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Large granular lymphocyte expansions in patients with Felty's syndrome: analysis using anti-T cell receptor Vβ-specific monoclonal antibodies

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SUMMARY

Felty's syndrome (FS), the association of rheumatoid arthritis (RA) and idiopathic neutropenia, remains an unexplained phenomenon. HLA-DR4 is found in over 90% of cases. Patients with FS may have a T cell lymphocytosis of $CD3^+CD8^+CD57^+$ large granular lymphocytes (LGL syndrome). In this study of 47 patients with FS, 19% had clear evidence for LGL expansions, while in total 42% had variable evidence for the LGL syndrome using currently available techniques. Of these T cell expansions, 76% were clonal, as demonstrated by Southern blotting and analysis with T cell receptor (TCR) β chain constant region probes. This technique may fail to detect clonal populations in some patients. Cytofluorographic analysis using antibodies specific for TCR Vβ chains identified patients with clonal LGL expansions with results comparable to those obtained with Southern blotting. No evidence for shared Vβ usage among expansions from different patients was seen. The role of LGL in RA and FS is currently unclear, but this technique offers a practical and accessible means of identifying patients with LGL expansions, as a starting point for further investigation.

Keywords Felty's syndrome LGL syndrome anti-TCR Vβ antibodies

INTRODUCTION

Felty's syndrome (FS) comprises a triad of rheumatoid arthritis (RA), leucopenia and splenomegaly [1] that is found in approximately 1% of RA patients. The splenomegaly is variable both over time and in extent, and never develops in some patients [2]. The mechanisms underlying the neutropenia are not clear, but appear multifactorial [3]. In favour, however, of a single cause are the immunogenetic data: since 95% of FS patients are $HLA\text{-}DR4^+$ compared with 70% of RA patients and 30% of the general population [4,5], and since the only known function of HLA molecules is the presentation of peptide antigen to T cells, this close association of FS with HLA-DR4 is suggestive of a specific antigen-dependent step in most cases of FS. Furthermore, an association has been found between RA in general and FS in particular with a form of T cell 'leukaemia', the large granular lymphocyte (LGL) syndrome, in which there is an expansion of peripheral blood T lymphocytes with a large granular morphology. LGL comprise approximately 15% of normal peripheral blood lymphocytes, although this increases with age [6]. LGL encompass two groups of cells. The first group are natural killer (NK) cells which do not rearrange the T cell receptor (TCR) genes and are $CD3^-$. True NK cell LGL leukaemia is rare. The second group are T cells which rearrange the TCR, are $CD3^+$ and, in the vast majority of LGL cases, are CD8 cells. Additional markers which are often expressed on T cell LGL are CD57 and CD16 and, less commonly, CD56. Of particular interest is that up to a third of patients with the T cell LGL syndrome have RA, and neutropenia is also common [7]. For this reason they often resemble FS. We and others have shown that a proportion of patients diagnosed as FS have additional evidence of the presence of the LGL syndrome [8–10].

This study was directed towards answering two question: (i) how common is the LGL syndrome among FS patients attending hospital rheumatology departments? (ii) how useful are MoAbs directed against the variable (V) region of the TCR β chain in identifying patients with clonal expansions of LGL compared with Southern blotting and hybridization with TCR Cβ probes?

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SUBJECTS AND METHODS

Patients and controls

Forty-seven FS patients (age 63 ± 13 years (mean ± s.d.),

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female : male 23 : 24) were recruited through hospital rheumatologists, predominantly in the South East of England. All fulfilled the 1987 ARA criteria for RA [11]. In addition they all had a current or past history of unexplained neutropenia (neutrophil count of $<2 \times 10^9/l$) persisting for at least 6 months. An additional eight patients with RA, current or previous neutropenia, and known to have the LGL syndrome were recruited from around the UK. All patients were North-European Caucasoids, except for one patient of Turkish-Cypriot origin.

The LGL syndrome was defined as the presence of $>1 \times 10^9/l$ LGL in the peripheral blood using morphological analysis, or the same number of $CD3^+CD57^+$ cells, using cytofluorographic analysis (see below), that had persisted for at least 6 months. Since lymphopenia can be present in the LGL syndrome, an alternative criterion is the presence of LGL or $CD3^+CD57^+$ cells comprising at least 25% of lymphocytes.

Since HLA type influences the TCR repertoire [12], the comparison of TCR Vβ repertoires using MoAbs was performed using 10 HLA-$DR4^+$ RA patients and 10 HLA-$DR4^+$ controls without rheumatic conditions, previously HLA-DR typed at UMDS. For comparison of the prevalence of the LGL syndrome two further groups were studied. Since the numbers of LGL increase with age [6] it was critical that the control groups should be of the same age range as the FS patients. The disease control group (23 patients) was a hospital out-patient (Guy's)-based group of randomly recruited RA patients (age 63 ± 11 years, female : male 17 : 6). The second normal group comprised individuals without rheumatic complaints (non-rheumatic controls (NC)) (age 60 ± 21 years, female : male 13 : 7). All of the RA and NC groups were North-European Caucasoids except for two RA patients (one Greek Cypriot, one Asian) and one control (Greek Cypriot).

Cell separation, immunofluorescence and cytofluorographic analysis

Peripheral venous blood was collected in preservative-free heparin and mononuclear cells separated on a Lymphoprep (Nycomed, Oslo, Norway) density gradient. Immunophenotyping was performed by flow cytometry (FACS) on a Becton Dickinson FACScan (Becton Dickinson, Oxford, UK) using double staining with PE- and FITC- conjugated MoAbs. These included: mouse IgG1 antibodies as negative controls, anti-CD3, anti-CD4, anti-CD8, anti-CD16, anti-CD56, anti-CD57, anti-TCR $\alpha\beta$ and $\gamma\delta$, FITC-conjugated goat anti-mouse (all from Becton Dickinson). Anti-Vβ MoAbs were all unconjugated: 2D9 (anti-Vβ2) [13], JOVI.3 (anti-Vβ3) [14], and six further MoAbs available commercially from T cell Sciences (Cambridge, MA) [15]: LC4 (anti-Vβ5.1), 1C1 (anti-Vβ5.2/3), W112 (anti-Vβ5.3), OT145 (anti-Vβ6.7a), 16G8 (anti-Vβ8), S511 (anti-Vβ12).

Haematological analysis

Full blood counts and leucocyte differential counts were provided by the Haematology Department, Guy's Hospital. Manual blood films stained with haematoxylin and eosin made from these samples were examined by M.B., for the presence of large granular lymphocytes. This was performed without knowledge of the FACS results.

Southern blotting and HLA-DR4 genotyping

Venous peripheral blood was drawn into EDTA and stored at −20°C. DNA was subsequently extracted by standard techniques, digested overnight with either EcoRI or HindIII (GIBCO-BRL, Bethesda, MD) and electrophoresed in a 0·6% agarose gel. The DNA was transferred to a nylon membrane (Hybond-N, Amersham, Aylesbury, UK) by ultraviolet

Table 1. Categorization of 47 patients with Felty's syndrome (FS) and eight patients with rheumatoid arthritis and known large granular lymphocyte (LGL) expansions, according to the level of the expansions in the blood

Group	Number of patients	$CD3^+CD57^+$ count ($10^9/l$)	Splenectomy	LGL morphology	No longer neutropenic	CD4 : 8 ratio	CD16 expression	CD56 expression	T cell receptor β-chain rearrangement
Group 1	3	1·5–3·4	2/3	3/3	1‡/3	0·06–0·34	0/3	0/3	2/3
(LGL 1–10)	7*	1·1–10·2†	0/7	7/7	1/7	0·02–0·27	5/7	2/7	7/7
Group 2	3	0·7–1·0	0/3	3/3	0/3	0·04–0·25	1/3	0/3	1/3
(LGL 11–14)	1*	1·0	0/1	1/1	0/1	0·43	0/1	0/1	0/1
Group 3	3	0·25–0·37	0/3	2/3	0/3	0·8–1·1	0/3	0/3	3/3
(LGL 15–17)									
Group 4	4	0·09–0·22	0/4	2/4 definite	0/4	0·32–0·73	0/4	0/4	0/4
(FS/LGL				2/4 +/−					
18–23)	2	0·51, 0·76	1/2	1/2 definite	2/2	1·4, 2·3	0/2	1/2	0/2
				1/2 +/−					
Group 5	32	0·01–0·32	0/32	6/32 definite	13/32	0·8–7·1	1/32§	2/32§	0/22
(FS 24–55)				6/32 +/−		(4/32 < 1·0)			

* Patients with previously known LGL expansions.

† One patient is included who was lymphopenic at the time of the study, but had previously had a lymphocyte count of $5·6 \times 10^9/l$, with morphological LGL comprising $1·6 \times 10^9/l$. The phenotype was $CD3^+CD8^+CD16^+CD56^+$, but the expansion in this individual did not express CD57.

‡ This patient had had a previous splenectomy, which could account for the resolution of the neutropenia.

§ Greater than 2 s.d. above the mean.

cross-linking, and hybridization, washing and autoradiography were carried out with ^{32}P-labelled constant region probes as described previously [8]. Two types of TCR Cβ probes were used. The first was isolated from a plasmid containing the probe as a cDNA insert [16]. A second 540-bp probe was produced by linear polymerase chain reaction (PCR) using primers internal to the Cβ region [17] using genomic DNA as the template. Following the PCR reaction the product was run out on an agarose gel, phenol/chloroform extracted and ethanol precipitated. Both the probes gave identical results. HLA-DR4 typing was performed by PCR or Southern blotting as described previously [5,18].

Statistical analysis

The Mann–Whitney *U*-test was used for comparison of TCR Vβ expression between groups of patients and controls.

RESULTS

The 47 FS patients recruited were categorized into five patient groups according to the level of LGL expansions identified (Table 1).

LGL group 1 (absolute LGL lymphocytosis)

FACS analysis identified 3/47 (6%) patients with an absolute $CD3^+CD57^+$ count of over $1 \times 10^9/l$. Seven of the additional eight patients with known LGL syndrome, recruited from around the UK, also fell within LGL group 1. The expansion in one of these patients was of morphological $CD3^+$ LGL that did not express CD57 (Table 1). As a consequence of the LGL lymphocytosis they all had a low CD4:8 ratio (0·06–0·34, normal CD4:8 ratio is 1·0 or over).

Southern blotting analysis (Fig. 1) showed that nine out of 10 had either a Cβ1 or Cβ2 rearrangement or both. LGL 10 had no definite extra band with either EcoRI or HindIII, and this is suggestive evidence that the expansion may be polyclonal (see also Vβ antibody results below).

LGL group 2

Three of 47 (6%) patients were identified with a raised $CD3^+CD57^+$ count above the normal range of $0{\cdot}4 \times 10^9/l$ ($0{\cdot}7-1{\cdot}0 \times 10^9/l$). They also had a markedly reduced CD4:8 ratio (0·04–0·25). One of the eight known LGL patients recruited also fell within this group.

In Southern blotting analysis for additional non-germ-line bands (Fig. 1), LGL 11 was negative with both enzymes, although a faint band had been visible with EcoRI on a previous occasion [8]. LGL 13 and LGL 14 were also negative. Sequencing data on LGL 13 [19] were highly suggestive of one or more clonal Cβ2 rearrangements. LGL 14 had a Vβ5.3 expansion of 24% of CD3 cells, and while sequencing data were not available, in the context of the other results it is potentially clonal.

Other anomalous results were identified from sequencing data in LGL 3 (Vβ2-Cβ2 = 82% of CD3 cells) and LGL 12 (Vβ3-Cβ2 = 54% of CD3 cells) [19]. In both cases the expansions were of a Cβ2 sequence, yet in neither case was an extra band demonstrated with HindIII.

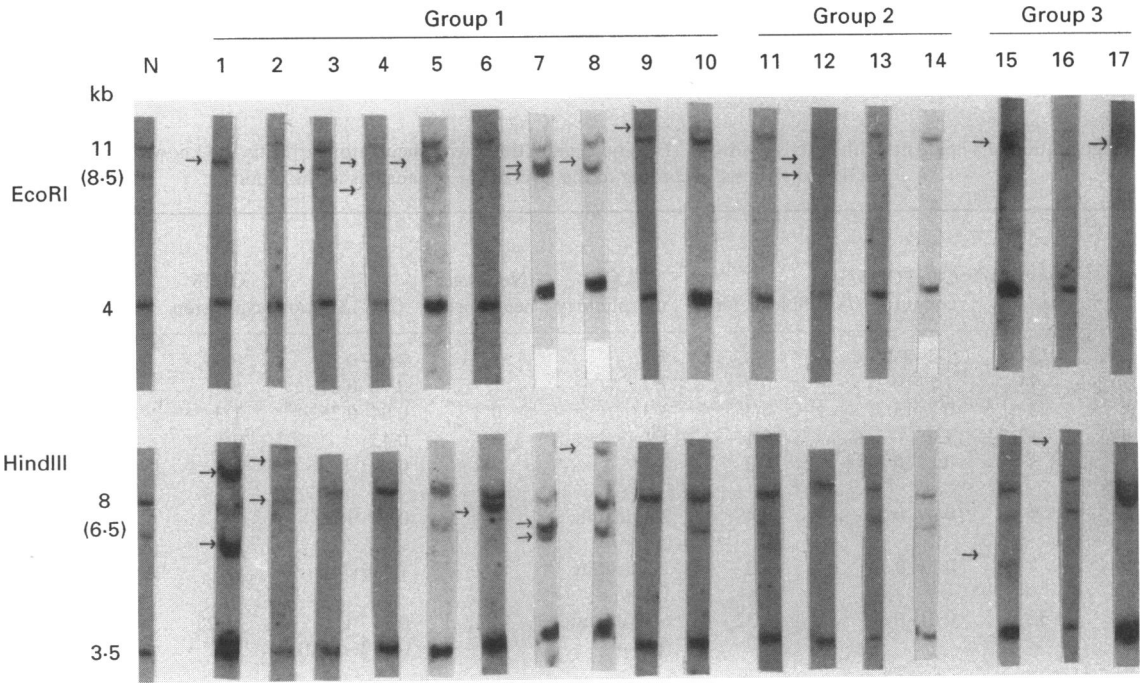

Fig. 1. Southern blot analysis of large granular lymphocyte (LGL) patients 1–17 after digestion of genomic DNA from venous peripheral blood with EcoRI or HindIII, and analysis with a T cell receptor (TCR) Cβ probe using autoradiography. Arrows delineate non-germ-line bands. Rearrangements to Cβ1 produce an alteration of the 11-kb band following restriction enzyme digestion with EcoRI (upper lane). Rearrangements to Cβ2 produce an alteration of the 8-kb band following restriction enzyme digestion with HindIII. The intensity of these altered and germ-line bands will depend on the proportion of clonal and non-clonal cell populations. The 8·5-kb band with EcoRI represents a partial restriction site that is not always seen.

LGL group 3

Of the remaining FS patients examined, 3/47 (6%) had additional non-germ-line bands (Fig. 1) and make up LGL group 3. Although these non-germ-line bands could represent $CD4^+$ expansions, these patients had a relatively low CD4:8 ratio (0·8, 0·9, 1·05), and a relatively raised percentage of $CD3^+CD57^+$ cells (45, 42, 30%), with the absolute number in the upper part of the normal range (0·34, 0·37, 0·25 $\times 10^9/l$). Taking these results together this is suggestive of 'occult' LGL clonality. This has not led to a rise in the absolute number of LGL beyond the normal range, nor of a substantial disturbance of the CD4:8 ratio, but is above the 2·5–5% level that is detected by Southern blotting.

FS/LGL group 4

Four other patients with a low CD4:8 ratio (less than 0·8) were identified. None had a raised absolute $CD3^+CD57^+$ level, but all were mildly or more severely lymphopenic (0·42–0·98 $\times 10^9/l$) with a per cent CD57/CD3 of over 25%. In addition, two other patients with a raised absolute $CD3^+CD57^+$ count were identified. Both, however, had CD4:8 ratios and neutrophil counts that were well within the normal range (Table 1). One had previously had a splenectomy which may cause an LGL lymphocytosis [7]. These six patients (FS/LGL group 4) cannot be clearly assigned to either the LGL or non-LGL groups using these criteria. None of the group 4 FS/LGL patients examined had additional bands on Southern blotting.

FS group 5

Of the remaining FS patients, 4/32 had a CD4:8 ratio of less than 1·0, and although 8/32 had a per cent CD57/CD3 of 25% or more, none had a raised $CD3^+CD57^+$ count. In no case did these findings coincide in any particular patient to suggest a clearcut LGL syndrome. Twenty-two of 32 were analysed by Southern blotting, and none had additional bands to suggest clonality.

Morphological analysis

Morphological analysis of the blood film was highly sensitive in identifying LGL expansions. Of the LGL patients in LGL groups 1–3, 16/17 (94%) were identified as having the LGL syndrome from the blood film. In group 4 morphological analysis of the blood film identified three of these six as having an increase in LGL, and the other three as having a borderline increase. Of the FS group 5 patients, 6/32 (19%) had increased morphologic LGL, with a further 6/32 having a borderline increase. Hence the theoretical 'false positive' rate using this technique in comparison with the other criteria used in this study (i.e. assuming FS group 5 patients are 'negative' for the LGL syndrome) was 12/47 (25%).

Analysis of TCR Vβ usage with monoclonal antibodies

The aggregate coverage of the repertoire by five anti-TCR Vβ antibodies with non-overlapping specificities (Vβ2, 5.1, 5.3, 8, 12) ranged from 16·7% to 24·4% (median = 18·5%) in the normal HLA-$DR4^+$ controls, 12·2% to 27·1% (median = 18·6%) in the RA control group, and 11·9% to 23·8% (median = 18·6%) in the 32 patients with FS alone (FS group 5). There were no significant differences between these results. There was also no difference in the percentage of peripheral blood $CD3^+$ cells expressing each of Vβ5.1, Vβ5.3, Vβ5.2/3, Vβ6.7a and Vβ12 among the FS, RA and normal groups (Table 2). The expression of Vβ2 was lower in the RA patients (5·6 ± 1·6 (mean ± s.d.)) compared with the FS (6·9 ± 1·3) patients (Mann–Whitney *U*-test, $P = 0·02$). Vβ8 expression was lower in the RA patients (4·1 ± 1·0) than in the DR4 controls (5·6 ± 1·6) (Mann–Whitney *U*-test, $P = 0·03$). These differences are not significant, however, after correction for the number of antibodies (Bonferoni correction).

Figure 2 shows the results using the above Vβ antibody panel for patients with LGL expansions. Expanded T cell populations were demonstrated in LGL 3 (Vβ2/CD3 = 82%), LGL 12 (Vβ3/CD3 = 54%) and LGL 14 (Vβ5.3/CD3 = 24%). In addition, LGL 4 had an expansion of TCRγδ-bearing T cells (γδ/CD3 = 82%). In other LGL patients a reduction in coverage by the panel was seen which was marked in some patients (e.g. LGL 5 1·2%, LGL 7 1·8%; see Fig. 2). LGL 10, who was examined only post-splenectomy, had a Vβ panel coverage of 18·8%, which was well within the normal range, and suggestive of a polyclonal rather than a clonal LGL lymphocytosis. This fits with the negative result with Southern blotting analysis

Table 2. Mean and s.d. of individual Vβ antibody results expressed as a percentage of CD3 cells in the Felty's syndrome (FS), rheumatoid arthritis (RA) and non-rheumatic control (NC) groups

Per cent of CD3	FS no = 32, mean ± s.d.	RA no = 10, mean ± s.d.	NC no = 10, mean ± s.d.	Mann–Whitney *U*-test significance at $P < 0·05$
Vβ2	6·9 ± 1·3	5·6 ± 1·6	7·0 ± 1·7	RA:FS $P = 0·02$ RA:NC NS
Vβ5.1	3·9 ± 1·0	5·8 ± 3·6	3·5 ± 0·8	NS
Vβ5.3	1·1 ± 0·3	1·3 ± 1·5	0·9 ± 0·4	NS
Vβ5.2/3	2·7 ± 1·2	2·9 ± 1·0	3·5 ± 1·8	NS
Vβ6.7a	2·8 ± 1·4	2·9 ± 1·8	3·1 ± 1·5	NS
Vβ8	4·6 ± 1·3	4·0 ± 1·0	5·6 ± 1·6	RA:FS NS RA:NC $P = 0·03$
Vβ12	2·3 ± 1·4	1·9 ± 1·0	2·2 ± 1·6	NS
Vβ panel	19·0 ± 2·5	18·7 ± 4·6	19·3 ± 2·5	NS

Significance between groups was assessed using the Mann–Whitney *U*-test with $P < 0·05$ taken as significant.

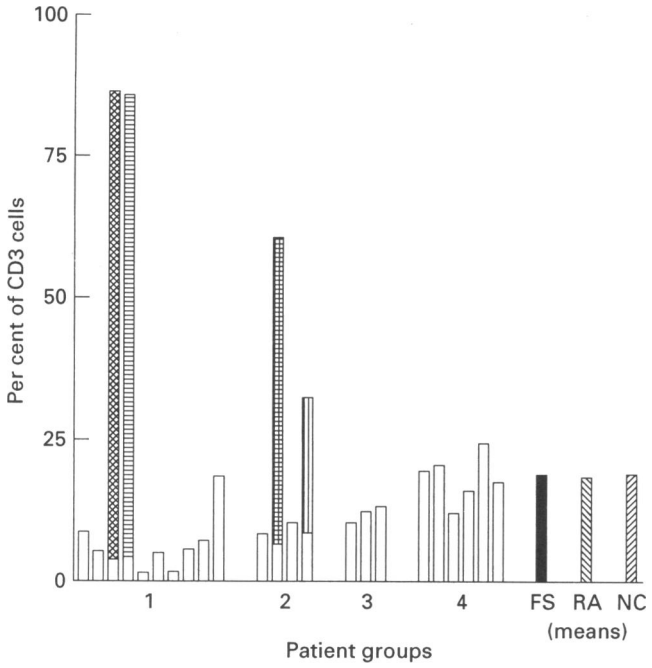

Fig. 2. T cell receptor (TCR) Vβ usage in individual large granular lymphocyte (LGL) patients (groups 1–4), and mean values in Felty's syndrome (FS) (group 5), rheumatoid arthritis (RA), and non-rheumatic control (NC) groups using a panel of monoclonal antibodies. Vβ panel (□) represents the aggregated percentage of CD3 cells expressing Vβ2, 5.1, 5.3, 8 and 12. Results for each LGL patient, and the mean values for the FS (■), RA (▧) and NC (▨) groups are given. Vβ2 in LGL3 and Vβ5.3 in LGL14, although part of the above panel, are identified separately. The expansion in LGL14 was found to be of $\gamma\delta$ T cells, which has been described [7] but is less common. Vβ3 expression in LGL12 is not part of the panel. ▩, Vβ2; ▤, $\gamma\delta$; ▦, Vβ3; ▥, Vβ5.3.

presented above. In LGL group 3, the most occult form of LGL expansions, the coverage by the antibody panel in the three patients overlaps the ranges of the FS, RA, and DR4 groups, but is at the very lowest end of the range, with only one FS patient having a lower result (11·9%, with the next lowest at 15·8%). The four LGL patients with expansions demonstrated by FACS analysis, and LGL 10 apparently without a clonal expansion, were sampled on at least two occasions 6 months apart, without significant change to the findings. The patients in group 4 had Vβ panel results within the normal range, except for one patient with 12·2%.

Comparison with controls

Examining the 23 RA patients, only one had evidence for the LGL syndrome. This patient (CD4 : 8 = 0·18, CD57% = 56%, $CD3^+CD57^+ = 0{\cdot}81 \times 10^9/l$, V$\beta$ panel = 9·85%) was a 66-year-old man with seropositive erosive RA for 20 years who had recently developed a severe gangrenous vasculitis and shortly after this analysis was commenced on cyclophosphamide. Three others had a moderate increase in $CD3^+$ $CD57^+$ cells (0·47, 0·53, $0{\cdot}54 \times 10^9/l$), but with a CD4:8 ratio of 1·0 or above. Of the 20 non-rheumatic controls, only two had an absolute number of $CD3^+CD57^+$ cells above the upper limit of the normal range of $0{\cdot}4 \times 10^9/l$ (0·46 and $0{\cdot}58 \times 10^9/l$). None of the controls had a CD4:8 ratio of under 1·0, and for the two patients mentioned above, it was 2·6 and 1·6, respectively. None therefore had evidence for the LGL syndrome.

DISCUSSION

We and others have demonstrated that up to a third of patients with FS have evidence for the LGL syndrome [8–10]. This work has now been extended by analysing a larger, well characterized, and clearly ascertained group of FS patients, and utilizing current technology in a novel way to identify clonal expansions. Out of 47 patients recruited, 9/47 (19%) had evidence for LGL expansions (groups 1–3). In practical terms this represents the most secure prevalence figure. If, however, group 4 is included, this figure becomes 15/47 (32%). If only currently neutropenic patients are included in the analysis, then 8/31 (26%) have the LGL syndrome, and if group 4 are included, this becomes 12/31 (39%). If the criteria are current neutropenia and an increase in morphological LGL then the figure is 13/31 (42%). This is likely to represent the maximum frequency of the LGL syndrome among FS patients using these techniques.

Only one of 23 RA patients and none of 20 control individuals had evidence for the LGL syndrome. Although we did not perform restriction fragment length polymorphism (RFLP) analysis on all these RA patients, studies which have looked for peripheral blood lymphocyte clonality using this technique have been negative unless cells were expanded *in vitro* before DNA extraction [20]. The presence of clonality using RFLP among control individuals is also not generally seen [21]. Examination of blood films from 20 RA patients of similar age range (M. Bhavnani, unpublished data), and 20 controls [10], failed to identify any with the LGL syndrome. The high frequency of LGL expansions seen in FS is not therefore seen in aged-matched RA or normal populations. Studies involving more sensitive means of identifying clonal expansions using the PCR have shown that occult clonal expansions of $CD8^+$ cells (but not $CD4^+$ cells) are common in normal older individuals [22,23] (and even in old mice [24]). This is more marked in RA patients, particularly in synovial fluid [25]. In normal individuals, clonal $CD8^+$ T cell expansions may accumulate as a consequence of a lifetime of chronic exposure to viral or autoantigens, and may reflect a physiological difference between CD4 and CD8 cells, rather than a pathological state. These findings raise the issue of what cut-off point determines whether a clonal expansion is 'abnormal' or a normal feature associated with older age. The RA and NC groups in this study were of similar ages to the FS patients. This is critical in demonstrating that FS patients differ both qualitatively and quantitatively from normal individuals in whom the expansions are lesser in frequency and degree. It would nevertheless be of interest to assess whether LGL expansions could be identified in all FS patients using these sensitive molecular clonality techniques.

While these techniques are clearly more sensitive than Southern blotting, the latter has previously been regarded as diagnostic for abnormal clonal T cell expansions, and remains the most commonly used technique in current use. In this study, using the restriction enzymes EcoRI and HindIII, Southern blotting failed to identify clonal expansions identified from sequencing analysis [19], in LGL 3, 11, 12, 13. The reasons for this were not clear. Relevant restriction enzyme sites were not found within the coding regions of these sequences [19]. Nor do

these sequences share a rearrangement involving a particular Vβ (or Vβ genes adjacent in the germ-line configuration) [19]. No germ-line HindIII TCR polymorphism has been identified. This situation in which known clonal populations do not yield additional bands on Southern blotting has been previously demonstrated with HindIII in T cell clones [26]. Although the combination of HindIII and EcoRI has been widely used to identify clonal populations [21], they will underestimate clonality if not combined with other restriction enzymes, e.g. BamHI, or by using additional probes.

A close correlation was seen between the results from Southern blotting and those from TCR Vβ antibody panel analysis. Although the Vβ antibodies used identified the expansions in only a minority of cases, a reduction in coverage by the Vβ antibody panel was seen in the other LGL patients (Fig. 2). This is likely to represent the presence of an expanded LGL population that does not express any of the five TCR Vβ segments used, with a consequent reduction in the coverage by the panel. Moreover, the Vβ panel results correlated more closely with the sequencing data which we are currently preparing for publication [19]. The significant advantage of the TCR Vβ antibody panel is the speed and ease of the technique compared with Southern blotting, and the avoidance of radiation exposure. Unlike the RFLP analysis, in which the only structural information about the expansions is Cβ1 or Cβ2 usage, the TCR Vβ antibodies provided additional information about TCR usage in these patients (see below). Furthermore, as more anti-human-Vβ MoAbs become available the power of this analysis will be enhanced. This latter technique may therefore be useful in other situations where clonal T cell expansions may be present. PCR-based techniques offer more structural detail, but are in general semiquantitative, and require complex analysis to identify low-level clonality of this type.

Since this study was commenced it has become apparent that there are differences between TCR Vβ usage by CD4 and CD8 cells [27,28]. Infections involving bacterial superantigens, however, can lead to alterations of both the CD4 and CD8 repertoires. Superantigens stimulate T cells by bridging MHC molecules and T cells via an external interaction, independent of the MHC peptide-binding groove, the TCR junctional region sequence, or CD4/CD8 restriction [29]. This can lead to the expansion and subsequent deletion of both CD4 and CD8 cells carrying the relevant Vβ chain(s) [29]. Early studies of RA found consistent alterations of Vβ14 and/or Vβ17 usage [30,31], suggestive of superantigen infection. Later studies, however, failed to corroborate these findings [32,33]. In this study, no pattern of TCR Vβ region usage was identified that was shared by different FS patients with LGL expansions. This does not therefore provide evidence in favour of a role for superantigens in driving these expansions. Although RA patients had a reduction in coverage by Vβ2 and Vβ8 compared with the FS patients and normal controls, these differences failed to reach statistical significance following correction for the number of antibodies examined. Expansions of T cells bearing Vβ2 and Vβ8 have been reported in Kawasaki's syndrome, and this has been proposed to be due to a superantigen [34], although others have failed to corroborate these findings [35]. In other studies of RA peripheral blood using anti-Vβ8-specific antibodies, no reduction was found [36,37].

T cell LGL are substantially increased in chronic viral infections such as cytomegalovirus (CMV) [38] and HIV [39]. They are also seen following splenectomy or bone marrow transplantation [40]. Functionally they are believed to represent anti-viral cytotoxic T cells [41]. The association between LGL expansions and RA/FS could therefore represent a marker of chronic viral infection, and this is an attractive hypothesis as a unifying explanation for RA. Since haplotypes containing HLA-DR4 may be associated with non-responsiveness to hepatitis B virus antigen mediated by CD8 cells [42], the class II association of RA/FS does not exclude a viral etiology. Although a direct suppressive effect of LGL on bone marrow neutrophil production has been difficult to demonstrate [43], the close association of the LGL syndrome with neutropenia is suggestive that the expanded LGL are involved. In RA, the association between persistent neutropenia, clonal LGL expansions, and HLA-DR4 also suggest a critical role for these expansions in the pathogenesis of FS. This remains a fascinating syndrome, and the identification of T cell LGL expansions in FS patients represents a significant advance in our appreciation of this condition. Further dissection of the role of these cells may yield important advances for our broader understanding of the pathogenesis of RA.

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