EXTENDED REPORT

γδ T cell subsets in patients with arthritis and chronic neutropenia

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Background: An abnormal distribution of subsets of γδ T cells, which are a component of the inflammatory infiltrate in arthritic synovium, has been demonstrated in the peripheral blood (PB) of patients with arthritis and neutropenia.
Objective: To evaluate whether the clinical manifestations of patients with arthritis and neutropenia are related to the specific γδ T cell subset predominant in the PB.
Methods: Flow cytometry of PB lymphocytes in six consecutive patients with chronic neutropenia and arthritis was performed. Variable (V) γ and δ gene families were analysed by polymerase chain reaction. cDNA was subjected to direct automated sequencing of T cell receptor (TCR) genes.
Results: Three patients had non-deforming and non-erosive rheumatoid factor (RF)$^+$ polyarticular rheumatoid arthritis, RF$^+$ oligoarticular arthritis, or RF$^-$ non-deforming oligoarticular psoriatic arthritis with persistent expansions of Vγ1$^+$/Vδ2$^+$, Vγ2$^+$/Vδ2$^+$, or Vγ1$^+$/Vδ $^{undetermined\ (2-\ 1-)}$ T cells, respectively. The other three patients, without persistent expansion of γδ T cells, had either non-deforming and non-erosive oligo- or polyarthritis with a balanced distribution of several Vδ and Vγ genes, or severe erosive RF$^+$ arthritis with deficiency of all but Vγ1$^+$/Vδ1$^+$ T cells.
Conclusions: γδ T cell lymphoproliferations in chronic neutropenia and arthritis use different Vγ and Vδ gene families, often forming T cell receptor (TCR) structures that are infrequent in normal adult PB. Arthritis with Vγ1$^+$/Vδ2$^+$, Vγ2$^+$/Vδ2$^+$, or Vγ1$^+$/Vδ2$^-$/Vδ1$^-$ γδ T cells in the PB is non-deforming and non-erosive, suggesting a protective effect of these cells, as opposed to a more pathogenic contribution of Vγ1$^+$/Vδ1$^+$ cells.

T cells bearing T cell receptor (TCR) γδ comprise 0.2–9% of the peripheral blood lymphocytes (PBL) in normal subjects.[1] Their functional programmes include cytotoxicity, lymphokine secretion, and regulatory effects on the functions of B cells, αβ T cells, natural killer cells, and macrophages.[2–4] Subsets of γδ lymphocytes, in particular those defined by expression of Vγ2 and Vδ2 genes in the TCR (Vγ2$^+$/Vδ2$^+$ γδ T cells), forming 50–70% of the γδ T cells in normal adult human PB, have a protective role in infectious diseases by recognising phosphorylated low molecular weight molecules produced by micro-organisms.[5–7] Moreover, discrete subsets within the γδ T cell population appear to regulate inflammatory and autoimmune diseases in experimental animals.[8 9] Interestingly, in this regard, rheumatoid arthritis (RA) is associated with alterations in γδ T cells.[10–14] Thus there is a general decrease in the number of γδ T cells in the PB of patients with RA.[10 11] Furthermore, some studies indicated that Vδ2$^+$ but not Vδ1$^+$ γδ T cells are relatively depleted in the PB, and Vγ1$^+$/Vδ1$^+$ γδ T cells are the predominant subset in inflamed arthritic joints, suggesting a differential contribution of each subset during the pathogenesis of this disease.[10–14]

One per cent of patients with RA have chronic neutropenia and splenomegaly, sometimes with repeated and severe infections, a condition known as Felty's syndrome (FS).[15] The neutropenia is thought to be due to effects of antineutrophil antibodies and/or cytotoxic T cells and cytokines.[15] Interestingly, a relative expansion of Vδ1$^+$ γδ T cells in the PB has been described in FS.[10] In addition, monoclonal lymphoproliferations of T cells (usually CD3$^+$CD8$^+$TCRαβ$^+$) occur in 1/3 patients with FS.[16] The group of patients with monoclonal T lymphoproliferations may, in fact, represent cases of CD3$^+$—large granular T lymphocytic disorder (T-LGLD) with RA, a condition also termed pseudo-Felty's syndrome (PFS).[15] Sporadic studies have shown, furthermore, that lymphoproliferations of γδ T cells, rather than CD3$^+$CD8$^+$TCRαβ$^+$ T cells may occur in a minority of patients with FS/PFS.[17–21] The specific functional repertoires of the different subsets of γδ T cells suggest they may potentially have beneficial or detrimental roles in disease manifestations in patients with neutropenia and arthritis—for example, by secreting cytokines after interacting with pathogenic micro-organisms, or by influencing antibody production.[1 2] In this study we examined the relation between the characteristics of the clinical syndrome and the nature of the specific subset undergoing expansion in patients with chronic neutropenia and arthritis.

PATIENTS AND METHODS

Patients

Six patients with chronic neutropenia and arthritis seen in the rheumatology and medical clinics at the Tel Hashomer and Wolfson medical centres were referred to one of us (IB) for evaluation of T cell subsets. Table 1 summarises their clinical characteristics and final diagnosis. Controls were normal subjects matched for age and sex.

Determination of T cell antigens

PBL were isolated from heparinised blood by Ficoll-Hypaque density centrifugation.[22] Cells (0.5–1×10^6) were spun down in 5 ml glass tubes in phosphate buffered saline (PBS) pH 7.4. Resuspended pellets were stained with saturating concentrations of primary murine monoclonal antibody (mAb) at 4°C for 30 minutes and washed twice in PBS + 0.2% sodium azide.

Abbreviations: FS, Felty's syndrome; mAb, monoclonal antibody; PB, peripheral blood; PBL, peripheral blood lymphocytes; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PFS, pseudo-Felty's syndrome; RA, rheumatoid arthritis; RF, rheumatoid factor; TCR, T cell receptor; T-LGLD, large granular T lymphocytic disorder

Table 1 Clinical syndromes in patients with arthritis and neutropenia

Patient	Age	Sex	Diagnosis	Spleen	Other	Treatment	N(/mm³)	Infection	Disease duration (y)	Status
1	70	M	FS/T-LGLD	E	Fever ANA weak+	MTX PRED NSAID	828–1000	Mild	8	A
2	63	F	T-LGLD	NE	Pleuropericarditis, cerebral ischaemia	None NSAID	400–860	Mild	5	A
3	50	M	T-LGLD	E	Psoriasis, haemolytic anaemia	NSAID	290–500	Mild	5	A
4	47	M	T-LGLD	E	Pleuropericarditis, cerebral ischaemia, ANA weak+ lymphoma*	MTX PRED NSAID	50–1000	Mild	13	D
5	59	M	PFS/T-LGLD	E		PRED NSAID	70–100	Severe	11	D
6	63	M	FS/T-LGLD	E		MTX PRED NSAID	100–500	Severe	7	D

M, male; F, female; E, enlarged; NE, not enlarged; N, neutrophils; A, alive; D, deceased; PRED, prednisone; ANA, antinuclear antibodies; MTX, methotrexate; NSAID, non-steroidal anti-inflammatory drugs.
Patients diagnosed with FS or PFS fulfilled ACR criteria for definite or probable RA.[22] T-LGLD diagnosis assigned clinically plus typical findings on blood smear or detection of increased CD57+ or CD16+ cells by FACS analysis.
*T cell lymphoma developed six months before death.

Fluorescein isothiocyanate conjugated goat antimouse-Ig $F(ab')_2$ fragment of antibody (Jackson ImmunoResearch Lab Inc, PA) was added for 30 minutes at 4°C. Cells were washed twice, resuspended in 0.5 ml of PBS, and analysed on an Epics profile II Coulter Electronic FACS as described previously.[22] The mAb used were anti-CD3, anti-CD4, anti- CD8 from Becton Dickinson, San Jose, CA. The mAb TCRδ1 (directed against constant region of TCRδ chain (Cδ)) was kindly provided by M Brenner (Harvard Medical School, Boston, MA). The mAb TCSδ1 (specific for Vδ1Jδ1) was purchased from T cell sciences (Cambridge, MA). The mAb against Vδ1 (mAb A13), Vδ2 (mAb BB3), and Vγ2(9) (mAb TiγA) were kindly provided by Dr L Moretta (Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy).[23] The mAb 23D12, which recognises Vγ1 gene products, was kindly supplied by Dr D Kabelitz.[24]

Polymerase chain reaction (PCR) analysis of TCR genes

The methodology has been previously described.[22 25] Briefly, total cellular RNA of PBL was extracted with guanidium thiocyanate and phenol (RNAzol B; Cinna Biotek Laboratories Int, Friends Wood, TX). cDNA transcripts were prepared with oligodeoxythymidine priming and avian reverse transcriptase in the presence of RNAse inhibitor (40 U/20 ml; Promega, Madison, WI). Vγ and Vδ gene family specific 5′ oligonucleotide primers were synthesised using an Applied Biosystems (Foster City, CA) DNA synthesiser, and paired with 3′ primers matched to Cγ or Cδ genes respectively.[22 25] The Vγ and Vδ gene family specific primers and probes were previously reported.[22 25] The denatured cDNA was amplified with Taq DNA polymerase, Taq polymerase buffer, and 1.5 mmol/l of each nucleotide in 35 amplification cycles. Negative controls included tubes without cDNA or irrelevant cDNA. The amplified products were electrophoresed on 1.55 agarose gels with ethidium bromide and visualised under ultraviolet light. To increase the sensitivity and specificity of the assay, the agarose gels were blotted onto nitrocellulose membranes, baked, and then hybridised with [^{32}P]ATP labelled oligonucleotide probes (5×10^5 ct/min of probe/ml) specific for the Cγ or Cδ regions, respectively, and internal to the TCR products, as described.[22] After washing, the blots were exposed to *x* ray film with an intensifying screen for 3–24 hours.

Sequencing of TCR genes

Samples of the PCR products corresponding to the expressed CδVδ2 genes were electrophoresed together with marker DNA on 1.2% agarose gel for estimating the amount of amplified DNA fragments to be sequenced. Excessive primers were subsequently removed from the PCR products with the DS primer remover kit (Adv. Gen. Technol. Corp), and the amount of DNA was determined by Gene Quant (Pharmacia LKB Biochrom Ltd, Cambridge, UK). The CδVδ2 amplified cDNA segments were sequenced in the 5′ and the 3′ directions. Sequencing by the automated DNA sequencer (Applied Biosystems 373A, Foster City, California, USA) was done as follows: 50–100 ng of amplified TCR cDNA segments were mixed together with 10 pmol of 3′ Cδ-specific oligomer for 3′ sequencing, or with 10

Table 2 T cell subsets in the peripheral blood. Results are shown as percentages

Patient No	CD3 $^+$	CD4 $^+$	CD8 $^+$	γδ $^+$	Vδ1 $^+$	Vδ2 $^+$
1	77.8	17.0	11.8	44.8	0.6	49.6
2	76	23	12	33	0.2	31
3	72	13	14	66	0.3	0.3
4	47.7	15.8	27.7	16.9	10.7	9.4
5	59.5	22.4	14.8	10.4	5.3	0.3
6	82.5	2.0	86.8	1.0	1.0	0.0
Normal (n=8)	68 (12.2)	43.7 (9.8)	24 (5.9)	3.4 (1.3)	1.2 (1.2)	1.8 (1.2)

Numbers are representative of two to three evaluations for each patient. For patient 4, subsequent evaluations showed a drop in γδ T cells to ~7% in repeated evaluations.

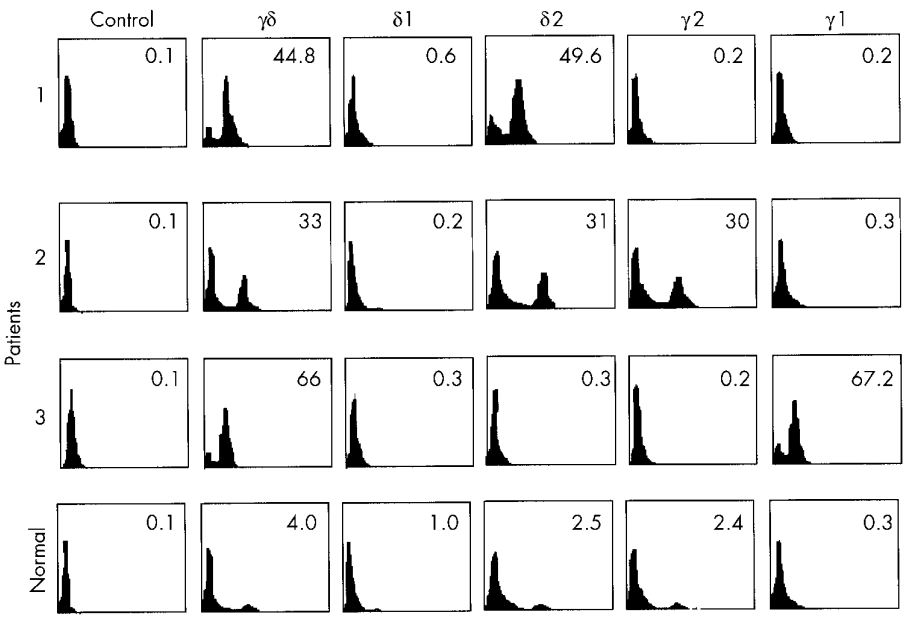

Figure 1 Analysis of T cell subsets in patient PBL. FACS analysis of patient PBL with mAb directed against the indicated antigens. Percentages of the indicated subsets are shown.

pmol of the 5′ Vδ2-specific oligomer for 5′ sequencing. One microlitre of each mixture was added to 39 μl of reaction premix of the Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems 373A) as recommended by the manufacturer. Cycling in the thermal cycler, purification of the extension products, and loading of the samples on the automated DNA sequencer was carried out by the automated DNA sequencing service unit (Biological Services, Weizmann Institute, Rehovot). The 5′ and 3′ sequences were aligned and the junctional sequences confirmed only when a perfect match was obtained between the 5′ and the 3′sequences.

RESULTS

γδ T cells in the PB

Table 2 summarises the percentages of $CD3^+$, $CD4^+$, $CD8^+$, γδ TCR^+ , $V\delta1^+$, and $V\delta2^+$ subsets in the PBL of the six patients and of eight normal subjects. Compared with the controls, the patients had a relative depletion of $CD4^+$ T cells in their PB ($p<0.008$), with normal or increased (in patient 6) $CD8^+$ T cells. Patients 1–3 had major and persistent expansions of γδ T cells (>30% of the T cells on at least two occasions over more than two months). Patients 4 and 5 had slightly or non-persistently expanded γδ T cells, whereas patient 6 had a normal number of these cells in the PB.

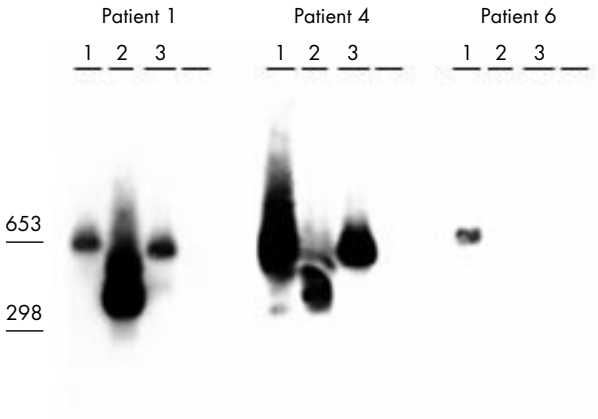

Figure 2 Vδ genes in patient PB. Southern blot representing DNA fragments amplified from PBL. PCR was employed using 5′ Vδ gene family specific primers (Vδ1, Vδ2, and Vδ3 corresponding to lanes 1, 2, and 3) matched to Cδ gene specific primers.

Vδ genes of the γδ TCR

The expanded γδ T cells in patients 1 and 2 were reactive almost exclusively with mAb to the Vδ2 gene (fig 1 and table 2), whereas the γδ T cells expanded in the PB of patient 3 did not react with mAb directed against Vδ2 or Vδ1 gene segments, suggesting they use other Vδ gene segments (for example, Vδ3) in the TCR δ polypeptide.[1 25] In patients 4 and 5 there was a more polyclonal expression of Vδ genes. Thus the PBL of patient 4 reacted almost equally with mAb to Vδ2 and Vδ1, and those of patient 5 contained at least two subsets of TCR $\gamma\delta^+$ cells (~50% reactive with mAb to Vδ2, ~50% $V\delta2^-/V\delta1^-$) (table 2). In contrast, the γδ T cells in patient 6, while comprising only 1% of the PBL, were exclusively reactive with anti-Vδ1 mAb (table 2 and data not shown).

cDNA prepared from mRNA of patients 1, 4, and 6 PBL was available for analysis of the expressed Vδ genes. In patient 1 there was strong expression of Vδ2 along with weaker expression of Vδ1 and Vδ3 genes (fig 2). In the PB of patient 4, Vδ 1–3 genes were expressed without striking predominance of a particular gene family. In patient 6, in contrast, only Vδ1, but neither Vδ2 or Vδ3 genes were detected (fig 2).

Vγ genes expressed in PBL

TCRγ genes in the three patients with persistent expansion of γδ T cells were characterised by FACS analysis (fig 1). The Vγ gene families used by the γδ T cell clones expanded in patients 2 and 3 were identified by mAb specific for Vγ2 or Vγ1, respectively (fig 1).

To analyse the TCRγ gene in patient 1, which was not recognised by the Vγ2 or 1 specific mAb used here (fig 1), TCR γ and δ genes expressed in a $V\delta2^+$ T cell clone (B8) isolated from the PB of this patient were studied.[18] cDNA prepared from clone B8 hybridised to a primer specific for consensus sequences of Vγ1–1.8, but not to Vγ2 or Vγ3 specific primers (fig 3A). Sequencing of the PCR amplified TCRγ genes revealed an in-frame rearrangement, wherein an unidentified member of the Vγ1 gene family is rearranged via Jγ2.3 to Cγ2 (not shown). Importantly, direct sequencing of B8 cDNA using Vδ2 and Cδ primers revealed it uses a Vδ2/Dδ2/Jδ1/Cδ gene rearrangement identical to that obtained by direct sequencing of cDNA prepared from non-selected lymphocytes directly isolated from the PB of patient 1, indicating this clone directly

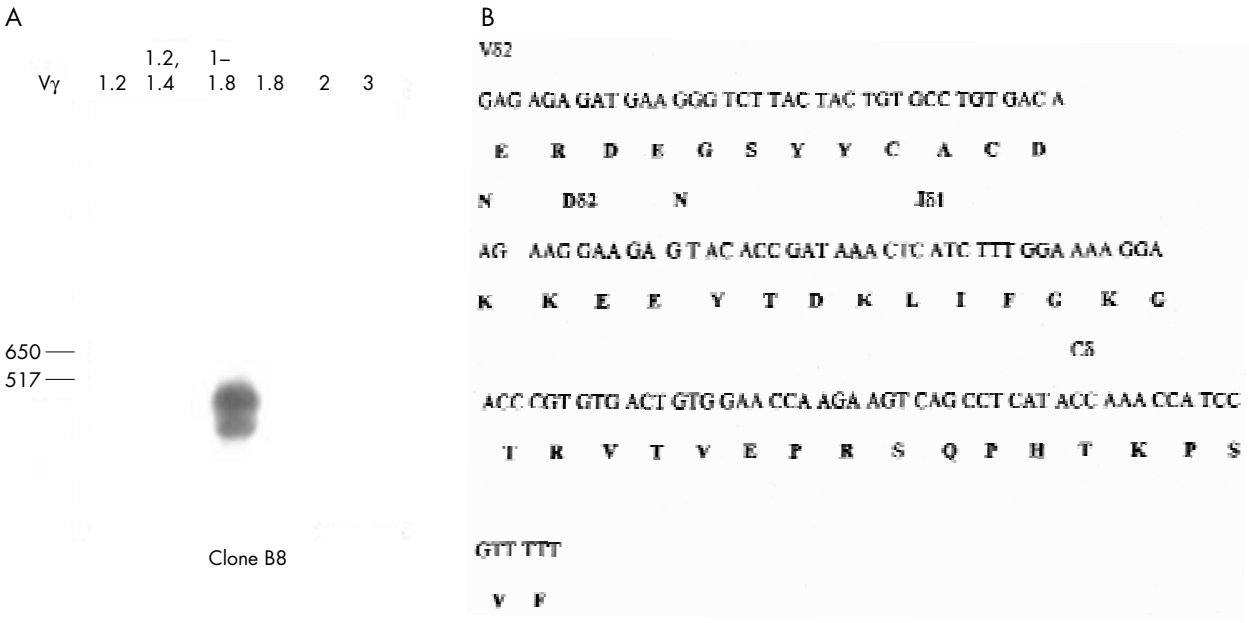

Figure 3 (A) Vγ TCR gene amplification in clone B8. Each lane represents hybridisation with a Vγ family specific probe, after amplification with family specific Vγ primers as indicated and a 3′ Cγ primer. (B) Nucleic acid and deduced amino acid sequence of patient 1 TCRδ genes. CδVδ2 amplified cDNA segments prepared from clone B8 and non-selected patient 1 PBL were sequenced in the 5′ and 3′ directions by the automated DNA sequencer ("Patients and methods"). The 5′ and the 3′ sequences were aligned and junctional sequences confirmed. The resulting matching sequences are aligned. The sequence has an in-frame Vδ2Dδ2Jδ1Cδ rearrangement and N region diversity. The deduced amino acid sequences are given below the nucleotide sequences.

derives from the expanded γδ T cell population in the PB (fig 3B). Together with the FACS analysis (fig 1), these results strongly suggest that the TCR Vδ2$^+$ chain of the γδ T cell lymphoproliferations in patient 1 pairs with TCRγ polypeptides using Vγ1 family genes.

Finally, TCRγ genes expressed in cDNA prepared from PBL of patients 4 and 6 were analysed. Figure 4 shows that Vγ1 and Vγ2 family genes were expressed in PBL of patient 4, whereas, in marked contrast, only members of the Vγ1 gene family were detected in the PBL of patient 6 (fig 4).

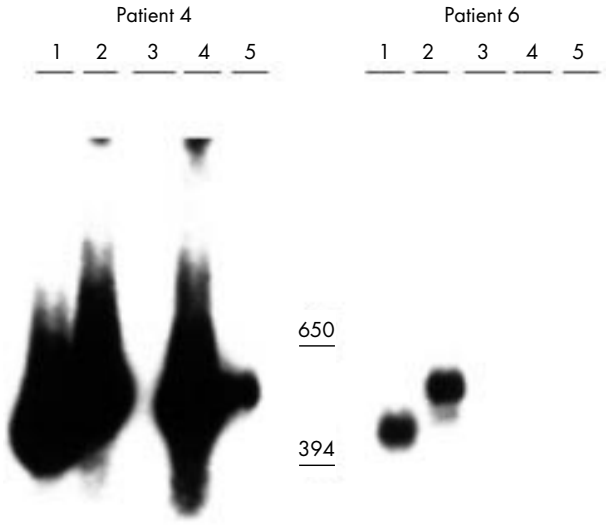

Figure 4 Vγ TCR gene amplification in PBL. Each lane represents hybridisation with a family specific Vγ probe, after amplification with a family specific 5′ Vγ primer and a 3′ Cγ primer. Five different Vγ primers (detecting Vγ1.2 in lane 1, Vγ1.2 and 1.4 in lane 2, Vγ1–1.8 in lane 3, Vγ1.8 in lane 4, and Vγ2 in lane 5) were used.

Relation of γδ TCR V genes to nature of arthritis

Although each of the three patients (1–3) with persistent γδ T cell lymphoproliferations had a clinically different form of arthritis (polyarticular rheumatoid factor (RF)$^+$ RA, oligoarticular RF$^+$ arthritis, and psoriatic arthritis respectively) none developed erosions or permanent deformities (table 3). The two RF$^+$ patients in this group had Vδ2$^+$ expansions coexpressed with either Vγ2 or Vγ1 family genes. In contrast, patient 3, with RF$^-$ psoriatic arthritis had an expansion of Vγ1$^+$/Vδ2$^-$/Vδ1$^-$ cells. Among the patients without persistent γδ T cell proliferation, patients 4 and 5 who had benign episodic arthritis or non-erosive polyarthritis respectively, had polyclonal γδ T cells as shown by the finding of Vγ (1, 2, or 3) and Vδ (1, 2, and 3) genes in the PB of patient 4, and by the equal distribution of Vδ2$^+$ and Vδ1$^-$/Vδ2$^-$ γδ T cells in patient 5 (tables 2 and 3). In contrast, patient 6, with a complete lack of expression of TCRγ and δ V genes other than those of the Vγ1 and Vδ1 families in the PB, had very severe deforming RF$^+$ RA.

DISCUSSION

The main conclusions of this study are that (*a*) γδ T cell proliferations in patients with chronic neutropenia and arthritis (FS or PFS) may derive from γδ T cell populations that are infrequent in normal adult PBL (for example, expansions of Vγ1$^+$/Vδ2$^+$ or Vγ1$^+$/Vδ2$^-$/Vδ1$^-$ cells); (*b*) the arthritis in these patients is usually non-erosive and non-deforming even in the presence of high titres of RF (as seen in patient 1); and (*c*) the clinical characteristics of the arthritic syndrome may be related to the particular γδ T cell subset undergoing expansion or predominating, in the PB.

In normal PBL, 50–70% of γδ T cells coexpress Vγ2 and Vδ2 in the TCR structure, and are the main subset that recognises and reacts to microbial antigens.[1–2 5–7] In contrast, two of three (67%) γδ T cell lymphoproliferations in our patients originate from cells that use TCR with variable regions encoded by combinations of V region genes (that is, Vγ1$^+$/Vδ2$^+$ or Vγ1$^+$/Vδ2$^-$/Vδ1$^-$ patients 1 and 3) which are relatively infrequent in normal PB (table 2). This result is consistent

Table 3 Predominant γδ T cell subset and arthritis

Patient No	Persistent expansion of γδ T cells	Predominant TCRγδ V genes	Arthritis	Deforming arthritis	Erosions	RF
1	+	$V\gamma1^+/V\delta2^+$	Poly	–	None	++
2	+	$V\gamma2^+/V\delta2^+$	Oligo	–	None	+
3	+	$V\gamma1^+/V\delta2^-/V\delta1^-$	Oligo	–	None	–
4	–	Polyclonal	Oligo	–	None	– –+
5	–	Polyclonal	Poly	–	None	–
6	–	$V\gamma1^+/V\delta1^+$	Poly	++	Severe	++

Oligo, oligoarthritis; poly, polyarthritis.
Presence or absence of erosions was determined by evaluation of roentgenograms of affected joints by a board certified rheumatologist or a trained radiologist, or both. RF+, low titre by latex fixation test (<1/160); RF++, high titre by latex fixation (>1/640).

with previous reports describing, although in less detail, the TCR of γδ T cell lymphoproliferations in arthritic patients with neutropenia.[12 13 19] For example, γδ T lymphoproliferations in a patient described by Hodges *et al*, and one of three patients described by Kuipers *et al*, used $V\gamma1^+/V\delta1^+$ TCR, which are characteristically seen in γδ T cells in synovial tissue, and in ~20% of PB γδ T cells.[1 12 13 19] An additional patient described by the latter authors, as discussed below in further detail, had an expansion of cells expressing another "unconventional" ($V\gamma2^-/V\delta1^-$) TCR.[19] Collectively, therefore, the data suggest the paradigm that γδ T cell lymphoproliferations in patients with FS or PFS derive from lymphocytes that are not usually expanded by common antigens from micro-organisms, but rather from other subsets whose antigenic targets have not yet been identified. In this regard patient 2 is exceptional, since her lymphoproliferations do use a $V\gamma2^+/V\delta2^+$ TCR (fig 1). Nevertheless, not all receptors using these genes recognise microbial antigens, and studies to determine whether it represents, at a structural and functional level, a conventional antigen-reactive $V\gamma2^+/V\delta2^+$ TCR are in progress.[5–7]

The arthritis in all three patients studied with persistent expansions of the γδ T cells was non-erosive and non-deforming, even in the presence of a high titre of RF (patients 1 and 2), which signifies more severe arthritis in patients with RA (table 3). This association suggests that the expanded populations of lymphocytes may attenuate autoimmune processes reflected in the synovium. Theoretically, the proliferating cells could exert their influence by secretion of cytokines, or regulating other immunocytes, including $CD4^+$ T cells.[2] In support of this concept, γδ clones were shown to induce apoptosis of $CD4^+$ cells in Lyme arthritis, and γδ T cells were also shown to exert a protective effect against TCRαβ mediated autoimmune orchitis, which is induced in the contralateral testis, in a rat model of infectious orchitis.[26 27]

In addition, recent studies have shown that certain γδ subsets protect against development of autoimmune myocarditis or uveitis after experimental induction of the disease in mice, whereas other subsets aggravated disease.[28 29] Our results, although limited by the small number of patients, enable a preliminary evaluation of the relation between the nature of the subsets of γδ T cells predominating in the PB and an arthritic syndrome developing in humans. In the three patients without persistent γδ lymphoproliferations, a balanced repertoire containing multiple Vδ and Vγ genes in the PB (patients 4 and 5) was associated with non-deforming arthritis, whereas depletion of Vγ2, Vδ3, and Vδ2 genes, with maintained Vγ1 and Vδ1 gene expression, was associated with very severe deforming arthritic disease (patient 6) (figs 2, 4 and tables 2 and 3). On the other hand, chronic lymphoproliferations of γδ T cells using Vδ2 or other, unidentified $V\delta2^-/V\delta1^-$ genes, along with either Vγ1 or Vγ2, was associated with relatively benign forms of arthritis in patients 1–3. Collectively, therefore, the results suggest that whereas $V\gamma1^+/V\delta2^-/V\delta1^-$, $V\gamma1^+/V\delta2^+$, and $V\gamma2^+/V\delta2^+$ clones might be protective during the development of the arthritic process, $V\gamma1^+/V\delta1^+$ clones might be detrimental (table 3). The immunoprotective mechanism may involve activation via the TCR in vivo because, for example, $V\gamma1^+/V\gamma2^+$ clones from patient 1 cells secrete lymphokines when activated via their $CD3^-$ TCR structure in vitro and can regulate immunoglobulin production by B cells.[18] Although the putative antigens and superantigens responsible for activating these clones are unknown, lymphocytes with unusual $V\gamma2^-/V\delta2^+$ TCR form a major proportion of the γδ T cells in cord blood, suggesting they may be recognising autoantigens.[30] In addition, $V\gamma2^-/V\delta2^+$ γδ clones have been detected invading muscle in polymyositis, further suggesting their regulatory involvement in autoimmunity.[31]

Studies of larger numbers of patients with chronic arthritis and neutropenia, in FS, PFS, or similar syndromes, relating the nature of the arthritis to the distribution of γδ subsets in the PB will be necessary in order to examine whether our findings are representative of the entire group of these patients. The relevance of defining these correlations is further enhanced by the fact that, in a clinical sense, patients 1–5 represent the majority of patients with PFS/T-LGLD, in whom arthritis is usually less severe than in FS, whereas patient 6, with the erosive arthritis, is exceptional in our group, and represents a clinically very severe case of FS.[15] Furthermore, patient 6, concurrent with the loss of $V\gamma2^+$, $V\delta3^+$, and $V\delta2^+$ cells, had a marked depletion of $CD4^+$ cells, a predominant population of $CD8^+$ T cells, and a markedly restricted TCRβ gene repertoire in the PB, suggesting oligoclonal dominance of the $CD8^+$TCRαβ lymphocytes (table 2 and data not shown). Such cells are thought to contribute to the pathogenetic mechanisms in arthritis, perhaps by recognising a common antigen.[16] Finally, it is noteworthy that the γδ T cell lymphoproliferations in patient 3 with RF^- psoriatic oligoarticular arthritis is unique in its expression of $V\delta2^-$ $V\delta1J\delta1^-$, although as yet undefined, Vδ genes in the TCRδ polypeptide in association with a Vγ1 gene family in the TCRγ chain.[32] Although this is the first description of γδ T cell lymphoproliferations in psoriatic arthritis, Kuipers *et al* described, a neutropenic patient with RA and ankylosing spondylitis whose γδ T cell lymphoproliferations used Vδ genes other than Vδ1, and Vγ genes other than Vγ2. [19] Together, these data suggest that γδ T cell lymphoproliferations associated with spondyloarthropathies may selectively involve $V\gamma2^-/V\delta1^-/V\delta2^-$ γδ clones. Thus definitions of the balance of γδ and other T cell subsets in neutropenic patients may have diagnostic and prognostic implications.

In summary, our findings, together with those previously reported, suggest unique features of the TCR in γδ T cell lymphoproliferations of patients with arthritis and chronic neutropenia, as well as correlations between the expression of certain Vγ or δ genes and the nature of the arthritic syndrome. Further structural and functional analysis of the T cells expanding in these unusual patients, which could best be obtained by pooling material from laboratories and clinics world wide, may help to clarify the role of γδ T cells in other autoimmune conditions as well.

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