Inflammatory vasculopathies targeting human medium and large arteries are debilitating and life-threatening diseases as they damage vital arteries and cause aortic aneurysms, cerebral ischemia, and aortic arch syndrome. Takayasu arteritis (TA) typically manifests in the aorta and its primary branches of young women, and giant cell arteritis (GCA) affects the aorta and its more distal branches in older individuals. The underlying process is a granulomatous inflammation accumulating within the vessel wall with typical granulomas and multinucleated giant cells or a diffuse lymphomonocytic infiltrate. Inflammatory cells access the artery through adventitial vasa vasmorum, not the macrovulum. In a patient subset, the arteritis histomorphology is that of perivasculitis, also called vasa vasa vortum, with smaller arterial branches in the adventitia as the primary target. How immune responses are compartmentalized to cause either of these disease architectures are identical or distinct is unknown. In both TA and GCA, CD4 T cells are the dominant lymphocyte population, accompanied by few CD8 T cells and rare NK cells and γδ-T cells. In GCA arteries, local immune responses are strongly biased toward interferon-γ production, with interleukin-4 essentially absent. Mechanisms through which the vessel wall edits the cytokine milieu are unidentified; possibilities include differential priming of T effectors or selective recruitment of committed effector populations. In GCA-affected arteries, T-cell populations are selected for specificity, with identical clonotypes isolated from anatomically distinct arteries. Priming of these clonotypes may occur in the lymph node. Alternatively, the local tissue site dictates which T cells are primed or recruited. In macrophages, functional commitment and topographical location in the vessel wall are closely correlated, emphasizing the potential impact of the microenvironment. Organs exposed to the outside world, such as the skin and the mucosal surfaces, have tissue-specific innate immune systems, with dendritic cells (DCs) functioning as potent immunosensors screening for local danger signals. Surprisingly, human macrovessels share with these tissues pathogensensing ability. Equipped with DC populations positioned at the media–adventitia border, medium and large arteries rec-
Deng et al  TLR4 and TLR5 Induce Distinct Types of Vasculitis 489

Oligonucleotides were purchased from Sigma-Aldrich (St Louis, Mo). Flagellin was (polyinosinic:polycytidylic acid), lipoteichoic acid (LTA), and CpG Lipopolysaccharide (LPS) (poly(I:C) (TLR3 ligand), LPS (3 μg/mL), flagellin (3 μg/mL), or CpG (50 μg/mL). Tissues were then harvested and used for RNA isolation. For kinase CD83 gene induction assays, tissues were stimulated with LPS (3 μg/mL) or flagellin (3 μg/mL) for 12, 24, or 48 hours. In selected experiments, temporal arteries were cultured with 1×10^6 human CD4 T cells for 3 days after stimulation with TLR ligands.

Quantitative Real-Time PCR
RNA transcripts were quantified by real-time PCR as described and adjusted to 2×10^5 copies of the housekeeping gene β-actin.15 Specific PCR primers are listed in Table I in the online data supplement, available at http://circres.ahajournals.org.

Immunostaining of Tissue Sections
OCT-embedded arteries were sectioned at 7-μm intervals and immunostained as described15 using the following primary antibodies: mouse anti-human CD3 (1:200, Dako, Carpinteria, Calif), mouse anti-human TLR4 (1:100; Biotechnology, Santa Cruz, Calif), mouse anti-human TLR5 (1:100; ImageA, San Diego, Calif), or mouse anti-human CCR6 (1:100; R&D Systems, Minneapolis, Minn). Isotype-matched primary antibodies served as control. Antibody binding was visualized with biotin-conjugated goat antimouse IgG (BD Pharmingen, San Diego, Calif). Tissue sections were stained with propidium iodine (Sigma) and examined by fluorescence microscopy. Sections from paraffin-embedded temporal arteries were dewaxed before being treated with mouse anti-human CCR6 antibody at 4°C overnight. To quantify tissue-invasive capability, T cells were stained with anti-CD3 antibodies, and their distance to the tissue surface was measured using Image J 1.36b software (NIH).

Human Temporal Artery–SCID Mouse Chimeras
Human temporal artery–SCID mouse chimeras were generated by subcutaneous implantation of temporal artery sections into NOD.CB17 Prkdc (SCID) mice (The Jackson Laboratory, Harbor, Me) as previously described.15 Three mice implanted with temporal artery tissues from the same donor were assigned to 2 treatment arms and a control arm. On day 7 after implantation, mice were injected with LPS (3 μg/mouse), flagellin (3 μg/mouse), or PBS. On day 8, PBMCs or PBMCs depleted of CCR6^+ T cells (3×10^6 cells/mouse) or CD4^+ T cells (10^6 cells/mouse) were adoptively transferred into the chimeras by IV injection. On day 15, arterial grafts were harvested for RNA extraction or embedded into OCT compound for immunostaining. The protocol was approved by the Emory University Institutional Animal Care and Use Committee.

Statistical Data Analysis
Results were analyzed using the 2-sided Students t test. Data are shown as means±SEM.

Results
TLR4 and TLR5 Ligands Activate Resident DCs in Temporal Arteries
To explore whether human arteries respond to different pathogen-associated patterns with distinct biological outcomes, human temporal arteries were stimulated with a panel of TLR ligands (Figure 1A). Based on previous studies establishing TLR expression patterns in human macrovessels,12 LTA (TLR2/6 ligand), poly(I:C) (TLR3 ligand), LPS (TLR4 ligand), flagellin (TLR5 ligand), and CpG (TLR9 ligand) were chosen as stimulatory agonists. Response patterns were quantified by the induction of CD83 transcripts, a sensitive measure of DC activation. In pilot experiments,

Materials and Methods
Human Temporal Arteries, Lymph Nodes, and Skin
Thirty-eight temporal artery specimens were collected by diagnostic temporal artery biopsy or harvested at postmortem examination. Normal arteries derived from patients with no clinical evidence for polymyalgia rheumatica or any other vasculitic syndrome, lacked inflammatory infiltrates, and had no changes indicating atherosclerosis. Vessels were cut into 5-mm pieces and used immediately for culture in vitro or engrafted into immunodeficient mice. For RNA extraction, arteries were immediately shock frozen and for immunohistochemical staining, specimens were embedded in OCT compound (Sikura Fine-Tek, Torrance, Calif). Human lymph nodes and skin were collected from surgical waste tissues and processed immediately after removal. All protocols were approved by the Institutional Review Boards, and appropriate consent was obtained.

Reagents
Lipopolysaccharide (LPS) (Escherichia coli, 0127:B8), poly(I:C) (polynosinic:polycytidylic acid), lipoteichoic acid (LTA), and CpG were purchased from Sigma-Aldrich (St Louis, Mo). Flagellin was purified as described previously17 or obtained from InvivoGen (San Diego, Calif).

Isolation of Cells
Human peripheral blood was obtained from healthy donors and mononuclear cells (PBMCs) were isolated by Ficoll–Paque (Pacigr, Manassas, Va). CD14^+ cells were purified with anti-human CD14 MicroBeads using the AutoMACS system (Miltenyi Biotec, Manassas, Va). Monocyte-derived DCs were generated from CD14^+ precursors at 37°C for 5 to 6 days in RPMI 1640 supplemented with 1600 U/mL recombinant human GM-CSF and 1000 U/mL recombinant human interleukin-4. CD4^+ T cells were purified with anti-human CD4 MicroBeads (Miltenyi Biotec) for T-cell migration assays or for T-cell adoptive transfers. CCR6^+ cells were depleted from PBMCs with phycoerythrin (PE)-conjugated mouse anti-human CCR6 antibodies (BD Pharmingen) and anti-PE microbeads.

In Vitro Organ Culture
Intact human lymph nodes, skin, or temporal arteries were cultured in RPMI 1640 (10% FCS) in 48-well flat-bottom plates and stimulated with different TLR ligands for 14 hours at 37°C: LTA (500 μg/mL), poly(I:C) (100 μg/mL), LPS (3 μg/mL), flagellin (3 μg/mL), or CpG (50 μg/mL). Tissues were then harvested and used for RNA isolation. For kinase CD83 gene induction assays, tissues were stimulated with LPS (3 μg/mL) or flagellin (3 μg/mL) for 12, 24, or 48 hours. In selected experiments, temporal arteries were cultured with 1×10^6 human CD4 T cells for 3 days after stimulation with TLR ligands.

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optimal stimulatory concentrations for each ligand were determined. Response patterns of temporal arteries were compared to that of 2 DC-rich tissues, human lymph nodes and skin (Figure 1A and supplemental Table II). LPS and flagellin emerged as the 2 strongest stimulators of arterial tissues with a 16- and 30-fold upregulation of CD83 transcripts, respectively (Figure 1A). Transcript induction translated into surface expression of CD83 protein (Figure 1C). LPS-mediated activation was maximal after 12 hours and rapidly declined at 24 and 48 hours. TLR5 ligation resulted in prolonged DC activation with CD83 transcripts at a plateau between 12 and 24 hours (supplemental Figure I). TLR3 and TLR9 ligands barely elicited a temporal artery response; both were efficiently detected by lymph nodes (supplemental Table II). The 3 tissues each displayed a unique TLR response profile. Skin was explicitly sensitive to TLR4 ligands, unresponsive to TLR5 agonists, reactive to TLR3, and minimally affected by CpG. Lymph nodes sensed each of the pathogen-derived patterns; however, CD83 induction varied widely among different agonists. Notably, the 3 tissues appeared to recognize each TLR ligand with differential efficiency. These experiments established selectivity in the TLR responsiveness of human organs and indicated that temporal arteries were particularly sensitive to TLR4 and TLR5 ligands.

Because arterial walls are complex structures, we examined which cells in temporal arteries carried TLR4 and TLR5 receptors. Tissue extracts contained high and comparable transcript levels for the DC markers CD11c and CD209 (DC-SIGN) but lacked the macrophage marker CD11b (Figure 1B). TLR4 and TLR5 transcripts were abundant and present in similar concentrations. Endothelial cells and VSMCs in vitro have been described as positive for TLR4 but immunohistochemical stains of normal arteries localized TLR4 proteins on spindly cells at the media–adventitia border (Figure 1D and 1F). von Willebrand factor–positive macroluminal endothelial cells had minimal TLR4 reactivity and the microendothelial cells of the vasa vasorum were consistently negative. Staining of serial tissue sections with anti-TLR4 and anti-CD209 or anti-TLR5 and anti-CD209 provided unequivocal evidence that the TLR-expressing cells were DCs (Figure 1F and 1G, insets).

**TLR4 and TLR5 Ligands Induce Differential Patterns of Adaptive Immune Responses in the Vessel Wall**

To determine whether the mode of activation affected evolving immune responses in the vascular wall, the effects of TLR4 and TLR5 ligands on T-cell immunity were compared. Temporal arteries from the same donor were engrafted into multiple SCID mice, and T-cell responses were probed by adoptive transfer of allogeneic CD4 T cells. LPS or flagellin pretreatment resulted in prompt and comparable T-cell recruitment and in situ activation (Figure 2A and supplemental Figure II). Similarly, stimulation of human temporal arteries in organ culture demonstrated that both TLR4 and TLR5 agonists initiated adaptive immune responses and facilitated recruitment of human T cells into the vessel wall. In the chimera system, concentrations of interferon-γ transcripts indicated similar efficiency of TLR4- and TLR5-sensing DCs in stimulating T cells (supplemental Figure II). However, spatial analysis of tissue-infiltrating lymphocytes in the arteries revealed fundamental differences in the immune responses initiated by TLR4 or TLR5 ligands. Immunohistochemistry demonstrated tissue-invading T cells in the media of LPS-stimulated vessels (Figure 2B and 2E) whereas the smooth muscle cell layer remained essentially T cell–free in TLR5-triggered arteries (Figure 2B). Conversely, CD3⁺ T cells accumulated in the adventitia of arteries exposed to flagellin (Figure 2C and 2F). In essence, tissue inflammation...
patterns were closely correlated with the pathogen-derived motif initially recognized by the vascular DCs. LPS, but not flagellin, endowed vascular DCs with the ability to recruit and/or instruct T cells to generate wall-penetrating infiltrates. Conversely, flagellin produced T-cell recruitment and activation patterns resembling perivasculitis.

**TLR4 Ligation Selectively Upregulates CCL20 Expression and Induces CCR6⁺ CD4 T-Cell Recruitment In Vitro and In Vivo**

As site-specific T-cell accumulation was determined by the initial mode of DC activation, we explored how TLR4 and TLR5 ligands differentially affected DC function. Using a gene array tailored for activated DCs, we compared a series of 58 gene transcripts following LPS or flagellin stimulation in vitro. In 4 independent experiments, the induction of 47 genes, including DC markers CD83 and CD86, was very similar under both activation conditions. A set of genes, including the chemokine CCL20, was differentially induced (Figure 3A). Compared to unstimulated DCs, LPS upregulated CCL20 expression 6.5-fold, whereas flagellin enhanced CCL20 transcripts only by 2.8-fold. Quantification of CCL20 transcripts by real-time PCR in LPS- or flagellin-treated DCs corroborated the gene array data (Figure 3B). CCL20 exclusively binds to CCR6, a receptor expressed on a specialized T-effector cell subset. To prove functional relevance, chemotraction of CD4 T-cell subsets was evaluated in migration assays using modified Boyden chambers. In vitro–generated DCs placed in the lower chamber remained untreated or were stimulated with LPS or flagellin. T cells positioned in the upper chamber were allowed to migrate toward the chemokine-producing DCs. After 2 hours, T cells attracted by untreated DCs included only 10% of CCR6⁺ CD4 T cells. Flagellin triggering of the DCs did not affect the representation of CCR6⁺ CD4 T cells. However, after LPS stimulation, DCs selectively attracted CCR6⁺ CD4 T cells enriching them to 32% (Figure 3C).
CCR6+ T Cells in the Vasculitic Infiltrates of GCA

To test whether CCR6+ T cells populate the panarteritic infiltrates in GCA, we analyzed inflamed temporal arteries from GCA patients and stained circumferential sections with CCR6-specific antibodies. CCR6 receptors were expressed on the majority of mononuclear cells but not giant cells and histiocytes within the inflammatory infiltrates (Figure 4F). Most lymphocytes infiltrating the lumen-occlusive intima stained positive. Adventitial infiltrates included a low frequency of CCR6+ cells (Figure 4E). CCR6+ cells accumulated at the intima–media junction surrounding giant cells and the fragmented elastic lamina (Figure 4F). Overall, the immunohistochemical analysis confirmed strong CCR6+ T-cell enrichment in the vasculitic infiltrates and the relevance of the CCL20-CCR6 axis in large-vessel vasculitis.

CCR6 Blockade Abrogates CD4 T-Cell Invasion and Prevents VSMC Damage

TLR4-mediated CCL20 induction and CCR6+ T-cell accumulation in the GCA lesions suggested a unique role of CCR6+ T cells in transmural arteritis. Human arteries were engrafted into SCID mice; chimeras were injected with LPS or sham treated, and CD4 T cells were adoptively transferred. These CD4 T cells were coadministered with anti-human CCR6 antibodies or isotype control antibodies. TLR4 ligation promoted accumulation of vessel wall infiltrates (Figure 5A). Disrupting the CCL20-CCR6 axis by coadministering anti-CCR6 abrogated the media-invasive phenotype of the developing vasculitis. In chimeras injected with CD4 T cells precoated with anti-CCR6 antibodies, T-cell infiltrates were restricted to the adventitia (Figure 5B and 5D). To quantify media invasion, the migration depths of individual T cells were measured by digital analysis of tissue sections stained for the T-cell marker CD3 (Figure 5D). Without LPS, few T cells were recruited to the arteries, with none migrating deeper than 50 μm. After TLR4 ligation, CD4 T cells invaded deeply into the wall, on average 300 μm, with the most advanced T cells passing almost 1000 μm. After antibody-mediated CCR6 blockade, the migration capacity of activated T cells was markedly impaired; CD4 T cells were retained in the adventitia, and only a few progressed into the smooth muscle cell layer.

To explore whether invading T cells caused VSMC damage, we measured the transcriptional activity for smoothelin, a molecule typically produced by healthy contractile smooth muscle cells. Loss of smoothelin-specific transcripts is a typical finding for arteries affected by GCA (Figure 5E). Adoptive transfer of anti–CCR6-coated CD4 T cells protected smoothelin production, indicating that CCR6 blockade shields the tissue from inflammatory damage (Figure 5F).

CCR6-Positive T Cells Possess Tissue-Invasive Capabilities in Vascular Inflammation

To confirm that a specialized CCR6+ T-cell subset is responsible for causing panarteritis, we compared the vascular inflammation patterns mediated by PBMCs containing the CCR6+ subpopulation or depleted of CCR6+ cells. Normal human arteries were engrafted, and chimeras were LPS-
conditioned or sham-treated. PBMCs for adoptive transfer were left unseparated or depleted of CCR6-expressing cells. Recruited cells assumed a characteristic distribution (Figure 6). T-cell recruitment into the vessel wall occurred only if unseparated PBMCs were transferred (Figure 6A). CCR6+ cell depletion essentially abrogated vascular infiltrate formation. Enumeration of media-residing cells demonstrated minimal infiltrates in the grafts of sham-treated chimeras and in grafts of chimeras injected with CCR6-depleted cell preparations. Dense cell infiltrates accumulated among the medial

Figure 5. CCR6+ T cells cause transmural vascular inflammation and smooth muscle cell injury. Temporal artery–SCID mouse chimeras were injected with LPS (3 µg/mL) or PBS (control) on day 7 after implantation; 24 hours later, CD4 T cells combined with anti-CCR6 antibodies (50 µg/mL) or isotype control antibody (50 µg/mL) were adoptively transferred. One week later, grafts were harvested and human T cells (brown) were identified by immunohistochemistry for CD3. A, Transmural T-cell infiltrates in grafts explanted from LPS-treated chimeras injected with CD4 T cells and isotype control antibody. B, T cells in grafts from chimeras treated with LPS and adoptively transferred with CD4 T cells plus anti-CCR6 antibodies. C, Minimal inflammatory infiltrates in arteries pretreated with PBS and adoptively transferred with CD4 T cells. Original magnification, ×200. D, T-cell tissue invasion was quantified by measuring the distance of infiltrating T cells from the surface of the artery using Image J1.36b software. Invasion distances for individual CD3 T cells were determined in 3 independent experiments. Mean invasion distances are indicated by bars. E and F, VSMC injury was quantified by measuring transcripts of smoothelin, a gene product produced by healthy contractile VSMCs. E, Smoothelin transcripts in GCA-affected (n=5) and noninflamed control temporal arteries (n=5). Results are presented as means±SEM. F, Recovery of smoothelin transcription by CCR6 blockade. Arteries were explanted from chimeras treated with LPS and adoptively transferred with CD4 T cells plus isotype control antibodies or anti-CCR6 antibodies. Smoothelin transcripts were quantified by quantitative PCR and are expressed as percent of transcripts present in sham-treated arteries. Data represent 1 of 4 independent experiments and are given as means±SEM.

Figure 6. CCR6+ T cells infiltrate into the vascular wall to cause panarteritis. Human artery–SCID chimeras engrafted with noninflamed temporal arteries from 5 different donors were injected with LPS (3 µg/mL) or PBS. Allogeneic PBMCs (3×10^7 cells/mouse), which were unseparated or depleted of CCR6+ cells, were adoptively transferred. Arteries were explanted 1 week later. A, CCR6 gene expression was quantified by real-time PCR. Results are presented as means±SEM. B through E, Fluorescence microscopy for propidium iodine–positive cells to determine positioning of wall-infiltrating inflammatory cells. Media VSMCs were identified through green autofluorescence. Numbers of propidium iodine–positive cells counted in a minimum of 10 high-powered fields in each of 10 independent cross-sections are presented as means±SEM. C through E, Positioning of tissue-infiltrating cells in arterial cross-sections. Green cells are VSMCs; red cells are nuclei of infiltrating cells; nuclei of VSMCs appear yellow because of the overlay of green and red. C, Vascular infiltrate in the absence of TLR stimulation. D, Vascular infiltrate generated by LPS pretreatment and adoptive transfer of unseparated PBMCs. E, Vascular infiltrate after LPS pretreatment and adoptive transfer of CCR6-depleted PBMCs.
VSMCs if unseparated PBMCs were transferred (Figure 6B). Direct visualization of tissue-invasive cells demonstrated marked differences in the architecture of vasculitic infiltrates. Without LPS pretreatment, cells failed to enter the smooth muscle cell layer (Figure 6C). Crowded cellular infiltrates accumulated between the smooth muscle cell lamellae if vascular DCs were activated through TLR4 ligation and the adoptive transfers provided CCR6+ cells (Figure 6D). Depletion of CCR6+ cells prevented infiltration of adoptively transferred cells into the media (Figure 6E). These experiments supported a selective role of CCR6+ T cells in causing panarteritis.

Discussion

Human medium and large arteries are populated by vascular DCs that sense pathogens and induce adaptive and vasculitic immune responses in the vessel wall.12 In the atherosclerotic plaque, wall-residing DCs sustain and amplify inflammation.18 Herein, we show that the role of vascular DCs extends beyond enhancing immunity but that the activation mode determines the architecture of the immune response emerging in the vessel wall. TLR4 ligation leads to transmural panarteritis, with CD4 T cells invading into the proximal wall layers and injuring VSMCs. Conversely, TLR5 triggering mediates the assembly of a perivasculitis with T cells accumulating in the adventitia. Our mechanistic studies show that TLR4-stimulated DCs preferentially produce CCL20, resulting in the recruitment and activation of a specialized CCR6+ T-cell subset. T-cell infiltrates in the medial smooth muscle cell layer are highly enriched for CCR6+ cells which may find optimal survival conditions in this microenvironment. Thus, vessel wall–embedded DCs not only initiate adaptive immune responses; they shape the architecture of inflammation, and different bacterial pathogens induce distinct vasculitis types. Categorizing patients with large-vessel vasculitis according to the patterning of inflammation may provide an important clue in the search for causative agents.

Human macrovessels display a broad spectrum of pattern-recognition receptors, but it is not known whether biological outcomes of recognizing “danger signals” is uniform or is specific for the stimulator. Human temporal arteries responded to a series of pathogen-derived motifs with a tissue-specific response, distinct from that of the skin and the lymph node. Intact arteries appeared to specialize in detecting bacterial products binding TLR4 or -5, yet the biological consequences were ligand-specific. TLR4 ligation resulted in the recruitment and activation of a specialized CCR6+ T-cell subset. T-cell infiltrates in the medial smooth muscle cell layer are highly enriched for CCR6+ cells which may find optimal survival conditions in this microenvironment. Thus, vessel wall–embedded DCs not only initiate adaptive immune responses; they shape the architecture of inflammation, and different bacterial pathogens induce distinct vasculitis types. Categorizing patients with large-vessel vasculitis according to the patterning of inflammation may provide an important clue in the search for causative agents.

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The TLR9 nonresponsiveness of temporal arteries (Figure 1 and supplemental Table II) is in line with the lack of plasmacytoid DCs in normal arterial walls, whereas that DC subtype is critically involved in amplifying inflammation in the atherosclerotic plaque.18

The present data implicate CCR6+ T cells as particularly relevant in mediating vasculitis, at least in GCA. Several lines of evidence link CCR6+ CD4 T cells to the panarteritic pattern of vasculitis. CCR6+ cells populate the inflammatory infiltrates in GCA-affected arteries (Figure 4). CCR6 anti-body blockade abrogates media-penetrating infiltrate formation, and CCR6+ T-cell depletion disrupts transmural infiltrates and restricts T cells to the adventitia (Figures 5 and 6).

Here, we propose that CCR6+ T cells are a potential target for therapeutic interventions in large-vessel vasculitis. So far, the CCL20-CCR6 axis has been implicated ensuring protective immune responses.21 CCL20 regulates turnover and positioning of CCR6+ immature DCs in peripheral tissues. In GCA lesions, DCs are greatly enriched and participate in forming the granulomas. Possibly, CCL20 production by wall-residing DCs facilitates further influx of DCs, enhancing their role in granuloma stabilization. Staining of GCA arteries with anti-human CCL20 antibodies ruled out its expression by the medial layer of smooth muscle cells or endothelial cells (data not shown). CCL20-CCR6 interactions shape intestinal immunity and lympho-organogenesis.22 Produced by intestinal epithelial cells, CCL20 orchestrates B-cell recruitment and the formation of lymphoid follicles in the gut. Besides B cells and immature DCs, multiple T-cell subsets express CCR6. CCL20 directs activated T cells into the skin during contact hypersensitivity.23 Asthmatic patients preferentially mobilize CCR6+ T cells during allergic responses.24 Recently, CCR6 has been identified on multiple Th17 cell subsets, as well as other regulatory T-cell subsets, specifically Foxp3+ T cells.25 CCR6 appears on Tr1 cells and on CD8+ effector memory T cells. CD8 T cells as well as Foxp3+ cells are explicitly infrequent in GCA lesions (data not shown), suggesting an unopposed proinflammatory role for CCR6+ DCs in this vasculitis. Blockade of CCR6 disrupted tissue damage in the artery; precisely, it restored production of smoothelin, a VSMC molecule linked to contractility. This observation identifies VSMCs as a direct target of T cell–mediated wall injury.

Corticosteroids, commonly used to treat GCA and TA, target the proinflammatory transcription factor nuclear factor κB26 CCL20 gene transcription and production are controlled by nuclear factor κB and sensitive to extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase inhibitors.27 Thus, present treatment may actually target the CCL20-CCR6 axis. With the devastating side effects of steroid therapy, a more sophisticated way of paralyzing CCR6+ T cells could revolutionize the therapy of large-vessel vasculitis.

As yet, the causative agents initiating GCA remain elusive. The data presented here suggest that innate immune reactions, shaped by vascular DCs, are not only inductive for arteritis but also determine the architecture and organization of the pathogenic response. The correlation between the mode of DC activation and the vasculitis pattern may provide useful
clues in the identification of disease instigators. Based on biopsy findings, patients should be categorized into those with panarteritis and periarteritis as a means of defining more homogenous subsets for pathogenic and therapeutic studies.

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Disclosures
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Online Figure I. Time kinetics and dose dependence of TLR4 and TLR5 stimulation of human macrovessels. Human temporal arteries from 3 donors were incubated with LPS at different concentrations (0, 0.3, 3 μg/ml) for 14 h (A) or at 3μg/ml for 12, 24, or 48 h (B). Human temporal arteries were incubated with flagellin for 14 h at different concentrations (0, 3 or 6 μg/ml) (C) or at 3 μg/ml for 12, 24, or 48 h (D). CD83 gene expression was quantified by real-time PCR. Data are from three independent experiments and presented as mean ± SEM.
Online Figure II. Efficiency of TLR4 and TLR5 agonists to facilitate T-cell recruitment and activation. Human temporal artery-SCID chimeras were treated with saline, LPS, or flagellin as described in Fig. 2. Purified human CD4 T cells were adoptively transferred one day later. Human artery grafts were retrieved 7 days later, and RNA was isolated. Gene expression of T-cell receptors (A) or IFN-γ (B) in the grafts was quantified by real-time PCR with human-specific primers. Data are from three independent experiments and presented as mean ± SEM.
Online Figure III. TLR4 agonists preferentially induce production of the chemokine CCL20 in vivo. Human temporal arteries were engrafted into SCID mice and injected with PBS, TLR4 ligands (LPS), or TLR5 ligands (flagellin) as described in Fig. 2. Human CD4 T cells were isolated and injected into the chimeras one day later. Artery grafts were retrieved after 7 days and processed for RNA isolation. Expression of transcripts specific for the chemokine CCL20 was analyzed by qRT-PCR. Data are from three independent experiments and presented as mean ± SEM.
### Online Table I. Human-specific primer pairs for mRNA quantification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense</th>
<th>Anti-sense</th>
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<tbody>
<tr>
<td>TLR4</td>
<td>5-CTGCAATGGATCAAGGACCA-3</td>
<td>5-TTATCTGAAAGGGTTTCAGCATTCC-3</td>
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<td>TLR5</td>
<td>5-TGCCCTTGAAGGCTTCGTTTATG-3</td>
<td>5-CCAAACCCAACCATGATGAG-3</td>
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<td>CD83</td>
<td>5-GTTATTGGAGGGGTGGTTAAGAGGG-3</td>
<td>5-GTGAGAGAGTCACTAGCCCTAATGTC-3</td>
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<tr>
<td>CD11c</td>
<td>5-ACCTACTTACCCCTACCTGTCAGG-3</td>
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<td>CD11b</td>
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<td>TCR</td>
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<td>IFN-γ</td>
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<td>β-actin</td>
<td>5-ATGGCCACGGCTTCCCAGC-3</td>
<td>5-CATGGTGAGTGCGGCGACACAG-3</td>
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## Online Table II. Responsiveness of human tissues to different TLR agonists

<table>
<thead>
<tr>
<th>Ligand</th>
<th>(µg/ml)</th>
<th>CD83 expression (fold induction)</th>
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<tr>
<td></td>
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<td>Lymph node</td>
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<tr>
<td>LTA</td>
<td>500</td>
<td>12</td>
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<tr>
<td>Poly (I:C)</td>
<td>100</td>
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</tr>
<tr>
<td>LPS</td>
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<tr>
<td>Flagellin</td>
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<td>398</td>
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<tr>
<td>CpG</td>
<td>50</td>
<td>5</td>
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</table>