Circulating TCR γδ cells in the patients with systemic lupus erythematosus

Ewa Robak¹, Jerzy Z. Błoński², Jacek Bartkowiak³, Hanna Niewiadomska⁴, Anna Syxa-Jędrzejowska¹ and Tadeusz Robak²-CA

¹Department of Dermatology and Venerology; ²Department of Hematology; ³Department of Molecular Biology and ⁴Department of Oncology, Copernicus Hospital, Medical University of Lódź, Poland

CA Corresponding Author
Department of Hematology, Medical University of Lódź, Copernicus Hospital, ul. Pabianicka 62, 93–513 Lódź, Poland
Tel./fax: +(48 42) 6846890
Email: robaktad@psk2.am.lodz.pl

SYSTEMIC lupus erythematosus (SLE) is a disorder with a wide range of immunological abnormalities. The results of the studies undertaken in the last decade indicated that SLE pathogenesis was mainly connected with the breakdown of the activation control of B and T cells, generating humoral or cell-mediated responses against several self-antigens of affected cells. The last studies demonstrate that the role of γδ T lymphocytes in autoimmune diseases can be especially important. Flow cytometry techniques were used to investigate the number and percentage of TCR γδ T cells and their most frequent subtypes in peripheral blood of 32 patients with SLE and 16 healthy volunteers. We also correlated TCR γδ cells number with the level of T CD3⁺, T CD4⁺, T CD8⁺, and NK (CD16) cells (cytometric measurements) and SLE activity (on the basis of clinical investigations). Our studies were preliminary attempts to evaluate the role of that minor T cell subpopulation in SLE. Absolute numbers of cells expressing γδ TCR in most SLE blood specimens were significantly lower than in the control group (P < 0.006). However, since the level of total T cell population was also decreased in the case of SLE, the mean values of the percentage γδ T cells of pan T lymphocytes were almost the same in both analysed populations (7.1% vs 6.3%, respectively). In contrast to Vδ2⁺ and Vγ9⁺ subtypes of pan γδ T cells, Vδ3⁺ T cells number was higher in SLE patients (20 × 10⁶ cells/μl) than in healthy control group (2 × 2 cells/μl) (P = 0.001). However, we found no differences between the numbers of pan γδ T lymphocytes and studied their subtypes in the patients with active and inactive disease. These cell subpopulations were doubled in the treated patients with immunosuppressive agents in comparison with untreated ones; however, data were not statistically significant. Our study indicated that Vδ3⁺ subtype of γδ T cells seems to be involved in SLE pathogenesis; however, we accept the idea that the autoimmunity does not develop from a single abnormality, but rather from a number of different events.

Key words: SLE, Lymphocytes, γδ T cells, NK cells, Disease activity

Introduction

Systemic lupus erythematosus (SLE) is a disorder with a wide range of immunological abnormalities. The disease is characterized by B cell activation and formation of autoantibodies against nuclear, cytoplasmic and cell surface antigens. However, increasing evidence indicates a critical role of T cells, particular CD4⁺ cells in inducing B cell hyperactivity. On the other hand, insufficient suppressor cell activity may be responsible for autoantibody overproduction. Defective concanavalin A-induced suppressor cell function and suppressor/cytotoxic responses to Epstein-Barr virus (EBV) have been demonstrated in SLE patients. Most human mature T cells express αβ T-cell receptors on the membrane (TCR αβ) in association with the signal transduction of CD3 complex. These TCR αβ T cells are the central lymphocytes in the immune system. They provide specific pathogen recognition and long-term memory all within the context of distinguishing foreign from self antigens.

Subpopulation of T-cell, which expresses TCR γδ receptors (γδ T cells) was identified 15 years ago but its significance in immune response and pathogenesis of different diseases is still poorly understood. TCR γδ receptors represent a disulphide-linked heterodimer composed of rearranged γ and δ chains.
homologous to the TCR αβ and Ig heavy and light chains, which contribute to specificity of T cells and B cells. Human γδ T cells range from 1% to 15% of peripheral blood lymphocytes and show a predilection for the red pulp of the spleen and the gastrointestinal tract. γδ T cells play a role in host epithelial surface control and early stage engagement in immune response against viruses, bacteria and parasites before the recruitment of αβ T cells. They are able to react with antigens both in a major histocompatibility complex (MHC-) restricted and MHC-unrestricted fashion.

The pathologic relevance of γδ T cells in human autoimmune diseases is suggested by their reactivity to highly conserved stress proteins and by the accumulation of γδ T cells in affected organs. The possible role of γδ T cells in autoimmune disease is also raised by their ability to recognise self antigens. Increased percentages of TCR γδ T cells have been found in the synovial fluids and synovia of patients with active rheumatoid arthritis. A number of studies suggest that γδ T cells play a role in the pathogenesis of systemic sclerosis. Increased numbers of γδ T cells have been found in perivascular areas of the skin and bronchoalveolar lavage samples, especially in patients with recently diagnosed disease. Clonal expansion of TCR γδ T cells has been also reported in the periphery of patients with systemic lupus erythematosus (SLE). Furthermore, Volc-Platzer et al. described preferential expansion of Vγ2/Vδ2 subset in lesions in chronic cutaneous lupus erythematosus.

In the present study we measured the number of total circulating TCR γδ T cells and their subpopulations in 32 SLE patients and in 16 healthy volunteers using monoclonal antibodies against pan-γδ, Vδ2, Vδ3, Vγ9 chain regions and flow cytometry techniques. We also correlated the number of γδ T cells with T CD3+, T CD4+, T CD8+ and NK (CD16) cells and SLE activity.

### Patients and methods

#### Patients

A total of 32 unselected patients with SLE, 30 women and two men all fulfilling the 1982 revised criteria defined by the American Rheumatism Association (ARA) were included in our study. Their mean age was 43.7 years (range 22–65 years). The mean duration of the disease was 84.2 months (range 3 months to 28 years). Ten patients had never been treated with steroids or any other immunosuppressive agents, 22 patients had been treated with prednisone and two of them with azathioprine for some time during the course of their disease, but 12 of them had not been treated for at least 4 weeks before the γδ T cell population analysis.

We included both patients with active and inactive disease into the study. Disease activity was scored during a visit to the outpatient clinic according to the method described by Liang et al. Each patient was assessed on two separate occasions, 2–4 weeks apart. The system of Systemic Lupus Activity Measure (SLAM) includes 24 clinical manifestations and eight laboratory parameters. The maximum score in this system amounts to 84 points. In our group of patients, the points ranged from 6 to 26. We considered the score of 0–10 points indicative of inactive disease, and a score of over 10 points indicative of active disease. This decision was based on our previous observations that patients with score 10 had no clinical symptoms of active disease such as photosensitivity, fever, polyarthritis, serositis, an elevated erythrocyte sedimentation rate or a high antinuclear antibody level (ANA). A similar distinction between active and inactive disease was also performed by other authors. Our group of patients included 9 patients with inactive and 23 patients with active disease. The clinical and laboratory features of SLE patients are presented in Table 1. The control group of 16 healthy volunteers was also studied. They were 12 women and 4 men, aged from 37 to 56 years (mean 48 years). Each underwent a thorough physical evaluation by one of the authors (ER).

#### Laboratory tests

On the day of blood sampling for T cells immunophenotyping, the following laboratory parameters were analysed: complete blood cell count (CBC), erythrocyte sedimentation rate, blood urea nitrogen and creatinine levels, fibrinogen level, partial thromboplastin time (PTT), liver function tests (GOT, GPT, bilirubin), immunoglobulins (IgG, IgA, IgM) and complement (C5, C4), urine and creatinine levels, and anti-DNA antibodies. Chest X-rays and ECG were also evaluated.
Immunophenotype analysis

Venous blood samples were collected at the time of clinical assessment into pyrogen-free tubes, containing anticoagulant (EDTA at a final concentration of 25 mM). General lymphocyte immunophenotyping and TCR diversity analysis were performed by standard two-colour immunofluorescence measurement. The details of the procedure are described elsewhere. Briefly a combination of phycocyanin (PE)-conjugated and fluorescein isothiocyanate (FITC)-conjugated monoclonal (MoAbs) was used. In polystyrene tubes 100 μl of whole blood were directly stained with 10 μl appropriate MoAbs in the dark at room temperature. IgG1 isotype control antibody conjugates were included in order to establish the background fluorescence. After incubation for 30 min, the samples were placed to Q-prep (Coulter) for lysis of erythrocytes and fixation of nuclear cells. At least 10,000 cells were then analysed on a Coulter Epics-XL flow cytometer (Coulter, Hialeah, FL, USA). Gate Check was used to gate lymphocyte population defined by FS/SS and anti CD14 and CD45RO MoAbs. Analysis was performed using XLv2 software.

Monoclonal antibodies

The direct staining of cells by monoclonal antibodies (MoAbs) was performed. We were able to use only commercially prepared MoAbs conjugated with proper fluorochrome-PE-conjugated UCHT1 (CD3⁺, pan T), IMMU510 (all γδ T cells), FITC-conjugated 13B8.2 (CD4⁺ T cells), B9.11 (CD8⁺ T cells), 3G8 (CD16⁺, mainly NK and at some percentage γδ T cells), IMMU510 (all γδ T cells), IMMU389 (Vδ2, specific domain of δ chain TCR), P11.5B (Vδ3, specific domain of δ chain TCR), IMMU360 (Vγ9, specific domain of γ chain TCR), were all supplied by Immunotech (A Coulter Company, USA). The anti-Vδ1 (Immunotech) was available as the plain proteins and was not applied for staining in whole blood samples together with other MoAbs. It was used for indirect phenotyping of isolated lymphocytes. In these experiments the goat/anti-mouse IgG (FITC and PE conjugated F(ab'₂)₂ fragments) was used for counterstaining.

Statistical analysis

The analysis of the results indicated that normal distribution was observed for almost all studied variables. We presented our calculations as a mean ± SD. The universal Mann–Whitney U test was used for determination of differences in quantity of studied cell populations. For phenotypic feature correlation between studied T and NK cell populations, measured simultaneously in the same patient, we used the Spearman rank test. Statistically characteristic changes were considered at $P<0.05$.

Results

The γδ TCR expression on peripheral blood T lymphocytes from 32 SLE patients and 16 healthy donors has been determined using standard double-colour immunofluorescence measurement. Their clin-

| Table 2. Characteristics of peripheral blood parameters in SLE patients and healthy donors (mean values ± SD and range in parentheses) |
|---------------------------------------------------------------|
| **Characteristic** | SLE patients | Controls | $P$ value |
|---------------------|--------------|----------|-----------|
| Hb g/dL             | 12.4 ± 1.5   | 14.2 ± 1.5 | NS        |
|                     | (9.7–17.3)   | (12.4–16.0) |           |
| Platelets (10⁶/L)   | 194.0 ± 68.9 | 258.4 ± 89.9 | 0.005     |
|                     | (30.0–369.0) | (156.5–426.4) |         |
| WBC/μL              | 4851.0 ± 2055.0 | 7148.0 ± 1635.0 | 0.008    |
|                     | (2320.0–10450.0) | (5540.0–10100.0) |     |
| Pan T (CD3⁺) cells/μL | 630 ± 330   | 1025.0 ± 256.0 | 0.003    |
|                     | (170–1360)   | (603.0–1415.0) |          |
| T CD4⁺ cells/μL     | 220 ± 140    | 500 ± 180  | 0.001     |
|                     | (440–570)    | (310–890)  |           |
| T CD8⁺ cells/μL     | 360 ± 230    | 450 ± 140  | NS        |
|                     | (90–830)     | (200–620)  |           |
| Ratio CD4⁺/CD8⁺ cells | 0.77 ± 0.54 | 1.24 ± 0.21 | 0.005     |
|                     | (0.25–1.50)  | (0.56–1.70) |           |
| NK cells/μL         | 120 ± 100    | 290 ± 170  | 0.004     |
|                     | (10–770)     | (60–550)   |           |
| Pan B (CD19⁺) cells/μL | 640 ± 540   | 530 ± 220  | NS        |
|                     | (95–3760)    | (300–1020) |           |
ical data are summarised in Table 1. Detailed characteristics of peripheral blood parameters, both for SLE patients and for the healthy donors, are shown in Table 2. The absolute numbers of WBC, pan T CD3⁺, CD4⁺, CD8⁺ cells and NK cells were significantly lower in SLE patients than in the control group. In contrast, the absolute numbers of cytotoxic/ suppressor (CD8⁺ CD3⁺) and pan B (CD19⁺) cells in both groups were similar.

The mean absolute values and percentages of γδ T cells and their subpopulations in peripheral blood of SLE patients and in healthy individuals are presented in Table 3. The mean absolute number of pan γδ T cells was lower in SLE patients (40 ± 30/μl) than in controls (67 ± 39/μl) (p=0.006). However, the percentage of γδ T cells of pan T cells was similar in both groups (7.1% ± 6.5% and 6.3% ± 3.9%, respectively) (P=0.7). A comparable decrease of circulating Vδ2 and Vγ9 subtypes of pan γδ T cells in SLE patients (20 ± 10 cells/μl and 20 ± 20 cells/μl, respectively) in relation to normal controls (50 ± 41 cells/μl and 49 ± 40 cells/μl, respectively) (P=0.003 and 0.005) was observed. On the other hand, the absolute number of Vδ3⁺ subtypes of γδ cells was significantly higher in peripheral blood of SLE patients (20 ± 10 cells/μl) than in control donors (2 ± 21 cells/μl) (P= 0.001). A similar difference was noted in the percentage of this subgroup in both groups (4.2% ± 5.7% vs 2.0% ± 0.1%, respectively; P=0.0003). Although the above calculated values for Vδ3⁺ cell subtypes were different for particular SLE patients (see ranges and SD in Table 3), we found in patients blood specimens a distinct positive correlation between peripheral blood absolute numbers of γδ T cells and the number of Vδ3⁺ cells (R=0.85, P=0.0001) (Fig. 1). This very high correlation coefficient for Vδ3⁺ lymphocytes provided additional strong evidence, that this γδ T subtype could play some role in SLE activity.

It should be noticed that γδ T cell levels practically did not fluctuate if the analytical tests for particular patients were repeated two or three times. However, the follow up of γδ T lymphocytes changes during disease development were not done.

Data presented in Table 3 showed that three-quarters of pan γδ T cells in the blood of healthy individuals expressed Vδ2 and Vγ9 TCR chains. Although the above result was obtained in separate staining measurements, we can univocally conclude that the subfraction Vδ2/Vγ9 is the most frequent in normal blood, as stated in several works.⁷ In SLE patients the proportion of Vδ2 and Vγ9 changed, but the usage of Vγ9 in TCR structure is still high. In both materials the percentage of the Vδ1 subpopulation was low. As we mentioned in Patients and Methods, the quantities of Vδ1⁺ were evaluated by the direct staining method. It is known that there are discrepancies of relative cell percentages determined by direct and indirect staining procedures. We observed 2 cells/μl (0.4%) in SLE patients and 11 cells/μl (1.1%) in healthy donors with Vδ1⁺ phenotype. These results confirmed data which could be calculated from Table 3 (pan γδ T cells minus Vδ2⁺ and Vδ3⁺ subfractions; other unique Vδ cells can be neglected). However, we have decided to present only the results (Tables 3 and

### Table 3. Analysis of γδ TCR expression on circulating T cells of SLE patients and healthy donors (mean of cells number/μl ± SD and range in parentheses)

| Cell Subpopulation | Number of γδ cells/μl | Percentage γδ T cells of pan T cells |
|--------------------|-----------------------|-------------------------------------|
|                    | SLE Mean ± SD (range) | Control Mean ± SD (range) | P value | SLE Mean ± SD (range) | Control Mean ± SD (range) | P value |
| Pan γδ TCR⁺        | 40 ± 30 (0–140)       | 67 ± 39 (19–154) | 0.006  | 7.1 ± 6.5 (1.2–26.9) | 6.3 ± 3.9 (1.5–13.1) | 0.7    |
| Vδ2 TCR⁺           | 20 ± 10 (0–100)       | 50 ± 41 (3–147)  | 0.003  | 3.6 ± 4.1 (0.15–19.6) | 4.5 ± 3.6 (0.3–12.5) | 0.03   |
| Vδ3 TCR⁺           | 20 ± 10 (0–100)       | 2 ± 2 (0–7)    | 0.001  | 4.2 ± 5.7 (0–21.4)  | 0.2 ± 0.1 (0.1–0.6)  | 0.0003 |
| Vγ9 TCR⁺           | 20 ± 20 (0–110)       | 49 ± 40 (3–131) | 0.005  | 4.1 ± 4.6 (0.4–21.2) | 4.1 ± 3.0 (0.3–11.1) | 0.1    |

**FIG. 1.** Correlation between pan γδ⁺ T cells number and the number of Vδ3⁺ cells in SLE patients.
According to our data the absolute numbers of cells weakly expressed CD8 antigen, as an activation effect of cells involved in autoimmune response (data not shown).

Our observation concerning V63+ cells’ expansion in SLE patients is the first demonstration that this subset of γδ T lymphocytes seems to be involved in SLE pathogenesis. Previously published data indicated the increase of pan γδ T cells in peripheral blood from patients with this disease. Other works characterised expanded subtypes as the polyclonal V61+ and V62+ or Vγ2/Vδ2+ oligoclonal subsets. These discrepancies probably arose as the result of different features of analysed disease in particular groups of patients, and indicated that changes of several parameters are involved in the disease development.

However, our results are to some extent unexpected, because most of the studies undertaken so far indicate that total levels of pan γδ T cells (calculated both as the cell number/μl and as the relative percentage of pan T CD3+ lymphocyte fraction) were significantly higher in peripheral blood of patients with autoimmune diseases than in healthy donors. Janadi et al. observed the expansion CD4+ CD29+ γδ T cells in the peripheral blood and synovial fluid of patients with rheumatoid arthritis (RA). The elevation of pan γδ T cells in the same materials of RA patients was stated by Keystone et al. It was also demon-

---

### Table 4. The frequency of γδ T cell subtypes in peripheral blood of patients with SLE dependent on disease activity and immunosuppressive treatment (mean of cells number/μl ± SD and range in parentheses)

| Cell Subpopulation | SLE activity | Immunosuppressive treatment |
|--------------------|--------------|-----------------------------|
| Active N=23        | Inactive N=9 | P value                     |
| pan γδ TCR+        | 41 ± 38      | 47 ± 49                     | NS                           |
| (4–125)            | (14–146)     |                             |                              |
| Vγ2 TCR+           | 19 ± 24      | 26 ± 27                     | NS                           |
| (1–99)             | (4–70)       |                             |                              |
| Vγ3 TCR+           | 22 ± 29      | 28 ± 29                     | NS                           |
| (0–108)            | (5–90)       |                             |                              |
| Vγ9 TCR+           | 21 ± 26      | 29 ± 27                     | NS                           |
| (1–107)            | (4–82)       |                             |                              |
| Treated N=22       | Untreated N=10| P value                     |
| pan γδ TCR+        | 49 ± 45      | 27 ± 24                     | NS                           |
| (4–146)            | (6–79)       |                             |                              |
| Vγ2 TCR+           | 24 ± 28      | 13 ± 11                     | NS                           |
| (1–99)             | (4–33)       |                             |                              |
| Vγ3 TCR+           | 28 ± 31      | 14 ± 18                     | NS                           |
| (0–108)            | (2–52)       |                             |                              |
| Vγ9 TCR+           | 27 ± 29      | 15 ± 15                     | NS                           |
| (2–107)            | (3–45)       |                             |                              |
strated in many investigations that the involvement of γδ T lymphocytes in the pathogenesis of autoimmune diseases was most likely, because their expansion was always significant. It has been discussed in the studies concerning coeliac disease, multiple sclerosis, autoimmune thyroid diseases, autoimmune liver disease and systemic sclerosis. However, in the cases of the above-specified diseases, the accumulation of γδ T cells occurred predominantly in pathologically changed tissues; their increases in peripheral blood of the same patients were less evident or even unnoticeable. We feel obliged to say that our study of peripheral blood cells was dictated by the pragmatic goal to find a simple, highly standardised test for some aspects of SLE diagnosis. Of course, we knew that the cell representation in blood was only the approximate image of the actual situation in affected tissues, but the reciprocal circulation of T lymphocytes in an organism could allow the description of real changes in γδ T cells, as it is observed in tumor infiltrating lymphocytes and peripheral circulation cell populations.

In the case of our SLE patients investigation, the decrease of pan γδ T cells number (and similar events for Vδ2+ and Vδ9+ subtypes) partly resulted from general pan T lymphopenia, which was more intensive than in other SLE studies. Another reason for that phenomenon could be connected with the capacity of γδ T lymphocytes to very strong infiltration and pathological damage of target tissues, as skin and kidneys. Such accumulation, particularly Vγ2/Vδ2 TCR expressed cells, was observed in disease-damaged skin of patients with chronic cutaneous lupus erythematosus and SLE. Probably in our case the Vδ3+ fraction responded to autologous immune antigens. The persistent treatment of the most patients with glucocorticoids could be also responsible for the lower numbers of γδ T cells in peripheral blood from SLE individuals than in specimens from control donors. Several published data demonstrated that long-continued immunosuppressive therapy determined the disappearance of expanded γδ T cell subset, in both target tissues, and the peripheral blood of patients with polymyositis or other autoimmune diseases. Spinozzi et al. demonstrated data obtained from in vitro experiments that all T lymphocytes bearing the γδ TCR (isolated both from SLE patients and healthy individuals) were susceptible to dexamethasone, and steroid-induced apoptosis was basic mechanism responsible for cells death. They also demonstrated that 6 month glucocorticoids treatment normalised the increased SLE γδ T cell subfraction in blood, simultaneously with clinical remission of the disease symptoms.

In our studies the analyses of immunosuppressive treatment influence on γδ T cell level were not such univocal. In all cell subtypes their amounts were two times higher in the treated patients than in untreated ones (but always lower than in specimens from control donors). It could be considered as a reconstruction process of the initial γδ T cells status, typical for normal blood. A relatively low number of studied cases can be responsible for statistical doubts. The reason why it was also observed for Vδ3+ T cells, which seem to compose the unique subtype in healthy individuals’ blood, is unclear. A simple look at the data presented in Table 4 may lead to the conclusion that the frequencies of total γδ T cells and their particular subtypes in SLE patients were not generally determined by disease activity, evaluated according to the method of Liang et al. But statistically important, objective data could probably be obtained...
during the analysis of affected tissues. We registered the cell distribution in peripheral blood, which probably reflected only some functional tendencies occurring in the tissue. The correlation between SLE activity and concentrations of some cytokines and their soluble receptors was detected in our previous investigations.26,27

Data from Table 2 and Figure 2 indicate, that although absolute amounts of pan T CD3+ cells, T helper CD4+ cells and NK cells were significantly reduced in SLE patients, variations of T CD4+ and NK cells were not correlated with changes of pan γδ T cells. In such case the influence (if any) of compared cell populations on SLE development is independent.

At present there is little known about the function of γδ T cells in SLE pathogenesis. The recent experiments confirm that they really play a significant role in autoimmune regulation in vivo, but some authors published contrary data, suggesting γδ T lymphocytes’ responsibility for down-regulation of autoimmune diseases.5,38 The work of Peng et al.39 was the first demonstration in vivo that mice with γδ T cells deficiency (TCRγδ/MRL/lpr) developed a significantly more severe lupus-like disease and their mortality was twice that in normal MRL/lpr mice. However, γδ T lymphocytes caused also down-regulation of the γδ T cell response to infection and thus they could intensify the autoaggressive testis in inflammation evoked by Listeria infection.40 Additionally it was shown that γδ T cells promoted the B cell mediated autoimmunity.41

All the above observations may indicate that different subtypes (or distinct clones) of γδ T lymphocytes realise separate, sometimes even opposite functions in induction of autoreactive immune responses, for example in SLE. It should be noted that their function is realised very early in ontogeny, and further disturbances of several metabolic pathways can change the final effects. We believe that the indicated observations do not reflect epiphemones, but univocal evidence of it has to be verified by additional experiments. We have initiated our further studies based on more cases, included cytometric analysis with cells isolated from affected tissues and proper controls. We have also extended our studies for molecular tests (RTFPCR detection of γδ TCR discrete, minor subpopulations, characterisation of their genomic structure and determination of clonality of expanded γδ T cells subtypes). Our study indicated that Vδ3+ subtype of γδ T cells seems to be involved in SLE pathogenesis; however, we accept the view that the autoimmunity does not develop from single abnormality, but rather from a number of different events.

REFERENCES

1. Steinberg AD, Klinman DM. Pathogenesis of systemic lupus erythematosus. Rheum Dis Clin North Am 1988; 14:25–41
2. Szaek AJ, Noessner JC, Sneed AF. Systemic lupus erythematosus. Int J Lab Invest 1992; 22:180–90
3. Linker-Israeli M, Quismorio FP Jr, Howitz DA. CD8+ lymphocytes from patients with systemic lupus erythematosus sustain, rather than suppress, spontaneous polyclonal IgG production and synergize with CD24+ cells to support autoreactive B cell responses. Arthritis Rheum 1990; 33:1216–23
4. Ishida H, Kamagai S, Uehara H, Sano H, Tagaya Y, Yodoi J, Imura H. Improved expression of high affinity interleukin 2 receptor on activated lymphocytes from patients with systemic lupus erythematosus. J Immunol 1987; 139:1070–4
5. Sakane T, Steinberg AD, Green I. Studies of immune functions of patients with systemic lupus erythematosus: 1. Dysfunction of suppressor T cell activity related to impaired generation of, rather than response to, suppressor cells. Arthritis Rheum 1978; 21:657–64
6. Tsikos GC, Magrath JT, Below JE. Epstein-Barr virus induces normal B cell responses but defective suppressor T cell responses in patients with systemic lupus erythematosus. J Immunol 1983; 131:1797–803
7. Bluestone JA, Khatri R, Sciammas R, Sperling AL. TCRγδ cells: a specialized Tcell subset in the immune system. Annu Rev Cell Dev Biol 1995; 11:507–53
8. Brenner MB, McLean J, Dakyns DR, Struminger JL, Smith JA, Owen FL, Seidman JG, Rosen F, Krangel MS. Identification of a putative second T-cell receptor. Annu Rev Immunol 1995; 13:1–40
9. Lew AM, Pardoll DM, Malay WJ, Powles BJ, Kruisbeek A, Cheng SE, et al. Characterization of T cell receptor gamma chain expression in a subset of murine thymocytes. Science 1986; 234:140–1
10. Davis MM and Bjorkman PJ. Tcell antigen receptor genes and T cell recognition. Nature 1986; 324:345–402
11. Bordewoude D, Gaulard P, Mason DW. Preferential localisation of human lymphocytes bearing gamma delta T cell receptors under the red pulp of the spleen. J Clin Pathol 1990; 43:461–4
12. Jarry A, Gerf-Bensussan N, Brousse N, Sch F, Guy-Grand D. Subsets of CD3gδ T cell receptor of γδ T and γδ CD4 lymphocytes isolated from normal human gut epithelium display phenotypical features different from their counterparts in peripheral blood. Eur J Immunol 1990; 20:1097–103
13. De Libero G. Sentinel function of broadly reactive human γδ T cells. Immunol Today 1997; 18:22–6
14. Hyday A. Autoimmunity is antigen-specific suppression now unexplained? Curr Biol 1995; 5:47–50
15. Hyday A, Gring L. γδ T cells regulate autoimmunity. Curr Opin Immunol 1997; 9:884–90
16. Kaufmann SH. Heat shock proteins and the immune response. Immunol Today 1998; 11:129–36
17. Stinissen P, Vandegoer G, Medrart R, Vandegraaf L, Nies J, Tuyls L, Hafler DA, Raus J, Zhang J. Increased frequency of γδ T lymphocytes from normal human gut epithelium: aspecialized T-cell subset in the immune system. J Clin Immunol 1989; 9:190–5
18. Volc-Platzer B, Anegg B, Milota S, Pickl W, Fisher G. Accumulation of γδ T cells in systemic sclerosis patients. J Clin Immunol 1994; 14:327–34
19. Yurovsky VV, Sutton PA, Schulze DL, Wigmuy FP, Nise RA, Howard BR, White B. Expansion of selected Vδ1+ γδ T cells in systemic sclerosis patients. J Immunol 1994; 153:881–91
20. Olive G, Gatenby PA, Sciejanszon FW. Restricted junctional diversity of T cell receptor delta gene rearrangements expressed in systemic lupus erythematosus (SLE) patients. Clin Exp Immunol 1994; 97:430–40
21. Yole-Platzer B, Anegg B, Mota S, Pickl W, Fisher G. Accumulation of γδ T cells in chronic cutaneous lupus erythematosus. J Invest Dermatol 1993; 100:845–915
22. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NE, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; 25:1217–25
23. Liang MH, Socher SA, Larson MLX, Schur PH. Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arthritis Rheum 1989; 32:1107–18
24. Robak E, Sya-Krzewiowska A, Stepich H, Robak T. Circulating interleukin 6 type cytokines in patients with systemic lupus erythematosus. Eur J Clin Immunol 1997; 28:821–3
25. Linker-Israeli M, Deans RJ, Wallace DJ, Prehn J, Otter-Chen T, Klinenberg JR. Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. J Immunol 1991; 147:17–23
29. Bartkowiak J, Blonski JZ, Niewiadomska H, Kukrzycka D, Robak T. Characterization of γδ T cells in peripheral blood from patients with B-cell chronic lymphocytic leukaemia. *Biomed Letters* 1998; 58:19–30

30. Gerli R, Agea E, Bertotto A, Tognellini R, Flegi L, Spinuzzi E, Velardi A, Gignani F. Analysis of T cells bearing different isotypic forms of the γδ T cell receptor in patients with systemic autoimmune diseases. *J Rheumatol* 1991; 18:1304–10

31. Spinuzzi F, Agea E, Bistoni O, Travetti A, Migliorati G, Moraca R, *et al*. γδ T lymphocytes bearing γδ T cell receptor are susceptible to steroid induced programmed cell death. *Scand J Immunol* 1995; 41:504–8

32. al-Janadi M, al-Balla S, al-Dalaan A, Raziuddin S. Cytokine production by helper T cell populations from the synovial fluid and blood in patients with rheumatoid arthritis. *J Rheumatol* 1993; 20:1647–53

33. Keytton F, Rutishauer C, Wood N, Snow KM, Hatow J, Purvis JC. Elevation of γδ T cell subset in peripheral blood and synovial fluid of patients with rheumatoid arthritis. *Clin Exp Immunol* 1991; 84:78–82

34. Halstensen TS, Scott H, Brandzeg P. Intraepithelial T cells of TCR γδ+ and Vδ1/Jδ1+ phenotypes are increased in coeliac disease. *Scand J Immunol* 1989; 30:665–72

35. Selmay K, Brosnan CE, Raine CS. Colocalization of lymphocytes bearing γδ T cell receptor and heat shock protein hsp 65+ oligodendrocytes in multiple sclerosis. *Proc Natl Acad Sci USA* 1991; 88:6452–6

36. Roura-Mir IC. γδ lymphocytes in endocrine autoimmunity: evidence of expansion in Graves' disease but not in type I diabetes. *Clin Exp Immunol* 1993; 92:288–95

37. Martins SE, Graham AK, Chapman RW, Fleming KA. Elevation of γδ T lymphocytes in peripheral blood and livers of patients with primary sclerosing cholangitis and other autoimmune liver diseases. *Hepatology* 1996; 23:988–93

38. Hohlfeld R, Engel AG, Li K, Harper MC. Polymyositis mediated by γδ lymphocytes that express the γδ receptor. *N Engl J Med* 1991; 324:877–81

39. Peng SL, Madaio MP, Hayday A, Craft J. Propagation and regulation of systemic autoimmunity by γδ T cells. *J Immunol* 1996; 156:5689–98

40. Mukasa A, Hiromatsu K, Matsuzaki G, O'Brien R, Borz W, Namoto K. Bacterial infection of the testis leading to autoggressive immunity triggers apparently opposed responses of alpha beta and gamma delta T cells. *J Immunol* 1995; 155:2047–56

41. Peng SL, Madaio MP, Hughes DE, Crispe IN, Owen MJ, Wen L, Hayday A, Craft J. Murine lupus in the absence of γδ T cells. *J Immunol* 1996; 156:4041–9

Accepted 24 January 2000