Vessel-Specific Toll-Like Receptor Profiles in Human Medium and Large Arteries

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Background—Inflammatory vasculopathies, ranging from the vasculitides (Takayasu arteritis, giant cell arteritis, and polyarteritis nodosa) to atherosclerosis, display remarkable target tissue tropisms for selected vascular beds. Molecular mechanisms directing wall inflammation to restricted anatomic sites within the vascular tree are not understood. We have examined the ability of 6 different human macrovessels (aorta and subclavian, carotid, mesenteric, iliac, and temporal arteries) to initiate innate and adaptive immune responses by comparing pathogen-sensing and T-cell–stimulatory capacities.

Methods and Results—Gene expression analysis for pathogen-sensing Toll-like receptors (TLRs) 1 to 9 showed vessel-specific profiles, with TLR2 and TLR4 ubiquitously present, TLR7 and TLR9 infrequent, and TLR1, TLR3, TLR5, TLR6, and TLR8 expressed in selective patterns. Experiments with vessel walls stripped of the intimal or adventitial layer identified dendritic cells at the media-adventitia junction as the dominant pathogen sensors. In human artery–severe combined immunodeficiency (SCID) mouse chimeras, adoptively transferred human T cells initiated vessel wall inflammation if wall-embedded dendritic cells were conditioned with TLR ligands. Wall-infiltrating T cells displayed vessel-specific activation profiles with differential production of CD40L, lymphotoxin-α, and interferon-γ.

Vascular bed–specific TLR fingerprints were functionally relevant, as exemplified by differential responsiveness of iliac and subclavian vessels to TLR5 but not TLR4 ligands.

Conclusions—Populated by indigenous dendritic cells, medium and large human arteries have immune-sensing and T-cell–stimulatory functions. Each vessel in the macrovascular tree exhibits a distinct TLR profile and supports selective T-cell responses, imposing vessel-specific risk for inflammatory vasculopathies. (Circulation. 2008;118:000-000.)

Key Words: arteries • immune system • inflammation

In contrast to microvessels, human macrovessels are complex organ systems composed of several different wall layers, each of which is arranged by different cellular and matrix components and specifically contributes to the tasks of the vessel. Structural organization of macrovessels is highly dependent on their mural thickness, which is controlled by body size and weight. Therefore, it has been challenging to explore macrovascular function in experimental models because large animals are needed to mimic human body dimensions.

Clinical Perspective p ●●●

Macrovessel diseases display stringent target tissue tropisms and selectively affect distinctive vascular territories. Polyarteritis nodosa targets small and medium-sized arteries of the abdominal circulation. Takayasu arteritis manifests in the aorta and its primary branches, favoring neck and upper-extremity vessels. Giant cell arteritis has a strong predisposition for the second to fifth aortic branches and is diagnosed by biopsy of the temporal artery, a vessel of the extracranial circulation. Tissue tropism for selected vascular regions is so characteristic that patterning of vascular involvement serves as an important diagnostic clue. Similarly, some arteries (coronary, carotid) are prone to develop atherosclerosis, now recognized as an inflammatory entity, whereas others (internal mammary arteries) are considered relatively resistant.

Concepts aiming to explain the predilection of selected arterial beds have assumed that vascular injury and subsequent inflammation are afflicted at the blood-wall interface, focusing attention on the macrocolumn and its endothelial lining. The intima is exposed to mechanical stress and circulating molecules, stressors that modulate arterial wall functionality. In addition, endothelial cells (ECs) have been implicated in numerous biological and physiological processes, ranging from antithrombotic effects to vessel tone.
regulation and control of inflammatory cell influx. However, in normal macrovessels, flow velocity may hinder adhesion and tissue migration of T cells and monocytes centrifugally into the wall. Inflammatory cells require low-flow conditions such as in capillaries to transit into the tissue space. The dominant macrovascular component is the media, a layer of concentrically arranged vascular smooth muscle cells (VSMCs) and elastic membranes providing pressure resistance and elasticity. The internal elastic lamina separates the intima and media, and the external elastic lamina defines the media-adventitia junction. Except for the human thoracic aorta, the media is avascular and thus rather inaccessible to inflammatory cells. However, in essentially all vasculitides, inflammatory infiltrates accumulate in the media, and the severity of media destruction largely determines patient outcome.

The outermost wall layer, the adventitia, is a concentric sheet of fibroblasts, poorly organized collagen fibers, and elastic bundles. Most important, the adventitia holds a microvascular network, or vasa vasorum, that supplies oxygen and nutrients to the wall. The adventitia functions critically in immune recognition and regulating vascular inflammation. Specifically, dendritic cells (DCs) in the adventitia recognize pathogen-derived molecular patterns and initiate adaptive immune responses in an otherwise immunoprivileged niche. Collar-induced occlusion of vasa vasorum causes rapid intimal hyperplasia, emphasizing the regulatory role of the adventitia in global vascular function.

The present study explored whether human macrovessels as organs settled by endogenous DCs participate in innate and adaptive immunity. More important, we examined whether arteries from distinct vascular territories display heterogeneity in their immune-regulatory functions, providing a possible clue toward the tissue tropisms of inflammatory vasculopathies. Innate and adaptive immune processes are involved in the atherosclerotic plaque and in large-vessel vasculitis. In the plaque, both myeloid and plasmacytoid DCs respond to microbial patterns and modulate tissue-destructive immune responses, including tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated T-cell cytotoxicity. In giant cell arteritis, CD83+ DC depletion abrogates vasculitis, attesting to the critical role of activated DCs in sustaining wall inflammation. Less is known about DC populations in normal, noninflamed arteries. Initial attention focused on a subintimal DC network in human aorta and carotid arteries first described by Bobryshev and Lord as S-100+ cells with a characteristic DC-like ultrastructure. Millonig and colleagues have described vascular DCs in the intima of normal, nonatherosclerotic arteries and have introduced the concept of vascular-associated lymphoid tissue. The present project has focused on the larger family of human macrovessels, including subclavian, mesenteric, iliac, and temporal arteries. These macrovessels are occupied by DCs embedded deep in the wall, specifically in the adventitia where they colocalize with vasa vasorum network and lymphatic vessels. Endowed with pattern-recognition receptors of the Toll-like receptor (TLR) family, adventitial vascular DCs empower the vessel wall with pathogen-sensing functions, which widen the role of macroarterial tissues far beyond the transport of blood and blood pressure regulation.

Methods

Tissue Specimens

Human arteries with intact wall structures were collected during postmortem examinations of 37 donors (mean age, 64 years; 25 men, 12 women). Samples were harvested within 8 hours of death from 6 vascular beds (temporal, carotid, subclavian, mesenteric, iliac, and thoracic aorta). Fresh blood vessels were obtained from diagnostic biopsies or vascular repair procedures and were immediately shock-frozen or embedded in optimal coherence tomography. On the basis of histomorphological examination, only samples with normal wall structure without atherosclerotic lesions and inflammatory infiltrates were selected. To ensure consistent high quality of vascular tissues, minimal β-actin transcript expression per 1 mm² was set at 6 × 10⁶ copies. Selective degradation of gene transcripts in the autopsied specimens was ruled out by direct comparison of TLR profiles in postmortem samples and tissues obtained during surgical procedures (Figure I of the online-only Data Supplement). Kinetic experiments in which aortic wall was stored for 30 hours after acquisition confirmed the stability of TLR expression profiles postmortem (online-only Data Supplement Figure II) as previously reported.

Quantitative Polymerase Chain Reaction

Total RNA was isolated from shock-frozen tissues with TRIzol (Invitrogen, Carlsbad, Calif), reverse transcribed into cDNA using avian myeloblastosis virus reverse transcriptase (Roche Applied Sciences, Indianapolis, Ind), and amplified with specific primer pairs (online-only Data Supplement Table I) as described previously.

Immunohistochemistry

Slides were prepared as previously described. Antibodies are listed in online-only Data Supplement Table II. After blocking with 5% normal goat serum, tissue sections were incubated with primary antibodies (CD11c, TLR2, TLR4, TLR5, CD86) for 1 hour, followed by biotin-conjugated secondary antibody for 30 minutes and ABC-Elite solution (Vector Laboratories, Burlingame, Calif). Binding was visualized with the chromagen 3,3'-diaminobenzidine (Dako, Carpinteria, Calif). For dual-color staining, slides were again blocked with serum and incubated with primary antibody (von Willebrand factor [vWF]) and then secondary (sheep anti-rabbit) antibody for 60 and 30 minutes, respectively. Sections were developed with ABC-alkaline phosphatase and visualized with red chromogens (Vector Laboratories). Endogenous alkaline phosphatase activity was inhibited with levamisole. Slides were counterstained with hematoxylin (Surgipath, Richmond, Ill). Control stains with isotype-matched primary antibodies were included in each experiment.

Blood Vessel Organ Culture

Arteries were cut into rings and placed into 48-well plates filled with complete medium (RPMI, 10% fetal calf serum). For “partial-wall” experiments, either the endothelial or adventitial layer was stripped off. Tissues were stimulated with lipopolysaccharide (LPS; 1 µg/mL, Sigma-Aldrich, St Louis, Mo) or flagellin (1 µg/mL, Invivogen, San Diego, Calif) or left untreated for 24 hours. Subsequently, arteries were shock-frozen for RNA isolation or embedded in optical coherence tomography for immunohistochemistry.

In Vivo Stimulation of Human Arteries

Arteries were subcutaneously implanted on the lower midback of NOD.CB17 Prkdc (SCID) mice. Seven days after implantation, chimeras were injected with LPS (3 µg/mouse IP) or PBS. Twenty-four hours later, CD4⁺ T cells from healthy donors were adoptively transferred into each mouse (20 × 10⁶/mouse IV). One week later, the artery grafts were recovered and shock-frozen for RNA isolation. For
DC depletion, arterial tissues were treated with GdCl₃ (300 μg/mL, Sigma-Aldrich) for 1 hour before implantation. All procedures were approved by the Animal Care and Use Committee of Emory University.

In Vivo T-Cell Tracking
CD4⁺ T cells were isolated from healthy donors and labeled with 2 μmol/L PKH67 green fluorescence dye (Sigma). Labeled cells were adaptively transferred into human artery–SCID chimeras as described.²⁷ Acetone-fixed tissue sections (7 μm) from explanted arteries were mounted in DAPI containing Vectashield (Vector Laboratories). Images from 2 fluorescence channels were merged with Adobe Photoshop 7.0.1. PKH67⁺ tissue-infiltrating cells were counted in 5 high-powered fields per section in 5 nonserial sections.

Data Analysis
Data were analyzed by 1-way and 2-way ANOVA with Sigma Stat 3.1 software (Systat Software Inc, San Jose, Calif). In the transcriptome profiling studies, relative TLR expression was obtained by normalization of each TLR to the median of all samples. Heat plots were generated with GeneSpring GX software (Agilent Technologies, Santa Clara, Calif).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Blood Vessel–Specific Expression Profiles for TLRs
The precise patterning in which inflammatory vasculitides target macrovessels suggests an active contribution of the vessel wall itself. Inflammation begins with the activation of innate immune responses, followed by formation of adaptive immunity. Pattern recognition receptors (eg, TLRs) initiate immune responses by sensing microbial motifs. We therefore established receptor expression profiles for TLR1 through TLR9 in tissue samples from 6 arterial territories (Figure 1). Transcripts for each TLR were quantified in each tissue by quantitative polymerase chain reaction (PCR) (Figure 1A). Remarkably, TLR profiles were heterogeneous and strongly correlated with the anatomic origin of the artery.

TLR expression levels were significantly different (P < 0.001) when the 6 vascular territories were compared. TLR2 had the highest copy numbers with significantly different expression among the 6 different arteries (P < 0.001). TLR4 also was abundantly expressed; levels in the 6 vascular beds were not statistically different. TLR7 and TLR9 were produced at low levels, with only iliac arteries containing intermediate amounts (P = NS). TLR1 demonstrated remarkable selectivity, dividing the arteries into high (iliac, aorta, carotid) and low (temporal, subclavian, mesenteric) expressers (P < 0.05, iliac versus any low-expressing artery). TLR1 and TLR6 had similar distribution patterns, with high expression in aortic, carotid, and iliac arteries and 5- to 10-fold lower transcript levels in mesenteric, subclavian, and temporal arteries. TLR5-specific sequences varied significantly among the arterial territories (P < 0.001). TLR3 displayed a selective expression pattern: Carotid and aorta were positive; temporal and iliac vessels were essentially negative (P < 0.005). Similarly, TLR8 expression varied widely, with temporal and iliac arteries positive but mesenteric and aorta negative (P < 0.05). Carotid samples were unique in that they contained similar levels of TLR3 and TLR8 transcripts (P = 0.2).

In a different approach to comparing expression of TLR family members, we used an artery-centric view (Figure 1B). The TLR profile of iliac arteries was distinct from that of all other vessels (P < 0.001) and had the broadest TLR spectrum, with 8 of 9 family members present (all except TLR3). Carotid arteries contained 7 of the 9 TLR types, missing TLR7 and TLR9. The signature pattern of the aorta was high levels of TLR1 through TLR6. The carotid and aortic patterns were statistically not different. The TLR portfolio was less diverse in mesenteric and subclavian arteries, which appeared alike. Temporal arteries had an unusual pattern, with high amounts of TLR2, TLR4, and TLR8 and intermediate results for TLR1, TLR5, and TLR6. The biggest difference in TLR profile, in both pattern and expression levels, was observed between iliac and subclavian vessels (P < 0.001). TLR profiles did not correlate with donor age, sex, or body mass index (data not shown).

TLR⁺ DC Distribution in the Major Human Arteries
TLRs typically are expressed on DCs but can appear on other cell types, especially monocytes, macrophages, and B cells. In addition, nonimmune cells in the arterial wall such as ECs and VSMCs could contribute to TLR expression. To explore which immune cells are represented in human macrovessels, we screened for cell-type–specific markers by quantitative PCR (Figure 2). All arteries expressed abundant transcripts for the DC marker CD11c. Arterial extracts were essentially negative for T-cell receptor transcripts, macrophage-specific CD11b transcripts, and the B-cell marker CD79A, with transcript levels < 10 copies per 200 000 β-actin transcripts. The exceptions were iliac vessels, in which low levels (10 to 100 copies) of these markers were detectable. These experiments essentially limited the spectrum of professional wall-residing immune cells to DCs. The 6 vascular beds displayed a clear hierarchy of CD11c expression (Figure 2). The aorta, the carotids, and the iliac arteries shared high levels of CD11c transcripts. Mesenteric, subclavian, and temporal vessels had lower levels of CD11c-specific sequences (P < 0.05 versus aorta). CD11c representation was unrelated to vessel diameter or elasticity; specifically, subclavian arteries resembled temporal arteries although subclavians have wall characteristics comparable to carotids.

The precise placement of CD11c⁺ cells in the wall layers was determined by double staining tissue sections for the endothelial marker vWF and the DC marker CD11c (Figure 2). All vessels had a circumferential ring of CD11c⁺ cells positioned at the adventitia-media border. CD11c⁺ cells within the medial VSMC layer were isolated and rare. Only the aorta and the carotid had an additional set of CD11c⁺ DCs within the intimal stratum. In temporal, mesenteric, iliac, and subclavian arteries, such intimal DCs were rarely encountered. Thus, adventitial CD11c⁺ DCs are a common feature of all human macrovessels (online-only Data Supplement Table III). Only aortic and carotid walls also possess an intimal DC network.
Macroarteries Sense TLR Ligands In Vitro and In Vivo and Respond With DC Activation and In Situ T-Cell Stimulation

To test the functional relevance of wall-embedded TLR-expressing cells, we concentrated on TLR2 and TLR4, receptors consistently encountered in all vessels. Immunohistochemistry for TLR2 and TLR4 localized positive cells at the media-adventitia border (Figure 3). Dual-color staining with the endothelial marker vWF distinguished TLR4+/H11001 cells from vasa vasorum ECs. ECs lining the macrolumen gave a low and inconsistent signal for binding of TLR4-reactive antibodies and were uniformly negative for TLR2. In addition, in all arterial sections, medial VSMCs lacked TLR2 and TLR4 expression. These results focused interest on adventitial DCs as TLR sensors.

To test vascular TLR functional relevance, arteries were exposed to TLR4 ligands either in organ culture or after the human vessels were engrafted into SCID chimeras. PKH67-labeled CD4+/H11001 T cells were adoptively transferred into human artery–SCID mouse chimeras. In vitro stimulation of arterial wall patches with LPS for 24 hours promptly resulted in transcriptional upregulation of the DC activation markers CD83 and CD86 (Figure 4A). Immunohistochemistry identified CD86+/H11001 cells exclusively in the adventitia (Figure 4B). Similarly, human arteries recovered from mouse chimeras upregulated CD83, CD86, and CCL19, the T-cell–attracting chemokine produced by activated DCs (Figure 4C). Explanted arteries contained high levels of T-cell receptor–specific transcripts, and immunohistochemistry visualized intramural CD4 T cells (Figure 4D), confirming that TLR
triggering paved the way for adaptive immune responses. Recruited T cells underwent in situ stimulation as evidenced by the induction of early and intermediate T-cell activation markers, including CD40L, lymphotoxin-α, and interferon-γ (Figure 4C). Subclavian arteries were the most potent in eliciting lymphotoxin-α production. In contrast, carotid arteries outperformed subclavian and iliac arteries in inducing interferon-γ production. All 3 arterial beds equally upregulated the early T-cell–activation marker CD40L.

Venous tissues were essentially nonresponsive to TLR ligands. In contrast to arteries, stimulation of mesenteric veins in organ culture with LPS failed to induce both CD83 and CD86 (online-only Data Supplement Table IV).

**Adventitial DCs Are Key Regulators of LPS Responsiveness**

To test pathogen-sensing functions of different wall layers, we studied TLR4 responsiveness in partially denudated vessels. The adventitial or intimal layers were carefully removed; denudation accuracy was confirmed with anti-vWF staining (Figure 5A). Denuded or intact arteries were stimulated with LPS in organ culture, and activation was assessed by quantifying CD86 induction. Denudation of macroluminal ECs did not diminish LPS-induced CD86 upregulation. Conversely, stripping of the adventitial layer essentially abrogated LPS sensing. Immunohistochemical studies in LPS-treated arteries confirmed that CD86 was expressed exclusively on adventitial CD11c⁺ cells (Figure 5B), identifying adventitial cells as critical mediators of TLR recognition. To provide further evidence for adventitial DCs as the sentinels of the artery, we proceeded with depletion experiments targeting phagocytic cells. Among the different cell types making up the normal vessel wall (VSMCs, ECs, fibroblasts, vascular DCs), only DCs are capable of phagocytosis. At branching points, some arteries may contain macrophages with phagocytic capability. Vessel segments examined in this study lacked wall-residing macrophages as documented by the absence of CD11b transcripts (Figure 2). To deplete phagocytes, human arteries were pretreated with gadolinium (III) chloride just before implantation. Chimeras were injected with LPS, followed by adoptive transfer of human CD4 T cells (Figure 5C). Phagocyte-depleted arteries were markedly impaired in responding to LPS (P<0.001, LPS versus gadolinium/LPS). DC activation markers...
CD83, CD86, and CCL19 showed minimal induction, assigning all 3 markers to phagocytic cells. In the absence of phagocytic cell activation, T-cell attraction (Figure 5C) and in situ T-cell activation (CD40L) were abrogated.

Heterogeneous TLR Expression Profiles Predict Differential Response Patterns to Pathogen-Derived Motifs

The TLR transcriptome comparative analysis (Figure 1) suggested vessel specificity and macrovascular bed heterogeneity. Essentially all arteries shared TLR4 expression (Figures 1 and 3) and were capable of sensing TLR4 ligands. Other TLRs such as TLR3 and TLR5 displayed marked variability with high- and low-expressing vascular territories. To examine whether differences in expression profiles translated into functional consequences, we compared responsiveness to TLR4 and TLR5 ligands in subclavian and iliac arteries that lack a subendothelial DC network. On the basis of transcript levels, both artery types were predicted to react to LPS but to display differential responsiveness to the TLR5 ligand flagellin.

Immunohistochemistry identified TLR5-positive cells exclusively in the adventitia in both arteries, with much higher frequencies in iliac vessels (Figure 6). Multifold induction of CD83 and CD86 transcripts demonstrated robust subclavian and iliac DC activation in response to LPS (Figure 6) (P<0.05 for both markers in both vessels compared with control). In contrast, flagellin was a powerful activator for iliac vessels (P<0.001) but was ineffective in stimulating subclavian DCs. Thus, TLR expression patterns not only provided a unique fingerprint for each arterial territory but also predicted the ability of the vessel to sense and react to pathogen-derived motifs.

Discussion

Besides their role in transporting blood, arteries have numerous physiological functions, including regulation of blood pres-
sure and blood coagulability. Many of these functions have been assigned to ECs lining the lumen. Much less is known about distal wall layers. The data presented here establish human macrovessels as lymphoid organs equipped with sophisticated DC networks that have a sensing function and monitor host exposure to pathogens through pattern recognition receptors. Remarkably, macrovessels from different vascular territories express vessel-specific profiles of pathogen-sensing TLRs, suggesting functional specialization in the surveillance task of arteries, with each region of the vascular tree dedicated to a selected spectrum of TLR ligands. This specialization of blood vessel territories mirrors the stringent target tissue tropism of inflammatory vasculopathies. Clinicians have long been puzzled by the selective distribution pattern of vasculopathies, including vasculitides and atherosclerosis. Here, we propose that vessel wall determinants may direct disease processes to unique sites. Conceptualizing macrovessels as a lymphoid tissue markedly extends the scope of the monitoring capabilities of the immune system and implicates macrovessels as immunomodulators that regulate not only local but also systemic immune responses.

The data presented here strongly support the hypothesis that large arteries sense circulating molecules through more than their EC layers. Determined by body size, structuring of deeper wall layers is different in small animals and humans. The number of aortic smooth muscle cell lamellae is tightly correlated to overall body size. Media thickness dictates the need for the adventitial vasa vasorum network, the major access route for cells to the vessel wall. Notably, lymphocytes egress from the blood into tissue through high endothelial venules after the blood has passed the capillaries. Vessel wall–residing DCs in the aorta and carotid artery have been suspected to contribute to vascular pathology. Millonig and colleagues have demonstrated their presence in healthy young individuals, long before atherosclerotic streaks appear, giving rise to the model of a vascular-associated lymphoid tissue, comparable to mucosa-associated lymphoid tissue.
Subendothelial DCs may advance atherosclerosis, but how they function is unknown. In our experiments, CD4 T cells did not selectively accumulate in the subintima. Whether T cells can reach subintimal DCs as long as the vessel wall is intact and avascular is unknown but would involve migration throughout the entire wall. This scenario obviously changes dramatically once a plaque is established, capillary support is remodeled, and the media and intima are easily accessible for T cells. In the plaque, highly activated DCs colocalize with T cells and produce T-cell–attracting chemokines.33

Partial-wall experiments assigned pathogen sensing in normal vessels primarily to adventitial DCs. TLR expression patterns identified wall-residing DCs as myeloid-like, recognizing bacterial products. Whether wall-residing DCs fall into several subcategories requires more detailed molecular profiling. So far, we have failed to find plasmacytoid DCs in normal vessels; they are abundant in the atherosclerotic plaque where they release interferon-α on triggering with TLR9 ligands.34

In the gene expression studies comparing whole-wall–derived transcript pools, cells others than DCs could obviously contribute to the TLR pattern. TLR4 expression has been described for ECs and VSMCs when associated with the atherosclerotic plaque. However, in normal, noninflamed arteries, VSMCs are consistently negative for TLR4 staining.35 Macroluminal ECs show weak and inconsistent binding of TLR4 staining, with no CD86 induction on LPS stimulation. The partial-wall experiments and in vivo experiments strongly implicated adventitial DCs as the major sensors of pathogen-related motifs. The questions arise as to why macrovessels participate in pathogen sensing and why vascular territories specialize in selected TLR portfolios. Studies with temporal arteries and other macroarteries described here unequivocally demonstrate that in their physiological state wall-embedded DCs do not initiate immune responses. TLR stimulation is necessary to render wall-residing DCs immunogenic. Thus, vascular DCs are primarily tolerogenic, protecting the artery from inflammatory attack. Considering the vital role of macrovessels and the small margin of benefit gained by vessel inflammation, this immunologic niche is definitely preferable. This niche appears to be not only defined by structural determinants such as accessibility but actively maintained by wall-resident DCs.

Vascular territory specialization in sensing pathogens may be related to anatomic positioning. Iliac arteries are near the gut; monitoring for flagellin may provide an advantage. This concept assumes far-reaching contact and communication between arteries and their tissue environment. Interestingly, veins that carry blood away from organs lack DCs (see also online-only Data Supplement Table IV). Alternatively, DCs placed around vasa vasorum may contribute to absorbing circulating endotoxins. Patients undergoing teeth cleaning display prolonged endothelial dysfunction after vascular tree exposure to subclinical bacteria.36 Traditionally, ECs have been implicated in sensing such danger signals; current data suggest that wall-embedded DCs play a major role in this vascular sensing function.

Experimental approaches of this study (in vitro organ culture and xenograft model) were focused on pathogen-sensing functions of the wall, with TLR ligands reaching wall-embedded cells through vasa vasorum. Flow conditions in the macrolumen were not investigated. Because TLR sensing was not affected by intima stripping but was essentially eliminated by adventitia removal (Figure 5), cells seated deep in the wall structure must be primarily responsible for TLR ligand recognition.

In vivo experiments demonstrating that human macrovessels are capable of supporting T-cell recruitment and in situ activation suggest broader immunoregulatory function of arteries. This places vascular DCs in a critical position. As guardians of the artery wall, they can protect the structural integrity of these vital and nonregenerative tissue structures. If appropriately triggered, however, vascular DCs can initiate and sustain vessel wall inflammation, assigning them a checkpoint role in vasculitis. Diversity in TLR profiles of human macrovessels provides a molecular framework for the selective targeting of vascular beds with the potential to identify unique instigators and therapeutic modalities for different vasculitides. Finally, vascular DCs may provide immunoregulatory functions affecting not only local but also systemic immune responses.

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Disclosures

None.
References


CLINICAL PERSPECTIVE

Inflammatoty disorders of arteries, including atherosclerosis and vasculitides, target certain regions of the vascular tree while sparing others. This phenomenon suggests that arterial beds respond to inflammatory stimuli with a vessel-specific pattern. The immune system initiates inflammatory responses after sensing “danger signals,” often products released by infectious microorganisms or by injured tissue. Here, we have tested human medium-sized and large arteries for their ability to sense danger signals and have compared arteries from different regions of the body (aorta, temporal, carotid, subclavian, mesenteric, and iliac arteries) for this function. Immune-sensing cells use receptors from the Toll-like receptor (TLR) family to recognize pathogen-associated patterns. Profiling of the 6 arterial beds for the expression of TLR1 through TLR9 demonstrated abundant expression of these receptors in all arteries; however, each arterial territory expressed a unique TLR portfolio. Pathogen-sensing receptors were localized primarily on cells in the adventitia of the artery. Specifically, dendritic cells placed at the adventitia-media border are responsible for this sensing function. In organ culture and human arteries engrafted into immunodeficient mice, we confirmed that pathogen-derived motifs stimulate dendritic cells embedded deeply in the vessel wall and facilitate recruitment and in situ activation of T lymphocytes. In essence, these data establish that human macrovessels participate in immune monitoring and that each arterial bed has a unique fingerprint of pathogen receptors. The territorial distribution of TLR may contribute to the tropism of inflammatory vasculopathies.
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