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A bias in the $\alpha\beta$ T cell receptor variable region gene usage in Takayasu's arteritis

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SUMMARY

Takayasu's arteritis (TA) is a chronic large vessel vasculitis with a predilection for the aortic arch and its branches. T lymphocytes may be important in the pathogenesis, as they have been found to infiltrate the vascular lesions. To elucidate further the role of T cells in the disease, we studied circulating $CD4^+$ and $CD8^+$ T cells, expression of the activation marker (HLA-DR), marker for naive (CD45RA) and primed (CD45RO) cells and the different variable α/β (AV/BV) gene segments on them. The TCR AV/BV repertoire was studied using a panel of 15 T cell receptor (TCR) V-specific MoAbs by flow cytometry in 18 patients and 23 age- and sex-matched controls. Patients had a higher percentage of AV12S1 ($P < 0{\cdot}05$), BV6S7 ($P < 0{\cdot}05$) and BV9 ($P < 0{\cdot}001$)-bearing $CD4^+$ cells. Patients also had a higher frequency of expansions, i.e. of T cell populations with an abnormally high TCR AV/BV gene usage. In patients' $CD4^+$ subset of cells, there were 22 expansions out of 231 analyses (9·5%), whereas in controls, four were expanded out of 310 analyses (1%) ($P < 0{\cdot}001$). For $CD8^+$ cells, the frequency of expansions was 32 in 231 analyses (14%) in patients and nine out of 304 analyses in controls (3%) ($P < 0{\cdot}01$). In addition, there was a correlation between $CD4^+$ expansions and disease activity; nine out of 10 patients with active disease in comparison with two out of eight patients with inactive disease ($P < 0{\cdot}01$) had an expansion. Some of the expanded populations in patients were phenotypically characterized and observed to be $HLA\text{-}DR^+$, $CD28^+$, $CD45RA^+$ and $CD45RO^+$, with a greater proportion being $CD45RO^+$. Patients had a higher percentage of expression of HLA-DR on both $CD4^+$ and $CD8^+$ T cells ($P < 0{\cdot}01$). The percentages of naive and primed $CD4^+$ and $CD8^+$ T cells, $\gamma\delta^+$ T cells and natural killer cells were comparable to those in the control group.

Keywords lymphocytes T cell receptor Takayasu's arteritis

INTRODUCTION

Takayasu's arteritis (TA) is a chronic inflammatory disorder that predominantly affects large arteries, with a predilection for the aortic arch and its branches. For this reason the disease is also often known as non-specific aortoarteritis. The clinical presentation can be constitutional during the acute inflammatory stage and/or result from end organ or limb ischaemia. The disease is uncommonly diagnosed during the acute inflammatory stage and most patients present after a latent period of variable duration, with features referable to obliterative and inflammatory changes in vessels [1–3].

The disease has a predilection for affecting young women, and also has a geographical clustering, with the largest number of cases reported from Japan, south-east Asia and Africa. Certain HLA associations have also been observed in these population groups [1–3].

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The etiology and pathogenesis of the disease are unknown. The initial associations with *Mycobacterium tuberculosis* have not been substantiated [4,5]. Instead, most of the present data indicate an autoimmune basis for the disease [1–3,6]. Histological findings in TA strongly suggest a cell-mediated autoimmune pathogenesis [6]. In the early stages there is a granulomatous inflammation and infiltration of lymphocytes and monocytes in the vascular wall. In more advanced stages the inflammatory process is less evident and the adventitial fibrosis and intimal smooth muscle proliferation lead to arterial stenoses, occlusions and aneurysmal dilatations. In a recent report it was shown that the infiltrating cells in the aortic tissue consisted of $\gamma\delta^+$ T cells, natural killer (NK) cells, macrophages, cytotoxic T lymphocytes and T helper cells, and that perforin was expressed in $\gamma\delta^+$ T cells, NK cells and cytotoxic T lymphocytes [7]. A study of peripheral blood lymphocytes in TA patients has shown an increase in $CD4^+$ cells and a decrease in $CD8^+$ cells [8] and an increased *in vitro* cytotoxicity against cultured human umbilical cord endothelial cells [9].

The specific recognition of antigen by T cells is dependent

Table 1. Salient clinical features of patients with Takayasu's arteritis

Patient	Age (years)	Sex	Duration of illness (months)	Disease activity	Type of disease	ESR (mm/h)	CRP (mg/dl)	Immunosuppressive treatment	TCR AV/BV expansions	
									CD4	CD8
1	8	F	42	Active	III	12	5·76	No	BV2, BV5S3, BV9	AV12S1
2	20	F	2	Active	II	55	<0·5	No	BV9, BV13S1	BV12, BV21
3	21	F	11	Active	III	64	3·8	No	BV2, BV9, BV13S1	BV12
4	25	F	12	Active	III	46	1·58	No	BV6S7	None
5	20	F	24	Active	III	54	3·03	No	BV9	None
6	25	F	12	Active	III	37	2·86	No	BV2, BV5S3, BV9, BV6S7 BV13S1	BV2, BV5S3, BV6S7, BV13S1
7	41	F	24	Active	II	50	4·85	No	None	None
8	21	F	24	Active	III	24	7·24	No	BV9, BV22	BV5S3, BV12
9	35	F	4	Active	I	66	6	No	AV12S1, BV8	AV12S1, BV8, BV17
10	40	M	38	Active	III	50	1·5	No	BV6S7	AV2S3, BV2, BV3
11	24	F	48	Not active	II	22	<0·5	No	BV9	BV12
12	28	M	42	Not active	I	15	<0·5	No	None	BV8, BV17, BV21
13	36	F	164	Not active	I	25	1·32	No	None	AV2S3, BV2, BV6S7, BV12, BV17
14	23	M	4	Not active	III	11	<0·5	No	None	BV6S7
15	30	F	12	Not active	I	21	0·9	No	None	AV12S1, BV3, BV17
16	32	M	36	Not active	III	33	<0·5	Steroids + azathioprin for 1 year	None	BV3, BV21
17	41	F	24	Not active	I	37	<0·5	No	None	None
18	26	M	72	Not active	III	20	<0·5	No	BV13S1	None

upon the T cell receptor (TCR), which recognizes antigenic peptide fragments bound to the MHC on the surface of antigen-presenting cells. In autoimmune diseases where the inciting antigen is unknown, a study of the T cell repertoire to find any bias in its usage may shed light on the inciting antigen. We have previously observed dramatic expansions of certain variable region α/β (AV/BV)-expressing T cell populations in systemic necrotizing vasculitis (Wegener's granulomatosis (WG) and polyarteritis nodosa) [10] and in temporal arteritis [11]. Selective expansions of T cell populations have also been observed in two other vasculitic disorders, Kawasaki's disease [12] and microscopic polyarteritis nodosa [13]. Thus it appears that in vasculitides, T lymphocytes are important in the pathogenesis and there is a bias in the TCR usage which may provide clues to their pathogenesis and help in designing better therapeutic strategies.

We thus decided to study circulating T lymphocytes, NK cells, expression of activation markers on T cells and the T cell repertoire in 18 patients with TA and 23 age-matched controls. The T cell repertoire was studied by flow cytometry using a panel of 15 TCR V-specific MoAbs, which covers about 42% of $CD4^+$ and 30% of $CD8^+$ T cells. We observed an alteration in the T cell repertoire of patients which was most pronounced in the $CD4^+$ subset. Patients had a higher percentage of AV12S1, BV6S7 and BV9 TCR-bearing $CD4^+$ cells. Patients also had a higher frequency of expansions in both the $CD4^+$ and $CD8^+$ subsets in comparison with that in controls.

PATIENTS AND METHODS

Patients

The patient group consisted of 18 patients with TA treated in the Departments of Immunology and Cardiology (SGPGIMS, Lucknow, India) between 1994 and 1995. All these patients fulfilled the ARA criteria for TA [11]. The disease was subgrouped as type I if the aortic arch or its branches were involved; type II if the abdominal, aorta or its branches were involved; type III if both the aortic arch and the abdominal aorta were involved [15]; and type IV if there was involvement of the pulmonary artery [16]. The disease was considered to be in an active stage if two or more of the following were present along with the other features of the disease: (i) constitutional features like fever, arthralgias, myalgias, weight loss (for which no other cause could be identified); (ii) painful arteries (carotodynia); (iii) elevated erythrocyte sedimentation rate (ESR) (> 30 mm/h); (iv) elevated C-reactive protein (CRP) (> 0·6 mg/dl). All patients were subjected to the following investigations at SGPGIMS—routine haematological, biochemical tests, rheumatoid factor, antinuclear antibody, ESR, CRP, C3, C4, digital subtraction angiography (DSA)/magnetic resonance imaging (MRI), ECG, 2DEcho and chest x-ray. Ultrasound abdomen and renal scan were done if required.

Determination of anti-neutrophil cytoplasmic antibodies (ANCA) to myeloperoxidase (MPO) and proteinase 3 (PR3) and the T cell studies were done in the routine and research immunology labs, respectively, of Karolinska Hospital (Stockholm, Sweden).

The salient presenting features of patients at the time of obtaining samples are summarized in Table 1. Especially included in this study were patients who had features of disease activity. There were thus 10 patients with active disease and eight with no signs of inflammation. After obtaining samples, patients with disease activity were put on an immunosuppressive regimen consisting of steroids and azathioprin. The mean age of patients was 27·6 years; 13 were females and five males. The commonest pattern of arterial involvement was of type III. The control group

consisted of 23 healthy Indian controls, preferably selected from the same area in which the patient was residing. The mean age of controls was 28·8 years; 11 were females and 12 males.

Monoclonal anti-TCR antibodies

The MoAbs used in this study have been described previously [10]. In addition, the following MoAbs were used: BV9 (AMKB1-2) purchased from Pharmingen (San Diego, CA); TCRBV20S1 (ELL 1.4), TCRBV21S3 (IG 125) and TCRBV22S1 (IMMU 546) purchased from Immunotech SA (Marseilles, France).

Analysis of lymphocyte subsets by flow cytometry

From each patient and control peripheral blood mononuclear cells (PBMC) were separated by the Ficoll technique and cryopreserved in 90% fetal calf serum (FCS) + 10% DMSO in liquid nitrogen and dry ice until further analysis. When samples were to be analysed, the frozen PBMC were rapidly thawed in a water bath at 37°C and washed in RPMI 1640 medium containing 10% FCS. PBMC were stained with the MoAbs in a V-bottomed 96-well microtitre plate. A triple staining technique was used which was essentially the same as described previously, with some modifications [10]. In brief, 5×10^5 lymphocytes were incubated at 4°C with a saturating amount of unlabelled TCR MoAb for 30 min. Cells were then washed twice in PBS (containing 0·2% bovine serum albumin (BSA) and 0·01% sodium azide) and then incubated with FITC-conjugated $F(ab)_2$ fragment of rabbit anti-mouse immunoglobulin for 30 min. Cells were then washed and blocked with normal mouse serum (NMS) for 20 min, washed once and incubated in a cocktail of CD4 PE-conjugated and CD8 PerCp-conjugated antibodies for 30 min. After washing three times in PBS, cells were transferred to test tubes and analysed in a flow cytometer (Becton Dickinson, Mountain View, CA) using the Lysis II program. Each population was analysed separately. The CD4 and CD8 populations were determined as $CD3^+/CD4^+$ and $CD3^+/CD8^+$, respectively. A T cell expansion was defined as a value more than mean + 3 s.d. of the value for the corresponding MoAb reactivity in the control group (excluding values which clearly lay outside a normal distribution for the control group) [17].

The frequency of expansions of the different TCR families was expressed as follows: the number of patients in which the expansions were present out of the total number of patients in which the analyses were done. The overall percentage of frequency of expansions in the CD4 and CD8 subsets was calculated by dividing number of expansions by the sum of all the different TCR AV/BV $CD4^+$ or $CD8^+$ MoAbs analysed.

Phenotypic characterization of expansions

Some of the expansions where adequate numbers of cells were available were phenotypically characterized. A triple staining was done with the MoAbs to TCR, CD4/CD8 and one of the following: HLA-DR, CD28, CD45RA and CD45RO, to see the percentage of the expanded population bearing the above markers [10].

HLA genotyping

To detect any correlation between T cell expansions and HLA types, the DQ and DR HLA typing of the patients was done as follows. Peripheral blood in EDTA was collected and the DNA extracted from the leucocytes. HLA genotyping was done by amplification of the second exon of DQA1, DQB1 and DRB1 by polymerase chain reaction (PCR), manual dot blotting and hybridization with sequence-specific oligo-probes and autoradiography, as described before [18].

Statistical analysis

Mann–Whitney *U*-test was employed to compare the percentages of the different subsets of lymphocytes between the patient and control groups. χ^2 was used for comparing two proportions. Fisher's exact test was employed when χ^2 could not be used. HLA associations of TCR expansions were studied by Fisher's exact test.

RESULTS

Lymphocyte subsets

The different lymphocyte subsets of patients and controls were comparable except for the expression of HLA-DR (Table 2). There was a higher percentage of HLA-DR-bearing $CD4^+$ and $CD8^+$

Table 2. Lymphocyte subsets in Takayasu's arteritis patients and healthy controls

	Patients Median (min–max)	Controls Median (min–max)	*P*
$CD3^+$ (%)	69·1 (39·6–83·9)	70·0 (42·0–93·1)	0·96
$CD3^+ CD4^+$ (%)	33·1 (6·8–52·9)	35·9 (14·8–51·1)	0·26
Percent of $CD4^+$ for			
CD45RO	61·0 (12·1–89·3)	43·9 (18·4–81·3)	0·34
CD45RA	33·7 (5·2–82·4)	30·0 (15·1–71·8)	0·62
HLA-DR	18·8 (6·6–50·4)	7·7 (2·5–27·9)	0·001**
$CD3^+ CD8^+$ (%)	22·8 (6·5–40·2)	18·0 (10·0–48·1)	0·43
Percent of $CD8^+$ for			
CD45RO	23·3 (2·9–87·7)	26·6 (4·3–53·2)	0·41
CD45RA	54·9 (13·4–88·2)	51·1 (14·2–85·6)	0·25
HLA-DR	34·8 (3·8–63·3)	13·3 (0·5–35·0)	0·007**
CD4:CD8	1·4 (0·2–4·3)	1·8 (0·5–4·6)	0·30
$CD3^+$ TCR $\gamma\delta^+$ (%)	4·3 (1·6–44·8)	3·4 (0·1–18·6)	0·22
$CD3^- CD16^+ CD56^+$ (%)	18·5 (3·7–37·0)	11·05 (1·9–31·4)	0·08

$^{**}P < 0{\cdot}01$.

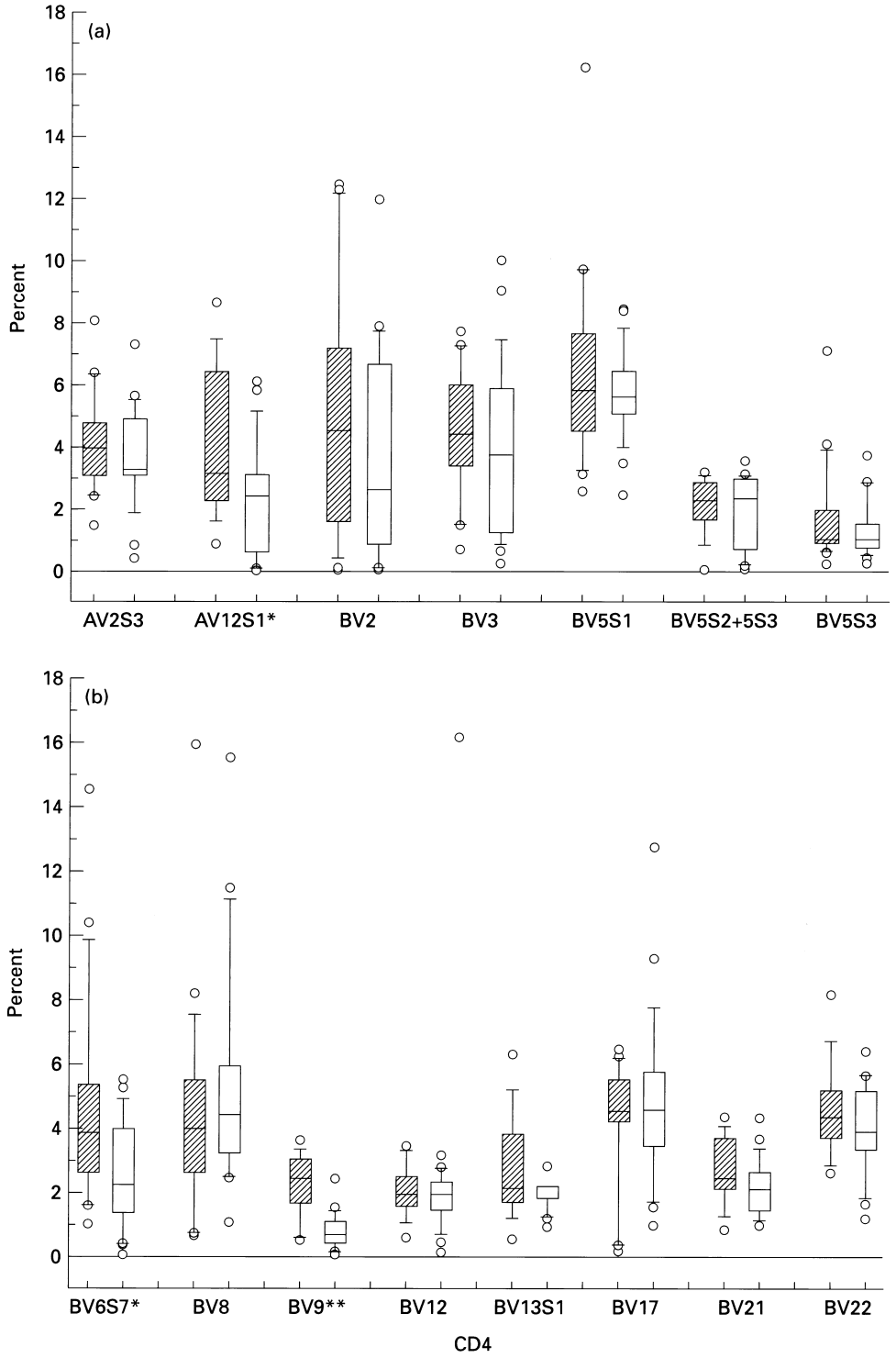

Fig. 1. Percentage of the various AV/BV T cell receptor (TCR)-bearing $CD4^+$ cells in patients (▨) and controls (□). The lower, mid and upper horizontal lines of the boxes represent 25th, 50th and 75th percentiles, respectively; vertical lines extend from the 10th to the 90th percentile. *****$P < 0{\cdot}05$; ******$P < 0{\cdot}01$.

T cells in patients in comparison with that in controls (Table 2). When comparing patients with and without active disease for expression of HLA-DR marker, it was observed that patients with active disease had a higher percentage of HLA-DR-bearing $CD4^+$ ($P < 0{\cdot}001$) and $CD8^+$ T cells ($P < 0{\cdot}01$). In patients with inactive disease, while the percentage of HLA-DR-bearing $CD4^+$ cells was still higher in comparison with that in controls ($P < 0{\cdot}001$), there was no difference in $CD8^+$/HLA-DR^+ cells ($P < 0{\cdot}05$).

Five patients had high percentages of $\gamma\delta^+$ T cells (> 15%); in three patients they were 15–25% of $CD3^+$ cells and in two other patients they were 33% and 54%, respectively. In comparison, only

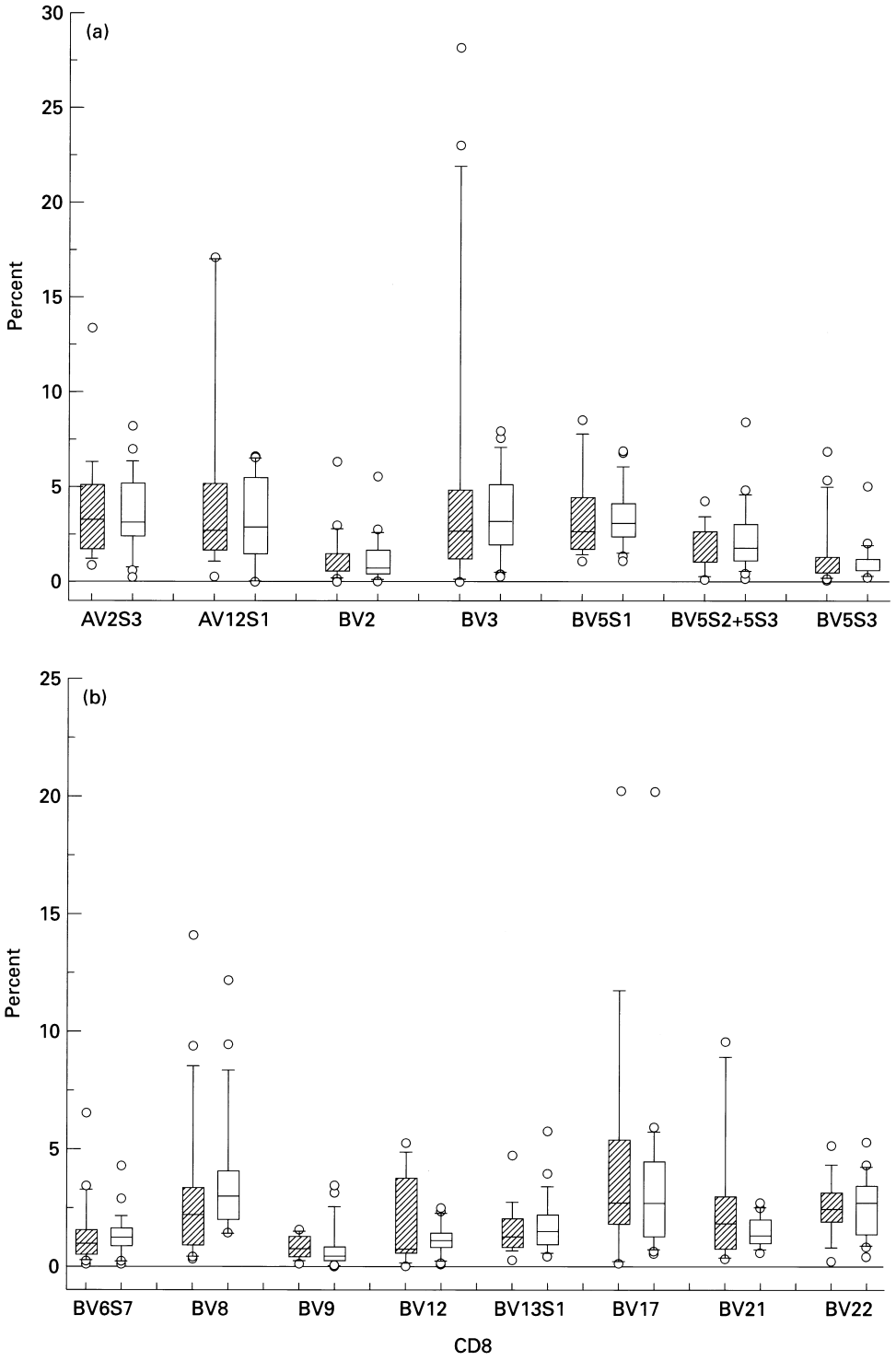

Fig. 2. Percentage of the various AV/BV T cell receptor (TCR)-bearing $CD8^+$ cells in patients (▨) and controls (□). The lower, mid and upper horizontal lines of the boxes represent 25th, 50th and 75th percentiles, respectively; vertical lines extend from the 10th to the 90th percentile.

one of the controls had a high percentage of 25% ($P < 0{\cdot}05$). However, when comparing the patient and control groups as a whole, the mean percentages of $\gamma\delta^+$ T cells were similar (Table 2).

TCR AV/BV gene usage

TCR AV/BV gene usage in patient and control groups for the $CD4^+$ subset is shown in Fig. 1a,b and for the $CD8^+$ subset in Fig. 2a,b. Patients had higher percentages of AV12S1 ($P < 0{\cdot}05$), BV6S7 ($P < 0{\cdot}05$), and BV9 ($P < 0{\cdot}001$) TCR-bearing $CD4^+$ cells. There were, however, no differences between patients and controls in any of the TCR families of the $CD8^+$ subset.

TCR AV/BV expansions

Expansions of one or more different TCR families were observed

Table 3. Frequency of individuals with expansions of the different T cell receptor (TCR) AV/BV-bearing subsets of T lymphocytes

	CD4		CD8	
MoAb	Patient	Control	Patient	Control
AV2S3	0/16	0/22	2/16	0/22
AV12S1	1/15	0/22	3/15	0/22
BV2	3/18	1/21	3/18	1/21
BV3	0/16	0/22	3/16	0/22
BV5S1	0/16	0/21	1/16	0/20
BV5S2 + 5S3	0/15	0/20	0/15	1/20
BV5S3	2/16	0/19	2/16	1/18
BV6S7	3/18	0/23	3/18	1/23
BV8	1/18	1/20	2/18	1/17
BV9	7/12	1/19	0/12	2/19
BV12	0/15	0/20	5/15	0/20
BV13S1	4/15	0/21	1/15	1/21
BV17	0/16	1/23	4/16	1/22
BV21	0/15	0/20	3/15	0/20
BV22	1/10	0/17	0/10	0/17

Table 4. Phenotypic characterization of expanded T cells

Patient no.	Expanded population	Percentage of expanded population bearing the CD markers			
		HLA-DR	CD28	CD45RA	CD45RO
1	BV2/CD4	100	100	53	50
	BV9/CD4	95	100	ND	ND
3	BV2/CD4	31	100	60	45
	BV9/CD4	75	93	38	72
4	BV657/CD4	95	47	20	70
6	BV2/CD4	36	100	33	46
8	BV22/CD4	76	85	11	83
	BV9/CD4	37	57	18	90
12	BV8/CD8	90	76	79	20
	BV17/CD8	90	73	23	70
13	AV2S3/CD8	27	75	15	78
18	BV13S1/CD4	29	97	15	87

ND, Not done.

in 15/18 patients and 13/23 controls. The frequency of individuals having expansions of the different TCR families is presented in Table 3. The difference was most pronounced in the $CD4^+$ subset. Overall, the frequency of expansions in the $CD4^+$ subset in patients was 22 out of 231 analyses (9·5%), in comparison with four out of 310 analyses (1%) for controls ($P < 0·001$). In the $CD8^+$ subset the expansions were 32 out of 231 analyses (14%) for patients and nine of 304 analyses (3%) for controls ($P < 0·001$).

In the patients' $CD4^+$ subset, the commonest expansion was of BV9, seen in seven patients, followed by that of BV13S1 in four and BV2 in three cases each (Table 1). The magnitude of the expansions varied between 3·0% and 15·0%. In the CD8 subset, the commonest expansions were of BV12 and BV17, being present in five and four cases, respectively. Most CD8 expansions were in the range of 5–20%, with two being more than 20% (23% and 42%).

There was a correlation between disease activity and $CD4^+$ expansions (Table 1). Nine out of 10 active disease patients had one or more $CD4^+$ expansions. A total of 20 expansions was observed of the $CD4^+$ subsets in these nine patients. In contrast, only two out of eight patients where the disease was not active had a $CD4^+$ expansion, and they had one expansion each ($P = 0·01$).

Phenotypic characterization of the expansions

In most of the cases, a high percentage of expanded populations had expression of activation marker HLA-DR (median value 75%) and the costimulatory molecule CD28 (median value 89%) (Table 4). However, there was heterogeneity in the percentage of cells expressing CD45RA and CD45RO (median values of 23% and 70%, respectively) (Table 4).

TCR expansions and HLA types

The TCR expansions and the DR and DQ HLA typing of patients are presented in Table 5. Though the sample size was too small to comment on the definitive associations of the TCR expansions and HLA genes, the following associations were observed: (i) in the CD4 population, BV13S1 was expanded in 3/4 $DR10^+$ patients ($P < 0·05$). BV9 was expanded in 4/10 $DR7^+$ and in 3/4 $DR10^+$ patients. Though BV9 association with DR7 and DR10 did not reach statistical significance ($P > 0·05$), it is worthwile to note it. A larger sample size may be required to define the exact significance of this association; (ii) in the CD8 population, BV12 was expanded in 4/5 $DR15^+$ patients ($P < 0·05$) and BV13S1 in 3/4 $DQA*0104^+$ patients ($P < 0·05$).

Autoantibodies

One patient had rheumatoid factor (RF) (patient no. 11) and one had antinuclear antibodies (ANA) (patient no. 12). None of the patients had ANCA.

DISCUSSION

The numbers of the activated $CD4^+$ and $CD8^+$ cells were higher in patients, while naive and primed $CD4^+$ and $CD8^+$ cells were comparable to those in the control population. Activated $CD4^+$ cells were higher in patients, with and without clinically active disease. This suggests that activated cells persist even when the disease is not clinically active. Although there was no difference in mean percentages of $\gamma\delta^+$ T cells and NK cells between patients and controls, 5/18 patients in comparison with 1/23 controls had very high levels of $\gamma\delta^+$ T cells (15–53% of $CD3^+$ cells). This may be of relevance to the disease, as a recent report has also shown the presence of $\gamma\delta^+$ cells in the vessel wall in TA [7].

We observed an alteration in the T cell repertoire of most of our patients. They had a higher usage of $CD4^+/AV12S1^+$, $CD4^+/BV6S7^+$ and $CD4^+/BV9^+$ T cells. A ×9 and a ×4·6 times higher frequency of expansions in the $CD4^+$ subset and the $CD8^+$ subset, respectively, were observed in patients in comparison with that in controls. The frequency of T cell expansions in controls was similar to that reported in the Scandinavian and Japanese healthy individuals.

Table 5. T cell expansions and HLA association of Takayasu's arteritis patients

No.	TCR AV/BV expansions		HLA typing			
	CD4	CD8	DR-DQ	DQA1-DQ-B1	DR-DQ	DQA1-DQB1
1	BV2, BV5S3, BV9	AV12S1	DR4-DQ8	A*0301-B*0302	DR7-DQ2	A*0201-B*0201
2	BV9, BV13S1	BV12, BV21	DR10-DQ5	A*0104-B*0501	DR15-DQ6	A*0102-B*0602
3	BV2, BV9, BV13S1	BV12	DR15-DQ?	A*0103-B*0302	DR14-DQ5	A*0101-B*0503
4	BV6S7	None	DR7-DQ9	A*0201-B*0303	DR11-DQ7	A*0501-B*0301
5	BV9	None	DR4-DQ8	A*0301-B*0302	DR7-DQ9	A*0201-B*0303
6	BV2, BV5S3, BV9, BV6S7	BV2, BV5S3, BV6S7, BV13S1	DR10-DQ5	A*0104-B*0501	DR7-DQ2	A*0201-B*0201
7	None	None	DR7-DQ2	A*0201-B*0201	DR7-DQ2	A*0201-B*0201
8	BV9, BV22	BV5S3, BV12	DR7-DQ2	A*0201-B*0201	DR8-DQ?	A*0501-B*0302
9	AV12S1, BV8	AV12S1, BV8, BV17	DR13-DQ7	A*0501-B*0301	DR15-DQ6	A*0103-B*0601
10	BV6S7	AV2S3, BV2, BV3	ND	ND	ND	ND
11	BV9	BV12	DR10-DQ5	A*0104-B*0501	DR15-DQ6	A*0103-B*0601
12	None	BV8, BV17, BV21	DR1-DQ5	A*0101-B*0501	DR14-DQ5	A*0103-B*0503
13	None	AV2S3, BV2, BV6S7, BV12, BV17	DR15-DQ6	A*0103-B*0601	DR3-DQ2	A*0501-B*0201
14	None	BV6S7	DR7-DQ2	A*0201-B*0201	DR14-DQ5	A*0101-B*0503
15	None	AV12S1, BV3, BV17	DR13-DQ6	A*0102-B*0604	DR7-DQ9	A*0201-B*0303
16	None	BV3, BV21	DR14-DQ5	A*0101-B*0503	DR4-DQ8	A*0301-B*0302
17	None	None	ND	ND	ND	ND
18	BV13S1	None	DR10-DQ5	A*0104-B*0501	DR7-DQ2	A*0201-B*0201

ND, Not done.

A bias in usage of certain TCR AV/BV genes has been observed in other vasculitides. We have previously carried out studies in temporal arteritis [11] and WG [10] and observed different TCR AV/BV expansions in these patients. It is noteworthy that these expansions were of a much larger magnitude than those observed in the TA patients. In temporal arteritis, which is also a large vessel vasculitis, at the onset of the disease expansions were observed in seven of nine patients [11]. The observed five $CD4^+$ T cell expansions were all of a magnitude of $>20\%$, whereas those observed by us in TA patients were 3–15%. In WG, we found dramatic expansions of both $CD4^+$ and $CD8^+$ T cells. Two $CD4^+$ expansions were about 50% of $CD4^+$ and five $CD8^+$ expansions were $>20\%$ of $CD8^+$ cells [10]. One explanation for the less dramatic expansions in our patients could be that in TA, the disease has been present for a significant period of time before it is clinically manifest, and disease-related T cells could decline over a period of time.

Different TCR AV/BV expansions were observed in TA patients (similar to our observations on WG and temporal arteritis [10,11]). The reason for this could be the patients' different HLA backgrounds. Some T cell expansions were observed to occur more frequently with certain HLA types. Though a larger sample size would be required to comment definitively on these associations, they are worth noting. The commonest HLA type was DR7, present in 9/16 patients, followed by DR15 in 5/16 and DR10 in 4/16 patients. The commonest expansions in the $CD4^+$ subset were of BV9 and BV13S1, and in the CD8 subset of BV12 and BV17. In the $CD4^+$ subset, BV13S1 was associated with DR10 ($P < 0{\cdot}05$) and perhaps BV9 with DR7 and DR10. In the $CD8^+$ subset, BV12 was associated with DR15 ($P < 0{\cdot}05$).

There was a correlation between the $CD4^+$ expansions and clinical disease activity. A similar correlation has been observed in temporal arteritis [11], where $CD4^+$ T cells constitute the major inflammatory cell population in the arterial wall lesions. In a recent study it has been shown that anatomically distinct lesions have identical T cell clonotypes [19]. This suggests antigen recognition by a subset of $CD4^+$ T cells and their potential relevance to disease. In TA the inflamed arteries are not as easily accessible as the temporal artery to biopsy, and thus it is more difficult to study the lesional T cell repertoire. Furthermore, TA is a chronic inflammatory disease. The composition of the inflammatory infiltrate is likely to change over time, with only an initial dominance of an antigen-specific response. Later there would be recruitment of T cells of highly diverse specificities. Finally, when the disease is usually clinically symptomatic, most of the cellular inflammatory infiltrate would be replaced by fibrosis.

Dramatic T cell expansions can occur by interaction with superantigens, such as staphylococcal and streptococcal toxins in toxic shock syndrome [20,21]. BV2 has been found to be higher in patients with Kawasaki's disease and microscopic polyarteritis [13,22] and is related to a superantigen [20,21]. We have also observed BV2 expansion in the $CD4^+$ subset in three TA patients. A restricted TCR repertoire within the context of specific MHC products has also been shown to conventional antigens [23–25]. The expanded population of T cells may shed light on the candidate antigens. They may also be important targets for immunotherapy, as the stimulated T cells might release increased amounts of T cell-derived cytokines, which may be important in pathogenesis of the disease.

T cell expansions can also be observed in healthy individuals [17,26–29]. They are usually in the $CD8^+$ subset and rare in the $CD4^+$ subset. Therefore, $CD4^+$ expansions are more likely to be related to disease. T cell expansions in healthy individuals in many cases express CD11b and CD57, but not CD28 [28,29]. Furthermore, T cell expansions of healthy individuals usually do not express activation markers such as CD25 or HLA-DR, and are heterogeneous with respect to CD45RA/RO expression [28,29]. The $HLA\text{-}DR^+$ and $CD28^+$ expansions observed by us in TA

patients are thus different from those in healthy individuals. A higher percentage of cells being $CD45RO^+$ would favour their being antigen-stimulated.

In conclusion, we have observed an alteration in the T cell repertoire in TA, in particular in $CD4^+$ cells, indicating an involvement of T cells in TA. There was a bias in the usage of certain TCR AV/BV families, with correlation between the $CD4^+$ expansions and disease activity. Further studies on these expanded T cell populations, especially of the $CD4^+$ subset, will delineate better their role in disease pathogenesis.

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REFERENCES

1 Eagle KA, DeSanctis RW. Diseases of the aorta. In: Braunwald E, ed. Heart disease. Philadelphia: WB Saunders Co., 1988;1546–76.
2 Kerr G. Takayasu's arteritis. Curr Opin Rheumatol 1994; **6**:32–38.
3 Weyand CM, Goronzy JJ. Molecular approaches toward pathologic mechanisms in giant cell arteritis and Takayasu's arteritis. Curr Opin Rheumatol 1995; **7**:30–36.
4 Sen PK, Kinare SG, Engineer SD, Parulkar GB. The middle aortic syndrome. Br Heart J 1963; **35**:610–8.
5 Lupi-Herrera E, Sanchez-Torres G, Marchushamer J, Mispireta J, Horwitz S, Vela JE. Takayasu's arteritis: clinical study of 107 cases. Am Heart J 1977; **93**:94–103.
6 Pariser KM. Takayasu's arteritis. Curr Opin Cardiol 1994; **9**:575–80.
7 Seko Y, Minota S, Kawasaki A *et al.* Perforin-secreting killer cell infiltration and expression of a 65-kD heat-shock protein in aortic tissue of patients with Takayasu's arteritis. J Clin Invest 1994; **93**: 750–8.
8 Sagar S, Ganguly NK, Koicha M, Sharma BK. Immunopathogenesis of Takayasu arteritis. Heart Vessels 1992; **7** (Suppl.):85–90.
9 Scott DGI, Salmon M, Scott DL, Blann A, Bacon PA, Walton KW, Oakland CDH, Slaney GF. Takayasu's arteritis: a pathogenetic role for cytotoxic T lymphocytes. Clin Rheumatol 1986; **5**:517–22.
10 Giscombe R, Grunewald J, Nityanand S, Lefvert AK. T cell receptor (TCR) V gene usage in patients with systemic necrotizing vasculitis. Clin Exp Immunol. 1995; **101**:213–9.
11 Grunewald J, Andersson R, Rydberg L, Gigliotti D, Schaufelberger C, Hansson GK, Wigzell H. $CD4^+$ and $CD8^+$ T cell expansions using selected TCR V and J gene segments at the onset of giant cell arteritis. Arthritis Rheum 1994; **37**:1221–7.
12 Leung DYM, Giorno RC, Kazemi LV, Flynn PA, Busse JB. Evidence for superantigen involvement in cardiovascular injury due to Kawasaki syndrome. J Immunol 1995; **155**:5018–21.
13 Simpson IJ, Skinner MA, Geursen A, Peake JS, Abbott WG, Fraser JD, Lockwood CM, Tan PLJ. Peripheral blood T lymphocytes in systemic vasculitis: increased T cell receptor Vβ2 gene usage in microscopic polyarteritis. Clin Exp Immunol 1995; **101**:220–6.
14 Arend WP, Michel BA, Bloch DA *et al.* The American College of Rheumatology 1990 criteria for the classification of Takayasu's arteritis. Arthritis Rheum 1990; **33**:1129–33.
15 Ueno A, Awane G, Wakahayachi A, Shimizue K. Successfully operated obliterative brachiocephalic arteritis (Takayasu) associated with the elongated coarctation. Jpn Heart J 1967; **8**:538–44.
16 Lupi HE, Sanchez TG, Horwitz S, Gutierrez FE. Pulmonary artery involvement in Takayasu's arteritis. Chest 1975; **67**:69–74.
17 Posnett DN, Sinha R, Kabak S, Russo C. Clonal populations of T cells in normal elderly humans: the T cell equivalent to ''benign monoclonal gammopathy'' (Published erratum appears in J Exp Med 1994; **179**:1077). J Exp Med 1994; **179**:609.
18 Sanjeevi CB, Zeidler A, Shaw S *et al.* Analysis of HLA-DQA1 and -DQB1 genes in Mexican Americans with insulin-dependent diabetes mellitus. Tissue Antigens 1993; **38**:72–77.
19 Weyand CM, Schönberger J, Oppitz U, Hunder NNH, Hicok KC, Goronzy JJ. Distinct vascular lesions in giant cell arteritis share identical T cell clonotypes. J Exp Med 1994; **179**:951–60.
20 Scherer MT, Ignatowicz L, Winslow GM, Kappler JW, Marrack P. Superantigens: bacterial and viral proteins that multipulate the immune system. Ann Rev Cell Biol 1993; **9**:101–28.
21 Kotzin BL. Superantigens and their role in disease. Hospital Practice 1994; **29**: 59–70.
22 Abe J, Kotzin BL, Jujo K, Melish ME, Glode MP, Koshaka T, Leung DYM. Selective expansion of T cells expressing T cell receptor variable regions Vβ2 and Vβ8 in Kawasaki's disease. Proc Natl Acad Sci USA 1992; **89**:4066–70.
23 Lehner PJ, Wang ECY, Moss PAH, Williams S, Platt K, Friedman SM, Bell JI, Borysiewicz LK. Human HLA-A0201-restricted cytotoxic T lymphocyte recognition of Influenza A is dominated by T cells bearing the Vβ17 gene segment. J Exp Med 1995; **181**:79–91.
24 Boitel B, Blank U, Mege D, Corradin G, Sidney J, Sette A, Acuto O. Strong similarities in antigen fine specificity among DRB1*1302-restricted tetanus toxin tt830–843-specific TCRs in spite of highly heterogenous CDR3. J Immunol 1995; **154**:3245–55.
25 Henwood J, Loveridge J, Bell JI, Gaston JSH. Resricted T cell receptor expression by human T cell clones specific for mycobacterial 65-kDa heat shock protein: selective *in vivo* expansion of T cells bearing defined receptors. Eur J Immunol 1993; **23**:1256–65.
26 Grunewald J, Tehrani MJ, Andersson R, DerSimonian H, Wigzell H. A persistent monoclonal T cell expansion in the peripheral blood of a normal adult male: a new clinical entity? Clin Exp Immunol 1992; **89**:279–84.
27 Fitzgerald JE, Ricalton NS, Meyer A-C, West SG, Kaplan H, Behrendt C, Kotzin BL. Analysis of clonal $CD8^+$ T cell expansions in normal individuals and patients with rheumatoid arthritis. J Immunol 1995; **154**:3538–47.
28 Grunewald J, Wigzell H. T cell expansions in healthy individuals. Immunologist 1996; **4**:99–103.
29 Moss P, Gillespie G, Frodsham P, Reyburn H, Bell J. Analysis of clonal expansions within CD4 and CD8 T cells. Immunologist 1996; **4**:104–103.