# Monocytes and macrophages in abdominal aortic aneurysm

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**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/EBPβ: 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

$^{18}$F-FDG: 18F-Fluorodeoxyglucose

$^{64}$Cu: Copper-64

$^{68}$Ga: 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 repair [9]. 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 CX3CR1 [6-8]. 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 properties [6-8].

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 cells [30], 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 macrophages [33].

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 fibrosis [34]. 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 macrophages [35-37]. 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 TGFβ, and are involved in matrix remodeling and tissue repair [35]. 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 subsets [38]. 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 markers [35].

Several studies reported the presence of macrophages in the aneurysmal aortic wall both in human aortic sections [40] 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 adventitia [45]. 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 controls [45]. Another study on human aortic aneurysmal sections identified M1 macrophages as $CD68^{+}$ $CD206^{-}$ cells and M2 macrophages as $CD68^{+}$ $CD206^{+}$ cells [47]. 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 experiment [48]. 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 II [48], and a role for ECM remodeling and elastin degradation products, particularly VGVAPG repeat sequence [49]. Other investigators addressed the status and implications of macrophage polarization after prolonged angiotensin II infusion in *Apoe*$^{-/-}$ mice [50]. 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 hemogloblin-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 $Ly6C^{hi}$ monocytes [38]. 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 cardiac [61] and vascular-associated resident macrophages [62]. 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 controls [45]. However, proliferation can occur in both tissue-resident macrophages and those derived from circulating monocytes after their infiltration into injured vessels [65]. 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 monocytes [26]. 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 mobilization [26]. 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*$^{+/+}$ backgrounds [70]. 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*$^{+/+}$ controls [70]. 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 macrophages [62]. 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 atherosclerosis [71], 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 circle [73]. 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) macrophages [74]. 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 infusion [22]. 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 aorta [26]. Inhibition of CXCL4/CCL5 heterodimer formation was associated with a reduction of monocyte/macrophage infiltration in an elastase-induced aneurysm [75]. However, deletion of CCR5 did not alter aortic disease in a model of CaCl2-induced AAA [76].

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 Gαi subunits, and desensitize receptor signaling [77]. Interestingly, RGS1 is highly expressed in human AAA tissue compared to non-aneurysmal vascular tissue [78]. *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 progression [78].

In addition to chemokines and cytokines, the selectin family is known for its role in immune cell interaction with the vessel wall [79]. L-selectin deficiency impaired both neutrophil and monocyte/macrophage recruitment and suppressed elastase-induced AAA formation [80]. 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 micro-dissections 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 stress [88]. 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 AAA [89] and periaortic fat volume correlated with the dimensions of both thoracic and abdominal aortas, suggesting that local fat accumulation may contribute to aortic remodeling [90]. 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 diet [91].

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 controls [94]. 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 controls [94].

## 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 response [95].

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 KFL5-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 *I11b* [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 development [118]. 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 IL1β [119].

A recent study identified a major role for NOS2 expression in VSMCs in mediating the development of thoracic aortic aneurysms [120]. 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 macrophages [38] and is found in AAA disease [121]. NOS2 inhibition did not affect aortic dilatation in a mouse model of elastase infusion [122] 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 aorta [125], particularly under conditions of reduced TGF-β signaling [69], and NOS2 production by those macrophages directly contribute to angiotensin II-induced aortic AAA and dissection, in part through enhanced MMP activation and ECM degradation [69].

*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 hemoglobin [126] accumulate in the aortic wall during AAA and can be engulfed by macrophages [47]. 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 IL1β 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 adventitia [41], 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) nanoparticles [67]. 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 plaques [143], and a PET/CT clinical study found an association between increased $^{68}$Ga-DOTATATE uptake in large arteries and the presence of cardiovascular risk factors [144]. Interestingly, a PET/MRI study in patients with carotid artery plaques revealed correlation between CD163 gene expression and $^{64}$Cu-DOTATATE uptake, suggesting more selective detection of alternatively activated macrophages by DOTATATE [145]. More recently, PET imaging using $^{18}$F-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 infarction [146]. There is currently no published study on the use of 64Cu-DOTATATE or $^{18}$F-Macroflor to image AAA. We believe that PET/MRI using 64Cu-DOTATATE or $^{18}$F-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 inflammation [148]. 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 monocytes [24].

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 degradation [75]. 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 settings [150]. It is noteworthy that the chemokine axis is highly redundant. The development of evasins (chemokine-binding proteins) with broad C, CC and CXC-binding properties [151] 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 arthritis [152]. IL6 is an important regulator of inflammation and macrophage activation during AAA [40], and Mendelian randomization approaches provided strong evidence for the involvement of the IL6 receptor pathway in human AAA [153] 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 IL1β has shown efficacy in experimental models of AAA [111]. Intriguingly however, a recent randomized, double-blind, placebo-controlled clinical trial using the monoclonal anti-IL1β 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 mm [150]. Although the results do not exclude the involvement of the IL1 pathway in AAA (IL1 receptor can be activated by other ligands than IL1β), and the lack of efficiency of canakinumab should prompt us to reconsider our understanding of IL1β 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 AAA [156]. 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 IL1α or IL1β cytokines in disease development. Another potential explanation of the disappointing clinical results is that anti-IL1β 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 $^{18}$FDG in AAA, indicative of high glycolytic activity, followed by the administration of a glycolysis inhibitor to limit macrophage activation [157].

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-environnement? 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 levels [1]. 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 models [17].

## 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.

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**Contribution statements**

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**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 intra-mural 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.

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**Blood**: Circulating monocytes

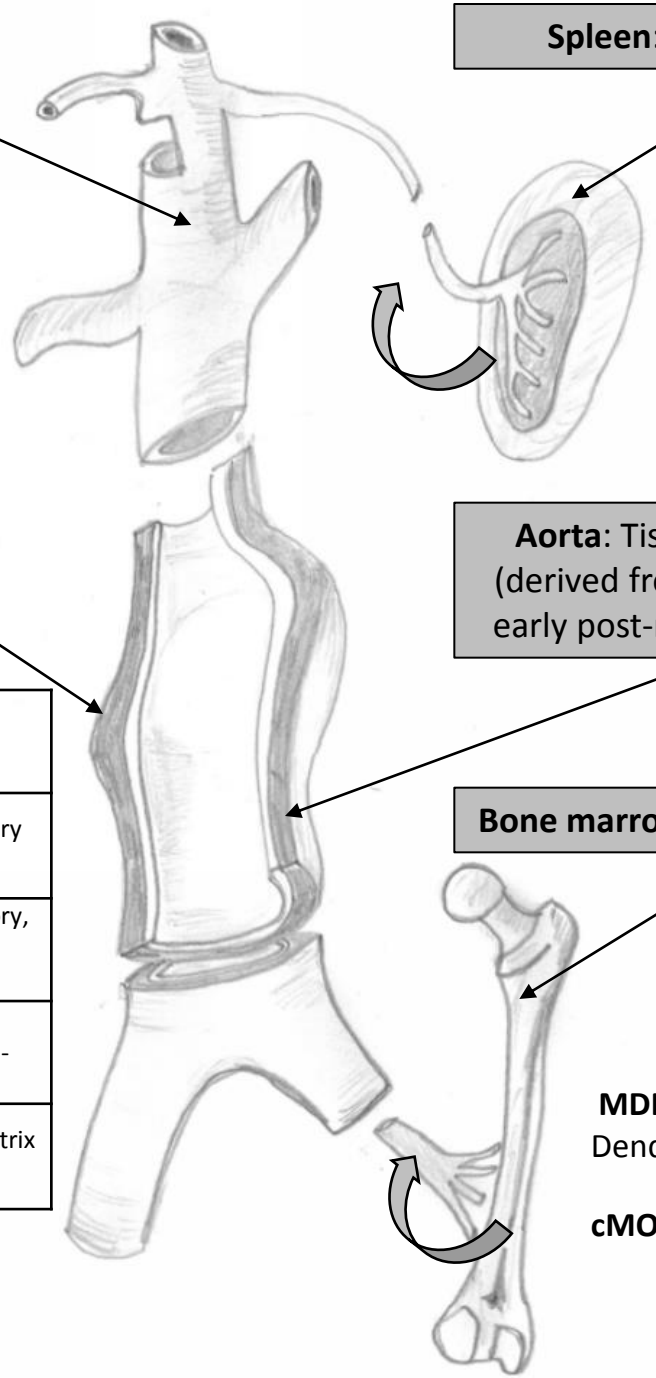
**Spleen**: Reservoir of monocytes

| Monocytes | Classical | Intermediate | Non classical | SatM |
|---|---|---|---|---|
| **Human** | CD 14$^{++}$ CD 16$^{-}$ | CD14$^{++}$ CD 16$^{+}$ | CD 14$^{+}$ CD 16$^{+}$ | ? |
| **Mice** | Ly6C high | Ly6C intermediate | Ly6C low | CD11b$^{+}$ Ly6C$^{-}$ Ceacam1$^{+}$ Msr1$^{+}$ |

**Abdominal aortic aneurysm:**
Monocyte-derived (and tissue resident?) macrophages

**Aorta**: Tissue resident macrophages (derived from embryonic precursors or early post-natal circulating monocytes)

| Macrophages | Main inducers | Main output products | Functions |
|---|---|---|---|
| **M1 like** macrophages | LPS, IFNγ , TNF | TNF, IL-6, IL1β, NOS2, CCL2 | Pro-inflammatory phenotype |
| **M2 like** macrophages | IL10, TGFβ | IL10, TGFβ | Anti-inflammatory, reparatory phenotype |
| **HA-Mac** macrophages | Hemoglobin, haptoglobin | HMOX-1, IL10 | Hemoglobin clearance, anti-inflammatory |
| **Osteoclast** like macrophages | TNF, M-CSF, RANKL, Calcium phosphate | Proteases | Extra-cellular matrix degradation |



**Bone marrow** : Production of monocytes

**HSC**: Hematopoietic Stem Cell

↓

**CMP:** Common Myeloid Progenitor

**MDP:** Macrophage and Dendritic cell Progenitor

↓

**cMOP:** Common Monocyte Progenitor

↓

**Monocyte**

**Granulocyte/ Macrophage Progenitor**

↓

**SatM Progenitor**

↓

**SatM**

**Figure 1**

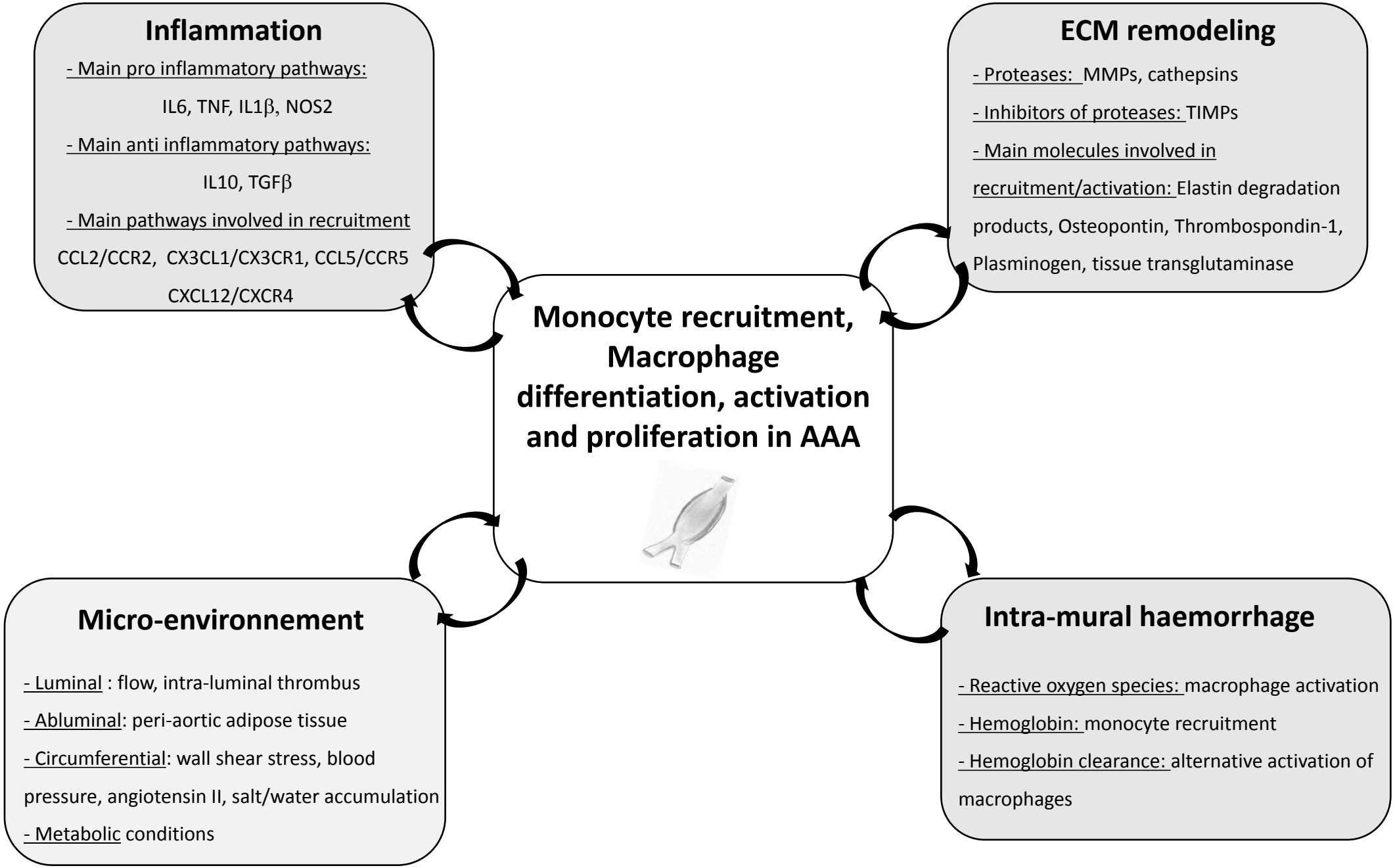

Inflammation
- Main pro inflammatory pathways:
IL6, TNF, IL1β, NOS2
- Main anti inflammatory pathways:
IL10, TGFβ
- Main pathways involved in recruitment
CCL2/CCR2, CX3CL1/CX3CR1, CCL5/CCR5
CXCL12/CXCR4
ECM remodeling
- Proteases: MMPs, cathepsins
- Inhibitors of proteases: TIMPs
- Main molecules involved in recruitment/activation: Elastin degradation products, Osteopontin, Thrombospondin-1, Plasminogen, tissue transglutaminase
Monocyte recruitment, Macrophage differentiation, activation and proliferation in AAA
Micro-environnement
- Luminal : flow, intra-luminal thrombus
- Abluminal: peri-aortic adipose tissue
- Circumferential: wall shear stress, blood pressure, angiotensin II, salt/water accumulation
- Metabolic conditions
Intra-mural haemorrhage
- Reactive oxygen species: macrophage activation
- Hemoglobin: monocyte recruitment
- Hemoglobin clearance: alternative activation of macrophages

**Figure 2**

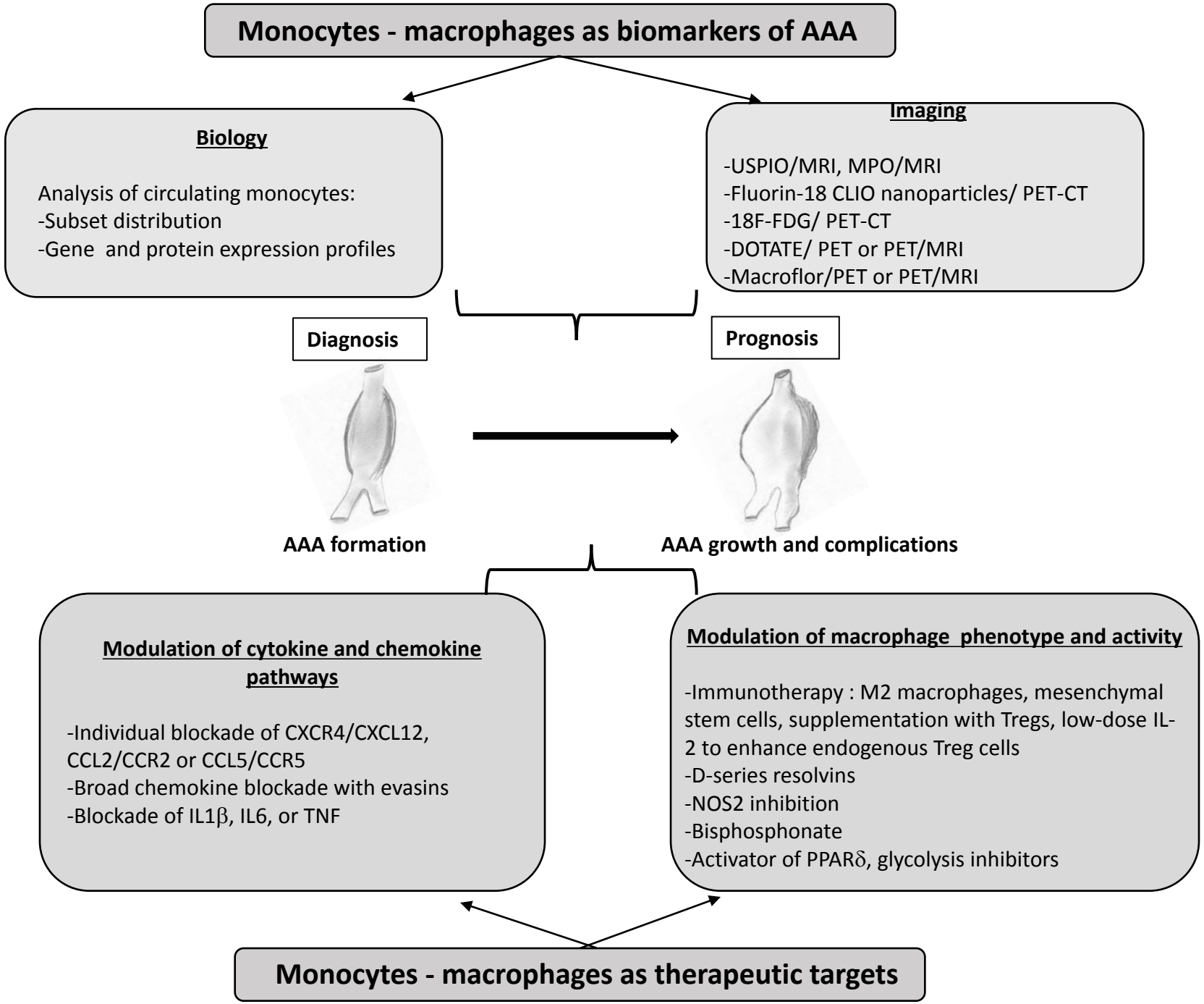

Monocytes - macrophages as biomarkers of AAA
Biology
Analysis of circulating monocytes:
-Subset distribution
-Gene and protein expression profiles
Imaging
-USPIO/MRI, MPO/MRI
-Fluorin-18 CLIO nanoparticles/ PET-CT
-18F-FDG/ PET-CT
-DOTATE/ PET or PET/MRI
-Macroflor/PET or PET/MRI
Diagnosis
Prognosis
AAA formation
AAA growth and complications
Modulation of cytokine and chemokine pathways
-Individual blockade of CXCR4/CXCL12, CCL2/CCR2 or CCL5/CCR5
-Broad chemokine blockade with evasins
-Blockade of IL1β, IL6, or TNF
Modulation of macrophage phenotype and activity
-Immunotherapy : M2 macrophages, mesenchymal stem cells, supplementation with Tregs, low-dose IL-2 to enhance endogenous Treg cells
-D-series resolvins
-NOS2 inhibition
-Bisphosphonate
-Activator of PPARδ, glycolysis inhibitors
Monocytes - macrophages as therapeutic targets

**Figure 3**

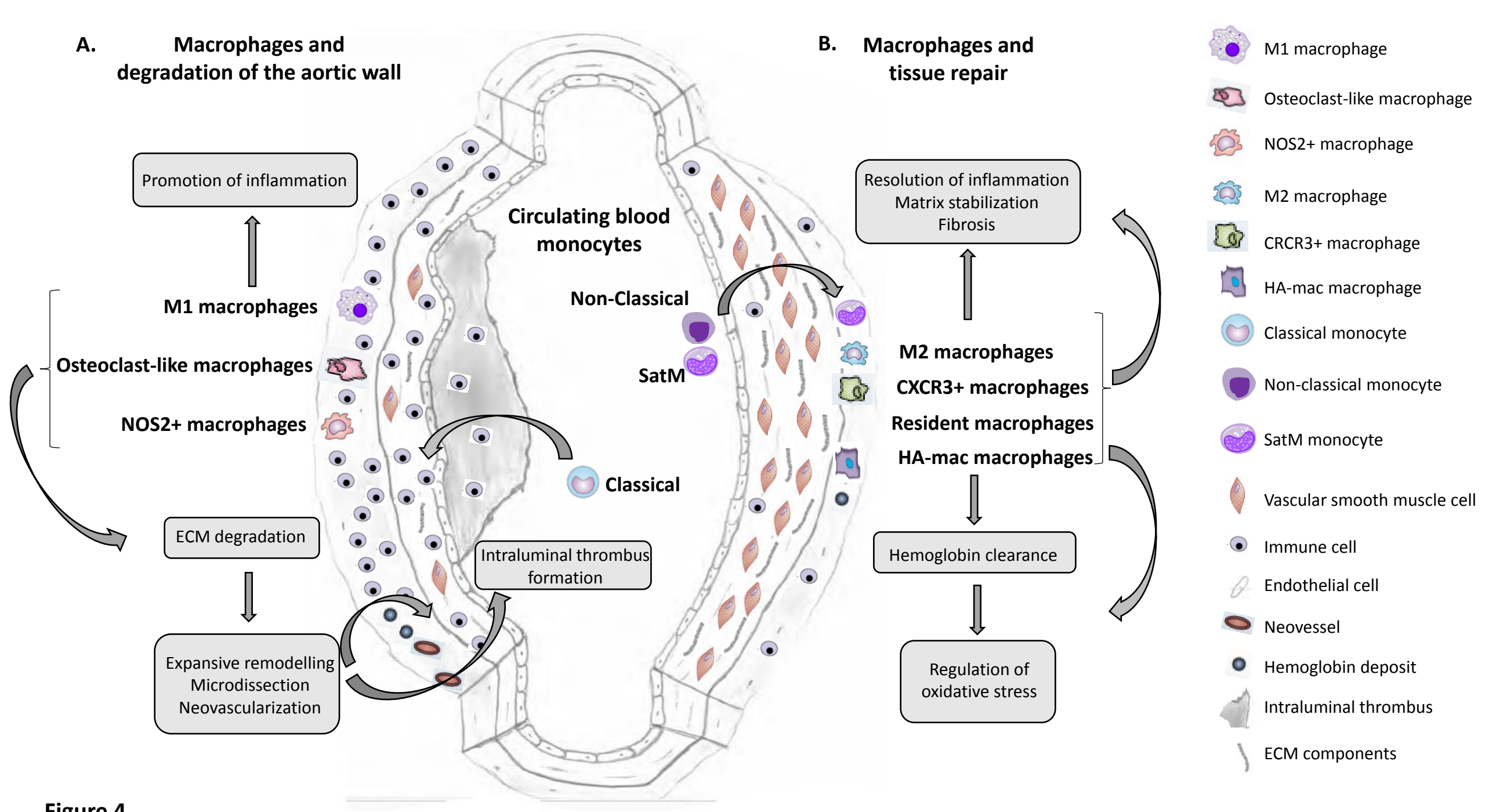

A.
Macrophages and degradation of the aortic wall
Promotion of inflammation
M1 macrophages
Osteoclast-like macrophages
NOS2+ macrophages
ECM degradation
Expansive remodelling
Microdissection
Neovascularization
Intraluminal thrombus formation
Circulating blood monocytes
Non-Classical
SatM
Classical
B.
Macrophages and tissue repair
Resolution of inflammation
Matrix stabilization
Fibrosis
M2 macrophages
CXCR3+ macrophages
Resident macrophages
HA-mac macrophages
Hemoglobin clearance
Regulation of oxidative stress
M1 macrophage
Osteoclast-like macrophage
NOS2+ macrophage
M2 macrophage
CRCR3+ macrophage
HA-mac macrophage
Classical monocyte
Non-classical monocyte
SatM monocyte
Vascular smooth muscle cell
Immune cell
Endothelial cell
Neovessel
Hemoglobin deposit
Intraluminal thrombus
ECM components

**Figure 4**