

Artery Tertiary Lymphoid Organs Contribute to Innate and Adaptive Immune Responses in Advanced Mouse Atherosclerosis

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Inflammation and Immunity in Arterial Diseases: Players and Layers

B Cells and Humoral Immunity in Atherosclerosis [*Circ Res.* 2014;114:1743–1756]

Mechanisms That Regulate Macrophage Burden in Atherosclerosis [*Circ Res.* 2014;114:1757–1771]

Artery Tertiary Lymphoid Organs Contribute to Innate and Adaptive Immune Responses in Advanced Mouse Atherosclerosis

The Arterial Media and Mechanisms of Immunoprivilege

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Sarajo Kumar Mohanta,* Changjun Yin,* Li Peng, Prasad Srikakulapu, Vineela Bontha, Desheng Hu, Falk Weih, Christian Weber, Norbert Gerdes,* Andreas J.R. Habenicht*

Abstract: Tertiary lymphoid organs emerge in tissues in response to nonresolving inflammation. Recent research characterized artery tertiary lymphoid organs in the aorta adventitia of aged apolipoprotein E-deficient mice. The atherosclerosis-associated lymphocyte aggregates are organized into distinct compartments, including separate T-cell areas harboring conventional, monocyte-derived, lymphoid, and plasmacytoid dendritic cells, as well as activated T-cell effectors and memory cells; B-cell follicles containing follicular dendritic cells in activated germinal centers; and peripheral niches of plasma cells. Artery tertiary lymphoid organs show marked neoangiogenesis, aberrant lymphangiogenesis, and extensive induction of high endothelial venules. Moreover, newly formed lymph node-like conduits connect the external lamina with high endothelial venules in T-cell areas and also extend into germinal centers. Mouse artery tertiary lymphoid organs recruit large numbers of naïve T cells and harbor lymphocyte subsets with opposing activities, including CD4⁺ and CD8⁺ effector and memory T cells, natural and induced CD4⁺ regulatory T cells, and memory B cells at different stages of differentiation. These data suggest that artery tertiary lymphoid organs participate in primary immune responses and organize T- and B-cell autoimmune responses in advanced atherosclerosis. In this review, we discuss the novel concept that pro- and antiatherogenic immune responses toward unknown arterial wall-derived autoantigens may be organized by artery tertiary lymphoid organs and that disruption of the balance between pro- and antiatherogenic immune cell subsets may trigger clinically overt atherosclerosis. (*Circ Res.* 2014;114:1772-1787.)

Key Words: adventitia ■ aging ■ atherosclerosis ■ autoimmune response

The lamina adventitia, which is the connective tissue surrounding arteries, has received little attention in mainstream atherosclerosis research. This review discusses adventitial

artery tertiary lymphoid organs (ATLOs) in aged apolipoprotein E-deficient (*ApoE*^{−/−}) mice and their potential role in human atherosclerosis immunity. Ever since the detection of T

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Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndrome
Apoe^{-/-}	apolipoprotein E-deficient
ATLO	artery tertiary lymphoid organ
cDC	conventional dendritic cell
CIA	collagen-induced arthritis
LDL	low-density lipoprotein
LN	lymph node
MS	multiple sclerosis
RA	rheumatoid arthritis
SLO	secondary lymphoid organ

cells in atherosclerotic plaques ≥ 2 decades ago initiated the era of research on adaptive immune responses in atherosclerosis by Jonasson et al,¹ it was generally assumed that the immune system responds to arterial wall inflammation either in intima plaques or systemically in secondary lymphoid organs (SLOs), such as lymph nodes (LNs) and spleen.²⁻⁶ However, the recent characterization of ATLOs in the aortic adventitia of *Apoe^{-/-}* mice⁷ calls for re-examination of these notions: results of our studies in aged mice afflicted with advanced atherosclerosis, and those of others in humans lead us to propose a paradigm change away from plaque- or SLO-triggered atherosclerosis immune responses to those carried out in the adventitia. This proposition is corroborated by our recent delineation of immune cell subsets in ATLOs, of ATLO's structures, the strict territoriality of ATLOs adjacent to plaques, the observation that ATLO stages and sizes correlate with disease severity, and the association of ATLO neogenesis with age. Similar to SLOs, ATLOs harbor all immune cell subsets to conduct key steps of primary immune responses.⁷ ATLOs are organized into distinct compartments, including separate T-cell areas; conventional dendritic cells (cDCs), monocyte-derived DCs, lymphoid DCs, and plasmacytoid DCs; B-cell follicles containing proliferating B centroblasts, centrocytes, and mantle zone B cells, as well as follicular DCs in activated germinal centers; and peripheral niches of plasma cells.⁷ ATLOs show neoangiogenesis, aberrant lymphangiogenesis, and extensive induction of high endothelial venules.⁷ Moreover, newly formed ATLO conduits that strikingly resemble LN- and spleen conduits connect the external lamina with T-cell areas, extend into germinal centers, and transport small molecular weight molecules.⁷ Here, we discuss the cellular and structural similarities and differences of ATLOs with SLOs and compare them with TLOs found in other chronic inflammatory diseases with a focus on bona fide human autoimmune diseases. Moreover, we consider the possibility that the characteristics of T- and B-cell responses in ATLOs may signify autoimmune reactions against unknown arterial wall-derived autoantigens in atherosclerosis. In light of the observation that ATLO-like aggregates also arise in the adventitia adjacent to atherosclerotic plaques of patients afflicted with atherosclerosis abdominal aortic aneurysm,^{8,9} the potential significance of the findings in ATLOs of *Apoe^{-/-}* mice for human atherosclerosis is considered. Finally, we introduce the concept that dichotomic, that is, both pro- and antiatherogenic, immune responses toward arterial wall-derived autoantigens may be organized by ATLOs,

and that disruption of the balance between these immune cell subsets may precipitate clinically overt atherosclerosis, including the acute coronary syndrome (ACS).

Players of Adaptive Immunity in Atherosclerosis

Since the discovery of T cells in atherosclerotic plaques,¹⁰ our understanding of adaptive immune cell lineages and subsets that contribute to atherosclerosis progression has made considerable progress. We will briefly describe innate and adaptive immune cells that participate in acute versus unresolvable inflammatory tissue reactions and emphasize the view that atherosclerosis represents a prototypic unresolvable inflammatory disease associated with mixed innate and adaptive immune reactions (Figure 1). Because the roles of innate immune cells for atherogenesis have previously been extensively reviewed, the current review will focus on adaptive immune cells that have been identified in SLOs to carry out primary antigen-specific immune responses and that have also been observed in ATLOs (Figures 2–4). The concept of dichotomically acting lymphocyte subsets that are presumed to maintain a well-tuned balance between proatherogenic (proinflammatory) and antiatherogenic (tolerogenic or immunosuppressive) subsets will be discussed. Interestingly, the phenotypes of lymphocyte subsets that may play major roles in TLOs and that participate in autoimmune diseases, such as multiple sclerosis (MS), are strikingly similar to those identified in ATLOs. We further stress the importance to study the aging/senescent immune system, the aging arterial wall, and the potential significance of peripheral programming and imprinting of T-cell subsets within the diseased arterial wall to understand atherosclerosis immunity better. Finally, we will propose that clinically apparent disease, such as the ACS, may be the result of a multistep process that may culminate in an acute impairment of the functionality of tolerogenic lymphocytes (Figure 5).

TLOs Arise in Response to Chronic, Nonresolving Inflammation of Peripheral Tissues in Adult Organisms

The immune system aims to identify and eliminate foreign antigen, while preserving self.¹¹⁻¹⁷ To accomplish this task, it uses highly plastic and diverse innate and adaptive immune cell subsets. A central tenet of immunity is that primary immune responses are carried out in SLOs, such as LNs, Peyer patches, and spleen, although the possibility that TLOs are also capable of activating naïve lymphocytes in chronic disease states has recently gained attention.^{7,11-15,18-22} After their recruitment to sites where antigen has been deposited, immune cells and the mesenchymal cells of the target tissue generate DC- and lymphocyte-activating cytokines. This inflammatory tissue environment is sensed by sentinel cDCs that initiate the primary immune response.²³ Tissue inflammation rapidly activates the DCs promoting efficient antigen-uptake, processing and presentation as antigen-peptide complexes in the context of surface major histocompatibility complex class-II molecules. Concomitantly, migration of the activated antigen-loaded DCs to the draining SLO T-cell areas is initiated. The overall outcome of these events is

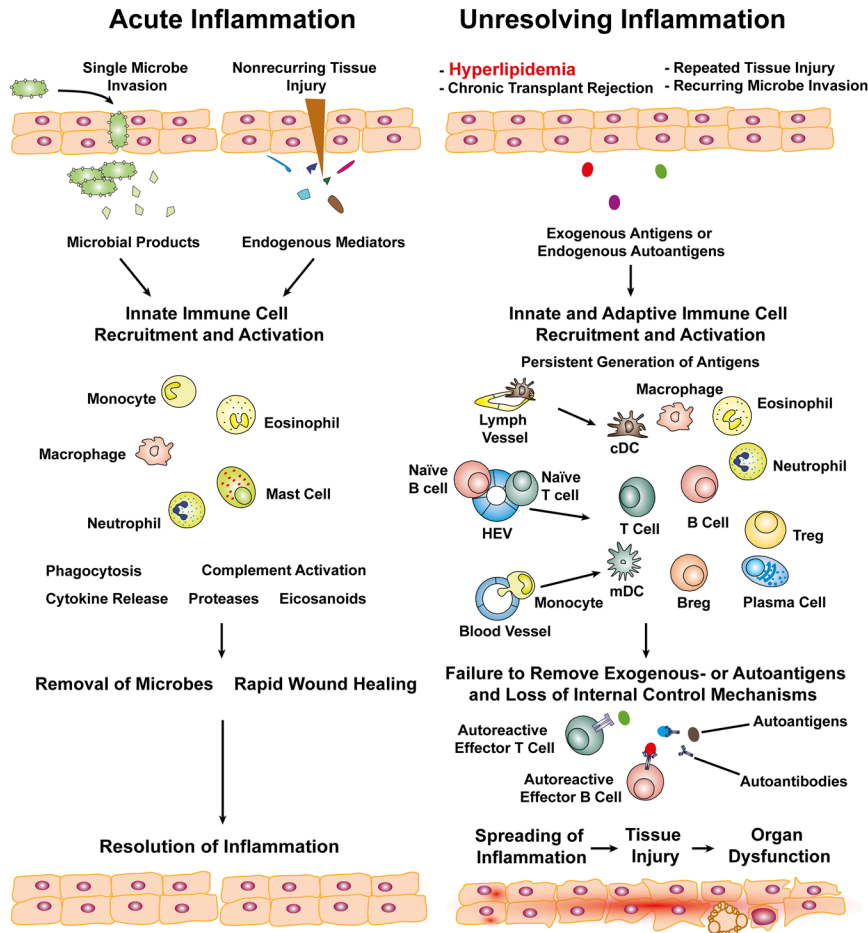


Figure 1. Chronic inflammatory diseases initiate mixed innate and adaptive immune responses and may cause tissue injury and organ dysfunction. Hyperlipidemia, repeated tissue traumata, chronic transplant rejection, and recurring microbe invasion cause vigorous mixed innate and adaptive immune responses, whereas single microbe invasion or nonrecurring tissue traumata cause a predominant innate immune response. TLOs (tertiary lymphoid organs) may form when autoantigens are persistently released from injured tissues. The failure to remove antigens may exhaust anti-inflammatory immune cells leading to hyperactivated, autoimmune B and T cells, which in turn can trigger a vicious circle of inflammation, tissue injury, and organ dysfunction. In addition to adaptive immune cells, activated innate immune cells may participate in tissue injury in nonresolving inflammation by persistent generation of endogenous proinflammatory mediators and destructive effector function. Breg indicates regulatory B cell; cDC, conventional dendritic cell; HEV, high endothelial venule; mDC, monocyte-derived DC; and Treg, regulatory T cell.

striking: Antigens are presented by DCs for extended periods of time to the continuously recirculating naïve T cells in SLO T-cell areas; the DC-epitope/cognate T-cell receptor interaction culminates in vigorous production of T-cell-activating chemokines and cytokines. This first step of priming is followed by initiation of T-cell proliferation during which functional avidity maturation of the T cell proceeds.^{24–26} Finally, imprinting events conclude the generation of unique tissue-specific T-cell effectors that have the ability to home into the inflamed target specifically.²⁷ In contrast, DC/T-cell interactions with noncognate T-cell receptors are short-lived, lasting only seconds followed by rapid emigration from SLOs into the blood stream.^{23,28–31} Given the structural and cellular similarities of SLOs and TLOs, it is conceivable that both lymphoid tissues have similar roles in conducting primary immune responses although direct evidence for this notion has yet to be obtained. Considering the scarcity of information on the effects of TLO-mediated immune responses, substantial work will be required to establish both the similarities and the dissimilarities of SLOs versus TLOs in disease affecting immune responses.³²

Differences Between SLOs and TLOs

A major difference in immune responses carried out in SLOs and those carried out in TLOs, however, is that immune responses in the latter involve a much larger component of innate immune cells and—in particular—macrophages.^{33,34}

The important question of whether ATLOs are capable of conducting primary immune responses deserves attention because clarification of this issue may reveal mechanisms of atherosclerosis autoimmunity as previously shown for experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice and MS and rheumatoid arthritis (RA) in humans.³⁴ To protect the host from injury, immune cells together with target tissue-derived parenchymal cells use a series of amazingly well-organized strategies: they constantly attempt to equilibrate the actions of hematopoietic and nonhematopoietic cells to achieve a finely tuned balance between destruction and protection by producing anti-inflammatory and immune-suppressing cells, such as regulatory T cells.^{35,36} As long as this balance is efficiently maintained, organ damage remains limited, antigen removal may proceed, and resolution of inflammation and wound healing may succeed.³⁷ After the naïve T-cell repertoire is established in the thymus, the adaptive immune system undergoes further developmental steps in SLOs and peripheral tissues. These are governed by territorialized cytokine-driven differentiation and transdifferentiation pathways shaping and programming highly specialized immune cell phenotypes in peripheral tissues in response to antigen and inflammatory cues. However, the immune system may be overwhelmed and become exhausted when the antigen load proceeds unimpaird,³⁸ such as in chronic infection, during graft rejection, in certain types of cancer, in autoimmune

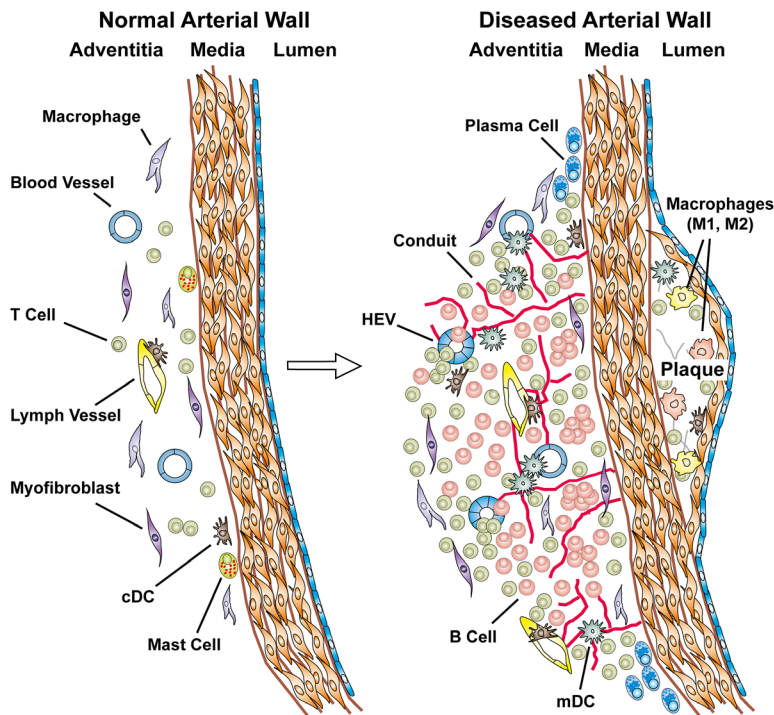


Figure 2. Atherosclerotic arteries harbor distinct immune cell infiltrates in plaques and adventitia. The adventitia of the normal arterial wall constitutively contains vasa vasora, lymph vessels, tissue macrophages, few T cells, mast cells, conventional dendritic cell (cDCs), and myofibroblasts. In response to transmural arterial wall inflammation in early stages of atherosclerosis, single T cells begin to infiltrate the adventitia. During disease progression, extensive reorganization of adventitia segments adjacent to atherosclerotic plaques can be observed in the abdominal aorta of *Apoe*^{-/-} mice resulting in several forms of ATLOs (artery tertiary lymphoid organs). Reorganization of the adventitia includes both the connective tissue-derived cells, such as myofibroblasts and neogenesis of lymph vessels, angiogenesis, conduit formation, and high endothelial venule (HEV) formation. Concomitantly, monocytes are recruited through blood vessels, cDCs are recruited through lymph vessels, and naïve T and B cells are recruited through newly formed HEVs. Moreover, survival niches are populated by plasma cells. In contrast to the adventitia, advanced atherosclerotic plaques are comparably acellular and show a limited set of adaptive immune cells. mDC indicates monocyte-derived DC.

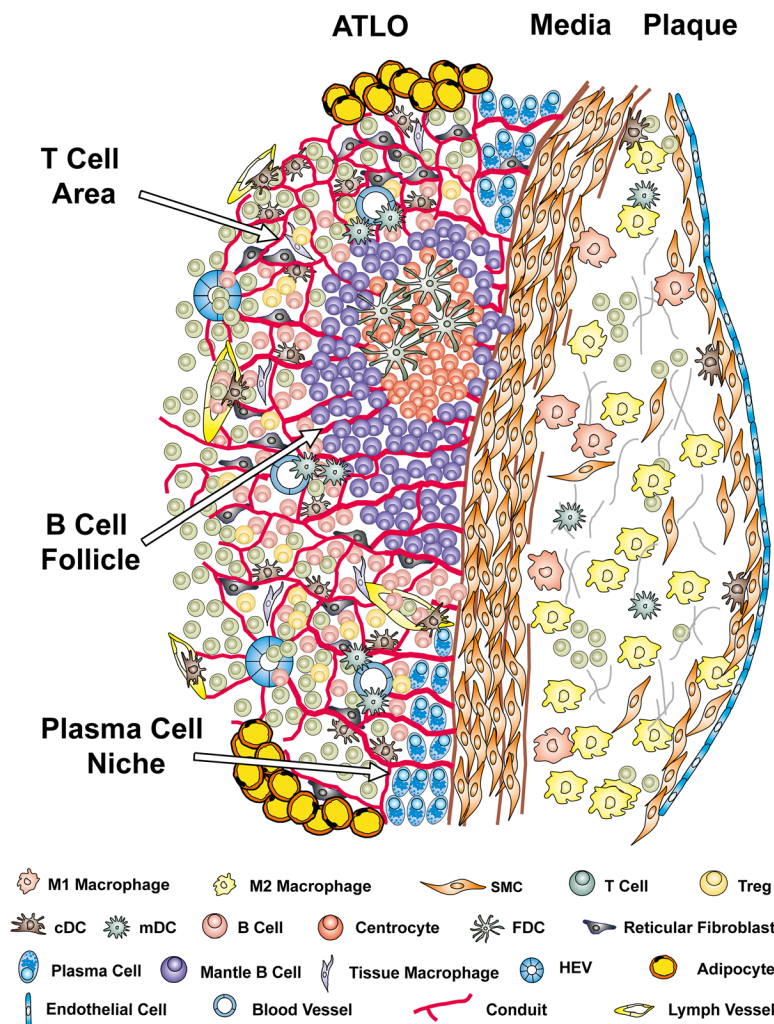


Figure 3. Well-structured artery tertiary lymphoid organs (ATLOs) arise adjacent to advanced atherosclerotic plaques in the abdominal aorta of aged *Apoe*^{-/-} mice. Cellularity, structures, and the territoriality of ATLO neogenesis indicate comprehensive T- and B-cell responses toward unknown arterial wall-derived autoantigens although these responses seem to maintain a balance between pro- and anti-inflammatory participants of both innate and adaptive immunity. Advanced ATLO stages are characterized by separate T-cell areas, activated B-cell follicles, and plasma cell niches in the periphery. Autoantigen presentation is indicated by the presence of follicular dendritic cells (FDCs) in activated germinal centers; the abundance of cDCs and monocyte-derived DCs (mDCs) in T-cell areas; B-cell affinity maturation is indicated by multiple centroblasts and their progeny in B-cell follicles; a balance between pro- and anti-inflammatory and T cells is indicated by multiple effector T cells and regulatory T cells (Tregs); newly formed conduits may maintain chemokine gradients and possibly guide autoantigen diffusion from the diseased arterial wall toward ATLO antigen-presenting cells. HEV indicates high endothelial venule; and SMC, smooth muscle cell.

and autoimmune-related diseases, and possibly in atherosclerosis (Table). Under such conditions, TLO neogenesis is induced in the connective tissue surrounding or within the diseased tissue, such as in the synovial tissue in RA, the meninges in MS, the adventitia in atherosclerosis (Figure 2), and the parenchyma of the thyroid gland in Graves' disease (Table). Translation of findings in experimental models and those observed in animal models of atherosclerosis to human diseases remains a major challenge.^{38,39}

Cellularity and Territoriality of Immune Cell Interactions and the Inflammatory Infiltrate of ATLOs and Atherosclerotic Plaques

Most chronic inflammatory and autoimmune diseases show a mixed innate and adaptive immune cell infiltrate with a large monocyte/macrophage component.⁴¹ Likewise, early atherosclerotic plaques harbor substantial numbers of monocytes/macrophage/foam cells in addition to a mixed, activated CD4⁺ and CD8⁺ lymphocyte infiltrate, cDCs, newly immigrated, induced monocyte-derived DCs, and a CCL17⁺ DC subtype that restrains regulatory T-cell homeostasis.^{6,7,87–93} When the immune cell composition of SLOs and ATLOs are compared with that of atherosclerotic plaques, major differences become apparent: Plaques have a rather limited set of immune cells (Figures 2 and 3). In addition, lymphocyte recirculation of adoptively transferred T cells is dramatically higher in ATLOs or SLOs when compared with plaques.⁷ Neoangiogenesis is a feature of advanced plaques, including those in aged hyperlipidemic mice, but we were unable to identify lymph vessels, high endothelial venules, or LN-like conduits in plaques. This lack of structural constituents that are required to organize effective lymphocyte and DC recirculation in SLOs raises the question whether an atherosclerosis-specific primary immune response can be efficiently conducted in the diseased intima. Evidence indicates that the phenotypes of innate immune cells in atherosclerosis are generated after their transendothelial migration into the intima of arteries of humans or mice.^{2,18,33,90} Like pro- or anti-inflammatory monocyte/macrophages, the adaptive immune system generates antigen-specific T- and B-cell effectors and their equally powerful tolerogenic antigen-specific regulatory T cells and their regulatory B-cell counterparts although atherosclerotic plaques are largely devoid of B cells. However, it is conceivable that both innate and adaptive immune cells in plaques may play critical roles during early stages of the disease and that adventitial immune cells may control atherosclerosis immunity during aging when the disease becomes more advanced and clinically apparent, such as in ACS. Thus, there is reason to think that the functional effect of immune cells in plaques versus that in the adventitia may switch from plaques to the adventitia during disease progression. In addition, the aging/senescent immune system deserves attention because it may affect both plaque and ATLO cellularity and the activation state of individual lymphocyte subsets. In RA, the evidence is robust that B-cell proliferation is associated with affinity maturation within the RA-associated TLOs in the synovial membrane or in salivary glands of patients afflicted with Sjögren syndrome,^{47,94} raising the possibility that

other forms of TLOs, including ATLOs, are also capable of mounting a primary B-cell response. If antigen persists beyond the critical time window of 12 to 24 hours, T-cell priming is initiated in LNs.^{23,34,95} Disturbance of a variety of factors—notably breakdown of tolerance—leads to tissue destruction and autoimmune injury.^{37,96} Thus, how tissue inflammation prompts the adaptive immune system to organize T- and B-cell immune responses through danger signal-activated antigen-presenting cells and how tissue inflammation gets out of control are areas of major interest to understand principles of adaptive immunity, autoimmunity, and atherosclerosis.^{16–18,22,38,39,87,96–102}

Does ATLO Neogenesis Imply an Atherosclerosis-Specific Autoantigen?

There are 4 major human disease conditions in which TLOs have been observed and each of them can be associated with and seem to require antigen: microbe infection; aberrant nonself highly antigenic tumor-antigen in certain types of cancer; in organ transplantation when epitopes on major histocompatibility complex/human leukocyte antigen complex molecules, that is, the transplantation antigens, are recognized by the immune system as nonself; and in autoimmune disease (Table). These data raise the fundamental question whether the formation of ATLOs implies and requires ≥ 1 atherosclerosis-specific autoantigen(s) or whether the immune system in atherosclerosis is activated in the absence of a disease-causing antigen? Unfortunately, the answer to this crucial question is presently not available for several reasons: many chronic inflammatory diseases, including atherosclerosis, show ≥ 1 components of autoreactivity, such as autoreactive antibodies and autoreactive T cells that may, however, be irrelevant for disease progression (Table). TLO research in human and in mouse chronic inflammatory diseases is at an early stage and experimental models to examine whether TLO formation requires antigen have not been established.^{2,103,104} However, one may argue that the presence of follicular DCs and proliferating B cells in germinal centers; and proliferating T lymphocytes in T-cell areas of ATLOs indicates the presence of an atherosclerosis-specific autoantigen (Figures 1–4). ATLO formation in atherosclerosis displays striking similarities with TLO formation in autoimmune diseases.^{22,105} The mechanisms underlying TLO neogenesis are in many respects similar to those identified in SLO development during ontogeny although TLO neogenesis requires a chronic inflammatory tissue reaction. In this regard, it is important to note that T-cell effector lymphocytes can be generated *in vitro* in distinct cytokine environments in the absence of antigen.^{28–30} Therefore, the important question whether ATLO neogenesis implies the local production of an autoantigen and whether such autoantigen is a precondition for ATLO formation remains unanswered (Figure 4). The immune system uses diverse strategies to detect and scavenge particulate or soluble antigens and initiate TLO neogenesis. DCs constantly patrol peripheral tissues as sentinels tracking down antigen-derived danger signals to carry antigen to SLOs and to initiate T-cell responses,¹⁰⁶ whereas LN or spleen follicular DCs bind soluble antigens

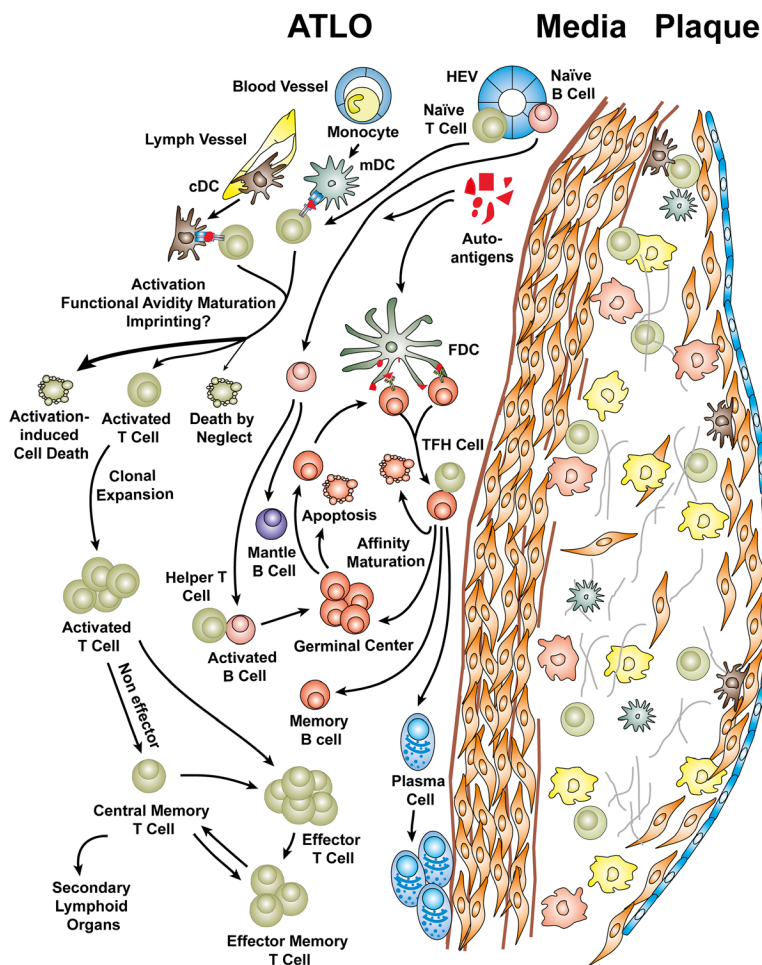


Figure 4. Hypothetical choreography of balanced T and B lymphocyte-mediated, autoantigen-specific autoimmune reactions in artery tertiary lymphoid organs (ATLOs). The structural and cellular organization of ATLOs and our unpublished data about recruitment, priming, and generation of T-cell effectors, as well as the identification of various B-cell subtypes, indicate both primary autoantigen-specific T- and B-cell responses. After recruitment of blood monocytes through vasa vasorum and their subsequent differentiation into monocyte-derived dendritic cells (mDCs), of naïve T and B cells through high endothelial venules (HEVs), and of conventional DCs (cDCs) through lymph vessels, autoantigen(s) is (are) presented by cDCs and mDCs to T cells and by follicular DCs to B cells, respectively. Priming of cognate naïve T cells may then result in activation,²⁶ functional avidity maturation,^{24,25} imprinting,²⁷ and clonal expansion of antigen-specific T-cell clones, which differentiate into effector T cells, effector memory T cells, and central memory T cells^{28,31}; other T cells that do not interact with DCs may return into the circulation; some DC-activated T cells may undergo either death by neglect or activation-induced cell death caused by overwhelming antigen exposure. Central memory T cells may leave ATLOs to home to secondary lymphoid organs (SLOs). Priming of cognate naïve B cells, with the help of either conventional helper T cells or T follicular helper cells, undergo activation and subsequently multiple rounds of proliferation during which multiple events of somatic hypermutation during affinity maturation of their variable immunoglobulin light chain genes occurs in germinal center reactions resulting in the generation of memory B cells or plasma cells. Some plasma cells may emigrate from ATLOs and home into the bone marrow, whereas memory B cells may home into SLOs and the bone marrow. The regulatory immune cell subsets are not depicted for ease of reading (Figure 5). FDC indicates follicular DC; and TFH, T follicular helper cells (functional effect on atherosclerosis controversial).

as immune complexes to initiate and organize B-cell affinity maturation in activated germinal centers. What, then, may be the advantage of TLO neogenesis within the target

organ of autoimmune responses versus antigen presentation in SLOs? First, low-grade chronic tissue inflammation may release only small amounts of autoantigen. However,

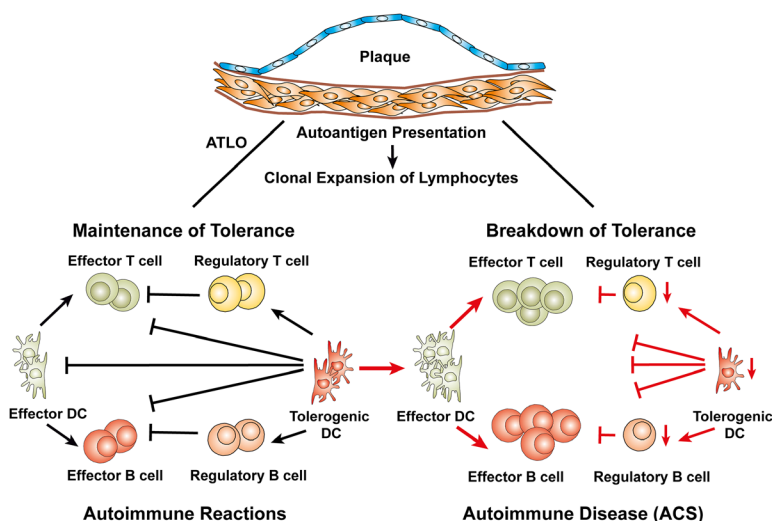


Figure 5. Hypothetical multistep development of unstable plaques including acute coronary syndrome (ACS) and aneurysm as manifestations of tolerance breakdown in autoimmune atherosclerosis. Multiple lines of evidence indicate that initiation of clinically relevant autoimmune disease requires breakdown of peripheral tolerance, including disturbances in the germinal center reaction. Although breakdown of tolerance is multifaceted and description of its mechanisms go beyond the scope of this review, a systemic activation of the immune system, such as a bacterial infection associated with enhanced circulating lipopolysaccharide, may contribute. As a result of peripheral tolerance breakdown, the balance of tolerogenic and effector lymphocytes in artery tertiary lymphoid organ (ATLO) or in the diseased tissue, such as the atherosclerotic plaque, may be disturbed. Atherosclerosis-imprinted effector T cells may home rapidly into the stable plaque and may activate quiescent macrophages, which will release a series of extracellular mediators. In addition, autoimmune B cells may be activated to produce autoimmune antibodies in germinal centers.¹¹³ These events may yield unstable plaques, plaque rupture, thrombosis, and tissue infarct. DC indicates dendritic cell.

Table. TLOs in Human Chronic Inflammatory Diseases

Disease	Target Tissue	Disease-Causing and Disease-Suppressing Antigen/in Brackets: No Evidence for a Disease-Causing Role	References
Autoimmune and autoimmune-related diseases			
Rheumatoid arthritis	Joint and synovial tissue	Not determined (rheumatoid factors and antibodies against citrullinated peptides)	40
Multiple sclerosis	Brain and meninges	Not determined (autoantibodies and autoreactive T cells against myelin and neuronal antigens, heat shock proteins)	34,41–43
Myasthenia gravis	Thymus	Nicotinic acetylcholine receptor	44,45
Hashimoto thyroiditis	Thyroid gland	Autoantibodies against thyroglobulin and thyroid peroxidase	46
Graves disease	Thyroid gland	Autoantibodies against thyroid stimulating hormone receptor, sodium iodide symporter, thyroglobulin, and thyroid peroxidase	46
Sjögren syndrome	Salivary gland	Not determined (rheumatoid factors, autoantibodies against SS-A/Ro, SS-B/La, α -fodrin, and salivary gland protein)	47–50
Systemic lupus erythematosus	Kidney and glomeruli, tubulointerstitium	Not determined (antibodies against Smith antigen and nuclear ribonucleoprotein)	51,52
Primary sclerosing cholangitis	Liver and bile duct	Not determined (antibodies against cardiolipin, lysosomal-associated membrane protein, catalase, bactericidal/permeability increasing protein, and cathepsin)	53,54
Crohn disease	Entire intestinal tract and lamina propria	Not determined (antibodies against pancreatic glycoprotein 2 and <i>Saccharomyces cerevisiae</i>)	55–59
Ulcerative colitis	Colon and lamina propria	Not determined (antibodies against neutrophil cytoplasmic antigen, high mobility group box 1 and box 2, and tropomyosin 5)	55,56,58,60,61
Chronic obstructive pulmonary disease	Lung and interstitium; peribronchial	Not determined	62,63
Chronic hypersensitivity pneumonitis	Lung and interstitium; peribronchial	Not determined	64
Idiopathic pulmonary fibrosis	Lung and interstitium; perialveolar	Not determined	65,66
Idiopathic pulmonary arterial hypertension	Lung and perivascular	Not determined (autoantibodies against glucose-6-phosphate-dehydrogenase; calumenin, heat shock proteins 27 and 70; lamin A/C; and tubulin β chain)	67–70
Disease with autoimmune component			
Atherosclerosis	Artery, adventitia; intima, and media	Not determined (autoantibodies against oxLDL, heat shock protein, and autoreactive T cells against heat shock protein 60)	9,71
Infectious disease			
Chronic hepatitis C	Liver and intrahepatic portal area	Not determined (autoantibodies against C-reactive protein and smooth muscle actin)	72,73
<i>Helicobacter pylori</i>	Stomach and gastric mucosa	Not determined (autoantibodies against mycobacterial heat shock protein)	74–76
Chronic Lyme disease	Joint and synovial tissue	Not determined	77
Transplant rejection			
Kidney failure	Kidney and intragraft tissues	Not determined (autoantibodies against insulin, cardiolipin, and nuclear antigen)	78
Lung allograft dysfunction	Lung and peribronchiolovascular area	Not determined (autoantibodies against κ - α -1-tubulin and collagen type V)	79–81
Cancer			
Mammary ductal carcinoma	Breast, milk duct, and medulla	Not determined (autoantibodies against tumor-associated tissue antigen)	82,83
Non-small-cell lung cancer	Lung and stroma	Not determined (autoantibodies against tumor-associated tissue antigen)	84–86

oxLDL indicates oxidized low-density lipoprotein; and SS, Sjögren syndrome.

in TLOs, autoantigen can be presented within close proximity to its generation avoiding dilution thereby lowering the antigen threshold to trigger an adaptive T-cell response. Second, the cytokine environment of TLOs may not only stimulate the tissue-resident cDC sentinels but also recruit blood monocytes and generate fully effective monocyte-derived DCs in situ.^{87,88,107} Third, immune complexes with unprocessed antigen can gain access to TLOs and bind to follicular DCs within germinal centers at higher concentrations when compared with follicular DCs of the more distant germinal centers of SLOs.^{19,47,105,107–113} Fourth, TLOs may be shielded from the centralized immune system because they may mount primary immune responses within territorialized tissue environments, whereas LNs and spleen drain large terrains (Figure 4). Fifth, antigen-specific T and B lymphocytes generated in TLOs may not only be activated, proliferate, and undergo avidity maturation, and affinity maturation, respectively, but also receive so-called imprinting signals to home specifically to the target tissue that originally accommodated the antigen during the primary immune response.^{28,30} Sixth, TLO neogenesis may be reversible after successful antigen eradication.

Functional Effects of TLOs Have Not Been Defined, Yet the Presence of ATLOs Raises New Questions About Atherosclerosis Immunity

Permissive conditions for SLO and TLO formation arise in connective tissues when mesenchymal lymphoid tissue organizer cells interact with immune cells termed lymphoid tissue inducer cells.^{21,114} Such conditions are met either at predetermined sites during ontogenesis of LNs, spleen, and gut-associated lymphoid tissues or at diverse locations in adult organisms during TLO neogenesis.¹³ It has been suggested that lymphoid tissue organizer cells can be generated in situ when lymphorganogenic cytokines, such as CXCL13 or CCL21, are produced: We observed that murine aortic smooth muscle cells in vitro acquire a lymphoid tissue organizer-like phenotype under distinct culture conditions.¹¹⁴ What has prevented to date a clear definition of the functional effect of TLOs in any autoimmune disease? There is ample evidence that breakdown of tolerance is required to convert clinically silent autoimmune reactivity to autoimmune disease, and this breakdown of tolerance may not occur during TLO formation per se. Importantly, breakdown of tolerance primarily occurs in the periphery.^{17,115} Under conditions of tolerance breakdown, identification of the triggers of lymphocyte activation, their emigration from SLOs or TLOs, and mechanisms of lymphocyte homing to attack the antigen-specific targets in human disease remains important issues of atherosclerosis research.^{10,18,95,116} Interestingly, ATLO formation strongly correlates with the size of atherosclerotic plaques in the abdominal aorta adventitia of aged *Apoe*^{-/-} mice. Thus, the initiation of autoimmune disease is the result of a multistep process in which TLO neogenesis seems required but not sufficient for disease initiation: Additional events, including toll-like receptor activation and breakdown of tissue barriers such as the blood–brain barrier in MS (Figure 5), are needed to trigger overt autoimmune disease. To facilitate local adaptive immune responses, TLOs generate and assemble conduits, high endothelial venules, blood vessels, and lymph

vessels to boost T- and B-cell recruitment and to promote their movement within T-cell areas or B-cell follicles. We proposed that these structures enhance the probability for T-cell receptor- or B-cell receptor-carrying lymphocytes to find their cognate autoantigen close to its generation.²² Although there is evidence from human autoimmune diseases indicating that TLOs mount specific T- and B-cell immune responses toward self-antigens,^{12,13,32,117} major issues of their formation and functional effect on disease progression remain to be explored: (1) What are the mechanisms leading to ATLO neogenesis and are these restricted to the atherosclerotic lesion and the adjacent media? (2) How do innate immune cells, that is, activated monocytes/macrophages, neutrophils, mast cells, and DCs, prompt adaptive immune responses within the target tissue's TLOs?³⁴ (3) Is atherosclerosis autoimmunity a late event after ATLOs have formed?^{108,109} (4) How does overt autoimmune disease arise from clinically quiescent autoimmune reactivity?⁹⁵ (5) Are immune responses in TLOs and SLOs distinct?¹¹⁸ (6) What is the relative share of autoreactive T and B cells versus innate immune cells to direct tissue destruction? (7) How is the apparent equilibrium between effector and tolerogenic arms of the immune system disturbed during disease progression?^{36,97,109,119} Answers to these questions will be crucial to develop immune-based therapies to treat autoimmune disorders and atherosclerosis (Figures 4 and 5).

Dissecting Inflammation, Adaptive Immune Responses, Autoimmune Reactions, and Autoimmune Disease in Atherosclerosis

Only few human autoimmune diseases, that is, Hashimoto thyroiditis, Graves disease, and myasthenia gravis, meet direct criteria to qualify as an autoimmune disease: isolation of a pathogenic autoantigen, isolation of a B-cell clone with exquisite specificity for autoantigen, demonstration that B-cell clones produce pathogenic autoantibodies in experimental transfer experiments and T cells responding to pathogenic autoantigen in an autologous mixed lymphocyte reaction, isolation of T cells carrying a T-cell receptor with specificity for autoantigens, and cloning of pathogenic T cells able to transfer disease to another individual.^{99,120–123} However, in addition, these diseases produce autoantibodies to other tissue-specific autoantigens whose functional roles remain to be demonstrated (Table). In our view, it is important to distinguish autoimmune reactivity and autoimmune disease in atherosclerosis strictly because the former may not be clinically relevant, whereas the latter may very well be. Moreover, direct autoimmune disease criteria (the so-called Witebsky criteria) are not yet met for atherosclerosis (see above and below). For example, clinical studies on MS show that many autoimmune antibodies and antigen-specific self-reactive T cells may not be disease-relevant indicating that the search for disease-causing autoantigens is similar to finding a needle in the haystack.^{16,22,34,95,112,117,121,124} Thus, it is not surprising that, despite considerable efforts, direct evidence for autoimmune-triggered organ damage by self-reactive lymphocytes in majority of clinically important human autoimmune diseases or autoimmune-related diseases, that is, RA, diabetes mellitus type I, MS, and atherosclerosis, has not been obtained.^{99,107,117,121,124} The view that atherosclerosis is associated with autoimmune

responses^{4,71,89} is largely based on evidence, such as the presence of autoantibodies directed against autoantigens such as heat shock proteins or low density lipoproteins (oxidized or native) and more recently by the demonstration of DC-like cells, including cDCs and monocyte-derived DCs, are present in atherosclerotic plaques.^{3,5,6,88,125,126} However, as noted above, autoreactive T and B cells, as well as autoantibodies, can be detected in a large percentage of the healthy population, and their presence often does not correlate with clinically significant disease. Therefore, the view that human atherosclerosis is caused or affected in its course by ≥ 1 autoimmune responses (T-cell and B-cell autoimmune responses)—although intriguing—requires more direct evidence and considerable efforts. Furthermore, unlike MS,³⁴ diabetes mellitus type I,³² and RA,¹¹ in which immune-based therapies have entered routine clinical practice, similar therapies for atherosclerosis are still lagging although blocking of central proinflammatory mediators (eg, interleukin-1) is currently under clinical investigation.¹²⁷ Why is progress into the autoimmune origin of atherosclerosis and—by the same token—other chronic inflammatory diseases in humans so difficult to achieve? One reason may be that it represents an extremely challenging task to isolate pathogenic autoantibodies for most human diseases, including atherosclerosis, because chronic inflammation is associated with the generation of multiple autoantigens, a phenomenon referred to as epitope spreading.¹²⁸ This makes identification of disease causing as opposed to irrelevant bystander autoantigens a complex task. However, the existence of polyclonal T-cell and antibody responses that may initiate a vicious circle of immune injury and inflammation is supported by a large body of circumstantial evidence in many of these diseases, including atherosclerosis.^{7,12,32,39,96,97,99,115,117,121,124}

ATLOs Are Aberrant Lymphoid Aggregates

TLOs and ATLOs share functional and structural features with SLOs, including separate T-cell areas and B-cell follicles, yet important differences are apparent in addition to those discussed above. Although the aortic adventitia of wild-type and *Apoe*^{-/-} mice contains a network of lymph vessels, the newly formed ATLO lymph vessels in aged *Apoe*^{-/-} mice show aberrant features (Figure 3).⁷ ATLOs contain large numbers of plasma cells, which are rare in LNs or spleen. The origin of these plasma cells in diseased arteries is not known but in analogy to RA they may derive from activated B-cell follicles or—alternatively—they may have migrated into the diseased adventitia using the inflammatory environment as survival niches. A major question about the organization of the B-cell adaptive immune response is the role of follicular DCs in the B-cell follicles. It has been shown that follicular DCs and affinity maturation of B cells require ongoing lymphotoxin β receptor signaling, as well as the presence of antigen.^{94,109} These data raise the important possibility that ATLO follicular DCs present arterial wall-derived autoantigens and that B-2 cells undergo affinity maturation giving rise to memory B cells and plasma cells (Figure 4). The presence of follicular DCs in ATLOs indicates (auto)-antigen in the adventitia of diseased arterial segments. However, it is not possible to conclude that affinity maturation and the appearance of follicular DCs demonstrate the presence of antigen, because extrafollicular affinity maturation has also been demonstrated

in mice. To understand the immune responses in ATLOs better, we used laser capture microdissection-based microarray analyses of ATLOs and compared transcriptomes of ATLOs directly with those of the draining renal LNs.¹²⁹ When ATLOs were compared with the aortic adventitia of wild-type mice, large numbers of immune-regulatory genes were acquired. These ATLO transcriptomes resembled those of SLOs, yet inflammation-regulating genes were expressed at significantly higher levels in ATLOs when compared with LNs.⁷

Territoriality of ATLOs Indicates Highly Localized Immune Reactions Toward Atherosclerosis-Specific Autoantigens in Aged *Apoe*^{-/-} Mice

Circumstantial lines of evidence that support a role of autoimmunity in atherosclerosis include disease-suppressing effects of natural antibodies, oligoclonal T-cell expansion toward potential autoantigens, protective roles of regulatory T cells, pro- and antiatherogenic effects of distinct B-cell subtypes, and inhibition of plaque growth by vaccination.^{3,5,6,33,35,36,98,103,104,116,126,130–136} However, major issues of atherosclerosis immune responses and autoimmunity remain unresolved: (1) Where are adaptive and autoimmune responses organized? (2) Which immune cells participate in arterial wall remodeling during different stages of the disease? (3) Is the adaptive immune response in atherosclerosis systemic or arterial wall specific? (4) Are there periods of heightened immune activation and what are the triggers of relapses and violent immune cell activities in the ACS? (5) What are the contributions of innate immunity carried out by subtypes of blood-derived monocyte/macrophages and foam cells, of DC subtypes, and of B-1 cells? (6) Are there antigen-specific CD4⁺ or CD8⁺ effector T cells that directly target structures of the arterial wall? (7) What causes dysfunction of the balance between effector cells and tolerogenic DCs, regulatory T cells, and regulatory B cells? And, most importantly, (8) What is the nature of the disease-triggering autoantigen(s)?

Relationship Between Single T-Cell Infiltrates in the Adventitia and Their Functional Effect on Atherosclerosis Remains Unknown

It has been widely assumed that T-cell responses are organized either in atherosclerotic plaques or in SLOs.^{4,125,137} Interestingly, the accumulation of leukocytes in the adventitia during atherogenesis was noted decades ago. Small round cell infiltrates were reported in the adventitia of patients afflicted with coronary artery disease.^{22,138} The relationship between single and early adventitial T cells and T-cell aggregates and ATLO formation is presently unclear. However, adventitial T cells seem to be an early event and can be observed throughout the artery tree, including coronary arteries, whereas ATLOs form late at distinct locations with a clear preference in the abdominal aorta. As judged from LN neogenesis during ontogeny, however, it is conceivable that T-cell infiltrates are the precursors and develop into T/B-cell aggregates and thus form what has been termed embryonic LN anlagen and by that token single T/B-cell aggregates in the adventitia

during atherosclerosis development may be termed TLO anlagen that will form ATLOs under to be defined permissive conditions.

ATLO Neogenesis in Mice and in Humans

Considerable work is required to identify ATLOs in human atherosclerosis: It is already apparent from our mouse studies that most atherosclerotic plaques are not associated with adjacent ATLOs. Thus, whether and where ATLOs form in human atherosclerosis remains to be addressed. In this context, it is of interest to note that TLO formation in MS is not observed in the brain parenchyma, but in the perivascular space of meningeal blood vessels. Several studies have examined the adventitia and its potential role in atherogenesis, which has been reviewed repeatedly.^{8,9,91,133,138–147}

T-Cell Density and Immune Cell Infiltrates in the Adventitia and in Plaques Change During Aging

Although early atherosclerosis is associated with significant T-cell infiltrates in the intima, T-cell density in plaques markedly decreases over time, whereas it dramatically increases in the adventitia during aging in mice. Furthermore, B cells which are absent in the normal aorta and in atherosclerotic plaques, form aggregates during intermediate stages of ATLO neogenesis, whereas advanced stages of ATLOs in mice are characterized by large B-cell follicles and ectopic germinal centers containing follicular DC networks with proliferating B-cell centroblasts. Notably, follicular DCs indicate adaptive B-cell responses, antigen-specific B memory cell formation, and affinity maturation of B cells. Therefore, the presence of follicular DCs in ATLOs and of proliferating B cells in ATLO germinal centers provide strong evidence for but do not prove a robust antigen-specific autoimmune response within the diseased arterial wall adventitia. Delineation of mouse ATLO cellularity suggests that the diseased artery is capable of organizing both T- and B-cell autoimmune responses, and that these responses are not observed in young animals. In *Apoe*^{−/−} mice, we observed preferential formation of ATLOs in the proximal part of the abdominal aorta but occasionally also in coronary and pulmonary arteries, in the adventitia of the innominate artery, in aortic valves, and rarely in the periarterial myocardium (unpublished observation). However, the formation of advanced plaques is not sufficient to trigger ATLO formation because atherosclerosis in *Apoe*^{−/−} mice is initiated and is most advanced in the aortic arch and its branches where ATLOs can only rarely be observed. The occurrence of ATLOs in the abdominal aorta adventitia is reminiscent of TLO formation in the meninges in MS. TLO formation requires long-lasting interactions between immune cells and mesenchymal cells involving a series of hematopoietic and connective-tissue-derived cytokines.^{20,21,112}

Immune Responses of Atherosclerosis: Plaque or Adventitia and Organ Specific or Systemic?

Although the response to injury hypothesis proposed 4 decades ago emphasized the role of smooth muscle cells for atherosclerosis progression,^{148–150} it is now established that adaptive

immune responses contribute to disease progression. The majority of investigators assume that these responses are carried out in the intima of the diseased arterial wall and in SLOs.^{2,6,136} Clusters of immune response-regulating cells in atherosclerotic plaques have also been termed vascular-associated lymphoid tissue.^{4,125} Moreover, other reports proposed that epitopes of heat shock protein 60, oxidized LDL, or LDL are culprit autoantigens, but the veracity of the conclusions drawn from these studies requires more evidence.^{116,126,130,134,135,151} Blood monocytes are systemically increased by hyperlipidemia in mouse models maintained under a Western-type diet.⁹⁰ Under conditions of acute myocardial infarction, spleen monocytes are rapidly mobilized into the heart. However, it is much less clear whether there are systemic alterations in the number, activation, or leukocyte subset composition during atherogenesis in hyperlipidemic mice maintained under normal mouse chow. Thus, despite extensive efforts, it is still largely unclear whether atherosclerosis is associated with organ-specific or systemic adaptive and autoimmune reactions. Systemic vaccination using various presumptive autoantigens, including LDL, oxidized LDL, and heat shock protein, in hyperlipidemic mice resulted in attenuation or acceleration of disease severity providing circumstantial evidence for systemic immune activity under these conditions in young mice. Moreover, T-cell-directed intervention in mice vaccinated with oxidized LDL also led to a decrease in atherosclerosis severity, but these mice generated a marked T-cell response against LDL epitopes rather than against oxidized LDL epitopes.^{116,130} Depletion of regulatory T cells using anti-CD25 antibodies or transgenic Foxp3 specific, diphtheria toxin-mediated ablation in hyperlipidemic mice increased atherosclerotic burden, whereas anti-CD20 antibody-induced B-cell depletion limited disease severity implicating antiatherogenic actions of regulatory T cells and proatherogenic actions of B-cell subsets.^{35,36,119} All these observations provide circumstantial evidence that T- and B-cell responses affect atherosclerosis systemically and dichotomically. Therefore, there is no doubt that systemic manipulation of the immune system at various levels affects atherosclerosis in the periphery, but this cannot be taken as evidence that the bona fide atherosclerosis immune response during the natural course of the disease in humans is organized systemically. Indeed, several recent studies, including our own, are more consistent with the view that atherosclerosis is an organ-specific disease whose adaptive immune responses are carried out locally. This suggestion is based on the cellular composition and structure of ATLOs in the adventitia of *Apoe*^{−/−} mice. In addition, age and in particular immune senescence is associated with some autoimmune diseases in both mouse and man.^{112,125,152,153} As we observed a large increase in adventitial immune cells in aged *Apoe*^{−/−} mice, the possibility that immune senescence contributes to atherosclerosis deserves attention.¹⁵³ The initial stages of ATLOs were noted at ≈52 weeks with preferential formation in the upper abdominal aorta, where aortic aneurysms are formed.⁹¹ Fully developed ATLOs emerge between 52 and 78 weeks exclusively in aortic segments burdened with advanced atherosclerotic plaques. In humans, atherosclerosis becomes clinically significant only when the late stages are reached,

often after short periods of violent progression of plaque growth, leading eventually to plaque instability and rupture. Unfortunately, such stages are difficult to study in mouse models because hyperlipidemic mice have a normal lifespan and rarely develop myocardial infarcts under steady state conditions and normal mouse chow, indicating that poorly understood secondary events are required to initiate clinically severe disease.²¹

Are ATLOs Whistle-Blowers of the Nature of Advanced Atherosclerosis Immune Responses?

The American Autoimmune Related Diseases Association (<http://www.aarda.org>) does not list atherosclerosis as an autoimmune or an autoimmune-related disease although it increasingly recognizes various chronic inflammatory diseases as conditions with a significant autoimmune component. Indeed, a large excess of 100 human disease conditions and clinical syndromes are acknowledged as autoimmune or autoimmune-related diseases, depending on ≥ 1 direct or a basket of indirect criteria. These include clinically important diseases, such as RA, MS, diabetes mellitus type I, Crohn disease, ulcerative colitis, and primary biliary cirrhosis. Despite major efforts, the culprit autoantigens in any of these diseases have not yet been determined. Moreover, TLOs were identified in only a small minority of human autoimmune diseases (Table). We view ATLOs as whistle-blowers of atherosclerosis autoimmune reactivity. Moreover, studies of ATLOs may uncover the functional effects of TLOs on disease progression and may also be a source of autoantigen-specific T and B cells.

Search for a Mouse Model of Atherosclerosis Autoimmune Responses

The well-established mouse models, experimental autoimmune encephalomyelitis and collagen-induced arthritis, have guided the implementation of current therapeutic strategies to treat MS or RA, respectively, in the clinic, and some of these strategies have even entered routine clinical practice.^{34,38,96} Although several attempts aimed to address aspects of atherosclerosis-associated autoimmunity in mice, it is our view that there is currently no robust mouse model to examine atherosclerosis autoimmune responses and ACS faithfully.^{4,5,154} Observational studies of human coronary artery disease across all age groups show that asymptomatic fibroatheromatous plaque buildup can continue for decades without developing into clinically overt disease, such as ACS. Initiation of disease requires the development of vulnerable plaques as evidenced by fibrous cap thinning, enlargement of the necrotic core, macrophage activation, plaque neoangiogenesis, plaque rupture, and thrombosis. However, the mechanisms of how stable plaques undergo prototypical alterations to become vulnerable plaques are not well understood, yet autoimmune T cells have been held responsible. However, some features of vulnerable plaques and myocardial infarcts have been induced in *Apoe*^{-/-} mice under distinct experimental conditions. One important parameter seems to be age, which has been shown to generate several but not all parameters of vulnerable plaques in the innominate artery of *Apoe*^{-/-} mice. Thus, aging of the arterial wall and senescence of the immune system should become a central part of mouse atherosclerosis research.^{154–156}

Autoimmune Reactivity Is Not Sufficient to Initiate Autoimmune Disease

Several caveats merit consideration about a pathogenic role of ATLOs in atherosclerosis. First, TLOs as organizers of adaptive immune responses may generate effector lymphocytes, T central memory cells, affinity-matured B memory cells, plasma cells, and their immunosuppressive counterparts in an apparent equilibrium.²² Therefore, it is not clear, whether, when, and how the balance between effector and tolerogenic lymphocytes is disturbed to cause autoimmune tissue injury in any autoimmune disease or in clinically apparent atherosclerosis, such as the ACS. It is conceivable that silent disease stages are characterized by a well-tuned balance between lymphocyte effector and suppressor activities, and that disease relapses and rapid disease progression is caused by the activation of effector lymphocytes, the recall of memory cells by additional antigen exposure,¹⁵⁷ and inhibition of suppressor activities. The important question what triggers the activation of immune cells, that is, the monocyte/macrophage system in autoimmune diseases and in ACS in atherosclerosis, has yet to be answered, but molecular mimicry—and recall responses of T and B memory cells—all merit attention.^{103,158} A large body of evidence supports the assumption that atherosclerosis involves T- and B-cell immune responses that promote or inhibit plaque growth.^{4,36,91,112,116,119,125,126,130,132,136,144,145,151,159} The presence of discrete T-cell areas and follicular DC networks within activated germinal centers of ATLOs provides first indirect evidence that transmural inflammation of the arterial wall generates atherosclerosis-specific antigen(s) locally that may initiate generation of T-cell and B-cell effector and memory cells. Similar to SLOs, organization of the ATLO immune response depends on the lymphotoxin β receptor.^{7,107–109,160} We assume that ATLOs generate and harbor proatherogenic and antiatherogenic T- and B-cell effectors in apparent equilibria, raising the question how this balance might be disturbed during disease progression. The complexity of the immune response in atherosclerosis precludes for now to predict what these mechanisms may be. For instance, the tolerogenic arms of the atherosclerotic adaptive immune response might be compromised (Figure 5). To examine this possibility at a cellular level, additional studies of arterial wall regulatory T cells and B-1 cells should be performed in addition to examining the local cDCs, which were recently shown to exert a protective role during early stages of atherosclerosis.⁸⁸ It is recognized that autoimmune diseases in humans develop in separable steps and that only in the late stages, when tolerance against autoantigen(s) breaks down, conversion to explicit self-reactivity associated with debilitating tissue destruction can be observed.⁹⁵ Acute phases of disease are often triggered by infections, and it has been suggested that toll-like receptor signaling may be involved in disease progression.^{95,97,118} Interestingly, the risk for ACSs and stroke are associated with preceding infections.¹⁶¹ Considering the well-recognized expression of innate pattern-recognition receptors in atherosclerotic lesions, one could envision pathological mechanisms leading to deregulated immune cell balances. Acute infections lead to systemic activation of immunity and weakening of the tolerogenic and regulatory controls may allow increasingly

unopposed activity of adaptive immune mechanisms in atherosclerosis. When proinflammatory signals persist for sufficient periods of time and are not resolved, they may affect destabilization and provoke rupture of atherosclerotic plaques adjacent to the ATLO.

Search for Human ATLOs

Human ATLOs have not yet been characterized in detail although ATLO-like adventitial aggregates containing T cells, B cells, and follicular DCs were reported in patients afflicted with atherosclerotic abdominal aneurysms.⁹ Adventitia leukocyte infiltrates are associated with clinically significant stages of atherosclerosis, and there were increased adventitial leukocytes in ruptured when compared with nonruptured plaques in a large cohort of patients afflicted with abdominal aorta atherosclerosis.¹⁶² Clearly, translational studies in human atherosclerosis are needed but where to look? At present, it is difficult to predict the location where human ATLOs may arise. However, TLOs do not necessarily emerge within the diseased target tissue but rather develop where the immune system finds the most favorable conditions to organize itself in an appropriate connective tissue environment—often in the perivascular space adjacent to the diseased parenchyma (Table). Likewise, in *ApoE*^{-/-} mice, ATLOs are only rarely found in the adventitia of the innominate artery or of the aortic arch where atherosclerosis is most prominent. The scarce evidence that human atherosclerosis is associated with ATLO neogenesis thus raises an important question: Is it conceivable that the aged *ApoE*^{-/-} mouse and the lack of ApoE per se are a special case of aberrant ATLO formation that is not representative for other mouse models or human atherosclerosis? Although this possibility cannot be ruled out completely at this time, we think that it is unlikely for the following reasons: We studied humanized aged ApoE3 and ApoE4 knockin mice to address some of these issues. These mice are normolipidemic when maintained under normal mouse chow, indicating that the human ApoE isoforms function properly as lipid transport proteins throughout the lifespan of the mice. However, under Western-type diets with added cholate, the transgenic mice become hyperlipidemic and subsequently develop a mild form of atherosclerosis. When we examined aged ApoE4 knockin mice that had been maintained under a cholesterol-rich diet, few mice developed small plaques in the suprarenal portion of the abdominal aorta and also developed early stages of ATLOs consisting of B-/T-cell aggregates adjacent to the small plaques. Mice did not form adventitial aggregates when maintained under normal mouse chow (Bontha V, unpublished data, 2013). These data are consistent with the view that ATLO neogenesis observed in aged *ApoE*^{-/-} mice is a result of combined aging, hyperlipidemia, and atherosclerosis rather than the lack of ApoE. In addition, it will be important to distinguish inflammatory single-cell T-cell infiltrates that can be observed throughout the arterial tree from bona fide early stages of ATLOs characterized by separate T- and B-cell areas.

Conclusions

The autoimmune hypothesis of atherosclerosis is based on circumstantial evidence. Accordingly, for now, atherosclerosis

should be categorized as a chronic inflammatory disease of the arterial wall with a significant autoimmune component. Additional work is needed to obtain more direct evidence for participation of autoreactive T and B lymphocytes during disease progression and during the clinically important stages of the disease when stable, clinically silent atherosclerosis proceeds to ACS. Robust experimental models of ACS are urgently needed. The study of murine and human ATLOs may provide new experimental approaches to isolate atherogenic or antiatherogenic autoantibodies and to clone atherogenic B-cell effectors or memory cells. Delineation of mechanisms underlying the generation and the subsequent disturbances of the equilibria between these subsets in ATLOs may provide clues for translational research into human atherosclerosis and open unprecedented avenues for immune-based therapeutics.

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Disclosures

None.

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