Giant Cell Vasculitis Is a T Cell–Dependent Disease

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ABSTRACT

Background: Giant cell arteritis (GCA) is a systemic vasculitis that preferentially targets medium-sized and large arteries. The etiopathogenesis of the syndrome is not known, and because of the paucity of information concerning the mechanisms of blood vessel wall damage, treatment options are limited. Clues to pathogenic events in this arteritis may derive from understanding the function of tissue-infiltrating cells. Arterial injury in GCA is associated with the formation of granulomas that are composed of T cells, activated macrophages, and multinucleated giant cells. To examine the role of T cells, we implanted inflamed temporal arteries from patients with GCA into severe combined immunodeficiency (SCID) mice and studied whether the vascular lesions were T cell–dependent.

Materials and Methods: Temporal artery specimens from patients with GCA were engrafted into SCID mice. The histomorphologic appearance of fresh arteries and grafts retrieved from the mice was compared by two-color immunohistochemistry, and the functional profile of tissue-infiltrating cells was analyzed by semiquantifying cytokine transcription with a polymerase chain reaction (PCR)–based assay system. The repertoire of tissue-infiltrating T cells was assessed for the presence of dominant T cell populations by using T cell receptor β-chain-specific PCR followed by sequencing. To investigate the role of T cells in the activation of tissue-infiltrating macrophages, T cells were depleted from the arterial grafts by treating the mice with T cell–specific antibodies and the production of monokines was monitored. To demonstrate the disease relevance of T cells expanding in the implants, T cells were isolated from tissue segments and adoptively transferred into mice implanted with syngeneic arteries. The in situ production of lymphokines was then determined.

Results: The inflammatory infiltrate penetrating all layers of the arterial wall persisted in the xenotransplants, indicating that the inflammatory foci represent independent functional units. Similar quantities of T cell– and macrophage-derived cytokines were detected in fresh and engrafted tissue. However, the diversity of tissue-infiltrating T cells decreased following implantation. T cells with identical T cell receptors were expanded in different mice that had been engrafted with tissue fragments from the same patient, indicating that T cell survival in the arterial wall was a nonrandom process. To confirm the disease relevance of these T cells, T cell depletion and reconstitution experiments were performed. Antibody-mediated elimination of T cells from the xenotransplants resulted in the attenuation of the production of the monokines, IL-1β and IL-6. Adoptive transfer of syngeneic tissue-derived T cells, but not of peripheral blood T cells, into engrafted SCID mice enhanced the transcription of IL-2 and IFN-γ in the implanted arteries.

Conclusions: The vascular lesions of GCA are maintained in human artery–mouse chimeras, indicating that all cellular and noncellular components necessary for the disease are present in the temporal artery. Activation of tissue-infiltrating T cells and macrophages depends upon an infrequent subpopulation of lesional T cells that have a survival advantage in the xenotransplants. The selective proliferation of these T cells in the arteries suggests that there is recognition of a locally expressed antigen. Therefore, these T cells should be candidate targets for the development of novel therapeutic strategies in GCA.

INTRODUCTION

Giant cell arteritis (GCA) is an inflammatory disease of medium-sized and large arteries. Injury to the arterial wall is caused by inflammatory infiltrates composed of T lymphocytes and macro-

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phages. Most patients have involvement of the proximal branches of the aorta, which possess well-defined internal and external elastic laminae. Morbidity and mortality are related to ischemic events such as blindness and stroke and to aneurysm formation (1,2). Inflammatory lesions in the vessel wall are often granulomatous and may include multinucleated giant cells (3). The cellular infiltrate of the arteries is generally centered on the medial layer with extension into the intima and adventitia and consists mainly of lymphocytes, histiocytes, macrophages, and infrequently, plasma cells. Multinucleated giant cells are often close to the fragmented internal elastic lamina or are located circumferentially along the destroyed elastic membrane.

Nonrandomness of incidence rates in different geographic regions and in different ethnic groups has been attributed to genetic factors contributing to disease pathogenesis. Female dominance among patients, a stringent restriction in age at disease onset, and tissue tropism may also be surrogates for genetic risk factors. Thus far, convincing experimental data have only been presented for one genetic system, the HLA-DR alleles (4,5). GCA is an HLA-associated disease with about 60% of patients expressing an allelic variant of the HLA-DR4 family. Sequence comparison of HLA-DR alleles in patients with biopsy-proven disease has demonstrated a shared-sequence polymorphism mapping to the floor of the antigen-binding site of the HLA-DR molecule (6,7). Amino acid positions 9, 28, and 30 of the HLA-DRB1 gene are strongly associated with GCA. All three amino acid positions contribute to binding pockets that accommodate side chains of an antigenic peptide bound in the HLA-DR molecule (8,9). These data provide evidence for an important role of antigen-binding in the pathogenesis of GCA.

The evidence for a pivotal role of T cells recognizing an antigen in the inflammatory lesion has so far been indirect. It has been described that selected $CD4^+$ T cells undergo clonal proliferation in the tissue (10). Clonally expanded T cell specificities with identical T cell receptor (TCR) β-chain sequences are present at distinct and independent inflammatory foci, suggesting that a single antigen recruits T cells to the wall of the inflamed arteries. However, the distribution and the nature of such an antigen remains unresolved. Possibilities include an arterial antigen that is locally expressed and gains immunogenicity, or an exogenous antigen that is deposited in the arterial wall. The concept of GCA as an antigen-driven immune response has been emphasized by studies analyzing the local production of cytokines (11). Cytokine mRNA could be detected in extracts from temporal arteries harvested from patients with GCA but not in tissue samples collected from patients with unrelated disease. Production of mRNA for interleukin-1β (IL-1β), IL-6, and transforming growth factor-β1 (TGF-β1) could be assigned to activated macrophages, and tissue-infiltrating T cells were shown to synthesize IL-2 and interferon-γ (IFN-γ) but rarely IL-4 or IL-5 mRNA. Lymphokine-secreting T cells represent a minority of the total T cell infiltrate and accumulate in the adventitia, suggesting the recognition of an adventitial antigen (12).

To allow for definite conclusions on the role of T cells in the vasculitic inflammation, we have established an animal model of the GCA. Temporal artery specimens from affected patients were engrafted into severe combined immunodeficiency (SCID) mice. These mice have defects in humoral as well as cell mediated immunity and can therefore serve as recipients for xenogeneic grafts (13,14). In these GCA–SCID mouse chimeras, the arteritis persists, indicating that the inflamed arterial wall represents a functionally independent unit. The inflammatory response is dependent upon a small subpopulation of T cells that displays a limited TCR repertoire and has a survival advantage in the tissue. Depletion of these T cells abrogates the synthesis of the proinflammatory monokines, IL-1β and IL-6. After adoptive transfer, these T cells home to the implanted tissue where they promote increased production of IL-2 and IFN-γ.

MATERIALS AND METHODS

Patients and Tissue Samples

Temporal artery biopsy specimens were obtained from patients undergoing biopsies for diagnostic reasons. All specimens showed inflammatory infiltrates establishing the diagnosis of GCA. Tissue specimens of 1–1.5 cm in length were cut into 3 to 5 equal pieces. One piece was embedded in OCT compound (Lab Tech Products, Naperville, IL) for subsequent immunohistochemical staining. The remaining pieces were frozen in media containing 10% dimethylsulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO) and 10% newborn calf serum (Life Technologies, Grand Island, NY) by computer-controlled freezing (Cryomed,

New Baltimore, MI) and stored in liquid nitrogen for later engraftment into SCID mice.

Animals

C.B.-17 scid/scid mice were kept under sterile conditions in a barrier facility. Prior to tissue implantation, serum samples were collected from all mice and IgG levels were determined by ELISA. Briefly, mouse serum was added to rabbit anti-mouse IgG (Cappel, Durham, NC) coated microtiter plates. Bound mouse IgG was detected with alkaline-phosphatase conjugated rabbit anti-mouse IgG (Sigma) and developed by using nitrophenyl phosphate (Sigma) as the substrate. IgG concentrations were determined by comparison to a standard curve. IgG concentrations of greater than 10 μg/ml were used to define leakiness.

Transplantation of GCA Tissue in SCID Mice

C.B.-17 scid/scid mice (6–8 weeks of age) were anesthetized by ether inhalation or intramuscular ketamine (1–2 μg/mouse) injection. Human tissue samples were placed subcutaneously on the back. Four weeks after engraftment, the mice were sacrificed and the biopsy specimens were retrieved and embedded in OCT compound. In selected experiments, engrafted mice were treated with daily intraperitoneal injections of a T cell–depleting antiserum for 3 days (ATGAM, Upjohn, Kalamazoo, MI; 3 mg/injection) and sacrificed at varying time intervals after the last injection.

Antibodies

The following antibodies were used for immunohistochemistry and flow cytometry: polyclonal rabbit anti-CD3 antibody, murine anti-CD68 monoclonal antibody (MAb) (KP1), murine anti-smooth muscle actin MAb, murine anti-MIB MAb (Ki-67), biotinylated anti-mouse Ig, biotinylated anti-rabbit Ig (all from Dako, Carpinteria, CA), murine anti-CD4 MAb, and murine anti-CD8 MAb (both from Becton Dickinson, San Jose, CA).

Immunohistochemistry

Twenty serial 5-μm sections were mounted onto gelatin-coated slides and stained with hematoxylin-eosin or MAb. For hematoxylin-eosin staining, sections were fixed in 10% formalin. All other slides were fixed in acetone and air dried. Prior to staining with MAb, the slides were fixed in 1% paraformaldehyde/PBS, pH 7.4. Sections were stained with an optimal concentration of the primary antibody for 30 min at room temperature. The slides were subsequently developed by incubation with biotinylated anti-mouse or anti-rabbit Ig, streptavidin-peroxidase, and finally with either amino-ethyl-carbazole (Sigma) or diaminobenzidine (anti-MIB stained sections) (Dako). Controls included staining with the secondary antibodies in the absence of primary antibodies.

Quantification of T Cell Recovery after Xenotransplantation

T cell infiltration was quantified in pre- and post-implanted temporal artery biopsy specimens of five patients by immunohistochemical staining with anti-CD3 MAb as described above. The total number of $CD3^+$ cells per slide were then counted. Tissue sections were scanned and the area of the artery specimen was quantified with an IBAS scanning system (Kontron, Munich, Germany). The number of infiltrating T cells was calculated per mm^2 of tissue for both pre- and post-implanted biopsy segments.

Semiquantification of Cytokine mRNA Expression in Temporal Artery Specimens

Total RNA was extracted by guanidinium thiocyanate phenol-chloroform extraction (RNAStat-60, TelTest "B", Friendswood, TX) from fresh temporal artery specimens and from specimens retrieved from SCID mice. cDNA was synthesized and β-actin-specific polymerase chain reaction (PCR) was used to adjust for total cDNA. Serial dilutions of adjusted cDNA were then amplified in duplicate with cytokine specific primers. All primer sets had been tested to be human-specific and to not cross-react with murine sequences. One primer in each set was designed to span an intron to avoid amplification of contaminating genomic DNA. The sequences of the primer sets are given in Table 1. In parallel, serial dilutions of a standard curve with known numbers of cytokine copies were amplified. The amplification conditions were as follows: 5 min denaturation at 94°C followed by 30 cycles of 1 min denaturation at 94°C, 2 min annealing at 55°C, and 2 min extension at 72°C. PCR conditions and cDNA concentrations were chosen to ensure

TABLE 1. Nucleotide sequences of oligonucleotides

	Primer or Probe	Primer or Probe Sequence
Cα	5′ sense primer	ATC ATA AAT TCG GGT AGG ATC C
	3′ antisense primer	GAA CCC TGA CCC TGC CGT GTA CC
	Biotinylated probe	AGC AAC AGT GCT GTG GCC TG
β-actin	5′ sense primer	ATG GCC ACG GCT GCT TCC AGC
	3′ antisense primer	CAT GGT GGT GCC AGA CAG
	Biotinylated probe	TAC AGG TCT TTG CGG ATG TC
IL-1β	5′ sense primer	GAC ACA TGG GAT AAC GAG GC
	3′ antisense primer	GGG ATC TAC ACT CTC CAG CTG
	Biotinylated probe	AGC TTT TTT GCT GTG AGT CCC GGA G
IL-2	5′ sense primer	ACT CAC CAG GAT GCT CAC AT
	3′ antisense primer	AGA CTT GTC TAC CTA ATG GA
	Biotinylated probe	CTG GAG GAA GTG CTA AAT TTA GCT
IL-6	5′ sense primer	GAT GTA GCC CCA CAC AGA CAG
	3′ antisense primer	CCT CAA ACT CCA AAA GAC CAG TGA TG
	Biotinylated probe	GAG AAA GGA GAC ATG TAA CA
IFN-γ	5′ sense primer	ACC TTA AGA AAT ATT TTA ATG C
	3′ antisense primer	ACC GAA TAA TTA GTC AGC TT
	Biotinylated probe	ATT TGG CTC TGC ATT ATT TTT CTG T
TGF-β1	5′ sense primer	AAG TGG ACA TCA ACG CGT TCA CTA
	3′ antisense primer	GCT GCA CTT GCA GGA GCG CAC
	Biotinylated probe	CAG TAC AGC AAG GTC CT G GCC CTG

nonsaturating conditions. Denatured amplified products were dot-blotted onto supported nitrocellulose membranes (Biorad Laboratories, Hercules, CA), prehybridized at 55°C for 3 to 5 hr, and then hybridized with biotinylated internal probes (200 ng/ml) (Biotin-On Phosphoramidite, Clontech Laboratories, Palo Alto, CA) at 42°C (β-actin- and all cytokine specific-probes except for IL-1β) or 55°C (TCR Cα- and IL-1β-specific probes) overnight. The sequences of the probes are in Table 1. Membranes were washed once in 2× SSC 0.1% SDS at 42°C for 10 min and once at 55°C in 2× SSC 0.1% SDS for 5 min. The second washing step was omitted for the IL-2- and IFN-γ-specific probes. Membranes were then blocked with blocking buffer (Boehringer Mannheim, Indianapolis, IN) for 30 min and incubated with streptavidin-alkaline phosphatase (Dako). The membranes were developed by a color reaction using 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro blue tetrazolium chloride (Boehringer Mannheim) and the optical density of the dots was scanned with an AMBIS Optical Imaging System (Scanalytics, Ballerica, MA). The copy numbers of cytokine-specific cDNA sequences in the tissue extracts were determined by comparison to the standard curve. Results are expressed for adjusted tissue as copy number per 200,000 β-actin sequences.

T Cell Receptor-β Gene Sequence Analysis

cDNA from implanted and nonimplanted tissue specimens were adjusted for TCR-AC gene transcripts and amplified with BV2, BV5S2, BV8, BV13S1, BV17, or BV18 specific primers and a BC primer (15). Because of the limited amount of cDNA available, approximately 30% of the total TCR repertoire was analyzed. The BV-specific primers were arbitrarily chosen. Radioactive amplified products were separated on 5% polyacrylamide sequencing gels and exposed on autoradiographic film. Dominant bands defined as containing at least 30% of the total amplification product and exceeding the sum of the neighboring bands were identified, eluted from the gel, and directly sequenced by using a modification of the genomic amplification with the transcript sequencing approach described by Stoflet et al. (16,17).

Adoptive Transfer Experiments

Mice were implanted with temporal artery specimens as described above. After 4 weeks, the tissue was retrieved, placed into tissue culture wells in the presence of RPMI-1640 supplemented with 10% FCS (Hyclone Laboratories, Logan, UT), 200 μM L-glutamine, penicillin/streptomycin (500 U/ml and 5 mg/ml, respectively) (Life Technologies), and recombinant human IL-2 (20 U/ml, Cetus Immune Corporation, Emeryville, CA). In parallel, a T cell line from peripheral blood mononuclear cells (PBMC) was established by polyclonal activation with immobilized anti-CD3 (OKT3, ATCC, Rockville, MD) and cultured in IL-2 containing medium. Then 5×10^6 T cells were adoptively transferred into SCID mice implanted with temporal artery tissues. To avoid an alloreactive reaction, temporal artery specimens and T cell lines from the identical patient were used. The tissue was retrieved 5 days after the adoptive transfer and analyzed for in situ cytokine transcription by semiquantitative PCR.

T Cell Proliferative Assays

T cell lines were established from tissue specimens explanted from SCID mice by incubation of the tissue fragment in IL-2 containing medium. T cells were harvested, washed in IL-2-free medium to deplete exogenous IL-2, and restimulated in 96-well round-bottomed culture plates at a concentration of 5×10^5 cells/ml with immobilized anti-CD3 or 1×10^6 cells/ml irradiated autologous PBMC preincubated with murine serum. The proliferative responses were assessed by ^{3}H-thymidine incorporation after 72 hr.

Statistics

All statistics were calculated using SigmaStat software (Jandel, San Rafael, CA). The Mann-Whitney Rank Sum test was used to compare the cytokine concentrations and the number of infiltrating T cells was compared with a paired *t*-test.

RESULTS

Giant Cell Vasculitis Is a Self-Sustained Inflammatory Disease that Persists in Xenotransplants

Temporal artery specimens from eight patients with typical GCA were engrafted into SCID mice. The presence of arteritis was monitored by histomorphological analysis of retrieved tissue. Lesions typical for GCA persisted for at least 2 months in the xenotransplants. Immunohistochemical staining of tissue explanted after 4 weeks is shown in Fig. 1. Typical lesions in GCA are characterized by the presence of $CD68^+$ macrophages and $CD3^+$ T cells, 70–80% of which express the CD4 marker (18,19). To document that the overall histomorphology of the arterial wall was maintained, the smooth muscle cells of the medial layer were identified with anti-smooth muscle actin antibodies (Fig. 1A). Tissue-infiltrating T cells were present in all samples (Fig. 1B) and included $CD4^+$ and $CD8^+$ subsets (Fig. 1C, D). Macrophages survived in the grafts and were identified with anti-CD68 antibodies (Fig. 1E).

Proliferating cells were identifed by staining for the cell cycle–dependent Ki-67 antigen to provide evidence of cell replenishment in the implanted arterial tissue (20). As shown in Fig. 1F, actively dividing cells, including T lymphocytes, were present in all layers of the engrafted temporal artery specimen. Inspection of the tissue sections indicated that the number of T cells in the tissue had decreased after 4–6 weeks of implantation. To quantify the T cell infiltrate in the grafts, nonimplanted and recovered tissue samples were compared for the density of the T cell infiltrate. Data are given in Table 2 and show that T cell recovery after implantation ranged between 31% and 115%. In 4 of the 5 tissues analyzed, T cell numbers decreased by 40–70%. Thus a substantial fraction of the tissue-residing T cells either died in the tissue or emigrated ($p = 0.08$). Concomitantly, a portion of the retained T cells proliferated and replenished the T cell infiltrate.

In summary, these experiments demonstrated that the arteritis persisted despite disruption of cell influx from the circulation and was maintained by self-replenishment of tissue-infiltrating cells. The inflamed arterial wall obviously represents an independent functional unit.

Production of Inflammatory Cytokines Continues in Xenotransplants with Vasculitic Lesions

To address the question as to whether the nature of the inflammatory response changed after xenotransplantation and separation from potentially important extra-arterial sources, the patterns

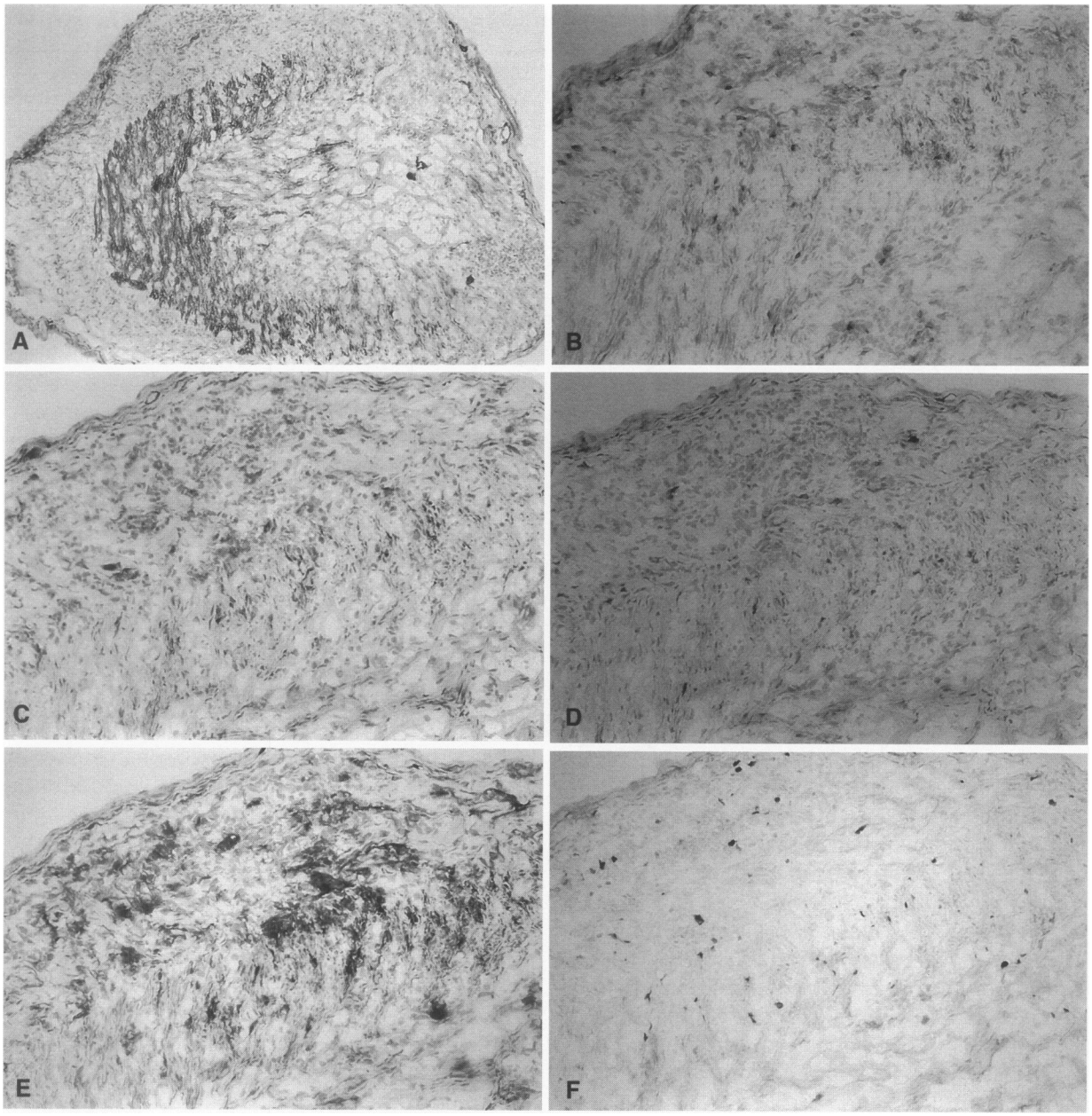

FIG. 1. Immunohistochemistry of temporal artery tissue of a GCA patient explanted from a SCID mouse

Temporal artery tissue was retrieved 4 weeks after implantation. Cryosections were stained with anti-smooth muscle actin (A), anti-CD3 (B), anti-CD4 (C), anti-CD8 (D), anti-CD68 (E), and anti-MIB (F). Immunoperoxidase stain, hematoxylin (A–E), or methylene green counterstain (F). A, ×50; B–F, ×200.

of cytokine mRNA produced in preimplanted and retrieved tissue fragments from the same patient were compared. Temporal artery specimens were cut into separate pieces, of which one was used as the preimplanted control and two to five pieces were implanted. Total mRNA was extracted from the preimplanted and the retrieved tissues. Transcripts of in situ produced cytokines were semiquantified by reverse transcriptase-PCR followed by oligonucleotide hybridization with cytokine specific primers. Primers were selected to ensure species specificity for human sequences. To account for differences in the amount of tissue, transcripts for β-actin were measured in all samples and cDNA concentrations were adjusted.

TABLE 2. Quantification of the T cell infiltrate after xenotransplantation by immunohistochemistry

Patient	Pre-implantation (CD3 cells/mm^2)	Post-implantation (CD3 cells/mm^2)	T Cell Recovery (% of Pre-implantation)
GCA-1	217	123	57
GCA-2	612	283	46
GCA-3	341	104	31
GCA-4	128	46	36
GCA-5	127	147	115

Previous work has shown that GCA is characterized by a defined cytokine pattern expressed in the tissue (11). Accumulated T cells synthesize IL-2 and IFN-γ mRNA and macrophages transcribe IL-1β, IL-6, and TGF-β1 mRNA. Comparative studies for these cytokines in nonimplanted and retrieved tissues from eight patients are summarized in Fig. 2. As shown in Fig. 2A, copy numbers for TCR-AC gene transcripts were essentially unaltered although T cell numbers declined, suggesting that the remaining T cells were activated and expressed higher amounts of TCR transcripts on a per-cell basis. In line with this interpretation is the fact that the production of IL-2 and IFN-γ mRNA persisted in the implanted tissue (Fig. 2A). After adjustment of cDNA from nonimplanted and recovered tissue to 200,000 copies of β-actin-specific transcripts, a median of 853 copies of IL-2-specific sequences was detected in nonimplanted fragments and 430 copies in engrafted tissue ($p = 0.59$). In nonmanipulated temporal artery specimens, 1,462 copies of IFN-γ transcripts were found, compared with 2,640 copies in the xenotransplants ($p = 0.23$). Thus, the production of T cell–derived lymphokines continued despite a decline in T cell numbers. This observation suggests that the migration of T cells out of the tissue was a nonrandom process and that T cells synthesizing lymphokines were retained in the inflammatory lesions.

Activated macrophages in the tissue generally produce IL-1β, IL-6, and TGF-β1. IL-1β production declined after engraftment (936 versus 242 copies), but this difference was not significant ($p = 0.09$). Functional competence of graft-residing macrophages was documented by the continuous expression of IL-6 (median of 1930 copies before implantation compared with 4191 copies after implantation) ($p = 0.44$) and of TGF-β1 mRNA (6964 versus 5060 copies) ($p = 0.97$) (Fig. 2B). These data suggest that no fundamental change occurred in the inflammatory process when the inflamed arteries were isolated and engrafted for 4–6 weeks. The persistence of the disease-typical inflammation provides a unique opportunity to study disease mechanisms in GCA.

Diversity of the TCR Repertoire in Vasculitic Lesions

Tissue-infiltrating T cells in GCA are highly diverse. Isolation of IL-2-responsive infiltrating CD4$^+$ T cells and sequencing of their TCR molecules have provided evidence that a small fraction of these T cells are functionally distinct in that they undergo clonal proliferation at the site of pathology (10). We have therefore proposed that T cells with potential disease relevance are infrequent. Functional properties and recruitment mechanisms of T cells that do not recognize antigen in the infiltrate are unknown, but these T cells most likely represent bystander T cells. We wanted to address the question as to whether T cell loss affected the T cell repertoire randomly or whether certain T cell specificities had a survival advantage in the engrafted tissue. To estimate the diversity of the TCR repertoire, TCR sequences from six arbitrarily selected BV gene families (BV2, BV5S2, BV8, BV13S1, BV17, and BV18) encompassing approximately 30% of the total repertoire were analyzed for the representation of different size classes. Nonimplanted and explanted tissue samples from four patients were compared. Adjusted cDNA was amplified with BV-BC-specific primer sets. Amplified products were radioactively labeled and size-fractionated on polyacrylamide gels. Representative examples are given in Fig. 3. Each band represents TCR sequences sharing a particular length of the third

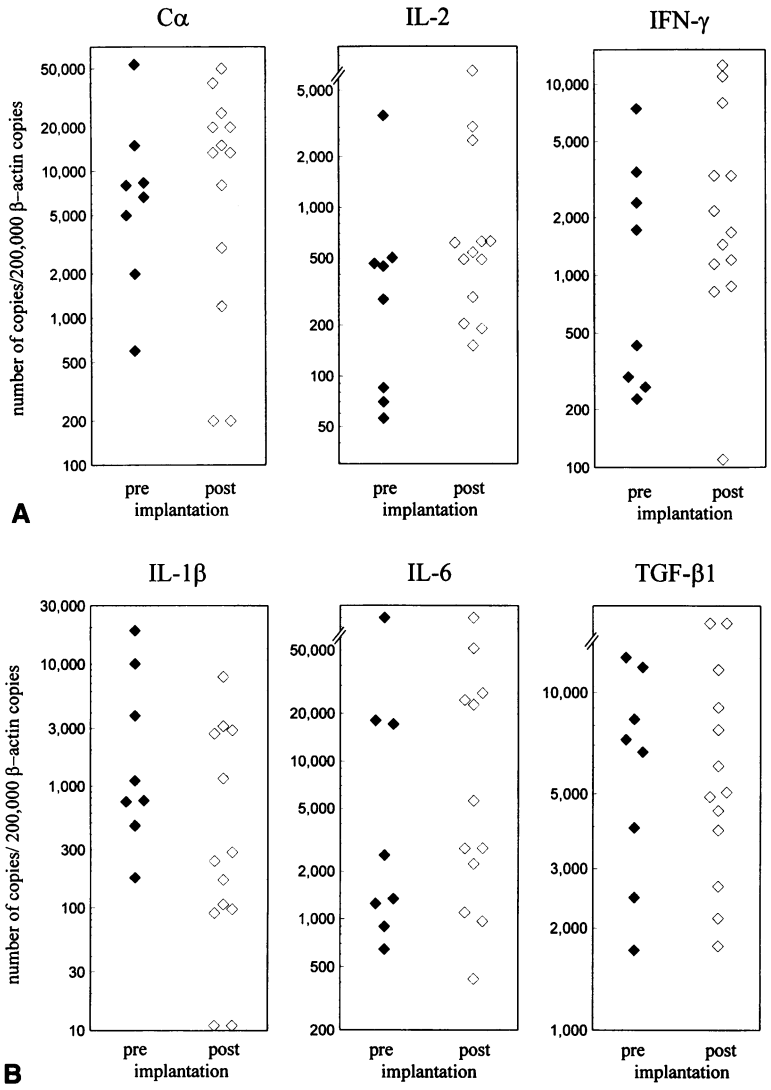

FIG. 2. Cytokine expression in fresh temporal artery specimens from GCA patients and in specimens explanted after 4 weeks

Biopsy specimens from eight GCA patients were cut into small fragments and either shock frozen or implanted into C.B.-17 SCID mice. Paired samples were analyzed in parallel. Total RNA was extracted, cDNA was adjusted for the number of β-actin copies, and cytokine sequences were semiquantified by PCR and subsequent oligonucleotide hybridization. Results are expressed as number of copies per 200,000 β-actin copies. (A) T cell–derived cytokines; (B) macrophage-derived cytokines.

complementarity determining region (CDR3). The reduction in the number of bands after tissue implantation indicated a reduction in the diversity of the TCR repertoire. Xenotransplantation induced a shift in the composition of the tissue-infiltrating T cell population. Not only did the number of detectable CDR3 size classes decrease, but selected bands emerged as dominant, suggesting that individual TCR specificities gained clonal dominance.

To confirm clonality as being the cause of the dominance of individual bands, these bands were eluted and directly sequenced. We have previously shown that an unequivocal TCR sequence is obtained with this approach, provided that the clonal specificity accounts for at least 30% of the transcripts in the band (21). A total of 176 suspicious bands were evaluated and 44 clonal sequences were found. These T cell clonotypes have likely undergone clonal proliferation in the tissue because of antigen recognition.

Selection of Identical T Cell Clonotypes in Different Mice Engrafted with the Same Temporal Artery Tissue

We hypothesized that if a common disease-relevant antigen in the tissue is recognized, T cell clones with identical TCR sequences would be selected in different mice carrying tissue from the same patient. Temporal artery segments from four patients implanted into 17 SCID mice were compared. Twelve TCR β-chain sequences were found repetitively in independent tissue sites. The N-D-N region sequences from these 12 clones are given in Table 3. Four of these 12

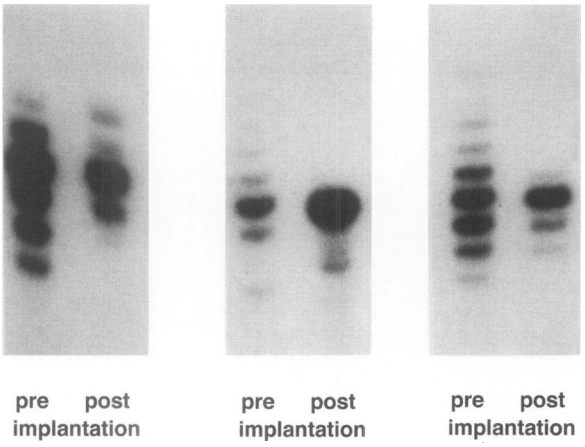

FIG. 3. TCR repertoire of tissue-infiltrating T cells before and after implantation

TCR sequences were amplified with BV-BC specific primer sets and separated on a 5% polyacrylamide gel. Results from three patients (GCA-1, BV2, left panel; GCA-2, BV8, middle panel; and GCA-3, BV2, right panel) are shown. The T cell infiltrate after implantation was less diverse as documented by the smaller number of TCR size classes and the emergence of dominant bands.

clonotypes were already detectable in the preimplantation tissue, but in all cases their frequency increased in the grafts.

These data indicated that within the diverse population of T cells infiltrating the vascular tissue selected T cells have a survival advantage. These T cells are present at different regions of the inflamed tissue as documented by their outgrowth in independent grafts. The most likely explanation is that they have encountered a common antigen at different sites in the arterial wall. To exclude that the selective expansion of these T cell specificities was induced by a reaction to murine antigens, T cell lines were established from grafts recovered 4 weeks after implantation. The proliferative response of these T cell lines to anti-CD3 antibodies and to murine serum proteins presented by autologous PBMC was tested. All tissue-derived T cell lines proliferated vigorously to anti-CD3, indicating that they were not anergic. However, they could not be stimulated with murine serum proteins presented by autologous PBMC (data not shown).

Whereas T cells with identical TCR β-chains were isolated from independent grafts, each patient had a unique set of such T cells and no sharing was observed between patients. With the exception of BV18, all BV gene segments were utilized by at least one patient. Similarly, BJ usage of the TCRs was heterogeneous. No sequence homologies in the CDR3s of the different clones were detected. However, the BV5S2-BJ2S3 combination, which was found in the specimens of two of the four patients, had previously been found in clonally expanded T cells isolated from temporal artery tissue of three of 13 GCA patients (22). Thus, there might be nonrandomness in the molecular structure of the selectively expanded T cells with the preferential use of selected BV-BJ combinations in antigen-driven T cells in GCA.

Arterial Inflammation Is Attenuated by T Cell Elimination and Amplified by Transfer of Tissue-Derived T Cells

To address the question as to whether T cells surviving in the xenotransplant are disease-relevant and are necessary to maintain the inflammation, GCA-SCID mouse chimeras were treated with a commercially available T cell–depleting antiserum (ATGAM). The effects of antibody treatment on the vasculitic infiltrates were monitored by immunohistochemistry. In initial experiments, doses and duration of antibody injections were established which could eliminate more than 90% of all tissue-residing T cells (data not shown). To assess the functional activity of lesional T cells and macrophages following antibody therapy, xenografts were retrieved between 2 and 10 days following antibody injection and the production of IFN-γ and IL-1β mRNA was analyzed. Results are shown in Fig. 4. In situ production of IFN-γ mRNA decreased within 2 days after antibody-mediated T cell depletion. In situ production of IL-1β mRNA also subsided, however, the decline was delayed and followed the disappearance of IFN-γ mRNA production. These data are consistent with the interpretation that IL-1β mRNA production in the vasculitic lesions is T cell–dependent.

To directly demonstrate the disease relevance of tissue-infiltrating T cells, T cell lines were established from tissue retrieved from GCA-SCID mouse chimeras. Tissue-derived T cells were then adoptively transferred into mice implanted with autologous temporal artery tissue. As controls, T cell lines established from the peripheral blood of the same patient were transferred into the GCA-SCID mouse chimeras. The xenografts were retrieved 5 days after the adoptive transfer and the in situ transcription of lymphokines was determined. Results from experiments examining tissue and T cells from two

TABLE 3. TCR β-chain sequences of T cell clones with identical sequences retrieved from independent mice implanted with temporal artery tissue from the same patient

Patient	BV					N-D-N						BJ						No. of Grafts Containing TCR Sequence
GCA-1	BV2	TCT	GCC	AGC	AGT	AGA	GGG	GCA	GGA	GGA		ACA	GAT	ACG	CAG	TAT	TTT	BJ2S3	2 of 3[a]
		C	A	S	S	R	G	A	G	G		T	D	T	Q	Y	F		
	BV5S2	TCT	GCC	AGC	AGT	TTG	CCG	GCT	AGC	GGT	ACT		GAT	ACG	CAG	TAT	TTT	BJ2S3	2 of 5
		C	A	S	S	L	P	A	S	G	T		D	T	Q	Y	F		
	BV13	TCT	GCC	AGC	AGT	TTA	CTA	CAG	CGA			AAA	AAC	ATT	CAG	TAC	TTC	BJ2S4	2 of 3
		C	A	S	S	L	L	Q	R			K	N	I	Q	Y	F		
	BV17	TCT	GCC	AGC	AGT	ATC	TTC	CCT	GGG	ACA	GGG								
		C	A	S	S	I	F	P	G	T	G								
						GGC	ATG	ACT	AGT	TAC	GAT				GAG	CAG	TTC	BJ2S1	2 of 3[a]
						G	M	T	S	Y	D				E	Q	F		
	BV17	TCT	GCC	AGC	AGT	GCA	AGG	GAC	AGG	CGG			GAG	ACC	CAG	TAC	TTC	BJ2S5	2 of 3
		C	A	S	S	A	R	D	R	R			E	T	C	Y	F		
GCA-2	BV2	TCT	GCC	AGC		ACG	GTG	GCA	GGG	AAT		AAT	TCA	CCC	CTC	CAC	TTT	BJ1S6	3 of 4
		C	A	S		T	V	A	G	N		N	S	P	L	H	F		
	BV8	TCT	GCC	AGC	AGT	TAC	CTG	ACA	GGG	TTA	GAC		ACT	GAA	GCT	TCC	TTT	BJ1S1	2 of 4
		C	A	S	S	Y	L	T	G	L	D		T	E	A	F	F		
	BV8	TCT	GCC	AGC	AGT	TTA	TTG	GGA	CAG	AGG	ATC			CCC	CAG	CAT	TTT	BJ1S5	2 of 4[a]
		C	A	S	S	L	L	G	Q	R	I			P	Q	H	F		
GCA-3	BV2	TCT	GCC	AGC		CCT	AGG	GCC					AAT	GAG	CAG	TTC	TTC	BJ2S1	2 of 4
		C	A	S		P	R	A					N	E	Q	F	F		
	BV5S2	TCT	GCC	AGC	AGT	ATC	TCG	GGA	GAC	ACT	CCG	ACA	GAT	ACG	CAG	TAT	TTT	BJ2S3	3 of 4
		C	A	S	S	I	S	G	D	T	P	T	D	T	Q	Y	F		
	BV17	TCT	GCC	AGC	AGT	ACC	TTG	ACA	GGG	ACA	GGA		TCA	CCC	CTC	CAC	TTT	BJ1S6	2 of 4
		C	A	S	S	T	L	T	G	T	G		S	P	L	H	F		
GCA-6	BV5S2	TCT	GCC	AGC		AGT	GTG	GGG	GCT	TCA	GGA				GCT	TCC	TTT	BJ1S1	4 of 4[a]
		C	A	S		S	V	G	A	S	G				A	F	F		

[a]TCR sequence was also found in the pre-implanted tissue, albeit at a lower frequency.

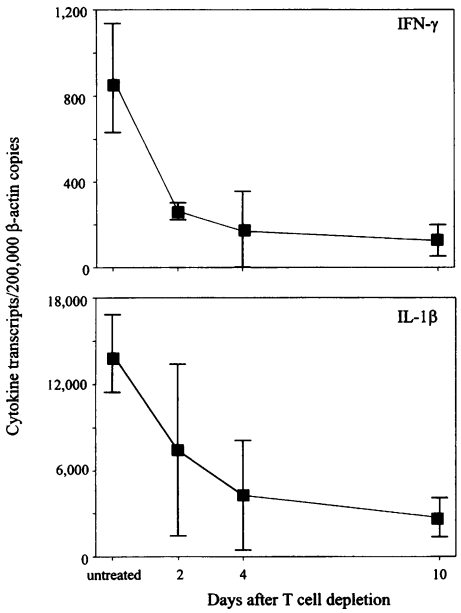

FIG. 4. In situ cytokine production in temporal artery grafts from GCA patients following cell depletion

Temporal artery specimens were engrafted into four mice. The mice were treated with a T cell-depleting antiserum (ATGAM). Tissues were harvested at different time points and in situ cytokine transcription was determined as described in Fig. 2. Results are shown for IFN-γ and IL-1β as the mean of three experiments.

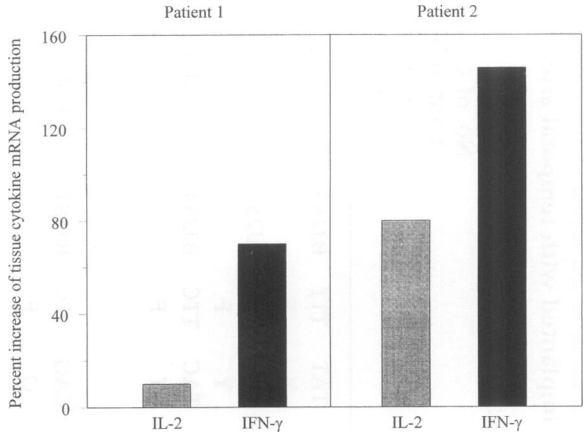

FIG. 5. Adoptive transfer of tissue infiltrating T cells with a survival advantage in xenografts

Temporal artery tissue was engrafted as described in Fig. 1. After 4 weeks, a T cell line was established from explanted tissue. T cells from this line were adoptively transferred into mice carrying implants of temporal artery tissue from the same patient. Control mice received T cells isolated from a peripheral blood lymphocyte line of the tissue donor. Tissue implants were retrieved 5 days after the adoptive transfers and in situ transcription of lymphokines was determined. Results are expressed as the percent increase of in situ cytokine production in the chimeras that had received tissue-derived T cells compared with that in the control mice.

patients are shown in Fig. 5. Adoptive transfer of T cells expanded from the explanted artery amplified the transcription of IL-2 and IFN-γ mRNA in the tissue implants compared with the adoptive transfer of peripheral blood-derived T cells. In one GCA patient, T cells selectively expanded in the grafts boosted IL-2 mRNA transcription only minimally but almost doubled the production of IFN-γ mRNA. Tissue-derived T cells from the second GCA patient enhanced the production of both IL-2 and IFN-γ mRNA. These results suggest that T cells that accumulate in the implanted tissue are disease-relevant. The elimination of these T cells disrupts the inflammation, whereas their adoptive transfer is associated with boosted T cell activation in the pathognomonic lesions.

DISCUSSION

Investigations into the pathological mechanisms of GCA have been complicated by the lack of an appropriate animal model for this vasculitic syndrome. The goal of the present study was to explore whether a human tissue–SCID mouse chimera model could be used to analyze the contribution of T cells and macrophages to the inflammation. We found that the phenotypic and functional characteristics of tissue-infiltrating cells were maintained over prolonged periods of engraftment. Intactness of the disease process after transplantation was documented by persistence of typical histomorphological findings and continuation of cytokine mRNA synthesis for monokines and T cell-derived lymphokines. The experiments presented here not only validate the GCA–SCID mouse chimeras as an appropriate model for this vasculitic entity but reveal several important aspects of the pathologic events in the arterial wall. In terms of the tissue-destructive immune response, temporal artery segments are functionally independent units that do not require the influx of cells, mediators, or antigens from the circulation of the patient. Although GCA has systemic manifestations and a disease variant, polymyalgia rheumatica, exists which lacks a histologically evident vascular infiltrate (2,11), the vasculitic inflammation itself appears

to be independent. All necessary components including immune cells and antigens are obviously present in the affected tissue.

In this respect, the vasculitic inflammation in GCA appears to be different from the synovial inflammation in rheumatoid arthritis. Rendt et al. (23) detected no to only a few T lymphocytes in synovial specimens from rheumatoid arthritis patients 6 to 10 weeks after engraftment. In contrast, inflammation persists in human psoriatic skin–SCID mouse chimeras (24). Apparently, human chronic inflammatory diseases differ fundamentally in their pathogenic mechanisms.

T lymphocytes and macrophages are the two important cellular components of the inflammatory infiltrate in GCA. Functional analysis of tissue-infiltrating macrophages by in situ hybridization and immunohistochemistry has provided evidence that different functional subsets of $CD68^+$ cells exist in the infiltrate (25). Most of the monokine-secreting macrophages can be located in the adventitia while macrophages in the media and intima mainly produce metalloproteinases and inducible nitric oxide synthase, respectively. Interestingly, circulating monocytes in patients with GCA are activated and secrete monokines. It could therefore be proposed that monocyte activation is a primary event preceding tissue infiltration. However, we have not found any evidence that the pool of tissue-residing macrophages had to be replenished from the circulation. Macrophages were abundant in xenotransplants as detected with anti-CD68 antibodies and remained functionally competent as demonstrated by the persistent synthesis of mRNA for IL-1β, IL-6, and TGF-β1. It is unclear how long human macrophages can survive in the tissue and whether they can multiply in inflammatory foci. In attempts to characterize the population of Ki-67-expressing cells in the tissue, we have found that the vast majority of proliferating cells lack the CD68 marker (data not shown). One would expect that macrophages, being terminally differentiated cells, have to be constantly replenished to maintain the inflammation. This appears not to be the case in GCA lesions where the macrophage infiltrate persists over time.

In previous studies, we have described a topographical relationship between IFN-γ-producing T cells and IL-1β/IL-6-producing macrophages in the adventitia. The experiments in the chimera model now conclusively demonstrate that activation of these tissue-residing macrophages is T cell–dependent. Depletion of human T cells by a T cell–specific antiserum in the GCA–SCID mouse chimeras suppressed the in situ production of IL-1β. IL-1β transcription was not completely abrogated, but it is likely that the low level of IL-1β transcription in the tissue was maintained by few residual T cells producing IFN-γ and not by a T cell–independent activation mechanism.

Equally important is the observation that tissue-infiltrating T cells are functionally diverse and that they either disappear or undergo clonal proliferation in the implanted tissue. After 4–6 weeks, lesional T cells had declined to 60% of preimplantation levels (Table 2). The finding that the production of IL-2 and IFN-γ mRNA persisted already indicated that T cells with the ability to produce these cytokines were enriched (Fig. 2). The fraction of T cells disappearing from the arterial segment is probably even higher but the proliferative activity of the retained T cells replenishes the infiltrate. Evidence for this model came from a shift in the TCR repertoire. The T cells infiltrating the temporal artery 4 weeks after implantation were less diverse, as shown by the loss of complexity in CDR3 size classes (Fig. 3). More importantly, certain clonal T cell populations had gained dominance and could be identified. Selection of these clonotypes was not a random process. Rather, identical TCR sequences emerged in independent tissue fragments implanted into different mice (Table 3). The plausible explanation for this finding is that a shared antigen expressed throughout the blood vessel wall is recognized by a small repertoire of T cells, thereby inducing clonal expansion of these specificities. The functional relevance of such T cells for the vasculitic inflammation could be documented in adoptive transfer experiments. In these experiments, the injection of T cells that had accumulated in the grafts into a GCA–SCID mouse chimera resulted in increased production of IL-2 and IFN-γ mRNA in the engrafted temporal artery tissue. These experiments cannot completely exclude the possibility that a murine blood–derived antigen is recognized in the human tissue. However, IFN-γ production in the implanted tissue remained stable over time and tissue-derived T cells did not respond to murine antigens in vitro, suggesting that these T cells truly recognize a disease-relevant antigen in the human arteries.

Provided that TCRs selected in distinct mice represent antigen-specific T cells reactive to the disease relevant antigen, they could be used as reagents to identify the antigen. However, we

have not seen shared T cell sequences in distinct patients. Rather, CDR3 regions of selected clonotypes exhibited considerable sequence polymorphism. This interpretation is supported by data collected in two other experimental approaches designed to isolate T cells of relevance from vasculitic tissue of GCA patients (10,22). IL-2-responsive $CD4^+$ T cell clones have been generated out of distinct tissue segments. TCR sequence studies demonstrated that identical TCRs were utilized in a low frequency of T cells, but identical TCR sequences could be isolated from independently analyzed sections of the inflamed arteries. Clonal expansion and presence at distinct sites of the inflammation were features of T cells utilizing different BV and BJ gene segments (10). More recently, we have systematically screened the repertoire of tissue-infiltrating T cells by investigating T cell populations sharing BV-BJ combinations (22). Clonal expansion of some T cells was a consistent finding in all patients analyzed. Again, a subset of clonally proliferating T cell specificities was detected in distinct regions of the artery. Utilizing these different strategies, we have so far collected information on a total of 17 patients with GCA. The repertoire of T cells recruited for antigen recognition in the arterial wall of these 17 patients was not restricted except that BV5S2-BJ2S3 T cell clones were encountered using all three approaches. This contrasts with the restriction in the expression of HLA-DR molecules in the patient group. A possible interpretation of these findings is that the TCR repertoire is sufficiently plastic to allow different individuals to recruit distinct T cells for antigen recognition in the disease process. This would imply that the restriction in the molecular TCR structure of disease-inducing T cells found in some of the animal models is not generalizable (25–28).

Several lines of indirect evidence have suggested that the vasculitic inflammation in GCA is an antigen-driven process, although the distribution and the nature of the putative antigen are unknown. Short of a demonstration of the antigen, the experiments in the GCA–SCID mouse chimera provide the most direct support for a pivotal role of antigen recognition in the pathogenesis of the vasculitic inflammation. Data presented here also indicate that the antigen is localized in the arterial tissue and that sufficient amounts of antigen are available in the engrafted tissue for at least 4–6 weeks to maintain the inflammation and to activate adoptively transferred T cells. These findings are best compatible with the model that an arterial self-antigen gains T cell stimulatory capacity in GCA patients. However, a persisting exogenous antigen cannot be completely excluded. To identify the antigen, T cell clonotypes selected in the xenotransplant might be the most powerful reagent since their proliferative activity should depend solely on the presence of the relevant antigen and the appropriate antigen-presenting cells.

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