Activation of Arterial Wall Dendritic Cells and Breakdown of Self-tolerance in Giant Cell Arteritis

Wei Ma-Krupa,[1,2] Myung-Shin Jeon,[1,2] Silvia Spoerl,[1,2] Thomas F. Tedder,[3] Jörg J. Goronzy,[1,2] and Cornelia M. Weyand[1,2]

[1]Department of Medicine and [2]Department of Immunology, Mayo Clinic, Rochester, MN 55905
[3]Department of Immunology, Duke University Medical Center, Durham, NC 27710

Abstract

Giant cell arteritis (GCA) is a granulomatous and occlusive vasculitis that causes blindness, stroke, and aortic aneurysm. CD4^{+} T cells are selectively activated in the adventitia of affected arteries. In human GCA artery–severe combined immunodeficiency (SCID) mouse chimeras, depletion of CD83^{+} dendritic cells (DCs) abrogated vasculitis, suggesting that DCs are critical antigen-presenting cells in GCA. Healthy medium-size arteries possessed an indigenous population of DCs at the adventitia–media border. Adoptive T cell transfer into temporal artery–SCID mouse chimeras demonstrated that DCs in healthy arteries were functionally immature, but gained T cell stimulatory capacity after injection of lipopolysaccharide. In patients with polymyalgia rheumatica (PMR), a subclinical variant of GCA, adventitial DCs were mature and produced the chemokines CCL19 and CCL21, but vasculitic infiltrates were lacking. Human histocompatibility leukocyte antigen class II–matched healthy arteries, PMR arteries, and GCA arteries were coimplanted into SCID mice. Immature DCs in healthy arteries failed to stimulate T cells, but DCs in PMR arteries could attract, retain, and activate T cells that originated from the GCA lesions. We propose that in situ maturation of DCs in the adventitia is an early event in the pathogenesis of GCA. Activation of adventitial DCs initiates and maintains T cell responses in the artery and breaks tissue tolerance in the perivascular space.

Key words: vasculitis • pathogenesis • T cells • polymyalgia rheumatica • Toll-like receptors

Introduction

Giant cell arteritis (GCA) is a systemic vasculitis that affects medium- and large-size arteries, most often the extracranial branches of the aortic arch and the aorta itself (1, 2). Arteritic lesions cause vascular stenosis and subsequent tissue ischemia (3). Severe complications of GCA include blindness, stroke, and aortic arch syndrome. The diagnosis of GCA is established by temporal artery biopsy, which typically shows granulomatous infiltrates, multinucleated giant cells, fragmentation of the elastic membranes, thinning of the media, and occlusion of the vascular lumen (4). Inflammatory lesions in GCA are composed of activated CD4^{+} T cells and macrophages. Selected CD4^{+} T cells undergo expansion in the artery, strongly suggesting antigen-driven responses (5). IFN-γ has been identified as a key cytokine and plays a critical role in regulating the effector functions of tissue-infiltrating macrophages (6, 7). Macrophages are committed

to distinct and nonoverlapping functional pathways (8), including the production of proinflammatory cytokines, the release of reactive oxygen intermediates (9, 10), the secretion of growth (11) and angiogenic factors (12), and the production of nitric oxide synthase (13). Intimal hyperplasia, the process that leads to vascular occlusion and ischemia, has been closely linked to both oxidative (9) and nitrosative stress (13) in the medial smooth muscle cell layer. Intimal hyperplasia is also associated with high tissue production of IFN-γ and the formation of multinucleated giant cells in the arterial wall lesions (14).

Clonal expansion of selected CD4^{+} T cells in the vascular wall suggests that adaptive immune responses drive the chronic inflammatory reaction (5). Interestingly, IFN-γ production by CD4^{+} T cells is limited to the arterial adventitia (7). The physiologic role of the adventitia is directly linked to the vasa vasorum, the capillary network supplying the wall structure. Because the vessel wall is otherwise avascular,

the adventitia provides the only physiologic port of entry for T cells and macrophages into this structure. We have found recently that the adventitia includes a cellular component that predisposes it to supporting T cell responses. Normal temporal arteries have a population of resident DCs that are positioned at the adventitia–media border (15). These adventitial DCs reside outside the external elastic lamina, which separates the adventitia from the smooth muscle cell–rich tunica media. The DCs typically form a ring of dendrites around the circumference of the artery. It is unknown how these adventitial DCs contribute to immunosurveillance, which types of antigens they sample, and which signals determine their maturation.

The spectrum of the GCA syndrome includes polymyalgia rheumatica (PMR), a variant that shares many clinical and epidemiological features with GCA, but lacks its vasculitic and ischemic manifestations (6, 16, 17). PMR is a syndrome of muscle pain and stiffness that affects the neck, shoulders, and pelvic girdle. PMR can be present in patients with GCA, but PMR occurs more frequently in the absence of fully developed GCA. In PMR, temporal artery specimens do not show an appreciable mononuclear infiltrate or any tissue damage by histological examination. However, a minute number of activated T cells and macrophages can be detected by reverse transcription–PCR (6).

Here, we report that activation of adventitial DCs is an early and critical event in GCA that precedes the invasion of T cells and macrophages. In normal arteries, adventitial DCs are immature. In situ triggering of Toll-like receptors (TLRs) is sufficient to induce the differentiation of DCs so that they are capable of stimulating T cells. In arteries from patients with PMR, adventitial DCs are no longer immature but are activated and capable of triggering T cells. Highly activated, chemokine-producing DCs are essential to maintain established GCA; depletion of CD83+ DCs abrogates the disease process. These data suggest a critical role of the innate immune system in the initiation and maintenance of vasculitis and assign a gatekeeper function to specialized DCs positioned in the arterial adventitia.

Materials and Methods

Patients and Temporal Arteries. Temporal artery specimens were collected from patients undergoing diagnostic temporal artery biopsy. 36 patients with typical histomorphology of GCA and 30 patients with the diagnosis of PMR and lacking histomorphologic findings of vasculitis were enrolled. At the time of the temporal artery biopsy, none of the patients were on >10 mg qd corticosteroids. 32 patients who had undergone diagnostic biopsy for suspected GCA but who had neither PMR nor GCA served as controls. Temporal arteries were divided into several sections and were shock frozen for RNA analysis and embedded in OCT compound (Sikura Fine-Tek) or in paraffin. The protocol was approved by the Mayo Clinic Institutional Review Board, and all patients gave informed consent.

Reagents. LPS (*Escherichia coli*, 0127:B8) and CFA were purchased from Sigma-Aldrich. Human TNF-α was obtained from R&D Systems. Mouse anti-CD83 Ab (IgG1 κ, clone HB15e; Research Diagnostics) was used for in vivo experiments. The

specificity of this antibody has been described previously (18, 19).

The following Abs were used for immunohistochemical procedures: mouse anti–human CD83 (1:1,000; Research Diagnostics), mouse anti–human CCL19 (1:300), goat anti–human CCL21 (1:300; both obtained from R&D Systems), rabbit anti–S-100 (1:500), mouse anti–human CD3 (1:300), mouse anti–human fascin (1:100), mouse anti–human CD11c (1:100), goat anti–rabbit Ig, rabbit anti–mouse Ig, and goat anti–mouse Ig (all obtained from DakoCytomation).

Immunohistochemical Analysis. The Abs used in this work and their optimal working dilutions have been described previously (10, 13, 15). Paraffin-embedded samples were cut into 5-μm sections and deparaffinized in 100% xylene. For fascin staining, the deparaffinized tissue was steamed in citrate buffer for 30 min to facilitate antigen recovery. Endogenous peroxidase activity was blocked, and the sections were incubated in 5% goat serum (Invitrogen). Slides were stained with anti–S-100 or anti–human fascin Ab at room temperature (RT) for 1 h in a humidified chamber. The sections were washed with tap water and incubated at RT with biotin-conjugated goat anti–rabbit Ig Ab for 30 min. To stain for S-100, the slides were washed with tap water and an avidin-biotinylated enzyme solution (ABC-peroxidase kit; Vector Laboratories) was added for 30 min. Red staining was produced using aminoethylcarbazole as the chromogen (Vector Laboratories) for 20–30 min. The Vector ABC–alkaline phosphatase kit was used with Vector blue for fascin staining. Sections were counterstained with hematoxylin solution (Surgipath) for 3 min.

OCT-embedded sections of temporal arteries were cut into 5-μm sections and fixed with acetone for 10 min, dried for 30 min, and soaked in 1% paraformaldehyde solution, pH 7.4, for 5 min. After blocking endogenous peroxidase and incubating with 5% normal rabbit or goat serum (Invitrogen), the sections were stained for 1 h with unconjugated primary Ab followed by biotin-conjugated rabbit anti–mouse or goat anti–mouse Ig Ab (30 min at RT). The sections were developed with ABC–alkaline phosphatase using blue or red as chromogens (Vector Laboratories) for CD83 or CCL19 detection, respectively, or with ABC-peroxidase using 3,3′-diaminobenzidine as the chromogen for CD3 and CD11c detection. The tissue sections were washed with tap water, and the slides were counterstained with hematoxylin (Surgipath) for 3 min. For all staining experiments, control stains with isotype-matched primary Ab were included. Tissue sections were viewed by light microscopy and were photographed using a microscopic system (model LSM-510; Carl Zeiss MicroImaging, Inc.).

PCR. Total cellular RNA was isolated using TRIzol reagent (Invitrogen), reverse transcribed into single-stranded cDNA using avian myeloblastosis virus reverse transcriptase (Roche Applied Sciences), and amplified using specific primer pairs. Sequences of the primers used in the paper are listed in Table I. Optimal conditions for all primers were established by amplifying cDNA samples from human PBMC-derived mature DCs, human-activated T cell clones, human tonsils, and activated human PBMCs. TLR2 and TLR4 sequences were amplified using a thermocycler (model 9600; PerkinElmer), and CD14, CD83, CCL18, IL-18, CCL19, CCL21, and TCR α-chain were amplified using an UNO II thermocycler (Biometra) using the following conditions: 30 cycles of denaturation at 95°C for 60 s, primer annealing at 55°C for 60 s, and primer extension at 72°C for 90 s. The reaction products were visualized on ethidium bromide–stained 1% LE or 3% MS agarose gels (both Roche Applied Sci-

Table I. *Primer Sequences*

CD14	5′-CACACTCGCCTGCCTTTTCC-3′
	5′-GATTCCCGTCCAGTGTCAGG-5′
CD40L	5′-GAAGGTTGGACAAGATAGAAGATG-3′
	5′-GCCCACTGTAACACAGATGTTG-3′
CD83	5′-GTTATTGGAGGGTGGTGAAGAGAGG-3′
	5′-GTGAGGAGTCACTAGCCCTAAATGC-3′
CCL18	5′-GGTGTCATCCTCCTAACCAAG-3′
	5′-GGAAAGGGGAAAGGATGATA-3′
CCL19	5′-CCAATGATGCTGAAGACTGCT-3′
	5′-GCCAAGATGAAGCGCCGCA-3′
CCL21	5′-CCCCAGGACCCAAGGCAGTGATGGA-3′
	5′-TGCAAGAGGACTGAGCGGTCACA-3′
IFN-γ	5′-ACCTTAAGAAATATTTTAATGC-3′
	5′-ACCGAATAATTAGTCAGCTT-3′
IL-18	5′-GACCAAGTTCTCTTCATTGACCA-3′
	5′-ATGTTATCAGGAGGATTCATTTC-3′
TLR2	5′-GGCCAGCAAATTACCTGTGTG-3′
	5′-AGGCGGACATCCTGAACCT-3′
TLR4	5′-CTGCAATGGATCAAGGACCA-3′
	5′-TTATCTGAAGGTGTTGCACATTCC-3′
β-Actin	5′-ATGGCCACGGCTGCTTCCAGC-3′
	5′-CATGGTGGTGCCGCCAGACAG-3′

ences) and digitally documented for further analysis (GelDoc 2000; Bio-Rad Laboratories).

The method to quantify tissue cytokine mRNA, including the sequences of the primers and probes, has been described in detail previously (20). mRNA for the chemokines CCL19 and CCL21, the cytokines IFN-γ and IL-18, and the cell surface markers CD83 and CD40L was quantified using the LightCycler PCR (Roche Applied Sciences). 1 μl cDNA was diluted in a total volume of 20 μl of SYBR green master mix (Roche Applied Sciences) as described previously (15). The PCR thermal cycling was as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles of amplification at 95°C for 0 s, 57°C for 7 s, and 72°C for 16 s. Melting curve analysis was accomplished by 95°C for 0 s, 60°C for 30 s, and 95°C for 0 s. HLA-DRB1 alleles were determined from DNA as described previously (21).

Depletion of CD83⁺ DCs in GCA-SCID Mouse Chimeras. All animal procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee. Temporal artery–SCID mouse chimeras were generated by implanting pieces of temporal arteries from patients with GCA subcutaneously on the back of NOD-SCID mice (Jackson Laboratories) as described previously (10, 22, 23). Three to five mice were each implanted with temporal artery from the same donor and assigned to the treatment

group or the control group. On days 9–11 after the implantation, the mice were injected i.p. with 200 μg anti-CD83 Ab or with control Ig each day. On day 17, the arterial grafts were recovered and shock frozen in liquid nitrogen for mRNA analysis or embedded in OCT compound and stored at −80°C for immunohistochemical analysis.

Activation of Arterial DCs in Vivo. Arteries obtained from individuals who had neither GCA nor PMR were implanted into SCID mice in accordance with the standard protocol. 7–10 d after the implantation, the mice were injected i.v. with 2 μg TNF-α (2.2 × 10⁵ U), 10 μg LPS, or buffer control, or they were injected i.p. with 100 μl CFA. 48 h after the injections, the artery grafts were explanted. The tissue was shock frozen for mRNA isolation or was embedded in OCT compound and stored at −80°C for immunohistochemical studies.

Adoptive Transfer of Alloreactive T Cells. Alloreactive T cell clones were generated from an HLA-DR4⁻ donor by stimulation with HLA-DRB1*0401⁺ stimulator cells and maintained as described previously (24). Human T cell clones were used for adoptive transfer experiments 14 d after their last in vitro restimulation. Temporal artery–SCID mouse chimeras were generated by implanting pieces of normal temporal arteries that were free of any inflammation into NOD-SCID mice. One artery was divided into three pieces to be implanted into three mice that were assigned to three different treatment arms. 6 d after implantation, group 1 was injected i.v. with 10 μg LPS. Group 2 received control buffer. 30 h later, 5 × 10⁶ cells of the HLA-DRB1*0401-specific human T cell clone were adoptively transferred into both groups of mice. Group 3 served as a control and was treated with control buffer on days 10 and 12. The arterial grafts were recovered 18 d after the original implantation and 6 d after the adoptive transfer. Tissues were shock frozen for RNA analysis or embedded in OCT compound and stored at −80°C for immunohistochemical studies.

Induction of a T Cell Response in Tissues from Patients with PMR. Temporal artery specimens were selected from patients who typed positive for the HLA-DRB1*0401 allele. Temporal artery–SCID mouse chimeras were generated with noninflamed arteries, arteries from patients with PMR, or arteries from patients with GCA. An additional group of mice received arteries from all three sources, and the tissues were implanted into distinct subcutaneous sites. The grafts were retrieved 7 d after the implantation and analyzed for in vivo transcription by quantitative reverse transcription–PCR or by immunohistochemistry.

Statistical Analysis. Results from the quantitative reverse transcription–PCR were compared using the Mann-Whitney Rank Sum test, and the datasets of the cotransplantation experiments were analyzed by the Student-Newman-Keuls method of the Friedman Repeated Measures analysis of variance (SigmaStat; SPSS).

Results

A Resident Population of DCs in the Arterial Adventitia. The adventitia functions as a support tissue for the vessel and provides the vasa vasorum and the capillary network that supply the wall structure with oxygen. In addition to the microvessels, the adventitia is composed of fibroblasts and it also contains a population of S-100⁺ cells (Fig. 1 A). These S-100⁺ cells express fascin and CD11c (Fig. 1, B and C) but not the monocyte/macrophage marker CD14, which is consistent with the phenotype of DCs. Adventitial DCs form a ring of extended dendrites at the outer edge of
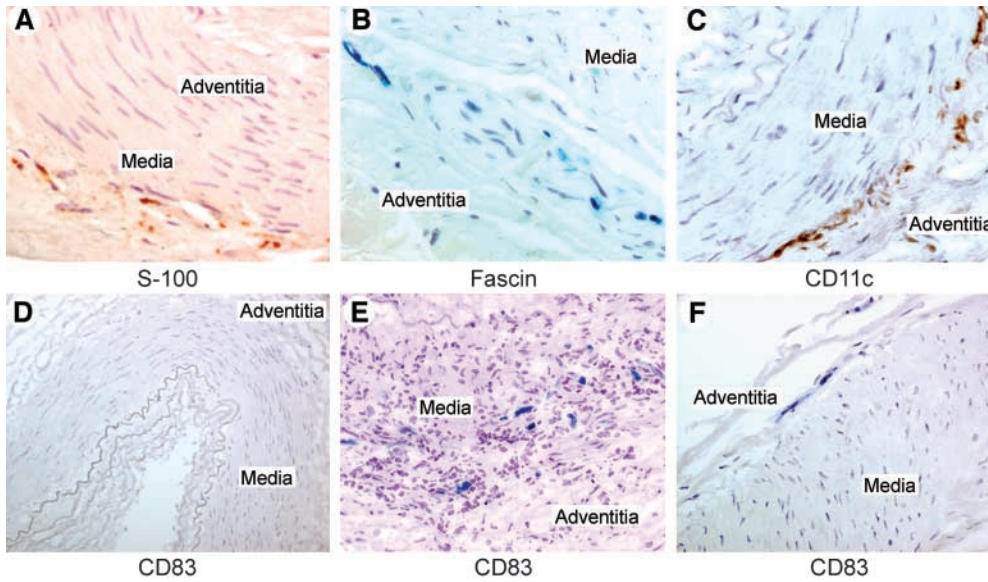
Figure 1. DCs in normal and inflamed temporal arteries. Temporal arteries were collected from diagnostic biopsies. Patients with granulomatous inflammatory infiltrates were categorized as having GCA. Patients were diagnosed with PMR if they had a typical clinical presentation but no vascular infiltrates. Normal temporal arteries were from patients who had neither PMR nor GCA. Paraffin-embedded arteries were stained with anti–S-100 (A), antifascin (B), or anti-CD11c Ab (C) to identify DCs. Frozen sections were stained with anti-CD83 Ab to assess DC activation (D–F). Normal temporal arteries contain a population of S-100^{+} (red), fascin^{+} (blue), and CD11c^{+} (brown) DCs positioned at the adventitia–media border (A–C). In noninflamed arteries, these DCs lack CD83 (D). Numerous CD83^{+} DCs (blue) participate in the granulomatous lesions in GCA (E). In arteries from patients with PMR, adventitial DCs have acquired the activation marker CD83 (F). Original magnification, 200 (D–F) and 400 (A–C).

the external elastic lamina. They lack CD83 (Fig. 1 D), indicating that they are in a resting state (19). Each arterial cross section contains 20–25 DCs. This DC population is constant in number and morphology in patients who undergo temporal artery biopsy to rule out vasculitis and who have neither GCA nor PMR.

In temporal arteries with vasculitis, DCs are greatly increased in number, acquire the CD83 activation marker, and populate the adventitia and also the media (15). They travel with the granulomatous infiltrates (Fig. 1 E). Phenotyping of arterial DCs in a cohort of temporal artery biopsies demonstrated that activated CD83^{+} DCs were also present in a subset of patients who lacked inflammatory infiltrates and who were diagnosed by a pathologist as not having vasculitis. Clinical analysis revealed that all patients with noninflamed arteries but with CD83^{+} DCs in the adventitia had PMR (Fig. 1 F).

The functional capabilities of DCs change fundamentally as the cells make the transition from the resting to the activated state. To examine the functional profile of vascular DCs in situ, we semi-quantified the production of chemokine transcripts in tissue extracts collected from 12 normal arteries, 18 arteries from patients with PMR, and 16 arteries from patients with GCA. As shown in Fig. 2, normal arteries were negative for CCL19-specific transcripts and expressed a low level of CCL21-specific sequences (median of 202 copies). In noninflamed arteries, there were no cells that stained with CCL19- or CCL21-specific Ab (unpublished data). In contrast, transcripts for both CCL19 and CCL21 were readily detected in arteries from patients with PMR, supporting the finding that DCs in these tissues are no longer resting. Concentrations of CCL19 and CCL21 mRNA copies in the arteries from the PMR patients did not reach the levels found in full-blown vasculitis. In the samples with fully developed GCA, copies for CCL19

were increased eightfold above controls (median of 1,543 copies), and copies for CCL21 were 24-fold higher (median of 7,662 copies) than in normal arteries. In immuno-
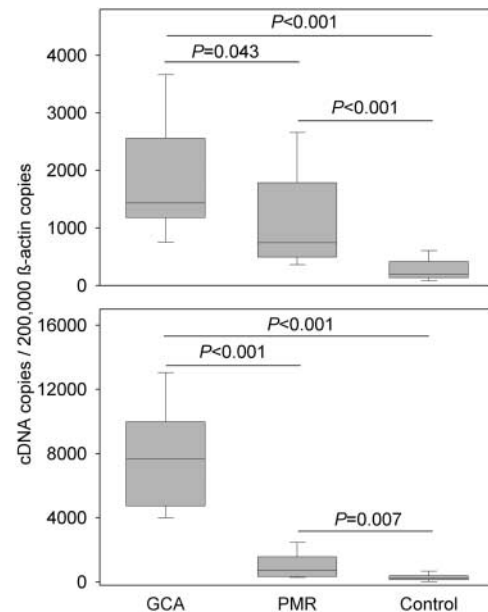

Figure 2. Tissue production of CCL19 and CCL21 in temporal arteries from patients with GCA or PMR and in normal arteries. Temporal arteries were collected, and cDNA was generated. Transcripts for β-actin, CCL19 (top), and CCL21 (bottom) were determined by quantitative PCR. cDNA concentrations were adjusted to 2×10^5 β-actin copies. The number of chemokine transcripts is presented as box plots, with the median and 25th and 75th percentiles as the box, and the whiskers signify the 10th and 90th percentiles. Normal arteries contained minimal copies of CCL19 and CCL21 mRNA. Abundant transcripts for both chemokines were present in arteries with GCA. Arteries from patients with PMR, despite the lack of inflammatory infiltrates, contained intermediate numbers of CCL19 and CCL21 mRNA transcripts.
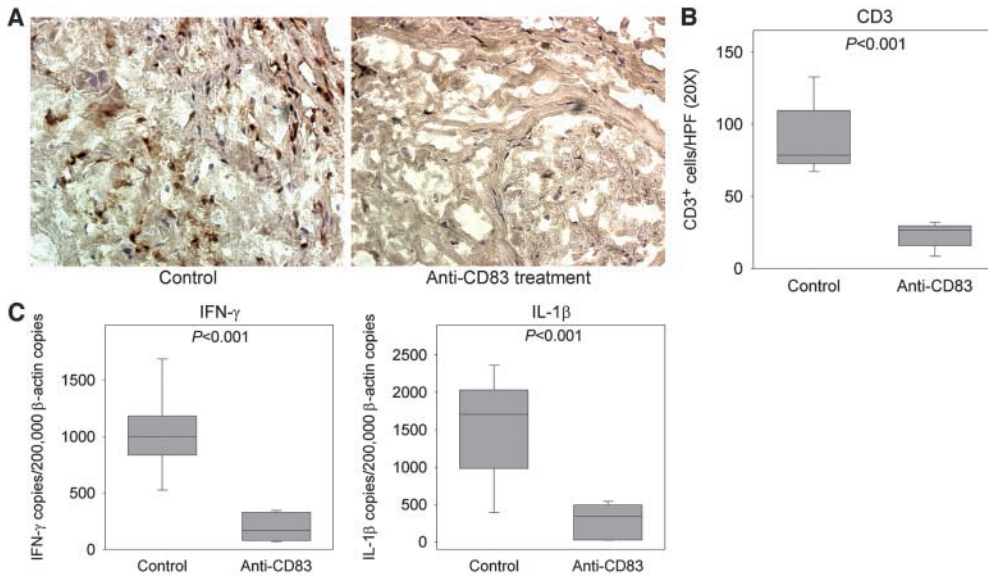
Figure 3. Therapeutic effects of depleting CD83+ DCs in GCA. Temporal artery–SCID mouse chimeras were generated by implanting SCID mice with segments from a GCA-affected temporal artery. The chimeras were injected on days 9–11 with anti-CD83 Ab or control Ig, and arterial grafts were harvested 1 wk later. Frozen tissue sections were immunostained with anti-CD3 Ab (A). The density of arterial wall T cell infiltrates was determined by counting CD3+ T cells (brown) on cross sections of the arteries (B). cDNA was generated from the explanted temporal arteries to quantify transcripts for β-actin, IFN-γ, and IL-1β by quantitative PCR. All cDNA concentrations were adjusted to 2×10^5 β-actin copies. Copy numbers of IFN-γ– and IL-1β–specific sequences are shown as box plots as described in Fig. 2. Results are from five experiments with tissues from five different patients. Treatment with anti-CD83 Ab induced massive loss of tissue-infiltrating T cells. In parallel, IFN-γ mRNA transcripts decreased to <20% of baseline, and IL-1β mRNA transcripts decreased by >75% (C). Original magnification, 400.

histochemical studies, the major source of CCL19 and CCL21 protein was CD83+ DCs (unpublished data).

These data suggest that adventitial DCs in normal arteries are immature and resting. In PMR, the arterial DCs have undergone activation despite the absence of an inflammatory infiltrate. Fully developed granulomatous lesions include a population of highly activated chemokine-producing DCs.

Treatment with CD83-Specific Antibodies Suppresses Arteritis. To investigate how DCs contribute to the disease process, we treated temporal artery–SCID mouse chimeras with
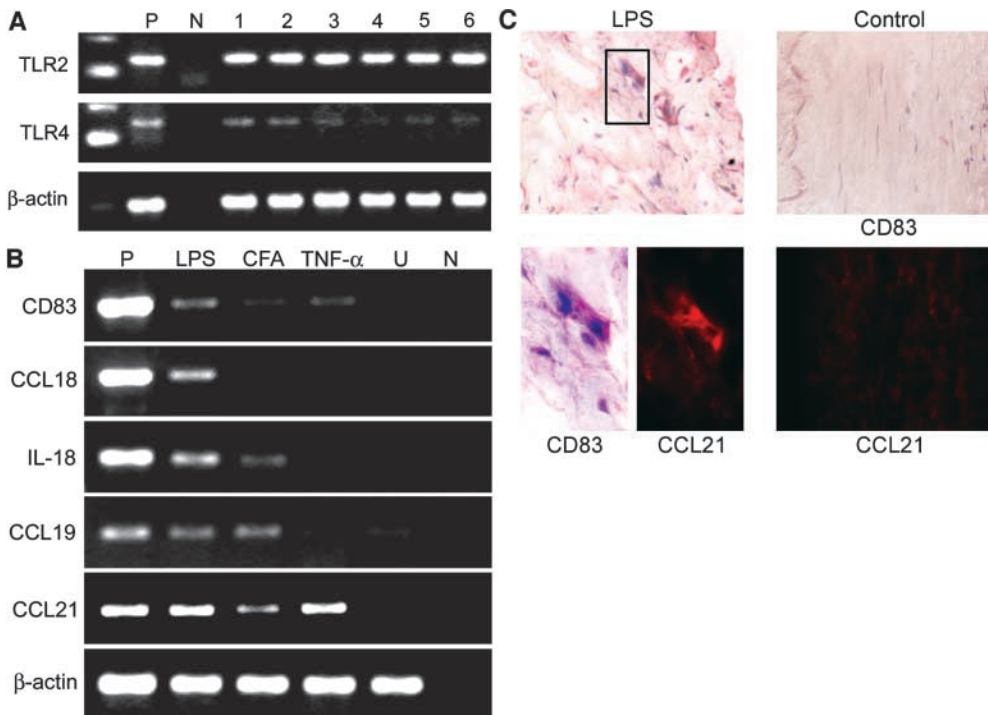

Figure 4. Functional characteristics of arterial wall DCs in normal arteries. Temporal arteries were collected from patients with neither GCA nor PMR. Tissue extracts from fresh shock-frozen samples were analyzed for TLR2- and TLR4-specific sequences by PCR. All negative arteries (marked as 1–6) contained mRNA transcripts for TRL2 and TLR4 (A). To test the responsiveness of arterial DCs to triggering with blood-born TNF-α or TLR ligands, we implanted pieces of arteries into SCID mice. 7–10 d after implantation, the SCID mouse chimeras were injected with 2 μg i.v. TNF-α, 10 μg i.v. LPS, or 100 μl i.p. CFA, and the arterial grafts were harvested 48 h later. Tissue extracts from the explanted grafts were analyzed for the mRNA transcripts of β-actin, CD83, IL-18, and the chemokines CCL18, CCL19, and CCL21. After stimulation with blood-born triggers, arterial wall DCs expressed CD83+ and began to produce an array of chemokines. The effect of TNF-α was limited to the induction of CCL21, whereas LPS induced the full spectrum of chemokines. One experiment representative of three is shown (B). Immunohistochemistry confirmed that arterial DCs from LPS-treated (left), but not control (right), arteries expressed CD83 (blue) and produced CCL21 (red) (C). Original magnification, 200 (except LPS-treated CD83 and CCL21 images, which were 600×). P, positive PCR control; N, untreated mouse control; and W, negative PCR control.

anti–CD83 Ab. Expression of the CD83 marker in the vas-culitic lesion was strictly limited to DCs (Fig. 1, E and F; reference 15). Temporal arteries from five patients were di-vided into three to five equal pieces. Mice carrying tissue from the same donor were injected with either control Ig or anti–CD83 Ab. Chimeras received Ab injections on days 9–11 after implantation, and the grafts were harvested 1 wk after the completion of the treatment. Histomorphological evaluation showed that anti–CD83 Ab treatment had a marked effect on the density of the T cell infiltrate (Fig. 3, A and B). After treatment with anti–CD83 Ab, the granu-lomatous microstructures were destroyed and few T cells were still present in the arterial wall. The remaining T cells were dispersed throughout the vascular tissue. To examine the effect on T cell and macrophage activity, cDNA was generated from tissue extracts, and transcript levels for IFN-γ and IL-1β were semi-quantified. After the adminis-tration of anti–CD83 Ab, levels of IFN-γ transcripts, re-flective of in situ T cell activation, decreased to <20% of those measured in the grafts from animals treated with con-trol Ig (Fig. 3 C). In parallel, the production of IL-1β, which is derived from macrophages in the vascular lesions, decreased to <300 copies. Thus, targeting CD83$^+$ DCs was highly effective in suppressing vasculitis, suggesting that T cell activation is strictly dependent on DC function.

Triggering of Arterial Wall DCs In Vivo. Considering the critical role of DCs in the vasculitic response, we wanted to explore whether DCs indigenous to the adventi-tia that are physiologically in a resting state could be stimu-lated to differentiate in vivo. DCs respond to their mi-croenvironment through a number of different receptors, of which TLRs play an important role (25). To determine the TLR profile expressed by adventitial DCs under nor-mal conditions, we amplified by PCR tissue extracts from normal temporal arteries with primers specific for TLR2 and TLR4. TLR2- and TLR4-specific sequences were present in all normal temporal arteries (Fig. 4 A). To exam-ine DC activation in vivo, temporal arteries from patients who had neither GCA nor PMR were engrafted into SCID mice, and the chimeras were injected with a panel of mediators known to activate DCs. The temporal arteries were explanted and analyzed for transcripts typically pro-duced by activated DCs.

As shown in Fig. 4 B, DCs in untreated arterial grafts re-mained immature, lacking CD83, IL-18, CCL18, CCL19, and CCL21. If the SCID mouse chimeras were injected with TNF-α, LPS, or CFA, the DCs began to differentiate, and each stimuli produced a different pattern of activation markers. Arteries harvested from the TNF-α–treated chi-meras contained CD83- and CCL21-specific transcripts. A broader spectrum of DC products, including CCL19, CCL21, and IL-18 could be obtained if the chimeras were treated with CFA. Optimal DC activation was achieved with LPS, which was the most efficient in the induction of CD83, IL-18, CCL18, CCL19, and CCL21. Immunohis-tochemistry confirmed that these products derived from ar-terial DCs. Fig. 4 C shows arterial tissue explanted from
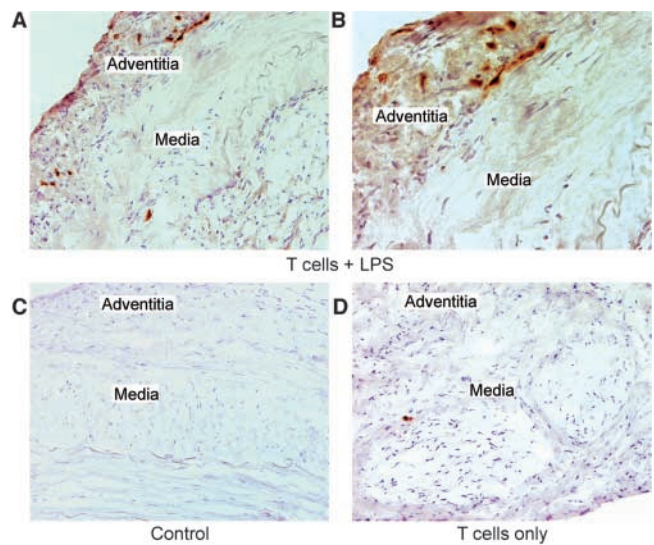

Figure 5. Activated arterial DCs are capable of stimulating T cells. Temporal artery–SCID mouse chimeras were generated by implanting normal temporal arteries from HLA-DRB1*0401$^+$ donors. 6 d after implantation, the chimeras received 10 μg LPS or PBS i.v., and 30 h later, 5 × 10^6 alloreactive human T cell clones specific for HLA-DRB1*0401 or T cell lines derived from arterial tissue of patients with GCA were adoptively transferred into the mice. Arterial grafts were explanted and embedded in OCT. To locate human T cells in the arterial grafts, frozen tissue sections were immunostained with anti-CD3 Ab. One experiment representative of five is shown. CD3$^+$ T cells (brown) were found in the arteries explanted from the chimeras that had been treated with LPS and had received the T cells. Tissue-infiltrating T cells accumulated along the adventitia–media junction (A and B). In the absence of LPS pretreatment, human T cells were rarely detected in the arteries (D). Grafts from animals that had received neither LPS nor T cells were free of human T cells (C). Original magnification, 200 (A, C, and D) and 400 (B).

LPS-treated and sham-treated SCID chimeras. LPS treat-ment induced the expression of CD83 and CCL21 selec-tively in DCs at the adventitia–media border. These exper-iments established that blood-born stimuli could reach DCs in the adventitia and induce maturation.

To investigate whether triggering of adventitial DCs was sufficient to establish T cell responses in the arterial wall, normal temporal arteries were selected from HLA-DRB1*0401$^+$ donors. The arteries were implanted into SCID mice, and 6 d later, the mice were injected with LPS. 30 h after the LPS injection, 5 × 10^6 HLA-DRB1*0401–specific alloreactive human T cell clones were adoptively transferred into the chimeras. The arteries, which were explanted 18 d after implantation, were enlarged and sur-rounded by highly inflamed perivascular tissue. Immuno-histochemistry demonstrated the accumulation of tissue-infil-trating human T cells in the graft (Fig. 5, A and B). Human CD3$^+$ T cells accumulated in the proximal adventitia and in the distal media, the site of DC localization. In contrast, adoptive transfer of alloreactive human T cell clones without prior LPS administration was not sufficient to induce the re-tention of human T cells in the artery wall (Fig. 5 D).

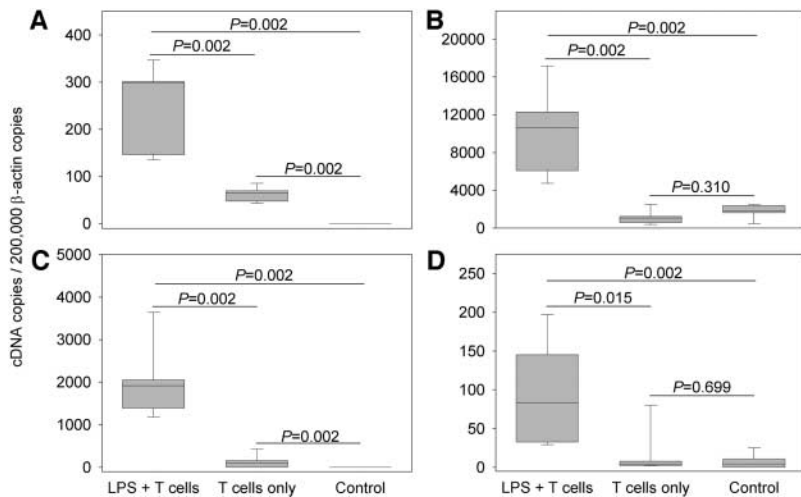To determine whether the cotreatment with LPS and human T cells had indeed resulted in the activation of tis–

Figure 6. Stimulation of adoptively transferred T cells by activated arterial DCs. Temporal artery–SCID mouse chimeras carrying HLA-DRB1*0401+ normal arteries were treated with LPS and adoptively transferred with alloreactive human T cells as described in Fig. 5. Explanted arterial grafts were shock frozen and used for the generation of cDNA. cDNA concentrations were adjusted to 2×10^5 β-actin transcripts. Tissue concentrations of β-actin, CD83 (A), IL-18 (B), IFN-γ (C), and CD40L (D) mRNA transcripts were determined by quantitative PCR. Pretreatment with LPS induced prompt activation of DCs as indicated by the up-regulation of CD83 and IL-18 mRNA transcripts. IFN-γ– and CD40L-specific mRNA transcripts were clearly induced in arteries that had been pretreated with LPS and that received human alloreactive T cell clones. In the absence of LPS stimulation, adoptively transferred T cells induced a minimal up-regulation of CD83, but they failed to undergo in situ activation.

sue-resident DCs and the recruitment and retention of allospecific T cells, markers of T cell and DC activation were quantified in explanted temporal arteries. As shown in Fig. 6, in the absence of LPS and T cell transfer, no CD83 and minimal levels of IL-18 were found. Adoptive transfer of the T cell clones alone caused a minor induction of CD83. When LPS stimulation preceded T cell transfer, both markers of DC activation, CD83 and IL-18, were up-regulated. Similar results were obtained for IFN-γ and CD40L, markers for T cell activation in the tissue. Production of IFN-γ as well as CD40L required the sequential treatment with LPS followed by the adoptive transfer of T cells. These experiments demonstrated that triggering of adventitial DCs was sufficient to induce T cell recruitment, retention, and stimulation in the arterial wall.

Induction of Vasculitis in Arteries with Activated DCs from Patients with PMR. The expression of CD83 on adventitial DCs and the production of chemokines in arteries from patients with PMR suggested that this disease entity is associated with DC activation, yet a detectable infiltrate of T cells and macrophages has not been established. To examine whether DCs in PMR arteries are indeed activated and have the potential to stimulate T cells, we developed an experimental system that allows for the in vivo transfer of T cells from GCA arteries into arteries lacking T cell infiltrates. Temporal arteries from patients who had unrelated diseases, patients who had PMR, and patients with typical granulomatous infiltrates of GCA were HLA-DR genotyped. We selected arteries that were HLA-DRB1*0401+. This allele is associated with both GCA and PMR and is present in 60–70% of the patients and in 20–25% of the controls. Temporal arteries were cut into two pieces, and SCID mice were implanted with a single arterial specimen from a control donor, a patient with PMR, or a patient with GCA, or they were implanted with a combination of arteries from all three patients. After 7 d, the grafts were explanted and examined for T cell activation. Representative results from three such coimplantation experiments are shown in Fig. 7.

T cells from the GCA lesions invaded the arteries from the HLA-DRB1–matched patients with PMR but could not be found in HLA-DRB1–matched normal temporal arteries (Fig. 7 A). Not only were T cells recruited to the PMR arteries, but they underwent in situ activation. As reported previously (6, 14), arteries with GCA contained high copies of IFN-γ; IFN-γ could not be found in negative arteries and arteries from patients with PMR (Fig. 7 B). Coimplantation of arteries from all three donor subsets resulted in the rapid induction of IFN-γ in the PMR arteries. Negative arteries were unaltered even when implanted together with inflamed arteries. The only source of human T cells in the SCID mice was the temporal arteries from the patients with GCA. T cells from these inflamed arteries moved to the PMR arteries but avoided the negative arteries. DCs and macrophages from the GCA artery apparently did not migrate to the normal artery in a sufficient number to sensitize this normal tissue for a T cell response. Although the evidence is indirect, the selectivity of the response to PMR arteries implicates the activated resident DCs in the PMR arteries as the driving force. Quantification of CD40L sequences, which are specifically produced by activated T cells, confirmed the migration of human T cells from the GCA to the PMR arteries, where they underwent stimulation (Fig. 7 C). Again, negative arteries remained free of activated T cells. Thus, adventitial DCs in patients with PMR have undergone activation and are capable of interacting with T cells, specifically T cells that have accumulated in the vascular lesions of GCA.

Discussion

Data presented here provide compelling evidence for a crucial role of DCs in the pathogenesis of GCA. First, we have demonstrated that an indigenous population of DCs resides in the adventitia of medium-size arteries. These DCs are immature and lack the ability to stimulate T cells. Analysis of temporal arteries from patients with PMR confirmed that activation of adventitial DCs is a critical early step in
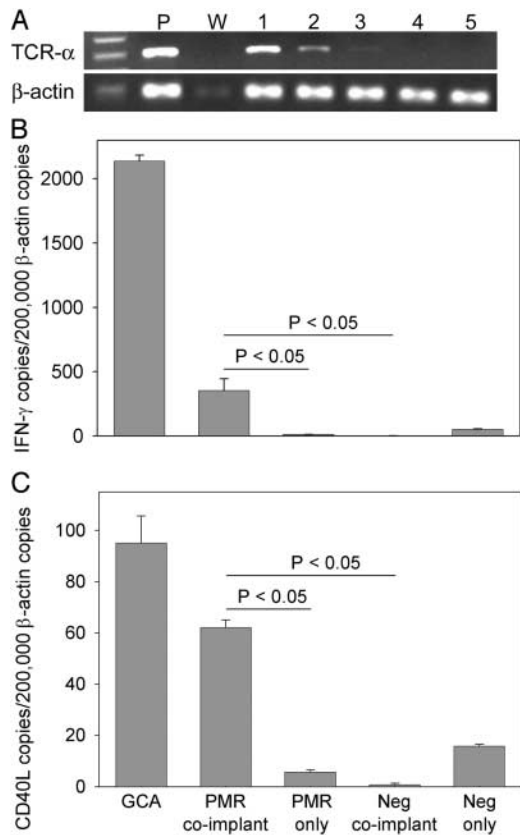
Figure 7. DC activation in PMR arteries is sufficient to activate disease-relevant T cells from GCA arteries. Donors of normal arteries and patients with PMR or GCA were typed for HLA-DR, and HLA-DRB1*0401+ arteries were selected. Arteries from patients with each diagnosis were implanted individually into SCID mice or three arteries (one from each disease category) were implanted into nonadjacent sites of the same mouse. After 7 d, the grafts were harvested, shock frozen, and used for the generation of cDNA. cDNA concentrations were adjusted to equal numbers of β-actin copies. One experiment representative of three is shown. Tissue-infiltrating T cells were detected by PCR analysis for TCR-α sequences (A). T cell activation in the tissues was assessed by quantifying mRNA transcripts for IFN-γ (B) and CD40L (C) by quantitative PCR. GCA arteries contained abundant TCR-α mRNA (lane 1). No T cells were detected in negative arteries (lanes 4 and 5), which remained free of TCR-α sequences even after coimplantation with inflamed arteries (lane 4). PMR arteries had a minimal signal for TCR-α if implanted individually (lane 3), but they accumulated TCR-α copies when combined with inflamed arteries (lane 2). No IFN-γ or CD40L could be detected in negative arteries. PMR arteries remained negative for IFN-γ and CD40L as long as they were isolated from arteries with typical vasculitic lesions. Once GCA and PMR arteries were combined in the same chimera, T cells from the vasculitic infiltrates migrated into the PMR arteries, where they produced IFN-γ and CD40L (B and C). Copy numbers of IFN-γ and CD40L are shown as mean ± SD for one representative experiment. P, positive PCR control; W, negative PCR control.

vasculitis that precedes the recruitment of T cells and macrophages into the vascular wall. Triggering of adventitial DCs by blood-born pathogen-associated molecular patterns, such as LPS, is sufficient to initiate in situ T cell stimulation and to break the immune tolerance in the perivascular space. Activated arterial DCs remain the key antigen-presenting cell in the vascular lesions of GCA, as shown by the depletion of CD83+ cells, which essentially abrogated vasculitis. We pro-

pose that adventitial DCs are primarily tolerogenic and protect the vessel wall from potentially devastating immune responses. Their activation is a seminal event in the establishment of a misplaced T cell response, leading to granuloma formation and arterial wall destruction.

DCs are powerful antigen-presenting cells, and it comes as no surprise that they have a role in stimulating T cells in granulomatous lesions. However, it was unexpected that treatment with anti-CD83 Ab virtually eliminated the inflammation in the artery. Highly activated macrophages are abundantly present in the arterial wall infiltrates (8, 26), and numerous microvessels are being formed (12). Nevertheless, interaction with T cells seems to be limited to DCs and does not involve endothelial cells, macrophages, or smooth muscle cells. CD83+ DCs are the only cell population in the vasculitic lesions that express CD86, thereby giving them the costimulatory molecules necessary to interact with tissue-invading T cells (15). The unique position of DCs in stimulating T cells even in chronic inflammatory lesions allows for unparalleled therapeutic possibilities.

Even more interesting than the contribution of fully mature DCs in established vasculitis is the role of immature DCs in healthy arteries. In healthy arteries, S-100+ fascin+ CD11c+ DCs typically reside at the adventitia–media border. The adventitia of large blood vessels has traditionally been considered a support tissue, but it may have an expanded role in the immunosurveillance of the perivascular space. Adventitial DCs have characteristic features of immature DCs: they lack CD83; they do not produce chemokines, including IL-18; and HLA class II molecules are not up-regulated (unpublished data). We tested the capability of arterial DCs to serve as allostimulators and found that they did not trigger in situ stimulation of adoptively transferred T cells. In principal, they were capable of allostimulation, and triggering by LPS was sufficient to turn them into competent partners for T cells. Considering their phenotype and their functional capabilities in vivo, arterial DCs may primarily function in preventing T cell responses. A similar functional profile has been reported for hepatic DCs, and it has been proposed that liver tolerogenicity is related to specialized DCs (27, 28).

Hallmark studies by Steinman and colleagues (29–32) have established that DCs are critically involved in the induction of peripheral tolerance. Although the underlying mechanisms are not entirely clear, it is assumed that preserving the immaturity of DCs is fundamentally important for tolerance induction. Under steady state conditions, DCs resident in peripheral tissues internalize self-antigens, followed by DC migration and presentation of self-peptides to T cells in the lymph nodes. Immature DCs may control peripheral tolerance by inducing the differentiation of regulatory human T cells (33, 34). The concept of controlling peripheral tolerance may be particularly important when it comes to the perivascular space. The vulnerability of blood vessels to inflammatory destruction and the potential lethal consequences of blood vessel damage emphasize the need for preventing immune responses in the perivascular space. Even when foreign and potentially harmful antigens are en-

countered in the vascular wall, it may be more beneficial to the host to prevent immune responses at any price. In that sense, the large arteries should be considered a territory desirable to be protected by immune privilege, comparable to the eyes or the testes (35, 36).

When DCs encounter antigen in the context of tissue injury and/or inflammation, they enter a program of maturation in which their phagocytic properties are down-regulated and their antigen-processing and -presentation machinery is up-regulated (37, 38). Also, they modify their profile of chemokine receptors and migrate toward secondary lymphoid tissues via lymphatics (39, 40). Such a reporting mechanism appears to be functional for temporal arteries as well because identical CD4$^+$ clonotypes have been isolated from the right and the left temporal arteries, suggesting central priming and seeding of T cells to the vasculitic infiltrate (5). Experiments in SCID mouse chimeras implanted with normal temporal arteries demonstrated that the immature state of arterial DCs could easily be overcome; circulating LPS was sufficient to drive tissue DCs into maturation and to prepare them to stimulate T cells. After arterial DCs were exposed to blood-born LPS, T cells were recruited to the artery and underwent stimulation, which was evident by the induction of CD40L and IFN-γ. Triggering with LPS induced retention of T cells in the arterial wall; under physiologic conditions, the vascular wall of medium-size arteries is free of any T cells and macrophages.

Once activated, tissue DCs leave the tissue site to initiate priming in the lymph node and to limit the duration of a localized inflammatory response. Surprisingly, the activation of arterial wall DCs, such as in patients with PMR or fully developed GCA, was not necessarily associated with their migration out of the tissue. Instead, DCs in the PMR arteries remained at the adventitia–media junction. In fully evolved arteritis, DCs were part of the granulomatous formation (15, 40). DCs in arteritic lesions express CCR7 (15) and release the CCR7-binding chemokines CCL19 and CCL21. This could possibly lead to a trapping of DCs in the lesions. PMR arteries do not contain CCR7 mRNA, suggesting a different mechanism of retaining activated DCs in the peripheral tissue.

Maturation of adventitial DCs could be a consequence of, not a cause for, T cell responses evolving in the arterial wall. To address this important question, we made use of an interesting clinical model. Temporal arteries from patients with PMR are free of inflammatory infiltrates (6). Arterial DCs in such arteries expressed the CD83 activation marker and produced CCL19 and CCL21 (Figs. 1 and 2). More importantly, when HLA-DR–matched temporal arteries from patients with GCA and PMR were coimplanted, T cells were recruited into the noninflamed PMR arteries and began to produce IFN-γ and CD40L. Under the same experimental conditions, temporal arteries from patients without PMR or GCA remained protected from T cell responses. These findings are best compatible with the model that the chain of events leading to vasculitis has been initiated in PMR but that T cells interacting with activated arterial DCs are not present. In contrast, in patients with fully developed GCA, such T cells accumulate in the arterial wall layers and orchestrate the generation of granulomas. This model is consistent with clinical observations and with prior papers on the nature of the subclinical inflammatory response in PMR (6, 14). Approximately 10% of patients diagnosed with PMR will eventually progress to GCA (17, 41). Also, IFN-γ is strictly absent from PMR arteries, but low levels of IL-2 are found (6). Whether the failure of activated DCs in PMR arteries to attract T cells reflects a genuine difference in the T cell repertoire of patients with PMR or GCA needs to be investigated.

When given the opportunity in the coimplantation experiments, T cells made the transit from the GCA artery into the PMR artery, suggesting that the relevant antigen is available in PMR arteries (42). These results confirm prior conclusions that T cell clones isolated from inflamed temporal arteries proliferate when driven with tissue extracts from GCA arteries and also from PMR arteries. The nature of such a shared antigen has remained elusive. As in most autoimmune syndromes, unidentified and/or unknown infections have been suspected to be the instigators. Multiple papers have suggested that viral or bacterial materials can be found in temporal artery sections (43–46), but attempts to confirm such papers have failed (47, 48). Data presented here would fit with an infection breaking the resting state of adventitial DCs and initiating disease. It seems that the site of infection does not necessarily need to be in the artery. Adventitial DCs were highly responsive to blood-born TLR ligands. Interestingly, stimulation with TNF-α could only partially activate the arterial DCs. CFA, known to trigger TLR2, was more potent that TNF-α, and LPS was the most effective in inducing DC activation, which was followed by T cell recruitment and stimulation. Under physiologic conditions, adventitial DCs do not express CD14, which is considered to be instrumental in mediating an LPS signal (49, 50). We have preliminary data that CD14 is up-regulated in the arterial wall after LPS injection (unpublished data).

GCA and PMR are currently managed with corticosteroid therapy (17, 51), which is highly effective and results in symptomatic improvement within hours to days. The promptness of the corticosteroid-induced improvement has been cited as a diagnostic criterion for both syndromes. Inhibition of NF-κB has been shown to be important in mediating immunosuppression in GCA (22, 52). Therefore, TLR-induced activation of the innate immune system should be explicitly sensitive to the therapeutic effects of corticosteroids. One of the shortfalls of corticosteroid therapy is the requirement to maintain immunosuppression over several years, thereby increasing the risk of unwanted side effects. Data presented here would encourage the development of alternative therapeutic interventions targeted at the DCs in early and late vascular lesions.

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