging to immune-paralysis: a slippery slope during aging for immune-adaptation. *Biogerontology* **17**, 147-157 (2016).
164. Campesi, I., Marino, M., Montella, A., Pais, S. & Franconi, F. Sex Differences in Estrogen Receptor alpha and beta Levels and Activation Status in LPS-Stimulated Human Macrophages. *J Cell Physiol* **232**, 340-345 (2017).
165. Bhatia, A., Sekhon, H.K. & Kaur, G. Sex hormones and immune dimorphism. *ScientificWorldJournal* **2014**, 159150 (2014).
166. Qiu, F., *et al.* Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? *Oncotarget* **8**, 268-284 (2017).
167. Wright, M.D. & Binger, K.J. Macrophage heterogeneity and renin-angiotensin system disorders. *Pflugers Arch* (2017).
168. Sarov-Blat, L., *et al.* Predominance of a proinflammatory phenotype in monocyte-derived macrophages from subjects with low plasma HDL-cholesterol. *Arterioscler Thromb Vasc Biol* **27**, 1115-1122 (2007).

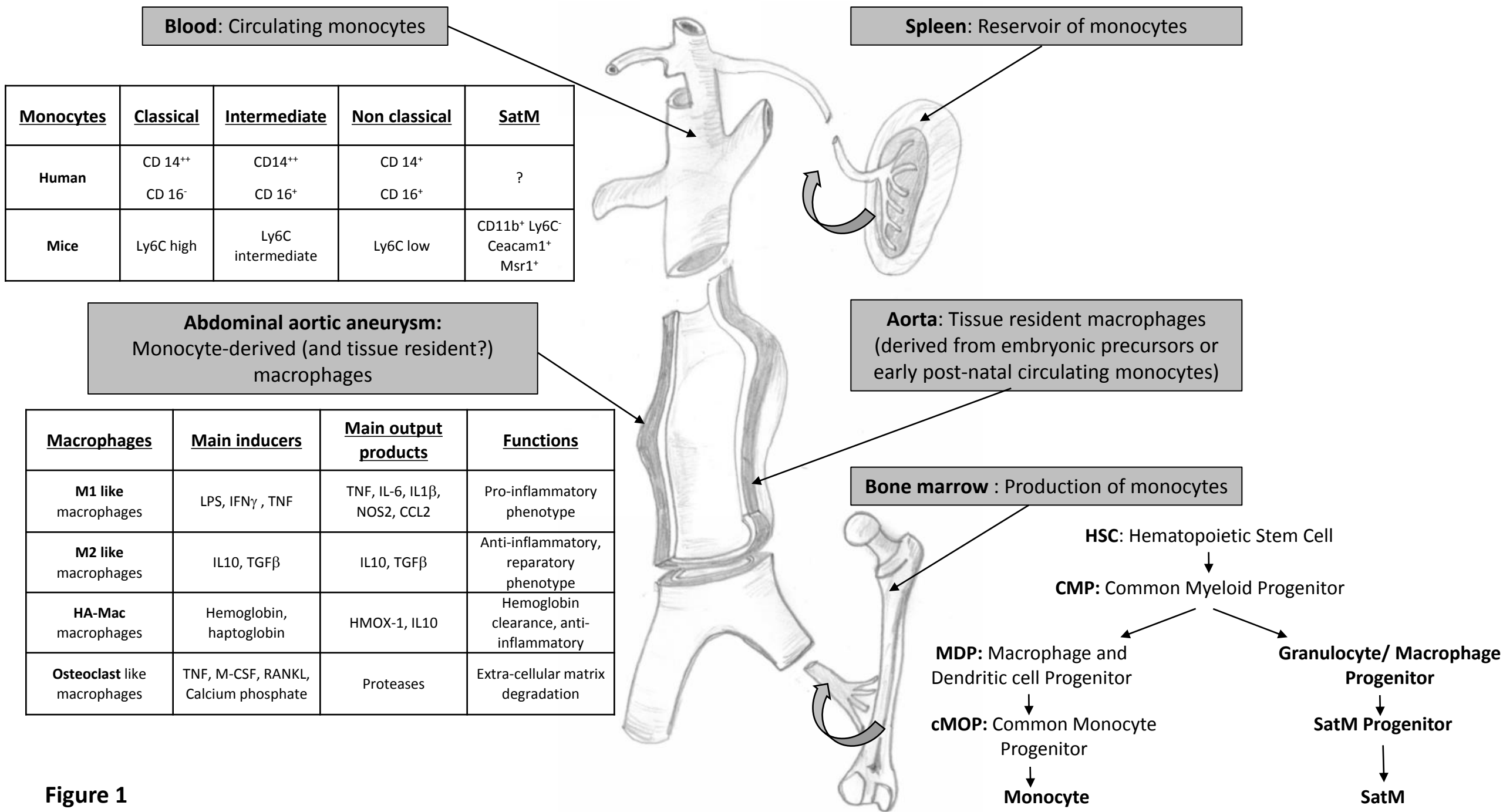


Figure 1

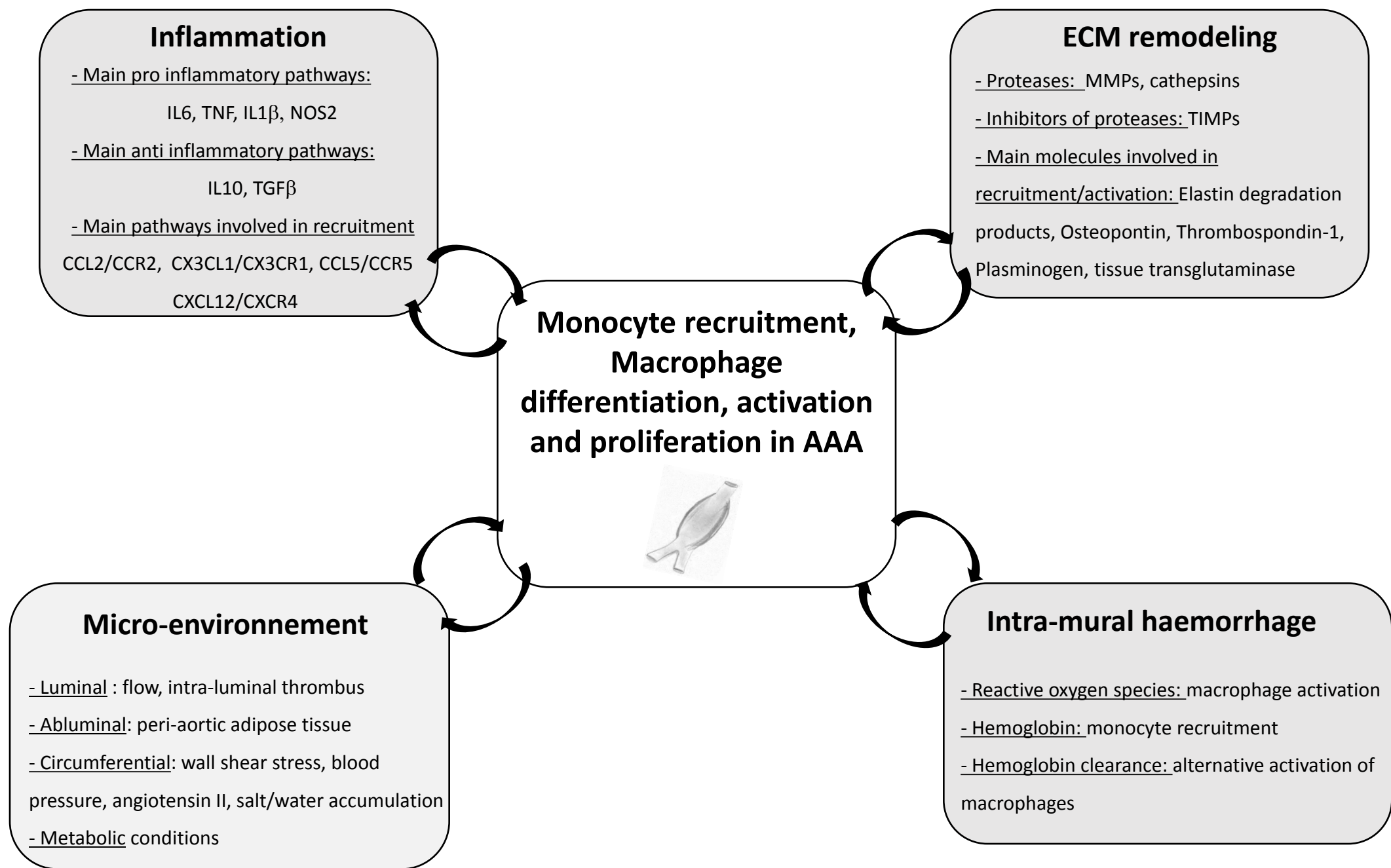


Figure 2

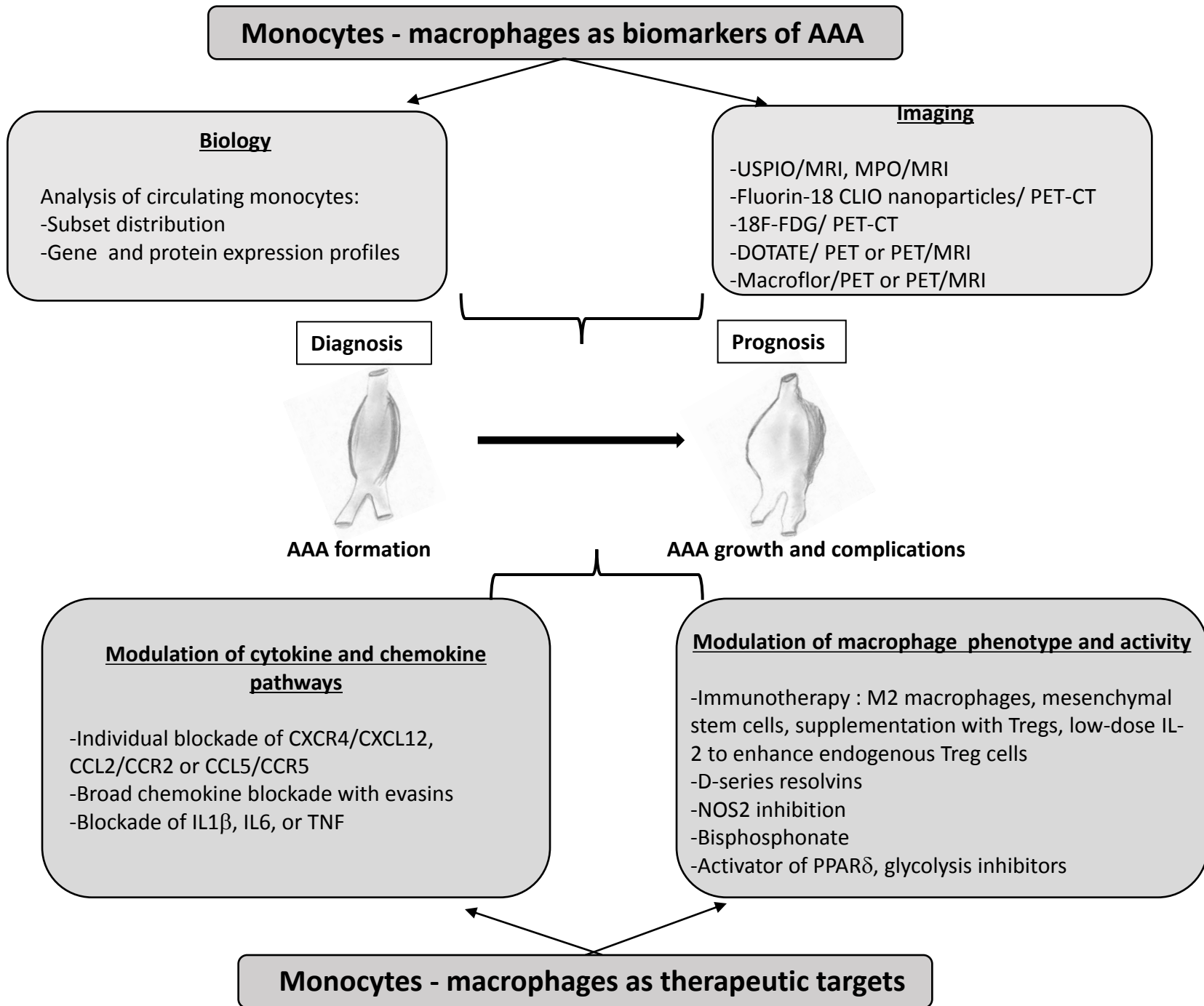


Figure 3

A. Macrophages and degradation of the aortic wall

B. Macrophages and tissue repair

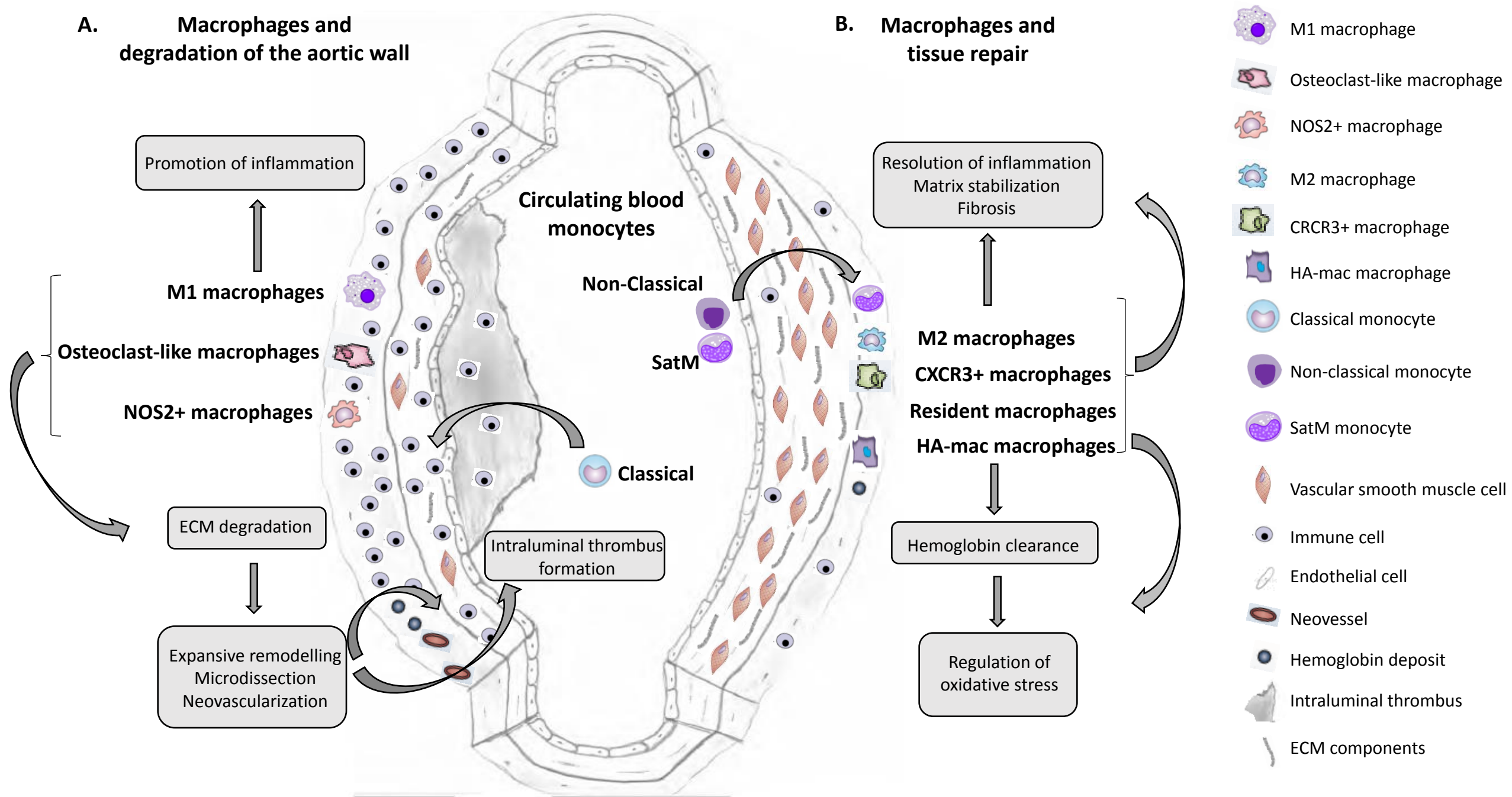


Figure 4