Inflammatory Cell Phenotypes in AAAs
Their Role and Potential as Targets for Therapy
Matthew A. Dale, Melissa K. Ruhlman, B. Timothy Baxter

Abstract—Abdominal aortic aneurysms (AAAs) are characterized by chronic inflammatory cell infiltration. AAA is typically an asymptomatic disease and caused ≈15,000 deaths annually in the United States. Previous studies have examined both human and murine aortic tissue for the presence of various inflammatory cell types. Studies show that in both human and experimental AAAs, prominent inflammatory cell infiltration, such as CD4+ T cells and macrophages, occurs in the damaged aortic wall. These cells have the ability to undergo phenotypic modulation based on microenvironmental cues, potentially influencing disease progression. Proinflammatory CD4+ T cells and classically activated macrophages dominate the landscape of aortic infiltrates. The skew to proinflammatory phenotypes alters disease progression and plays a role in causing chronic inflammation. The local cytokine production and presence of inflammatory mediators, such as extracellular matrix breakdown products, influence the uneven balance of the inflammatory infiltrate phenotypes. Understanding and developing new strategies that target the proinflammatory phenotype could provide useful therapeutic targets for a disease with no current pharmacological intervention. (Arterioscler Thromb Vasc Biol. 2015;35:1746-1755. DOI: 10.1161/ATVBAHA.115.305269.)

Key Words: aorta ▪ aneurysm ▪ inflammation ▪ lymphocytes ▪ macrophages

Abdominal aortic aneurysms (AAAs) are permanent dilations of the abdominal aorta that, if left untreated, can lead to fatal aortic rupture. Death results from exsanguination into the retroperitoneum or abdominal cavity and may be rapid. Approximately 15,000 deaths because of aneurysm rupture are reported each year in the United States. Most AAAs are often diagnosed serendipitously because they are asymptomatic until the time of rupture. Therefore, screening programs have been used to identify the disease in high-risk populations. They include male sex, increased age (>65 years), and a positive family history. A smoking history is one of the major risk factors associated with aneurysm formation. Smoking history predicts a larger aneurysm size at diagnosis as well as a higher risk of aneurysm progression with continued smoking. Other associated risk factors for AAA formation include white race, presence of other aneurysms, and atherosclerosis.

An aortic aneurysm is defined as a 50% increase in aortic diameter. Many screening studies have assumed that an infrarenal aortic diameter >3.0 cm is an aneurysm. Surgical intervention is not recommended until the aorta reaches a diameter of 5.5 cm in men and 5.0 cm in women, where the risk of rupture exceeds the risk of repair. Currently, there are no pharmacological therapies for reducing AAA progression, so patient management is a matter of watching and waiting until the aorta reaches a size where repair is indicated. Animal studies using statins, β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and tetracyclines have been shown to have a beneficial effect by delaying progression of aortic dilation. Despite this, none of these drugs have proven benefit; the only known approach to reduce aneurysm progression is smoking cessation.

The pathogenesis of AAA is a highly complex process that is undoubtedly multifactorial with, an as yet uncertain, genetic contribution. Histological features of AAA include smooth muscle cell apoptosis, elastin fragmentation, as well as chronic adventitial and medial inflammatory cell infiltration. Elastin fragmentation is triggered by upregulation of various elastin-degrading enzymes, such as matrix metalloproteinases (MMPs), cysteine proteases, and serine proteases. These elastin and extracellular matrix (ECM) fragments recruit inflammatory cells to the artery wall causing an innate immune response that attempts to resolve the damage. The adaptive immune response is associated with aneurysm progression through antigen-specific antibody production. In this review, we focus on the dynamic phenotypes of inflammatory cells present in AAA tissue and how they exacerbate disease progression.
The immune system is a complex arrangement of many cell types and molecules interacting to maintain homeostasis. The first basic distinction is the difference between innate and adaptive immunity. The innate immune system comprises all of the immune responses present from birth, whereas the adaptive immune system consists of those responses generated after exposure to specific new antigens. The innate immune system is considered the first line of defense against invading microorganisms and is made up of the cells and molecules that act as first responders in the face of an insult. It has been conserved throughout evolution with similar molecular components found in simple eukaryotes and more complex organisms, including humans. The adaptive immune system developed later in evolution and can acquire what is considered immunologic memory. After exposure to a novel pathogen, the adaptive immune system tailors its response while maintaining self-tolerance (except in autoimmune diseases). These responses are typically controlled with an acute phase response.

### Inflammatory Cells in Atherosclerosis

Atherosclerosis is an inflammatory disease beginning in the intima of large and medium sized arteries caused by accumulations of low-density lipoproteins. A wide range of inflammatory cell types has been found in advanced atherosclerotic lesions including but not limited to macrophages/monocytes, lymphocytes, and dendritic cells. Infiltration of these inflammatory cells occurs primarily in the neointima, aiding in the inward rather than outward remodeling in atherosclerosis. Glagov et al were the first to describe compensatory outward remodeling that partially compensated for intimal expansion. A recent study proposed that IL-1 is an important factor that enhances outward remodeling, protecting the artery from stenosis. Inhibition of the IL-1 pathway actually enhanced macrophage infiltration and caused further narrowing of the arterial lumen. MMPs assist in the outward remodeling process and are implicated as a cause of AAA formation, but in atherosclerosis, MMPs are predominantly expressed in the atherosclerotic plaque, leading to plaque instability. In contrast, Figure demonstrates that the inflammatory lesions in AAA tend to occur in the outer layers of the media and adventitia, where they may be expected to have a greater impact on outward remodeling. The presence of inflammatory cells and their associated cytokines and proteases may protect from arterial narrowing by promoting outward remodeling. Taken to the extreme, this inflammatory response may promote overcompensation in the outward remodeling process by causing aneurysm formation.

### Inflammatory Cells in AAAs

The transmural inflammation seen in AAA involves a variety of inflammatory cell types, where macrophages and lymphocytes are the most prominent with mast cells and neutrophils migrating to a lesser extent. Ocana et al demonstrated that both T- and B-lymphocytes are present in AAA tissue, where the majority of the infiltrating lymphocytes are cluster of differentiation 4 positive (CD4+) T cells. No natural killer (NK) cells were detected in tissue from AAA patients; however, peripheral blood levels of NK cells were significantly higher in AAA patients compared with controls. The presence of B and T lymphocytes suggests antibody-mediated humoral immunity and potentially an autoimmune process driving AAA formation or progression. Previous animal studies have reported that without the presence of CD4+ T cells, AAA formation does not occur. Furthermore, experimental AAA formation is mediated by interleukin (IL)-17, a cytokine notably produced by proinflammatory CD4+ T cells. These proinflammatory CD4+ T cells exacerbate AAA disease progression and play a role in creating chronic inflammation in the damaged aortic wall (Figure). Macrophages have also been shown to play a key role in AAA progression. Macrophages are key components of the inflammatory process. Tumor necrosis factor (TNF-α), IL-6, IL-1β, and interferon (IFN)-γ are proinflammatory macrophage-associated cytokines studied as biomarkers of AAA progression. Although all of these cytokine levels are elevated in patients with AAA compared with controls, only IFN-γ levels had a positive correlation with AAA progression. Macrophages are recruited to injury sites by ECM degradation products and numerous chemokines. These chemokines, monocyte chemotactic protein (MCP)-1, IL-8, and TNF-α, are colocalized to infiltrating macrophages. Macrophages recruited to AAA tissue begin a positive feedback loop creating chronic inflammation, but they may not always be harmful in AAA development (Figure). Macrophages, like T cells, display plasticity and have the ability to polarize to various phenotypes, such as the classically activated (M1) phenotype or the alternatively activated (M2) phenotype. M1 macrophages are typically characterized by proinflammatory cytokine production and initiate tissue degradation, whereas M2 macrophages are implicated in inflammation resolution and tissue repair. An M1/M2 imbalance may be occurring in AAA, where strategies targeting a stronger M2 response may prove a potential therapeutic target to reduce chronic inflammation in AAAs.

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### Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAA</td>
<td>Abdominal aortic aneurysm</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>NK</td>
<td>Natural killer</td>
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<tr>
<td>STAT4</td>
<td>Signal transducer and activator of transcription 4</td>
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<tr>
<td>T eff</td>
<td>T effector</td>
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<tr>
<td>Th</td>
<td>T helper</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>T reg</td>
<td>T regulatory</td>
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### Brief Review: Innate and Adaptive Immune System

The immune system is a complex arrangement of many cell types and molecules interacting to maintain homeostasis. The first basic distinction is the difference between innate and adaptive immunity. The innate immune system comprises all of the immune responses present from birth, whereas the adaptive immune system consists of those responses generated after exposure to specific new antigens. The innate immune system is considered the first line of defense against invading microorganisms and is made up of the cells and molecules that act as first responders in the face of an insult. It has been conserved throughout evolution with similar molecular components found in simple eukaryotes and more complex organisms, including humans. The adaptive immune system developed later in evolution and can acquire what is considered immunologic memory. After exposure to a novel pathogen, the adaptive immune system tailors its response while maintaining self-tolerance (except in autoimmune diseases). These responses are typically controlled with an acute phase response.
Diabetes mellitus is an important risk factor for the development of atherosclerotic lesions. Irreversible formation of advanced glycation end products is implicated as a cause of accelerated atherosclerosis. The interaction of advanced glycation end products with mononuclear phagocytes induces a proinflammatory macrophage phenotype, resulting in production of various proinflammatory cytokines, such as TNF-α and IL-1β. Interestingly, diabetes mellitus is negatively associated with AAAs. This negative correlation may be related to the formation of advanced glycation end products and alterations to ECM proteins. Golledge et al found that aortic tissue from patients with diabetes mellitus have decreased activities of MMP-2 and MMP-9. Modification of collagen lattices by glycation or treatment with glutaraldehyde reduced MMP activity. These findings suggest that modification of ECM proteins reduces protease activity, potentially preventing aortic wall degeneration and aneurysm formation.

**Inflammatory Cells in Diabetic Atherosclerosis and AAAs**

Diabetes mellitus is an important risk factor for the development of atherosclerotic lesions. Irreversible formation of advanced glycation end products is implicated as a cause of accelerated atherosclerosis. The interaction of advanced glycation end products with mononuclear phagocytes induces a proinflammatory macrophage phenotype, resulting in production of various proinflammatory cytokines, such as TNF-α and IL-1β. Interestingly, diabetes mellitus is negatively associated with AAAs. This negative correlation may be related to the formation of advanced glycation end products and alterations to ECM proteins. Golledge et al found that aortic tissue from patients with diabetes mellitus have decreased activities of MMP-2 and MMP-9. Modification of collagen lattices by glycation or treatment with glutaraldehyde reduced MMP activity. These findings suggest that modification of ECM proteins reduces protease activity, potentially preventing aortic wall degeneration and aneurysm formation.

**CD4+ and CD8+ T Cells**

A review of the cell types involved is critical to understanding the role of inflammation in AAA development and progression. T cells are a heterogeneous group of lymphocytes with a diverse classification system and multitude of physiological actions. They are initially classified based on surface expression of CD4 or CD8 molecules. CD4+ cells recognize antigens presented by major histocompatibility complex class II, whereas CD8+ cells recognize antigens presented by major histocompatibility complex class I, important in cell-mediated toxicity. Most modulatory T cells express CD4, whereas most cytotoxic T cells express CD8. The CD4+ T cell has been found to be the predominant cell type in human aneurysm tissue. Through its profile of secreted cytokines, the CD4+ T cell indirectly controls matrix metabolism by recruitment of macrophages and regulation of ECM and protease synthesis.

**T-Cell Phenotypes: Th1, Th2, Th17, and Treg**

CD4+ T cells can be further subdivided into the T helper (Th) or T effector (Teff) subsets: Th1, Th2, and Th17 and the regulatory subset: T regulatory (Treg) cells. Each subset is classified by the cytokine profile required for stimulation, their secreted products, and their physiological actions. In human disease, there is rarely polarization to one specific cell phenotype but an imbalance between proinflammatory and anti-inflammatory CD4+ cells may enhance aneurysmal disease progression.

**Th1**

The Th1 cell has been linked to many chronic auto-inflammatory disorders including rheumatoid arthritis and systemic lupus erythematosus. Th1 cells are characteristicly activated by IL-12, triggering the signal transducer and activator of transcription 4 (STAT4) and T-bet pathway to produce IFN-γ, TNF-α, and TNF-β (Table 1). This leads to activation of macrophages and an internal autoregulatory loop to potentiate Th1 development and inhibit alternate T cell differentiation. Through this cycle, IFN-γ activates macrophages and enhances inflammatory cell recruitment through augmenting cytokine, chemokine, and adhesion molecule expression. Macrophages then produce additional IL-12, which promotes...
ApoE−/− model of AAA, where Rag1 deficiency had no effect on γ lymphocytes and IFN-γ together, the animal models have failed to elucidate a clear role of combination of hemodynamics and hypercholesterolemia. Taken in AAA (Table 2).48,49,54–57 Madhur et al65 found aneurysm formation in the ApoE−/− model of AAA, where Rag1 deficiency had no effect on reducing aneurysm size.59 This was also true for IFN-γ and its downstream transcription factor STAT1 in the ApoE−/− model.40,61 These data highlight differences in the models. The CaCl2 model relies on an inflammatory response for aneurysm formation, whereas the ApoE−/− model depends to a much greater extent on a combination of hemodynamics and hypercholesterolemia. Taken together, the animal models have failed to elucidate a clear role of lymphocytes and IFN-γ in AAA (Table 2).

Th2

The Th2 cell, along with its cytokine profile, is largely considered anti-inflammatory. Interestingly, the Th2 cell can also be associated with inflammatory processes, including IgE-mediated antibody responses, allergy, and asthma.48,55,57 IL-4 drives the differentiation of CD4+ T cells into the Th2 phenotype. Through the STAT6 and GATA-3 pathway, the Th2 cell secretes IL-4, IL-5, IL-10, and IL-13 (Table 1).48,49,54–57 IL-4 and IL-10 limit the cytotoxic potential of macrophages and decrease expression of proinflammatory mediators and MMPs. IL-13 enhances development of anti-inflammatory M2 macrophages but also increases MMP expression.54,55

The Th2 cell profile has been implicated as a culprit in aneurysmal disease in both human and murine studies.64 Schönbek et al64 found increased levels of Th2-associated cytokines and low expression of Th1-related cytokines, particularly IFN-γ, in human tissue. They suggested that IL-4 overexpression prevents Th1 differentiation and that IL-4 and IL-5 induced chemotraction of neutrophils contributes to excessive elastolytic activity. Shimizu et al59 showed that in allografted mice aortas, AAA development was more severe in IFN-γ−/−-deficient mice, whereas mice deficient in IL-4 were protected from AAA. Contradictory to the murine data, IL-4 has been shown to stimulate production of ECM proteins in human fibroblasts and suppress MMP expression (MMP-1 and MMP-9) in human alveolar macrophages.67,68 Chan et al56 suggested an alternate source for the Th2-associated cytokines; another member of the T-cell family, the natural killer (NK) T cell.56 NK and NKT cells produce both IL-4 and IFN-γ and have a profile that is neither Th1 nor Th2; referred to as Th0. Unlike most immune cells whose function seems to decline with age, the NK and NKT cells increase in number and produce greater IL-4 with advancing age. The important role of the Th2 cell may be specific to the murine transplant model, as other murine models show Th1 predominance. The conflicting data between the Th1 and Th2 profiles may relate to technical differences in how measurements were made, differences in animal models, and the late disease state at which human aneurysm samples are obtained (Table 2).

Th1

A more recently identified distinct subclass of T helper cell, the Th1 cell, has generated interest with regard to its potential role in AAA. This cell type is unique from Th1 and Th2 cells based on both its cytokine profile and differentiation pathway.53 The Th1 cell is stimulated by IL-23, IL-1, and IL-6, which primarily induce retinoic acid receptor–related orphan receptor γt and STAT3, leading to secretion of IL-17 (Table 1).49,53,57,62 IL-17 has 6 known isoforms, A through F, of which Th17 cells secrete IL-17A and F. IL-17A, its primary cytokine, mediates many immune and inflammatory diseases and is critical for vascular superoxide production.63 The Th17 cell promotes macrophage recruitment to the vascular wall, whereas deficiency of IL-17 seems to reduce aortic macrophages in murine models.52,65

Madhur et al66 found aneurysm formation in the ApoE−/− murine model to be unchanged when comparing control mice with IL-17 knockout mice. This suggested that IL-17 did not have a critical role in this murine model. In contrast, Sharma et al39 showed that IL-23 and IL-17 expression was increased in

### Table 1. Th Cell Differentiation, Function, and Role in Disease

<table>
<thead>
<tr>
<th>Stimulating factors</th>
<th>Pathway</th>
<th>Secreted products</th>
<th>Role in disease</th>
<th>Th1</th>
<th>Th2</th>
<th>Th17</th>
<th>Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12</td>
<td>STAT4, T-bet</td>
<td>IFN-γ</td>
<td>Macrophage activation, †inflammatory cell recruitment</td>
<td>IL-4</td>
<td>IL-4</td>
<td>IL-23, IL-1, IL-6</td>
<td>IL-2, TGF-β</td>
</tr>
<tr>
<td>Pathway</td>
<td>STAT6, GATA-3</td>
<td>IL-4, IL-5, IL-10, IL-13</td>
<td>Limit macrophage cytotoxicity, †MMPs and cytokines (IL-4/10), †MMPs (IL-13)</td>
<td>IL-6</td>
<td>IL-17α and F</td>
<td>†Macrophage recruitment</td>
<td>IFN-γ → [\text{T}_{\text{eff}}] proliferation, IL-12, and IL-15 secretion from Th17, and macrophages, remove autoreactive T cells</td>
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<tr>
<td>Secreted products</td>
<td>IL-23, IL-1</td>
<td>RORγt, STAT3</td>
<td>†Macrophage recruitment</td>
<td>IL-5</td>
<td>STAT5, Foxp3</td>
<td>[\text{T}_{\text{eff}}]</td>
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<tr>
<td>Role in disease</td>
<td>IL-6</td>
<td>IL-17A and F</td>
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<td>IL-10 and TGF-β</td>
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Foxp3 indicates forkhead box P3; IFN-γ, interferon-γ; IL, interleukin; MMP, matrix metalloproteinase; RORγt, retinoic acid receptor–related orphan receptor γt; STAT, signal transducer and activator of transcription; \[\text{T}_{\text{eff}}\], T effector; Th, T helper; and TGF, transforming growth factor.
Table 2. Th Cell Differentiation in Human and Experimental AAA

<table>
<thead>
<tr>
<th></th>
<th>Human AAA</th>
<th>Th1</th>
<th>Th2</th>
<th>Th17</th>
<th>Treg</th>
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<tbody>
<tr>
<td>ApoE−/− AngII model</td>
<td>Increased</td>
<td>Decreased</td>
<td>Increased</td>
<td>Decreased</td>
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<tr>
<td>CaCl2 model</td>
<td>Increased</td>
<td>Undefined</td>
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<tr>
<td>Elastase-perfusion model</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Increased</td>
<td>Undefined</td>
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</table>

AAA indicates abdominal aortic aneurysm; AngII, angiotensin II; Th, T helper; and Treg, T regulatory.

human AAA. Using an elastase-perfusion murine model, deletion of IL-17 or IL-23 attenuated aneurysm development and decreased proinflammatory cytokine production. Furthermore, mesenchymal stem cell treatment, at the time of aneurysm induction, decreased IL-17 production and reduced aortic dilation. Recently, Wei et al. showed a decrease in AAA incidence and increased mouse survival in the ApoE−/− and elastase-perfusion murine models. Digoxin was found to antagonize receptor–related orphan receptor γt, inhibit Th17, and augment Treg cells. This growing body of evidence suggests an important role of the Th17 cell in promoting inflammation and enhancing aneurysmal disease.

Treg cells are a unique subclass of CD4+ T cells that function to counteract the proinflammatory effects of the T effector subclasses and are important in immune tolerance. IL-2 and transforming growth factor-β stimulate Treg cells, which produce IL-10 and transforming growth factor-β through the STAT5 and forkhead box P3 pathway (Table 1). The Treg assumes the critical function of curtailing the inflammatory process, limiting collateral damage, and allowing matrix repair to begin. Known functions of Treg cells include blocking further proliferation of Teff cells, inhibiting the inflammatory cascade by blocking TNF-α and IFN-γ secretion from effector cells and invading macrophages, and removal of autoreactive T-cell clones generated in response to matrix degradation products. Because one of the most important functions of the Treg cells is to limit the proliferation of Teff cells, a relative increase in Teff cells indicates a loss of control of the inflammatory response. Yin et al. showed a reduction in the proportion of Treg cells in AAA patients. Increasing the Treg population, by injection of splenic Treg cells from a donor mouse, protected ApoE−/− mice from aneurysm formation. An imbalance in the proportion of the Treg cell population or dysfunction of the Treg cell has been implicated in other chronic inflammatory processes, including chronic obstructive pulmonary disease, lupus, scleroderma, and organ rejection. Importantly, matrix destruction, which leads to end stage disease in each of these processes, seems to represent collateral damage from an uncontrolled inflammatory response. Normally functioning Treg cells have the potential to limit matrix damage by inhibiting T cell proliferation, blocking TNF-α and IFN-γ secretion, and removing autoreactive T cells.

In summary, the majority of CD4+ cells are effector T cells whose specific role is largely confined to regulation of the acute inflammatory response as part of their central purpose in the adaptive immune system. With an acute insult, these cells are pivotal to the normal host defense. As part of this initial and aggressive response, the Teff cells secrete and induce the secretion of proteases allowing the inflammatory cells to migrate into tissues to establish contact with the injurious agent. In normal conditions, the Treg allows for resolution of this response once the threat has been curtailed. In chronic inflammatory processes such as AAA, this proinflammatory and proteolytic milieu is not adequately opposed by anti-inflammatory mechanisms. The result is significant and progressive destruction of the ECM.

Macrophage Phenotypes: M1 and M2

Macrophages play crucial roles in the innate and adaptive immune responses and have been studied in various diseases since their discovery in the late 19th century by Élie Metchnikoff. Like other immune cells, macrophages respond to various stimuli in their microenvironmental milieu. These highly plastic cells play dual roles in initiation and resolution of inflammation. The macrophage population consists of 2 major phenotypes, M1 and M2. M1 macrophages respond to stimuli that enhance and sustain ongoing inflammation via production of proteolytic enzymes and proinflammatory mediators. Initial arterial injury leads to recruitment of M1 macrophages. Normally, these infiltrating macrophages would later convert to M2 macrophages, promoting tissue repair and wound healing. This M1/M2 balance is vital to proper wound repair and resolution of the inflammatory response. If the M1 phenotype continually predominates, chronic inflammation occurs. Conversely, if the M2 phenotype predominates, ongoing infection or poor wound healing may result. In certain cancers, this M2 imbalance has been shown to be detrimental, actually leading to tumor growth. Histologically, AAA tissue shows marked inflammatory cell infiltrates. Evidence suggests a chronic inflammatory milieu where the M1 phenotype is not adequately balanced by the M2 phenotype, consistent with progressive aneurysm expansion.

M1

M1 macrophages respond to environmental stimuli and sustain ongoing inflammation via production of proteolytic enzymes and proinflammatory mediators. The classical M1 macrophage phenotype can be activated in vitro by proinflammatory cytokines, including IFN-γ and TNF-α. IFN-γ primes the macrophages for activation but is inadequate alone to produce the M1 phenotype. A secondary signal, such as TNF-α or lipopolysaccharide, is required for the activation of toll-like receptor 4 resulting in M1 macrophage polarization. This phenotypic polarization triggers production of various
M1 markers, such as inducible nitric oxide synthase, TNF-α, IL-1β, and other proinflammatory mediators (Table 3). Characteristic cell surface markers, including those associated with antigen presentation, such as CD80 and CD86, can further identify these cells as M1 macrophages. The M1 macrophage products may produce a positive feedback loop resulting in chronic inflammation and significant tissue damage.

In AAAs, examination of these M1 markers in human tissues and in experimental animal models has yielded noteworthy results. Many studies have focused on the discovery of novel biomarkers in AAA patient serum. Through these studies, researchers have identified some potential targets, which are associated with the M1 phenotype. Although human studies of macrophages in AAA have been limited to examination of end stage disease tissue or circulating monocytes, key findings have emerged. Circulating monocytes from AAA patients displayed enhanced adhesive activity to the endothelial cell wall and increased MMP-9 production. Although these monocytes were not studied specifically for M1 or M2 markers, their presence suggests a systemic inflammatory response, which would be expected because of the presence of high levels of MMP-9 resulting in tissue breakdown. Hance et al demonstrated that monocyte chemotaxis to AAA tissue can be directly linked to breakdown of the ECM, specifically via a six-peptide sequence (VGVAPG) found mainly in elastin. Experimental animal studies have shown that blocking the presence of the VGVAPG sequence with a monoclonal antibody reduces monocyte/macrophage recruitment limiting further ECM breakdown. These ECM breakdown products act as proinflammatory mediators, further recruiting monocytes and promoting their differentiation into M1 macrophages. Once initiated, the resolution of this inflammatory response is unlikely.

Various cell surface markers are associated with M1 macrophage polarization. CD14 acts as a coreceptor with toll-like receptor 4, which is required for M1 polarization through the IFN-γ and lipopolysaccharide activation pathway. Recent studies showed that patients with AAAs have increased levels of CD14+CD16+ monocytes compared with control patients, suggesting these monocytes may be associated with the chronic inflammatory process of AAA. CD16, a low affinity Fc receptor for IgG antibodies involved in antibody-dependent cytotoxicity, is also associated with an M1 macrophage polarization. Experimental aneurysm models indicated that CD14 deletion reduced inflammatory cell infiltration therefore reducing AAA incidence. With the increase in CD markers associated with increased proinflammatory processes, it is clear that the M1 phenotype plays a major role in AAAs, at least in the latter stages of disease when tissue samples are obtained.

Examination of proinflammatory cytokines in AAAs has been more extensive and has led to many treatment strategies focused on their antagonism. M1-associated proinflammatory cytokines TNF-α, IL-6, IL-1β, and IFN-γ were all increased in human aneurysmal tissue and serum (Table 4). IFN-γ is one stimulus that activates M1 macrophage polarization, and deletion of IFN-γ in experimental mouse models inhibited aneurysm formation and macrophage infiltration. Another M1-associated cytokine, TNF-α, stimulates M1 macrophage polarization resulting in further TNF-α production. Genetic deletion of TNF-α or antibody-mediated sequestration with Infliximab reduced macrophage infiltration and aneurysm formation in a murine model. Similar deletion studies focused on M1-associated cytokines, IL-6 and IL-1β, have yielded comparable results. Without these M1 polarizing cytokines, aneurysm formation is dramatically reduced and macrophage infiltration is minimized. These data are now being further investigated in a translational study using Canakinumab, an IL-1β neutralizing antibody, in patients with small AAAs with a goal of inhibiting aneurysm expansion (NCT02007252).

M2

In contrast to classically activated M1 macrophages, alternatively activated M2 macrophages are associated with wound repair and inflammation resolution. M2 activation is achieved by IL-4 or IL-13, both antagonizing the actions of IFN-γ. IL-4 and IL-13, associated with Th2 cells, bind to the IL-4 receptor and signal M2 polarization through STAT6. Polarization of macrophages to an anti-inflammatory M2 phenotype results in production of anti-inflammatory cytokines, including IL-10 and transforming growth factor-β. The M2 macrophage is identified by markers, such as mannose receptor (CD206), arginase I, and CD163 (Table 3). These anti-inflammatory markers have been identified in tumors, where tumor-associated M2 macrophages suppress the natural immune response to cancer cells. However, in diseases associated with chronic inflammation, such as AAA, enhancement of the M2 response could potentially limit the ongoing inflammatory response.

A few recent studies have examined one of the most common M2-linked markers, CD206. CD206 regulates the levels of glycoproteins released after inflammatory responses, aiding in wound resolution. Examination of CD206 in human AAA tissue has revealed contrasting results. Boytard et al

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**Table 3. Macrophage Differentiation, Function, and Role in Disease**

<table>
<thead>
<tr>
<th>M1</th>
<th>M2</th>
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<tbody>
<tr>
<td><strong>Stimulating factors</strong></td>
<td>TNF-α, IFN-γ, LPS</td>
</tr>
<tr>
<td><strong>Pathway</strong></td>
<td>STAT1, AP-1, NF-κB</td>
</tr>
<tr>
<td><strong>Secreted products</strong></td>
<td>TNF-α, IL-6, IL-1β, iNOS, MCP-1</td>
</tr>
<tr>
<td><strong>CD markers</strong></td>
<td>CD80, CD86, CD16, CD14</td>
</tr>
<tr>
<td><strong>Role in disease</strong></td>
<td>Proinflammatory, cytotoxicity, microbicidal activity, tumor suppression</td>
</tr>
</tbody>
</table>

*AP indicates activator protein; Arg, arginase; CREB, cAMP response element–binding protein; FIZZ, found in inflammatory zone; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MCP, monocyte chemotactic protein; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator–activated receptor; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α; and Ym1, Chi3l3 (Chitinase 3-like-3).*
demonstrated that CD206+ macrophages are present in the intraluminal thrombus but absent in the heavily damaged aortic adventitia. Conversely, Dutertre et al. found CD206+ macrophages to be present in the damaged aorta, potentially signaling wound resolution. An experimental aneurysm model using ApoE−/− mice revealed increased CD206 staining in the more severely affected tissue. These contradictory data indicate the further need for M2 phenotype clarification and examination of additional M2 markers. Despite the conflicting results, targeting the M1/M2 imbalance may offer an interesting therapeutic aim in aneurysmal disease.

Neutrophils and Mast Cells

Neutrophils are quickly recruited to sites of injury and are characteristic of acute inflammation. These cells are most commonly found in the intraluminal thrombus in AAAs. Although intraluminal thrombus are frequently found in human AAAs, animal models fail to recapitulate formation of an intraluminal thrombus. However, examination of neutrophils in AAA has elucidated important findings. In an elastase-perfused model of aneurysm formation, depletion of polymorphonuclear neutrophils reduced aortic dilation in an MMP-independent manner. Human AAA studies revealed a negative correlation of neutrophil catalase activity and aortic size. These studies indicate a role for neutrophils in aneurysm formation, which may depend on regulation of reactive oxygen species.

Mast cells are inflammatory cells associated with immediate hypersensitivity and chronic allergic reactions. These cells have been found in human AAA tissue, primarily residing in the media and adventitia. Genetic deletion of mast cells protects against aneurysm formation in both the elastase-perfusion and CaCl₂ murine models of aneurysm formation. MMP-2 and -9, as well as other ECM degrading enzymes, can be activated by mast cell secreted chymase, promoting aneurysm formation. Although much of the focus on aneurysm formation has been on T cells and macrophages because they are the predominant inflammatory cells, interactions between these and other inflammatory cells also play a role in aneurysm formation.

Discussion

AAA is a complex disease where an improper balance of T cell or macrophage phenotypes may worsen the disease process. This imbalance that occurs with the numerous cell types involved in AAAs enhances the chronic inflammatory aspect of the disease. CD4+ T cells display many different phenotypes, most of which have been found in AAA tissue. Studies targeting the switch of T cells from a proinflammatory phenotype to an anti-inflammatory phenotype, such as upregulation of Treg cells, create exciting new strategies for targeting AAA progression. Macrophage phenotype polarization is a promising new field that may prove beneficial in identifying key regulators of chronic inflammation in AAA. Whether macrophages in AAA tissue exhibit a stronger M1 or M2 phenotype and altering the M1/M2 balance are being explored. Understanding and addressing the imbalances in the immune system associated with AAA offer new and exciting translational strategies.

It is difficult to determine the cause of AAA in humans because of the chronicity of the disease and the problem of obtaining specimens at different stages of the disease. Therefore, investigators have attempted to make use of animal models with similarities to the human disease. Validations of these animal models must be performed by correlating these studies with human tissue obtained from patients with end stage disease. Correlating data obtained from murine models to human AAA tissue has yet to yield useful therapeutic inflammation to date. Therefore, development of humanized animal models may help to alleviate some of the differences seen in the human and mouse disease process. Opportunities to obtain human AAA tissue are becoming more limited because of the transition from open to endovascular aneurysm repair. Clinical trials must develop protocols to examine as many parameters of the disease as possible, including serially obtaining precise imaging and circulating biomarkers. By correlating biomarkers with changes in aneurysm shape and size, it may be possible to develop a greater understanding of which bioactive molecules and cell types promote aneurysm growth.

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Disclosures

None.

References

7. Lederle FA, Johnson GR, Wilson SE, Chute EP, Hye RJ, Makaroun MS, Barone GW, Bandyk D, Moneta GL, Makhoul RG. The aneurysm detection and management study screening program: validation cohort

Table 4. Macrophage Differentiation in Human and Experimental AAA

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human AAA</td>
<td>Increased⁶³,⁶⁴,⁶⁵</td>
<td>Increased⁷,⁷⁶</td>
</tr>
<tr>
<td>ApoE−/− AngII model</td>
<td>Increased⁹²</td>
<td>Increased⁹³</td>
</tr>
<tr>
<td>CaCl₂ model</td>
<td>Increased²⁶,³³,⁴⁰</td>
<td>Undefined</td>
</tr>
<tr>
<td>Elastase-perfusion model</td>
<td>Increased⁹²</td>
<td>Undefined</td>
</tr>
</tbody>
</table>

AAA indicates abdominal aortic aneurysm; and AngII, angiotensin II.


This review of the immune system in relation to experimental and human abdominal aortic aneurysms is timely considering the burgeoning literature in this area. Abdominal aortic aneurysm is now recognized as an inflammatory disorder and there is an enormous interest in translation of new discoveries into medical therapies for small abdominal aortic aneurysms. Interpretation of these data will require a thorough understanding of the immune aspects of this disease. As such, we feel the article should be of interest to a broad readership, including clinicians, clinician scientists, and scientists studying and treating aneurysmal disease.

**Significance**
Inflammatory Cell Phenotypes in AAAs: Their Role and Potential as Targets for Therapy
Matthew A. Dale, Melissa K. Ruhlman and B. Timothy Baxter

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