Distinct Vascular Lesions in Giant Cell Arteritis Share Identical T Cell Clonotypes

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Summary

Giant cell arteritis (GCA) is a spontaneous vasculitic syndrome that specifically targets the walls of medium and large arteries. Vascular lesions are characterized by patchy granulomatous infiltrates composed of T cells, macrophages, histiocytes, and giant cells. To test the hypothesis that a locally residing antigen recruits T cells into the vessel walls, we have analyzed T cell receptor (TCR) molecules of tissue infiltrating T cells. A total of 638 $CD4^+$ T cell clones were isolated from temporal artery specimens of three patients with GCA. Analysis of TCR molecules for the usage of $V\beta1$-$V\beta20$ revealed that all TCR $V\beta$ elements were represented, demonstrating that interleukin 2 (IL-2)–responsive T cells infiltrating the tissue are highly diverse. To detect expanded T cell specificities, we made use of the patchy character of the inflammatory disease and compared the TCR repertoire of T cells established from independent vasculitic foci of the same artery. Sequence analysis of TCR $V\beta$ chains documented that individual TCR specificities were present in multiple copies, indicating clonal expansion. T cells with identical β chains were isolated from distinct inflammatory foci of the same patient. These specificities represented only a small fraction of tissue-infiltrating T cells and involved the $V\beta5.3$ gene segment in the two patients sharing the HLA-DRB1*0401 allele. The third complementarity determining region of clonally expanded TCR β chains was characterized by a cluster of negatively and positively charged residues, suggesting that the juxtaposed antigenic peptide is charged. The sharing of identical T cell specificities by distinct and independent regions of the granulomatous inflammation suggests that these T cells are disease relevant and that their repertoire is strongly restricted. These data suggest that an antigen residing in the arterial wall is recognized by a small fraction of $CD4^+$ T cells in the inflammatory process characteristic for GCA.

Giant cell arteritis (GCA)[1] is a vasculitis of unknown etiology that occurs primarily in older individuals (1). Typically, inflammatory infiltrates are found in the walls of medium- and large-sized arteries (2). Several lines of evidence emphasize a role of T cells in the pathogenesis of GCA. The cellular infiltrate is dominated by macrophages and T cells. Immunohistochemical experiments have shown that $CD4^+$ T lymphocytes are the major inflammatory cell population, whereas $CD8^+$ T cells are less frequent, and B lymphocytes are essentially absent (3). Granulocytes typically found in other vasculitides are apparently not involved in the arteritic lesions characteristic for GCA (2). Recent genetic linkage studies have demonstrated an association of GCA with alleles at the HLA-DRB1 locus, in particular, HLA-DRB1*04 variants (4). Whereas the molecular basis for this genetic predisposition is unknown, it is striking that all patients with GCA share a sequence motif in the second hypervariable region (HVR) of the HLA-DRB1 gene.

The HLA-DRB1 gene encodes for the highly polymorphic HLA-DR $\beta1$ chain, which on cell surfaces associates with a nonpolymorphic HLA-DRα chain. The major function of this α–β complex is to present peptides to $CD4^+$ T lymphocytes (5). The shared sequence motif identified in all patients with GCA translates into a region of the β-pleated sheet of the antigen binding site of the HLA-DR molecule (4, 6). It has therefore been hypothesized that the inflammatory process in GCA represents a local immune response to an unidentified antigen (7). Further support for a local activation of T lymphocytes came from the recent demonstration of locally produced IL-2 and IFN-γ mRNA (8). Since disease-relevant antigens in GCA are not known, it is difficult to directly test this hypothesis. Analysis of the local T cell infiltrate, however, may provide information on the diversity of accumulated T cell specificities and may also be useful in identifying the relevant antigens.

[1] *Abbreviations used in this paper:* GCA, giant cell arteritis; HVR, hypervariable region.

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Several studies in animal models have suggested a strong correlation between TCR V gene usage and T cell antigen recognition specificities (9–13). For example, in experimental allergic encephalomyelitis, a rat and mouse model of multiple sclerosis, the induction of disease has been correlated to T cell clones specific for myelin basic protein peptides. The antigen specificity in this model has been strictly correlated with TCR V gene usage and junctional diversity (14). More recently, TCR sequences with an identical sequence motif in the junctional region have been obtained from demyelinated human lesions from patients with multiple sclerosis (15). These studies suggest that the TCR repertoire involved in the pathogenesis of an autoimmune disease may be restricted and that the identification of disease-relevant polymorphisms may allow one to address the question of whether an antigen-driven expansion of T cells is involved in an inflammatory response.

The inflammatory response in GCA is remarkable in that the involvement of a given artery is characteristically focal and segmental (2). Here we have made use of the multifocal arrangement of the inflammation and have compared the diversity of the TCR repertoire of T cell clones derived from distinct segments of the same artery. Sequence analysis of TCR β chains demonstrated that selected T cells were present in multiple copies, indicating clonal proliferation in vivo. The repertoire of T cells involved in the inflammatory process was highly restricted within one individual. Anatomically distinct lesions were characterized by identical T cell clonotypes. These findings provide strong, albeit indirect, evidence for the model that GCA is triggered by the specific recognition of an antigen.

Materials and Methods

Patients and Tissue Samples. Temporal artery specimens were obtained from three patients undergoing biopsies for diagnostic reasons. All specimens showed an inflammatory infiltrate by standard histological techniques, confirming the diagnosis of GCA. All patients were characterized for their HLA-DRB1 alleles by allele-specific amplification and subsequent oligonucleotide hybridization as recently described (16, 17). In brief, DNA was extracted from PBMC and amplified with primer sets specific for the sequence polymorphism in the HVR1 of the HLA-DRB1 alleles. Amplified sequences were subsequently hybridized with oligonucleotides specific for polymorphisms of the HVR2 and HVR3. The sequences of the oligonucleotides and the amplification conditions have been described (16, 17). The HLA-DRB1 alleles of the three different patients were as follows: patient GCA-1, HLA-DRB1*0403/13; patient GCA-2, HLA-DRB1*0401/15(16); and patient GCA-3, HLA-DRB1*0401/11.

T Cell Cloning. The specimens were cut into small fragments of ~1 mm in length. The different fragments were dispersed and placed into tissue culture in the presence of recombinant human IL-2 (20 U/ml; Cetus Corp., Emeryville, CA). T cells had migrated out of the tissue within 18 h after the initiation of the cultures. Cultures were resuspended, and cells in suspension were directly cloned without additional in vitro activation in the presence of irradiated syngeneic feeder cells and IL-2–containing medium by limiting dilution. T cell lines and T cell clones were obtained from about two thirds of the tissue segments cultured, suggesting that about one third of the segments did not contain a T cell infiltrate. Established T cell clones were screened for the expression of CD4 and CD8 markers by FACS® analysis (Becton Dickinson & Co., Mountain View, CA). The majority of T cell clones in all three patients expressed the CD4 marker (data not shown). The frequency of growth-positive wells in patient GCA-1 suggested that a significant number of these cultures were not clonal. Only cultures that were negative for $CD8^+$ cells were further analyzed.

Vβ Gene Segment Analysis of T Cell Clones. Total RNA was extracted from $CD4^+$ T cells by guanidium thiocyanate phenol chloroform extraction using a commercially available kit (RNA Stat; Tel Test, Friendswood, TX). cDNA was synthesized by priming with oligo dT (15 mer) and reverse transcribing with avian myeloblastosis virus reverse transcriptase. Vβ gene segment usage was determined by PCR with 22 Vβ-specific primers and a Cβ-specific primer as recently described (18, 19). Amplified products were separated on 2% agarose gels and stained with ethidium bromide. We have recently shown that in the vast majority of human T cell clones only one rearranged Vβ element can be demonstrated under these conditions (19). Because the limiting dilution cloning approach, in particular in patient GCA-1, cannot exclude biclonality, Vβ elements identified in single wells were counted as independent clones. Sequencing of TCR β chains showed that the Vβ19-specific primer set, which had been reported to be Vβ specific (18), coamplified Vβ7.2. The assignment to Vβ7 and Vβ19 (see Table 1) has been corrected according to the sequence data.

Sequence Analysis. cDNA from selected T cell clones was amplified with a Cβ primer and the appropriate Vβ primer attached to a T7 promoter. The amplified product was transcribed by using a T7 RNA polymerase and then directly sequenced by reverse transcriptase–mediated dideoxysequencing as recently described (20, 21).

Oligonucleotide Hybridization for CDR3 Polymorphism. Biotin-conjugated oligonucleotides specific for CDR3 of the Vβ7.2 T cell clone C3-13 (ATCTGTATCCCTGTCCCGACG) from patient GCA-1 and the Vβ5.3 T cell clone MN 3-43 (GCCATAGTTAGACAGTCCCGG) from patient CGA-2 were designed. cDNA from PBMC and from T cell lines established from the distinct tissue segments was amplified with the appropriate Vβ-specific primer sets. The amplified templates were adjusted. Serial dilutions were vacuum blotted onto supported nitrocellulose membranes, prehybridized, and hybridized with the CDR3-specific probe and an internal biotinylated Cβ probe (AGAGAGCGACCTCGGGTGGGA), respectively, at 55°C. Blots were washed 10 min at 42°C and 10 min at 55°C in 2 × SSC 0.1 SDS, blocked with BSA blocking buffer, and developed with streptavidin-alkaline phosphatase. Signals were scanned using an imaging system (AMBIS, San Diego, CA). CDR3-specific signals were normalized to the Cβ-specific signal. Serial dilutions of cDNA from T cell clones expressing the CDR3 sequence were used as internal standard to estimate the frequencies of these clonotypes in the peripheral blood and the different tissue specimens.

Results

TCR Gene Usage of Tissue Infiltrating $CD4^+$ T Cells. To analyze the diversity of T cell specificities recruited to the inflammatory lesions, the Vβ gene segment usage of IL-2–responsive T cell clones derived from inflamed temporal arteries was determined. Results are summarized in Table 1. A total of 638 $CD4^+$ T cell clones were isolated from vasculitic foci of three different GCA patients. All Vβ elements,

Table 1. *TCR Vβ Gene Segment Usage by CD4+ T Cell Clones Derived from Vascular Infiltrates in GCA*

Vβ element	GCA-1		GCA-2		GCA-3	
	n	Percent	*n*	Percent	*n*	Percent
1	17	4.6	11	7.0	10	8.8
2	29	7.9	11	7.0	9	8.0
3	8	2.2	3	1.9	0	0.0
4	27	7.4	0	0.0	7	6.2
5.1	24	6.5	10	6.4	10	8.8
5.2–5.3	24	6.5	18	11.5	12	10.6
6	47	12.8	6	3.8	18	15.9
7	17	4.6	2	1.3	4	3.5
8	22	6.0	10	6.4	2	1.8
9	13	3.5	8	5.1	2	1.8
10	25	6.9	3	1.9	0	0.0
11	7	1.9	3	1.9	0	0.0
12	7	1.9	7	4.5	1	0.9
13.1	31	8.4	12	7.6	15	13.3
13.2	11	3.0	3	1.9	0	0.0
14	9	2.5	13	8.3	3	2.3
15	3	0.8	0	0.0	1	0.9
16	8	2.2	1	0.7	1	0.9
17	9	2.5	16	10.2	6	5.3
18	12	3.3	12	7.6	8	7.1
19	0	0	0	0.0	1	0.9
20	18	4.9	8	5.1	3	2.7

except Vβ19, were represented in CD4+ T cells from patient GCA-1. In patients GCA-2 and GCA-3, some Vβ segments were not found at all, such as Vβ10, Vβ11, Vβ15, and Vβ19, which are known to be infrequently used Vβ elements. The only remarkable finding was the nonusage of Vβ4 in GCA-2, and Vβ3 and Vβ13.2 in GCA-3. The Vβ diversity of tissue infiltrating CD4+ T cells resembled that found in peripheral blood. Vβ gene segments frequently encountered in peripheral T cells, such as Vβ2, Vβ5, Vβ6, and Vβ13.1, were the dominant Vβ elements in the T cell clones isolated from the tissue. Thus, IL-2R+ CD4+ T cells in the vasculitic lesions are highly diverse without evidence for restrictions in the utilization of TCR Vβ gene segments.

Clonal Expansion of Selected T Cell Specificities. The heterogeneity of preactivated tissue infiltrating CD4+ T cells might reflect the heterogeneity of the circulating pool of preactivated T cells or may indicate a polyclonal T cell response to a variety of antigens. To identify potentially disease-relevant T cells, we made use of the characteristic arrangement of vasculitic foci in GCA. The inflammatory infiltrates are generally arranged in patches with alternating areas of normal and inflamed tissue. We compared the Vβ usage in distinct inflammatory patches from the same artery. The results for three

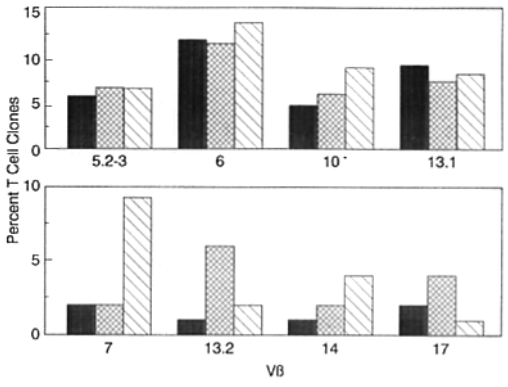

Figure 1. Vβ gene segment usage of CD4+ T cell clones derived from distinct tissue segments. CD4+ T cell clones were established from three distinct tissue segments of an inflamed temporal artery of patient GCA-1: segment 1 (*solid bars*), segment 2 (*cross-hatched bars*), and segment 3 (*diagonal bars*). TCR Vβ gene segment usage was determined by PCR with Vβ-specific primers. Vβ5.2-3, 6, 10, and 13.1 were evenly distributed as were the Vβ elements not shown. Vβ13.2- and Vβ17-expressing T cells were enriched in segment 2, whereas Vβ7 and 14 were more frequently found only in segment 3.

Table 2. *Nucleotide Sequences of TCR β Genes from Clonally Expanded T Cell Specificities*

Patient	T cell clone	Vβ	N-D-N region	Jβ	No. of identical sequences
GCA-1	CT3-13 CT3-26 CT3-248 CT1-210 CT3-252 CT3-253 CT3-258 CT1-237	(7.2) AGC AGC	CGT CGG GAC AGG GAT ACA GAT	GGC TAC ACC TTC (1.2)	8/11
GCA-2	MN3-202 MN3-204	(14) AGC AGT	AAC GAC CCC AGG AGA	CCC CTC CGC TTT (1.6)	2/10
	MN2-205 MN2-216	(14) AGC AGT	GTA CAG GGG TTC CAA GGC	ACT GAA GCT TTC TTT (1.1)	2/10
	MN3-61 MN3-220	(14) AGC AGT	TTA GAA CAG GGG ACA	GAG ACC CAG TAC TTC (2.5)	2/10
	MN1-59 MN1-212 MN3-43	(5.3) AGC AGC	CCG GGA CTG TCT	AAC TAT GGC ACC TTC (1.2)	3/11
	MN1-226 MN2-246	(5.3) AGC AGC	TTT CAA GGG	AGC AAT CAG CCC CAG CAT TTT (1.5)	2/11
GCA-3	DU1-18 DU1-72	(5.3) AGC AGC	CCG GAC AGG GAA GGG	AGC ACA GAT ACG CAG TAT TTT (2.3)	2/10

segments from the artery of patient GCA-1 are shown in Fig. 1. Use of some Vβ families was more pronounced in some vasculitic foci, but not others. Uneven distribution among the three different biopsy segments was found for Vβ7, Vβ13.2, Vβ14, and Vβ17. In comparison, the Vβ distribution for Vβ5.2-3, Vβ6, Vβ10, and Vβ13.1 are shown, all of which were frequently used and evenly distributed between the different segments.

We hypothesized that the overrepresentation of some Vβ elements in individual inflammatory foci resulted from clonal expansion of selected T cell clonotypes. To screen for clonally expanded specificities, TCR β chains from T cell clones expressing the unevenly distributed Vβ segments were sequenced. 11 of the Vβ7$^+$ T cell clones utilized the Vβ7.2 element. Among the set of 11 T cell clones, 8 expressed the identical TCR β chain, indicating that they had originated from a single precursor. No identical TCR sequences were found for the three other unevenly distributed Vβ elements, Vβ13.2, Vβ14, and Vβ17. Sequence analysis demonstrated that all T cell clones utilizing the evenly distributed Vβ elements Vβ5.2, Vβ10, and Vβ18 expressed unique TCR β chain sequences. Thus, in this patient, clonal expansion was limited to Vβ7.2$^+$ T cells.

Using the same approach, clonally expanded T cell populations were identified in patient GCA-2 (Table 2). In this patient, five expanded clonotypes were identified; three of them used the Vβ14 element and two used the Vβ5.3 element. No clonal expansion was seen in Vβ2$^+$, Vβ13.1$^+$, Vβ17$^+$, and Vβ18$^+$ clones, although these Vβ elements were frequently found and made up about one third of the inflammatory infiltrate. In patient GCA-3, a sufficient number of T cell clones was established from only one segment. Sequence analysis demonstrated clonal expansion again for a Vβ5.3 T cell clone.

Our data suggest that our sequencing strategy was successfully biased to identify expanded clonotypes in all three patients. Even if we missed an expanded specificity in the Vβ elements that were not sequenced, the T cells which had undergone clonal expansion were a minority among all tissue infiltrating IL-2R$^+$ T cells. Clonal expansion was restricted to T cell clones expressing Vβ7.2 in patient GCA-1, Vβ5.3 and Vβ14 in patient GCA-2, and Vβ5.3 in patient GCA-3. Thus, patients GCA-2 and GCA-3 shared clonal proliferation within Vβ5.3$^+$ T cells. These two patients also have in common the HLA-DR allele, B1*0401, whereas patient GCA-1 expressed a distinct disease-linked B1 allele, the B1*0403 variant. These data suggest that the Vβ restriction of clonally expanded cells may be linked to the HLA-DRB1 allele.

Clonally Expanded T Cell Specificities Are Residing in Distinct Inflammatory Foci. Using the patchy nature of the vasculitic process was helpful to define a microenvironment and to identify clonally expanded T cell specificities. Comparing the composition of tissue infiltrating T cells from distinct inflammatory patches could provide further evidence for the distinction of disease-relevant T cells and bystander T cells. To approach this question, we compared the representation of CD4$^+$ T cell clones isolated from independent inflammatory foci. In the two patients in whom we obtained sufficient numbers of T cell clones from multiple fragments, we found that T cells with identical TCR β chains were isolated from independent segments of the inflamed temporal arteries (Table 3). The clonally expanded Vβ7.2$^+$ T cell clone in GCA-1 and two different Vβ5.3$^+$ T cell clones in GCA-2 were

Table 3. *Distribution of T Cell Clonotypes with Identical TCR β Sequences*

	Fragment 1	Fragment 2	Fragment 3
Vβ7.2	CT1-210	–	CT3-13
	CT1-237		CT3-26
			CT2-248
			CT3-252
			CT3-253
			CT3-258
Vβ14	–	MN2-205	–
		MN2-216	
	–	–	MN3-202
			MN3-204
	–	–	MN3-61
			MN3-220
Vβ5.3	MN1-59	–	MN3-43
	MN1-212		
	MN1-226	MN2-246	–

localized in different patches of the inflammatory disease. These findings represent a definte proof that clonal expansion occurred in vivo and was not an in vitro amplification. Use of identical T cells in different regions of the disease demonstrates that the repertoire of T cells is strongly restricted. It also raises the question of where these T cells are primed and expanded. Detection of identical T cells in distant areas of the inflammatory process makes it unlikely that they are primed in the arterial wall. It is more likely that selected T cell specificities are primed outside of the artery, proliferate, and then migrate into different sites of the artery where they recognize their specific antigen on the surface of an antigen-presenting cell.

Expanded T Cell Clonotypes Are Not Detectable in the Peripheral Blood Compartment. To address the possibility that the isolation of several T cell clones with identical sequences resulted from a restriction within the total TCR repertoire of the patients, we cloned $CD4^+$ T cells from the peripheral blood of patient GCA-2 after in vitro activation with immobilized anti-CD3 for 18 h and sequenced the TCR β chain from $V\beta5.2/5.3^+$ clones. We did not find evidence for clonal expansion nor did any of those T cell clones utilize a TCR sequence that we had identified in the tissue infiltrating T cells, suggesting that these clonal specifities were not overrepresented in the peripheral blood (data not shown). To estimate the frequencies of clonotypes in the tissue and peripheral blood compartments, we used oligonucleotide hybridization dot blot assays for CDR3-specific sequences. Results for the sequence derived from a $V\beta5.3$ clonotype of patient GCA-2 are shown in Fig. 2. The probe was specific for CDR3-specific sequences and did not crosshybridize on a T cell clone sharing the same $V\beta$-$J\beta$ combination. Two distinct tissue segments from GCA-2 contained the CDR3 sequence, whereas the signal for the PBMC sample of patient GCA-2 was not different from the control PBMC sample. The frequencies of T cell clones expressing the CDR3 sequence was calculated by parallel hybridization with a $C\beta$-specific probe (Fig. 2). Serial dilutions of T cell clones expressing the CDR3-specific sequence were used as standard curve. 25 and 17%, respectively, of all $V\beta5.2\text{-}3^+$ clones in tissue segments 1 and 2 expressed the sequence compared with <3% of $V\beta5.2\text{-}3^+$ PBMC from patient GCA-2 and the control individual. These data demonstrate that these clonotypes are locally accumulated and/or expanded and are not overrepresented in the PBMC.

Characteristics of Clonally Expanded $CD4^+$ T Cells. The clonal expansion of selected T cells and their presence at distinct sites of the inflamed artery suggests that these T cells are potentially disease relevant, whereas the accumulation of nonexpanded T cells may only reflect their homing pattern. Comparison of the TCR molecules of these two T cell populations may provide information on the nature of the antigens

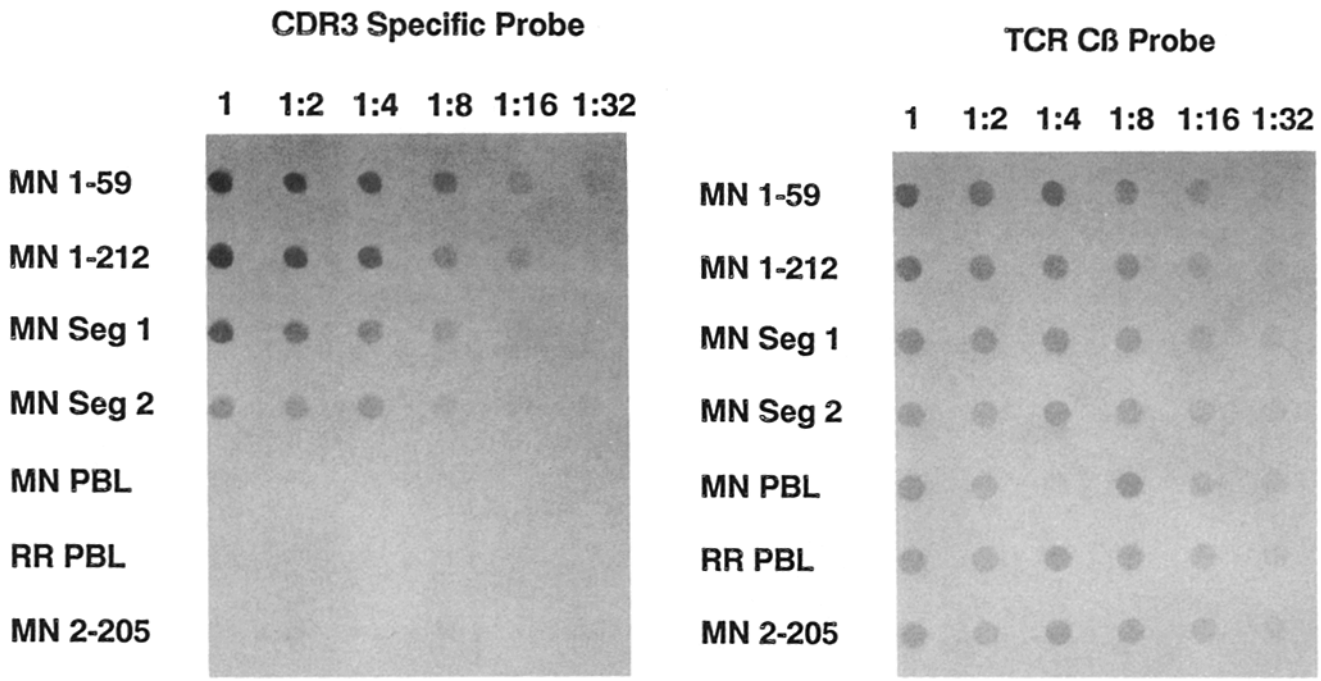

Figure 2. Representation of CDR3-specific sequences in PBL and temporal artery specimens. T cell lines were established from two segments, segment 1 and 2, of the temporal artery specimen of patient GCA-2 (lines *3–4*). cDNA was amplified with a $V\beta5.2\text{-}3$–specific primer set and compared with the amplified products derived from PBL from patient GCA-2 (line *5*) and a control individual (line *6*). T cell clones MN 1-59 and MN 1-212 (lines *1–2*), which have identical TCR sequences and were established from segment 1, served as positive control. T cell clone MN 2-205, which expressed an unrelated CDR3 sequence, was used as negative control (line 7). Results are shown for serial dilutions of the amplicons after hybridization with a CDR3-specific probe (*A*) and a $C\beta$-specific probe (*B*). CDR3-specific sequences were found in both tissue specimens, but not in a significant concentration in the PBL population from the same patient.

Table 4. *CDR3 Sequence Motifs in Expanded Clonotypes*

Patient	T cell clone	Vβ	N-D-N region	Jβ
GCA-1	CT3-13	7.2	R R D R D T D	Q Y T F (1.2)
GCA-3	DU1-18	5.3	P D R E G	S T D T Q Y F (2.3)
GCA-2	MN3-202	14	N D P R R	P L H F (1.6)
GCA-2	MN2-205	14	V Q G F Q G	T E A F F (1.1)
GCA-2	MN3-61	14	L E Q G T	E T Q Y F (2.5)
GCA-2	MN2-246	5.3	F Q G	S N Q P Q H F (1.5)
GCA-2	MN3-43	5.3	P G L S	N Y G T F (1.2)

that are specifically recognized. To address this question, we analyzed the usage of amino acid residues in the CDR3 region of clonally expanded and nonexpanded T cells isolated from the inflamed tissue.

The N-D-N region of three of the seven expanded T cell clones contained a high number of charged residues (Table 4). This was particularly evident for the Vβ7.2$^+$ T cell clonotype which used a repetitive sequence of Arg and Glu. The total net charge for the three T cell clones with charged residues in the CDR3 was different. However, two clones had a DRD or DRE motif in the central position of the CDR3, suggesting that the interacting protein includes charged amino acids. A second motif was identified in expanded TCR molecules of patient GCA-2. Three of the five TCR used the QGS/T motif. While the DRE motif is charged, amino acids in the QGS motif may function as hydrogen donors in the interaction with a charged peptide.

The distribution of amino acids in the CDR3 of nonexpanded T cell clones was very similar to amino acid use described by Prochnicka-Chalufour et al. (22) (Table 5). Gly, Thr, and Ser were frequently used. Negatively and positively charged residues accounted for slightly more than 20% of all amino acids. The clonally expanded T cells were characterized by a preferential usage of charged amino acids. In particular, Asp, Arg, and Gln were frequently encountered and may be involved in hydrogen bonds. This structure of the

Table 5. *Usage of Amino Acid Residues in the CDR3 of CD4$^+$ T Cells Isolated from Inflammatory Infiltrates*

	Amino acids (Percent TCRβ-CDR3)		
Amino Acid	Clonally Expanded T cells	Nonexpanded T cells	Prochnicka-Chalufour et al. (22)
Aspartic Acid	13.6	4.0	10.6
Arginine	13.6	9.5	5.7
Glycine	13.6	16.0	17.7
Glutamine	11.4	4.7	5.3
Threonine	9.0	10.2	8.5
Glutamic Acid	6.8	7.3	3.9
Serine	6.8	8.4	11.3
Proline	6.8	6.2	1.4
Asparagine	6.8	6.5	8.1
Leucine	4.5	5.0	6.7
Phenylalanine	4.5	0.7	1.0
Valine	2.3	3.3	2.1
Alanine	0.0	5.8	7.1
Tyrosine	0.0	3.3	4.6
Lysine	0.0	4.4	0.3
Isoleucine	0.0	1.5	2.1
Tryptophan	0.0	3.3	2.5

CDR3 suggests that the antigenic peptide recognized has negatively and positively charged residues.

Discussion

To evaluate the contribution of $CD4^+$ T cells to the inflammatory infiltrate in GCA, we have analyzed the diversity of tissue infiltrating $CD4^+$ T cells. Data described in this report demonstrate that a highly heterogeneous population of $CD4^+$ T cells is recruited to the vasculitic infiltrates. A small percentage of T cells have undergone clonal expansion, suggesting an antigen-specific response. The composition of the T cell infiltrate in this vasculitic response proposes the model that a small fraction of disease-relevant T cells are surrounded by T cells attracted to the inflammatory site by antigen-nonspecific mechanisms. Most importantly, identical clonotypes had infiltrated the tissue at independent sites, indicating that the TCR repertoire for a putative disease relevant antigen in GCA patients is limited.

GCA represents a unique model of an inflammatory disease in humans. In general, the patients have sudden onset of disease, and by the time temporal artery tissue is obtained for diagnostic reasons, the disease is in an early and acute stage (1). For other chronic inflammatory diseases, it has been argued that the composition of the inflammatory infiltrate changes over time with an initial dominance of an antigen-specific response but recruitment of highly diverse T cell specificities during chronic disease (23, 24). All patients analyzed in this report underwent biopsy within 2 wk after onset of symptoms. Thus, the $CD4^+$ T cell population isolated from the inflamed arteries should be representative of the acute disease process.

To focus on relevant T cells, we selected for IL-2–responsive $CD4^+$ T cells. It is a reasonable assumption that all $CD4^+$ T cells proliferating to low doses of IL-2 have been recently activated. The wide spectrum of IL-2R–expressing T cells accumulated in the vasculitic lesions indicates that recruitment mechanisms are not selective. Similar observations have been described for antigen-specific responses to exogenous antigens. Doherty et al. (25) have determined the frequencies of influenza virus–specific T cells in different target tissues of virus-infected mice and have described that ~50% of locally infiltrating cells secrete lymphokines, whereas only 1% are antigen specific. Recently activated T cells, as well as memory cells, are apparently equipped to migrate into tissue in search of their relevant antigen (26, 27). Areas of inflammation thus enrich for such T cells and include a random representation of currently activated $CD46^+$ T cells in the circulation.

To define a T cell population that is potentially relevant to the disease process, we have chosen to define local clonal expansion of a T cell clonotype as a criterion. The fact that T cells with identical TCR β chains were isolated from different and nonadjacent segments of tissue emphasizes the interpretation that replicate copies of the expanded T cells did indeed develop in vivo and were not simply an artifact of in vitro T cell isolation. There are several possible explanations for encountering replicate copies of the same T cells in different tissue segments. In the first model, T cells are primed outside of the artery and are independently recruited to the inflammatory foci. Alternatively, T cells first replicate within the inflammatory vasculitic infiltrate. In this model, T cells would have to leave the arterial wall after antigen contact in the inflammatory lesions and recirculate. Our data on the representation of these clonotypes in tissue and in peripheral blood (Fig. 2) do not exclude that these cells are present in the blood compartment, however, the frequency must be very low compared with the frequencies in the tissue. In both models, the repertoire of disease-relevant T cells triggered by an autoantigen or exogenous antigen would have to be highly restricted and involve only a few clonotypes.

Clonal expansion was limited to T cells utilizing Vβ7.2 in the HLA-DRB1*0403$^+$ patient and Vβ5.3 and Vβ14 in the HLA-DRB1*0401$^+$ patients. This restriction supports the hypothesis that the clonally expanded T cell are specific for an antigen. There was no evidence for an overall overrepresentation of those Vβ families, and the composition of the inflammatory T cell infiltrate does not suggest the action of a superantigen. Several animal models of autoimmune diseases have suggested that the autoimmune response is triggered by a limited number of T cell specificities that recognize an autoantigenic peptide in restriction to MHC class II molecules. In general, these autoimmune diseases have been induced by immunization with autoantigens or by antigens closely related to autoantigens in the presence of adjuvants (14, 28, 29). It remains to be proven whether the pathomechanism implied in these animal models are functional in chronic inflammatory diseases which spontaneously arise in susceptible patients. Recent studies by Oksenberg et al. (15) suggest that this indeed might be the case. These authors have observed identical clonotypes in different plaques and in different patients with multiple sclerosis. In these studies, T cells were not isolated. Therefore, the question cannot be directly addressed whether the TCR share an antigen specificity. However, homology data of the CDR3 suggested that these TCR may be directed to the myelin basic protein. In contrast to multiple sclerosis, GCA has the advantage that tissue is readily available at very early stages of the disease. Our data support the model that the repertoire of disease-relevant T cells during the early stages of the disease is highly restricted, suggestive of an antigen-specific response. The nature of this antigen in GCA is unresolved. The arrangement of the granulomatous lesion in the media of the artery and the destruction of the elastic lamina has raised the hypothesis that elastic fibers represent a target antigen of the disease. The clinical presentation of patients with GCA is not easy to reconcile with this model. Patients present with an acute onset of the disease, suggesting an exogenous antigen. The disease usually has a self-limiting course and goes into spontaneous remission after 1–2 yr, suggesting that the immune response in GCA patients is not directed to a widely present autoantigen such as elastin. In support of this view, we have shown that the disease-implicated T cell clones do not proliferate in response to elastin (data not shown).

Analysis of the amino acid use in the CDR3 of the putatively relevant T cell clones may allow one to speculate on

the nature of the stimulating antigen. Several features were unique for the T cell clones isolated from the temporal artery. Three out of the seven T cell clones used a repetitive stretch of positively and negatively charged amino acids. Most striking was the finding of the RRDRDTD motif in the $V\beta7.2^+$ T cell clones. This clustering of charged residues suggests that the corresponding antigenic peptide may be charged as well. The second set of T cell clones was characterized by the motif QGS/T. Whereas it cannot be predicted that these T cell clones recognize the same antigen, it is possible that these amino acids are involved in hydrogen bonds with a charged antigenic peptide. These observations may be helpful to define candidate antigens in narrowing down the spectrum of antigens potentially recognized by these TCR specificities. Ultimately, the nature of the antigen has to be demonstrated in direct biological assays. The clonally expanded T cells isolated from the inflammatory lesions represent unique reagents to search for the relevant antigen.

Current therapies of GCA are focused on the use of corticosteroid which cannot shorten the disease process and, in many patients, is accompanied by serious side effects. Suppressing the inflammatory response in GCA patients with more specific means would improve our management of these patients. One of the conclusions of this study is that the fraction of disease-relevant T cells is probably very small. This might explain why experimental approaches using $V\beta$ repertoire studies in human autoimmune disease have been of very limited success (24, 30–33). However, the ability to define disease relevance for a small subset of T cells that cannot be detected by crude approaches, such as $V\beta$ analysis, would open the possibility to spare the majority of T cells and target the minor subset that appears to initiate and maintain the chronic destructive inflammation.

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