EXTENDED REPORT

Selective T cell receptor decrease in peripheral blood T lymphocytes of patients with polymyalgia rheumatica and giant cell arteritis

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Objectives: To investigate the phenotype and T cell receptor (TCR) use in peripheral blood T cells in patients with polymyalgia rheumatica (PMR) and giant cell arteritis (GCA).

Methods: Circulating T lymphocyte phenotype and TCR repertoire were studied by flow cytometry using specific monoclonal antibodies in 23 healthy controls and 37 patients with PMR/GCA.

Results: Patients with active PMR/GCA showed an inverse relation between naive and memory CD4+ T cells and unchanged expression of activation surface markers compared with controls. CD4+ TCR BV expansions were seen in 12 (52%) controls and in 8 (22%) patients with active disease ($p=0.03$). Within the CD8+ subset, the frequency of expansions was similar between groups. Most T cell expansions remained stable over time. Seventeen of the 23 patients with active PMR/GCA disclosed a simultaneous CD4+ and CD8+ T cell depletion for at least one particular BV family with a clear predominance of BV5S2/S3.

Conclusions: The phenotype of circulating T cells in patients with PMR/GCA is similar to that found in aged healthy subjects, except for the surface markers of naive and memory cells and a striking non-activated phenotype. Specific BV expansions in CD4+ and CD8+ T cells, which remain stable over time, are frequent in aged subjects, including patients with PMR/GCA. TCR BV changes in patients with active disease seem to be also age related, except for the significant decrease in certain BV families in both CD4+ and CD8+ T cell subsets, which may favour the participation of a superantigen stimulation in PMR/GCA.

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are two closely related syndromes affecting elderly people.¹ GCA is a granulomatous vasculitis affecting medium and large sized arteries, and the inflammatory lesions are characterised by the presence of mononuclear cells, multinuclear giant cells, and fragmentation of the internal elastic laminae.^{1–3} Around 50% of patients with GCA have PMR, a clinical syndrome characterised by pain and stiffness in the neck, shoulder, and pelvic girdle.⁴ The main factors that determine the development of a full vasculitic syndrome are yet unknown.⁵

GCA and PMR represent HLA-DR4 associated diseases.^{6,7} The HLA genes may affect disease pathogenesis through several mechanisms. One of the major biological functions of the HLA molecule is the antigen presentation to T cells, the so-called peptide selection model. In GCA, this model would be supported by the demonstration of selected CD4+ T cell populations expanded in the arterial wall that proliferate in vitro when cultured with tissue extracts from inflamed temporal artery.⁸ The HLA-DR polymorphisms may also shape the selection of the T cell repertoire and determine the severity of the manifestations of a disease as has been shown in patients with rheumatoid arthritis.⁶ Unfortunately, and despite the relatively high frequency of PMR and GCA, the influence of the HLA molecules on the diversity of the peripheral T cell repertoire in these diseases has not been studied in a large sample of patients.

Antigens are presented to T cells as peptides in association with major histocompatibility cell (MHC) molecules, and the specificity of T cells resides within the T cell receptor (TCR). Therefore, analysis of TCR use in human diseases may provide some clues about the nature of the triggering agents.⁹ Accumulating evidence indicates that GCA represents the consequences of a local immune response to a

disease-inducing antigen, and it is considered the best example of a T cell mediated vasculitis.^{10,11} CD4+ T cells are one of the dominant cellular types in the vasculitic lesions, which also express activation surface markers, and undergo clonal proliferation in the inflammatory lesions of the temporal artery.^{8,12,13} However, much less is known about the role of circulating T cells in patients with GCA and PMR. One of the most striking features of these two conditions is the development of the disease, almost exclusively, in people aged over 50 years. Thus, there is evidence for age associated changes in the circulating T cell compartment that might favour the development of autoimmune disorders in the elderly.^{14–17}

The purpose of this study was to investigate the phenotype and TCR use in CD4+ and CD8+ peripheral blood T cells in a large sample of patients with PMR and GCA.

PATIENTS AND METHODS

Patients and controls

During a two year period, 23 consecutive patients with active PMR/GCA before steroid treatment and 14 patients with PMR/GCA in clinical remission after steroid treatment (mean (SD) time after diagnosis 2.4 (1.9) years) were recruited into the study. Six of the 14 patients with controlled disease had not been receiving steroids for at least six months, and the remaining eight patients continued to receive low dose prednisone. In four patients with active PMR/GCA, follow up samples were taken after six months of treatment, when

Abbreviations: GCA, giant cell arteritis; mAb, monoclonal antibody; MHC, major histocompatibility cell; PCR, polymerase chain reaction; PMR, polymyalgia rheumatica; TCR, T cell receptor

Table 1 Main demographic and routine laboratory data in patients with polymyalgia rheumatica (PMR)/giant cell arteritis (GCA) and healthy controls

	Healthy controls (n = 23)	PMR/GCA before treatment (n = 23)	PMR/GCA after treatment (n = 14)
Age (years)	73 (8)	73.2 (6.9)	74 (6.2)
Sex (F/M)	14/9	11/12	9/5
PMR/GCA (n)	0/0	18/5	6/8
Leucocytes (×10 ⁹ cells/l)	5.6 (1.1)*	7.4 (1.9)*	8.8 (2.6)
Lymphocytes (%)	30.5 (6.1)*	24.3 (8.8)*	25.2 (9.9)
Lymphocytes (×10 ⁹ cells/l)	1.7 (0.4)	1.7 (0.6)	2.1 (0.7)
Haemoglobin (g/l)	137 (18)*	125 (18)*†	138 (16)†
Packed cell volume	0.41 (0.06)	0.38 (0.05)†	0.41 (0.05)†
Platelets (×10 ⁹ cells/l)	190 (35)*	275 (108)*†	223 (69)†
ESR (mm/1st h)	7.5 (5.3)*	48.5 (34.5)*†	14.4 (13.5)†
CRP (mg/l)	4 (1)*	43 (37)*†	6 (6)†

The results are expressed as mean (SD)

Sex (F, female; M, male); ESR, erythrocyte sedimentation rate (normal <15 mm/1st h); CRP, C reactive protein (normal <5 mg/l).

*Healthy controls versus PMR/GCA before corticosteroid treatment: p<0.05; †PMR/GCA before corticosteroid treatment versus PMR/GCA after treatment: p<0.05.

the patients were in clinical remission. Twenty three healthy controls matched for age and race were studied in parallel. Table 1 summarises the main clinical and laboratory characteristics of the study cohort. PMR was diagnosed according to the criteria proposed by Chuang *et al.*¹⁸ All the patients with GCA had a positive temporal artery biopsy.¹⁹ Corticosteroid treatment for PMR was started with prednisone 10 mg daily (5 mg twice daily) and for GCA was begun with prednisone 40–60 mg daily (15–20 mg three times a day). After initial control of the disease, the dose of corticosteroids was reduced according to clinical disease activity.

Routine laboratory assessment

All the blood samples were collected between 7 30 am and 9 30 am, before the morning dose of the daily treatment. Total white cell, lymphocyte and platelet counts, haemoglobin, erythrocyte sedimentation rate and C reactive protein were measured by routine techniques (table 1).

Preparation of cells and HLA typing

Peripheral blood mononuclear cells were purified from EDTA venous blood by Ficoll gradient centrifugation (Amersham Pharmacia Biotech, Uppsala, Sweden). DNA was extracted with DNAzol reagent (InVitrogen, Grand Island, NY) according to the manufacturer's instructions. Patients and controls were characterised for their HLA-DRB1 alleles by allele-specific polymerase chain reaction (PCR) amplification with a Dynal All Set sequence-specific primers (SSP; Dynal AS, Oslo, Norway).

FACS analysis

Peripheral blood T lymphocyte phenotype and TCR BV usage were determined by flow cytometry in whole blood samples. The following monoclonal antibodies (mAbs) were used for staining: FITC conjugated mAb specific for CD7, CD45RA, CD45RO, HLA-DR (Pharmigen, San Diego, CA), CD25, CD28, CD57, BV2 (Labgen, Labclinics, Barcelona), BV3S1, BV5S1, BV5S2/5S3, BV6S7, BV8, BV13S1/13S3 (T cell diagnostics, Woburn, MA), BV12 and BV17 (Endogen, Woburn, MA), phycoerythrin conjugated anti-CD4 and anti-CD8, and FITC conjugated anti-CD3 (Becton Dickinson, San Jose, CA). Whole blood samples were stained within six hours of venepuncture with two colour direct immunofluorescence

reagents for 30 minutes at 4°C. Thereafter, erythrocytes were lysed using FACS Brand Lysing Solution (Becton Dickinson). After two washings with phosphate buffered saline, cells were resuspended and analysed on the day of processing using a Becton Dickinson FACScalibur and CellQuest software. Twenty thousand events were analysed. TCR BV-specific expansion was defined as BV specificities expressed as a value of more than 20% or at a frequency greater than the mean+3SD of a young healthy population.

Statistical analysis

Analysis was performed with the STATISTICA software package for Macintosh (Macintosh, Apple Computer Inc, Cupertino, CA, USA). We used the two tailed Student *t* test for the continuous variables with a normal distribution, and non-parametric tests (median and Mann-Whitney U test) for those without a normal distribution. The χ^2 test or the Fisher exact test was used to compare the dichotomous variables.

RESULTS

Demographic features and main laboratory data

Table 1 shows that patients with active disease had a higher leucocyte count ($p = 0.001$) and a lower percentage of total lymphocytes ($p = 0.01$) than healthy controls. As expected, all the measures reflecting the inflammatory process were higher in patients with active disease than in healthy controls and patients in clinical remission ($p < 0.05$).

Circulating lymphocyte subsets

The percentages and absolute numbers of the main lymphocyte subsets were comparable except for the percentage of CD4+ T cells between patients with active and controlled disease (table 2). Despite a higher percentage of CD4+ T cells in patients with active untreated disease ($p = 0.03$), absolute counts and the CD4/CD8 ratios were similar between the different groups. In agreement with a previous report,²⁰ percentages and absolute numbers of CD8+ T cells were similar between the different study groups. The frequencies of NK cells (CD3–CD16+CD56+) and T cells with a killer phenotype (CD3+CD16+CD56+) were also similar in patients and controls.

Peripheral T lymphocyte phenotype

Analysis showed that the T cell phenotype in both groups of patients was very similar (table 3). A slight but significant increase in the percentage of CD4+CD7+ T cells ($p = 0.03$) was seen in patients with active PMR/GCA compared with healthy controls. Although other markers have been recently

Table 2 Main circulating T lymphocyte subsets in patients with PMR/GCA and healthy controls

	Healthy controls	PMR/GCA before treatment	PMR/GCA after treatment
CD3	68.4 (10.4) [1154 (312)]	71.1 (8.2) [1237 (474)]	71 (12.5) [1423 (630)]
CD4	40 (8.7) [669 (186)]	44.8 (8.3)* [757 (275)]	37.5 (11)* [765 (304)]
CD8	28.4 (8.9) [484 (218)]	26.3 (11.3) [479 (317)]	27.5 (12.6) [608 (387)]
CD4/CD8 ratio	1.7 (1.4)	2.1 (1.2)	1.9 (1.6)
CD3+/CD16+CD56+	3.8 (4) [64 (67)]	2.1 (1.2) [36 (21)]	1.9 (1.6) [39 (33)]
CD3–/CD16+CD56+	14.3 (7.2) [241 (121)]	13 (4) [224 (69)]	17.8 (6.5) [367 (134)]

The results are expressed as mean (SD) percentage and absolute numbers are shown in square brackets.

*PMR/GCA before corticosteroid treatment versus PMR/GCA after treatment: p<0.05.

Table 3 Phenotype of circulating T lymphocyte subsets in patients with PMR/GCA and healthy controls

	Healthy controls	PMR/GCA before treatment	PMR/GCA after treatment
CD4/CD7	79.7 (9.2)* [540 (176)]	85.3 (8)* [653 (270)]	81.8 (11.1) [636 (277)]
CD4/CD28	87.7 (13.9) [598 (218)]	92.5 (9.9) [705 (287)]	86.4 (16.7) [675 (303)]
CD4/CD57	10.7 (10.9) [66 (64)]	6.5 (8) [48 (59)]	10.3 (10.2) [69 (60)]
CD4/CD45RA	30 (11.7)* [209 (113)]*	40 (14.9)* [316 (192)]*	36.2 (17) [289 (185)]
CD4/CD45RO	72.4 (13.1)* [472 (122)]	60.3 (14.7)* [445 (154)]	62.7 (17.1) [475 (226)]
CD4/CD25	4.9 (2.4) [34 (20)]	5.5 (4.1) [40 (30)]	4.8 (3.7) [39 (35)]
CD4/HLA-DR	3.3 (1.9) [21 (15)]	2.3 (1.4) [17 (11)]	4.6 (4.8) [32 (33)]
CD8/CD7	89.2 (6.3) [439 (211)]	91.6 (5.9) [437 (284)]	89.6 (13.3) [520 (375)]
CD8/CD28	35.8 (12.2) [172 (89)]	33.6 (14.8) [142 (77)]	26.6 (14.5) [155 (153)]
CD8/CD57	49 (13.1) [243 (144)]	43.8 (17.2) [242 (252)]	50.3 (17.5) [324 (299)]
CD8/CD45RA	64.3 (7.8) [315 (163)]	68.8 (10.9) [329 (231)]	75 (14.6) [435 (333)]
CD8/CD45RO	39.6 (8.6) [191 (86)]	34.5 (10.6) [165 (121)]	28.9 (18.8) [186 (208)]
CD8/CD25	1.3 (0.8) [6 (5)]	1.3 (1.1) [6 (6)]	1.2 (0.7) [6 (5)]
CD8/HLA-DR	5.3 (3.4) [24 (18)]*	3.4 (2.2) [16 (17)]*	5.6 (6) [37 (48)]

The results are expressed as mean (SD) percentage and absolute numbers are shown in square brackets.

*Healthy controls versus PMR/GCA before corticosteroid treatment: $p < 0.05$.

described for naive and memory cells, we used CD45RA and CD45RO respectively as cellular surface markers.²¹ Within the CD4+ subset, patients with active disease showed a significant increase in the percentage ($p = 0.03$) and absolute numbers ($p = 0.03$) of CD45RA, and conversely a decrease in the percentage of the CD45RO marker ($p = 0.005$). Surprisingly, patients with active disease did not show a higher expression of surface markers indicating T cell activation (CD25 and HLA-DR). Within the CD8+ subset, the only significant difference was a slight increase in the absolute numbers of HLA-DR ($p = 0.046$) in healthy controls compared with the group of patients with active disease.

Peripheral T lymphocyte TCR analysis

TCR BV gene usage

The TCR repertoire of circulating T lymphocytes was analysed with nine BV-specific mAbs, which account for about 40–50%

Figure 1 Frequency of TCR BV expansions in CD4+ and CD8+ in patients with PMR/GCA and healthy age matched controls. Peripheral blood TCR BV use was determined by flow cytometry. TCR BV specific expansion was defined as BV specificities expressed at a frequency greater than the mean + 3SD of a young healthy population or as a value of more than 20%. Patients with active disease showed a lower frequency of CD4+ TCR BV expansions than healthy controls or patients with controlled disease after steroid treatment.

of the total T cell repertoire.²² Patients with active disease had a higher percentage of CD4+/BV3+ T cells ($p = 0.02$) than healthy controls. However, there were no differences between patients and controls in any of the TCR BV families of the CD8+ subset (table 4). Furthermore, no significant difference between patients with active and controlled disease was found (data not shown).

TCR BV expansions

We identified T cell expanded populations in both patients and controls. CD4+ TCR BV expansions were seen in 12/23 (52%) healthy subjects (1.1 expansion/individual) and in 5/23 (22%) patients with active disease (1.4 expansion/individual). The difference between these two groups was significant ($p = 0.03$) (fig 1). Patients with PMR/GCA with controlled disease after steroid treatment showed CD4+ TCR BV expansions in 7/14 (50%) cases, with a total of nine expansions (1.3 expansion/individual), although this difference was not significant compared with patients with active disease ($p = 0.07$). Within the CD8+ subset, expansions were present in 11 (48%) healthy aged controls (1.1 expansion/individual) and in 11 (48%) patients with active disease (1.6 expansion/individual) ($p = \text{NS}$). Patients with PMR/GCA after steroid treatment showed CD8+ T cell expansions in 7 (50%) patients (1.7 expansion/individual).

Table 4 TCR BV expression on T cells in patients with PMR/GCA and healthy controls

mAb	CD4+ T cells			CD8+ T cells		
	Healthy controls	PMR/GCA before treatment	p	Healthy controls	PMR/GCA before treatment	p
BV2	10.1 (1.9)	10.3 (1.9)	0.9	5 (4.6)	5.1 (3.1)	0.6
BV3	4 (2.1)	5.5 (1.5)	0.02	6.8 (6)	8.4 (7.5)	0.2
BV5S2/S3	4.4 (7.6)	2.4 (0.5)	0.08	3.4 (2.8)	2.2 (1.3)	0.1
BV5S1	5.1 (1.4)	5.3 (1.3)	0.3	3.6 (3.9)	3.1 (2.6)	0.9
BV6.7	4.6 (1.8)	5 (2.6)	0.9	1.6 (1.4)	2.4 (2.3)	0.3
BV8	5.1 (4.8)	4 (1.2)	0.3	2.9 (2.4)	8.7 (15.6)	0.09
BV12	2.1 (1.1)	2.6 (1.7)	0.2	2.1 (1)	2.2 (1.2)	0.9
BV13.1	7.6 (5.7)	6.7 (3.1)	0.9	4.2 (2.8)	6.7 (6.1)	0.1
BV17	6.4 (3.2)	6.1 (1.3)	0.6	5.4 (5.5)	4.3 (3.9)	0.4

The results are expressed as mean (SD).

Figure 2 Distribution of TCR BV expansions in patients with PMR/GCA and in healthy age matched controls. TCR expansions of one or more BV families were seen in patients and controls in both CD4+ T cell subsets (A) and CD8+ T cell subsets (B).

TCR expansions of one or more BV families were seen in patients and controls. Figure 2 shows the distributions of such expansions. Within the CD4+ and CD8+ subsets the distribution of the BV expansions followed a random pattern with no significant differences between healthy controls and both groups of patients. Although the sample size was probably too small to for definite conclusions to be drawn, no apparent associations between BV expansions and a particular HLA-DRB1 allele were found (data not shown).

Stability of TCR BV expansions

In four patients we had the opportunity to study blood samples during the active phase of the disease before steroid treatment and after six months of treatment while they were in clinical remission. Before steroid treatment these four patients carried six expanded populations, four within the CD4+ subset and two in the CD8+ T cells (fig 3). After six months of treatment and when they were in clinical and laboratory remission, four of the expanded populations

remained stable over time, two of them (both in patient B) increased more than 30% of the initial value, and a new expanded population appeared during the disease course in patient D.

Selective decrease of certain BV families in patients with active disease

Patients with active PMR and GCA disclosed TCR BV expansions in both CD4+ and CD8+ T cell subsets.²²⁻²⁴ The magnitude of such T cell expansions (in many cases over 10-20% of a particular BV family) might favour the possibility of superantigen stimulation.²⁵ Superantigens have the property to stimulate a massive proliferation of certain TCR BV families and afterwards they induce the death of a large proportion of these stimulated T cells.²⁵⁻²⁶ As previously shown, CD4+ T cell expansions and especially CD8+ T cell expansions were common in both groups of patients and aged healthy controls. However, simultaneous T cell expansions in both T cell subsets were only present in one patient at the

Figure 3 Longitudinal analysis of TCR BV expansions in patients with active PMR/GCA. Before steroid treatment four patients with PMR/GCA carried six expanded populations, four within the CD4+ (black squares) subset and two in the CD8+ (black circles) T cells. After six months of treatment, four of the expanded populations remained stable over time, two increased more than 30% (patient B), and a new expansion appeared (patient D).

Table 5 HLA-DRB1 association and TCR BV families that simultaneously decreased (<25th centile of young healthy controls) in both CD4+ and CD8+ T cell subsets in active untreated patients with PMR and GCA

Patient No	<25th Centile	HLA-DRB1 typing
1	BV5S1, BV13.1, BV17	DRB1*04/*08
2	BV8	DRB1*13/*15
3	BV5S2/S3, BV5S1, BV17	DRB1*01/*11
4	BV5S2/S3, BV8	Not done
5	BV2, BV5S2/S3, BV5S1	DRB1*07/*12
6	BV5S2/S3, BV6.7, BV12	DRB1*01/*15
7	BV12	DRB1*07/*11
8	BV5S2/S3	DRB1*08/*13
9	BV6.7, BV8	DRB1*01/*07
10	None	DRB1*01/*13
11	BV12	DRB1*07/*08
12	None	DRB1*13/*16
13	BV8	DRB1*01/*09
14	BV5S1, BV12	DRB1*07/*10
15	None	DRB1*04/*07
16	BV5S2/S3	DRB1*04/*10
17	None	DRB1*04/*15
18	BV5S2/S3	DRB1*01/*04
19	BV6.7, BV13.1	DRB1*01/*03
20	BV2	DRB1*07/*15
21	None	DRB1*07/*12
22	BV6.7	DRB1*04/*11
23	None	DRB1*03/*12

time of the analysis (patient B, BV6.7) and did not show a significant decrease on follow up (fig 3).

Although PMR and GCA are well known entities, the delay to diagnosis varies between two and four months, mostly depending on the clinical presentation of these syndromes.^{2 4 27 28} Therefore, based on the surprising lack of activation surface markers in patients with active disease and on previous results,²⁹ we decided to search for those BV families that were underrepresented in both T cell subsets in patients with active disease. We arbitrarily chose a value lower than the 25th centile of a healthy young population. Seventeen of the 23 patients with active PMR/GCA disclosed a simultaneous CD4+ and CD8+ T cell depletion for at least one particular BV family (table 5). All the BV families except BV3 showed a significant decrease in at least one patient. However, there was a clear predominance of BV5S2/S3 (seven patients) followed by BV5S1, BV6.7, BV8, and BV12 (four patients each). No significant association with any particular HLA-DRB1 molecule was found for BV5S2/S3 (table 5). Furthermore, BV5S2/S3 was the only element that did not disclose any single expansion in circulating CD4+ or CD8+ T cells (fig 2).

DISCUSSION

There is evidence that the circulating T cell compartment undergoes age associated changes in the distribution of T cell subsets.³⁰ Therefore, PMR and GCA, two related syndromes that show an extremely close association with age, provide a unique case in which to understand the possible influence of these changes on disease. Our data showed a very similar circulating T cell phenotype among the three study groups, although there were several significant findings. Firstly, patients with active disease showed an inverse relation of naive and memory CD4+ T cells as compared with healthy aged matched controls. Secondly, and more striking, was the finding that patients with active PMR/GCA did not show a higher expression of surface markers indicating activation of T cell subsets than controls. Despite a significant increase in acute phase reactants, the comparison with healthy subjects and also with patients with controlled disease did not show

the presence of an activation phenotype in T lymphocytes from patients with active disease. Finally, the severe depletion of CD8+/CD28+ circulating T cells observed in our patient population and also in the aged controls compared with young healthy subjects (data not shown), may contribute to the development of disease in two different ways. Firstly, it may reflect a possible infective agent, especially a viral infection, as responsible for these syndromes. An increasing amount of epidemiological, clinical, and laboratory evidence supports this hypothesis.¹ Secondly, and not mutually exclusive from the first hypothesis, the loss of expression of CD28 with aging may contribute to a state of immunodeficiency that may make a susceptible person prone to develop a disease.³¹

The recognition of certain antigens by specific T cells depends on the TCR, which recognises antigen peptides bound to the MHC on the surface of antigen presenting cells. In most autoimmune disorders the triggering antigen is unknown and, therefore, the study of the TCR repertoire use may shed important light on the inciting antigen. Recent studies have demonstrated that the TCR repertoire of circulating T cells is not as extensive as previously thought.^{32 33} Monoclonal antibody and PCR based analyses have shown that circulating T cells include clonal populations, especially within the CD8+ subset.^{22 32-34} The physiological consequences of this phenomenon are not completely understood, but it has been proposed that these clonal populations are the result of repetitive antigen exposure and might lead to reduced immunoresponsiveness.³⁵ For this reason, we investigated any skewing in the TCR VB repertoire in our patient group. Our results showed a significant TCR BV expansion of T cells expressing specific TCR BV domains at onset of the disease, after control of the disease with corticosteroids, and also in healthy controls. TCR BV specific expansions constituted a variable, although usually significant, percentage of the total T cell repertoire in both CD4+ and CD8+ T cells, with a majority of expansions exceeding 10% of the T cell repertoire. However, we found no apparent preference for any particular BV gene segment used by the expanded T cell populations. There are several explanations for this finding. One might be the different HLA backgrounds of our patient group. Thus, we did not observe a significant relation between certain HLA-DRB1 alleles and TCR BV expansions. Additionally, other HLA molecules may be involved in the pathogenic mechanisms of these syndromes, or it may be that the size of our sample was not sufficiently large to detect specific associations between HLA and expansions. Another possible explanation is that the triggering agents are multiple and differ between patients. An alternative explanation is that at the time of diagnosis we were missing relevant antigen-specific T cells and only detected bystander T cells. Despite the short time between the onset of symptoms in patients with PMR/GCA and the diagnosis of these syndromes, it is possible that relevant T cells are already undetectable by conventional techniques. This may be the case with flow cytometry, which cannot detect differences in TCR BV use during a conventional antigen-specific T cell response. As previously reported in patients with PMR/GCA, more sensitive techniques such as PCR based methodology are necessary to detect differences in the TCR BV gene usage.²²

The TCR repertoire has been examined in patients with PMR and GCA in two recent studies.^{22 23} In both of them patients with PMR and GCA carried multiple expanded T cell populations, especially within the CD8+ T cell subset. Although a significant number of these selected clonotypes decreased in size with high dose steroid treatment, all of them persisted despite successful control of the disease with treatment.²⁴ In contrast with previous studies,^{22 36 37} we did not find a clear correlation between T cell expansions and

Figure 4 Microbial superantigen hypothesis for the development of PMR and GCA. Ag, antigen.

disease activity. A new analysis carried out in patients with PMR and GCA six months after steroid treatment, when they were asymptomatic, showed no significant changes in the distribution of the TCR BV expansions, suggesting that these expanded populations probably are not directly involved in the disease process.

T cell expansions at disease onset may be explained by specific interactions between T cells that recognise antigens, resulting in the proliferation of T cells expressing a particular TCR element. Dramatic T cell expansions occur more frequently with superantigens.²⁶ Superantigens have been implicated in the pathogenesis of other vasculitic syndromes, such as Kawasaki's disease, and other autoimmune disorders, such as rheumatoid arthritis, myasthenia gravis, or Crohn's disease.^{38–41} Superantigens interact with the immune system by binding in an unrestricted way to the MHC class II proteins and activating T cells through the TCR BV region. Therefore, they cause a massive proliferation of both CD4+ and CD8+ T cells bearing a specific BV element and, then, death of a large proportion of these T cells. Another characteristic of superantigens is that they can activate both CD4+ and CD8+ T cell subsets and, as a consequence of the massive activation and proliferation of T cells, they are responsible for the production of large amounts of proinflammatory cytokines.²⁶ Hypothetical superantigen stimulation may explain most of the results of the present study (fig 4). It can be postulated that patients with PMR and GCA have encountered a viral or bacterial superantigen specific for a particular BV(s) element(s). In this regard, several micro-organisms have been implicated in the development of PMR and GCA.^{42–46} This encounter would have led to the activation of most T cells bearing this particular BV(s) element(s), a few of which behave as autoreactive T cells, and because of a cross reaction with self antigens would have homed to the arteries or to the synovia, where they help to develop and sustain the inflammatory process. The characteristic strong proliferation of a particular BV element in response to superantigen stimulation is

usually followed by a swift decline in the cells expressing the relevant BV.²⁶ Possibly, the delay in the diagnosis of these two syndromes explains the lack of activation surface markers in our patients with active disease and the simultaneous CD4+ and CD8+ T cell depletion of certain BV families found. The initial superantigen response would stimulate the production of high amounts of proinflammatory cytokines, which are responsible for the main systemic manifestations of these syndromes.^{47–49} Afterwards, a few of the millions of proliferating T cells could include several autoreactive T cells that would proliferate against self antigens.

Different strategies have been used to obtain information about T cells from the inflammatory infiltrate of 17 patients with GCA.^{8 12 13} The repertoire of these T cells was not clearly restricted (expressed oligoclonal T cells bearing BV2, BV8, BV13.1, BV17, and BV18) except for BV5S2-BJ2S3 T cell clones that were encountered using three different approaches.¹³ Furthermore, these T cells had a proliferative response against temporal artery tissue extracts from patients with GCA and PMR but not against normal temporal artery.⁸ The development of PMR or GCA almost exclusively in aged subjects would probably depend on many factors related to aging, such as immunosenescence or endocrinosenescence.^{15 16 30 50 51} Moreover, the evolution to a predominantly musculoskeletal disorder, such as PMR, or to a predominantly vascular disease, like GCA, may be determined, at least in part, by genetic factors or by the previous existence of cardiovascular risk factors.^{52–55}

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