The systemic and SmD1\textsuperscript{83–119}–autoantigen-specific cytokine memory of Th cells in SLE patients

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Objectives. The aim of the study was to analyse the cytokine memory of T-cells derived from systemic lupus erythematosus (SLE) patients and healthy donors enriched for autoantigen-specific T-cells by in vitro stimulation with SmD1\textsuperscript{83–119}, a common autoantigen in SLE.

Methods. Autoreactive CD3\textsuperscript{+} T-cells derived from 37 SLE patients and 14 healthy donors were enriched by repetitive ex vivo stimulation of peripheral blood mononuclear cells (PBMCs) with SmD1\textsuperscript{83–119}. For control, PBMCs were stimulated only with interleukin-2 (IL-2). After two rounds of antigenic stimulation, cultures were stimulated with PMA/ionomycin to probe the cytokine memory by intracellular cytokine staining. Frequencies of cytokine-expressing T-cells were analysed and, in SLE patients, compared with disease activities and autoantibody levels.

Results. Comparing the cytokine memory in the cultures, SLE patients displayed higher frequencies of tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\))\textsuperscript{+} T-cells than healthy donors and the frequencies correlated with disease activity. Frequencies of SmD1\textsuperscript{83–119}–specific TNF-\(\alpha\)\textsuperscript{+} T-cells and of memory T-cells expressing interferon-\(\gamma\) (IFN-\(\gamma\)) correlated with serum anti-dsDNA antibody levels. The frequencies of IL-10 expressing SmD1\textsuperscript{83–119}–specific T-cells were lower among PBMCs of SLE patients. Relatively higher frequencies of IL-10\textsuperscript{+} T-cells in SLE patients correlated with low disease activities, and low anti-dsDNA and anti-SmD1\textsuperscript{83–119} antibody concentrations in culture supernatants.

Conclusions. The memory of autoreactive SmD1\textsuperscript{83–119}–specific and unspecific stimulated peripheral Th cells for re-expression of cytokines is shifted towards more cells expressing TNF-\(\alpha\) and less IL-10\textsuperscript{+} cells, when compared SLE patients with normal donors. This shift towards proinflammatory memory effector Th cells correlates with disease severity and humoral autoimmunity.

Key words: SLE, T-cell cytokines, Cytokine memory, IL-10+ T-cells, Autoantigens.

Introduction

In systemic lupus erythematosus (SLE), the increased number of activated and affinity-maturated T-cells as well as the presence of autoreactive T-cells suggests an impaired T-cell effector function that seems to be restricted to a distinct panel of autoantigens [1]. The impaired effector function is usually best exemplified by the pattern of cytokines produced [2]. Therefore, characterization of T-cell cytokine expression, especially of autoantigen-specific T-cells, is central to understand T-cell help in the etiopathogenesis of lupus.

T-cell cytokines are involved in the control of B- and T-cell interaction and, therefore, are target candidates as regulators of pathogenicity in SLE. Interferon-\(\gamma\) (IFN-\(\gamma\)) stimulates B-cells [3, 4], induces B- and plasma-cell migration into the inflamed tissue [5] and plays a pivotal role in directing cell-mediated immune responses. The increased expression of IFN-\(\gamma\) in autoreactive T-cell clones indicates a role for IFN-\(\gamma\)+ T-cells in lupus pathogenesis [4]. Interleukin-4 (IL-4), termed ‘B-cell growth factor’, also induces B-cell migration [6] and class switch to the immunoglobulin (Ig) isotypes G1/G4 over-represented in SLE [7, 8]. IL-10 induces differentiation of B-cell blasts into plasma cells and also participates in the control of isotype switching towards IgG1 and IgG3 [8] isotypes also over-represented in SLE [9]. Furthermore, IL-10 has a regulatory function in lupus, (summarized in [10, 11]) especially when produced by T-cells, and recently, autoantigen-specific IL-10 expressing T-cells were shown to decrease anti-dsDNA antibody generation in vitro [12]. Tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) is an important proinflammatory cytokine in lupus and other autoimmune diseases [13]. Furthermore, it is a survival factor for plasma cells [14, 15]. Naive and central memory T-cells do not produce effector cytokines except IL-2. Upon primary activation, naive T-cells become instructed for expression of cytokines. Repetitive stimulations with the corresponding antigen result in a cytokine memory with a faster recall expression of previously instructed cytokines without the requirement of the original instructing signal. This allows a stable effector function [16, 17]. Therefore, T-cells expressing effector cytokines such as IFN-\(\gamma\), IL-4, IL-10 and TNF-\(\alpha\) exhibit effector/memory cells previously instructed by antigens or, in SLE, by autoantigens.

Studying the T-cell cytokine memory among all freshly isolated memory Th cells, most authors have not found any differences between healthy donors and lupus patients [18–20]. This is probably related to the low frequencies of autoreactive T-cells, which is considered to be \(<1\) per 10\textsuperscript{5} T-cells [21, 22]. Furthermore, the missing differences in the T-cell cytokine memory may reflect the heterogeneity of the patients, or the
40 ml of venous blood were collected using heparinized patients and healthy donors. rIL-2 were added to the cultures. Some days later (median [Roswell Park Memorial Institute (RPMI) 1640 (all supplements (BSA) and split: One fraction was suspended in growth medium twice in Phosphate Buffer Saline (PBS)/Bovine Serum Albumine Pharmacia Biotech AB, Sweden) density centrifugation, washed due to extended culturing and repeated restimulation. The T-cell cytokine memory was correlated with clinical findings and autoantibody concentration in the culture supernatants.

Material and methods

Patients

This study was approved by the Ethics Committee of the Charité University Hospital, and is in compliance with the Helsinki Declaration. Blood samples were obtained, after written consent from 37 patients with SLE and 14 healthy donors. Patients fulfilled the American Congress of Rheumatology (ACR) classification criteria for SLE [23]. Most of the patients received immunosuppressive drugs (14 received azathioprine, three cyclophosphamide, four mycophenolate, six hydroxychloroquine, two methotrexate, one leflunomide, one ciclosporin-A, and three patients had a combination of two different immunosuppressive drugs). Two patients received steroids in a dosage >10 mg/day. The disease activity was measured by SLE Disease Activity Index (SLEDAI) as determined elsewhere [24]. Three patients were tested twice at 6-month intervals; one of them with different disease activity, the others remained stable.

Antigen

The SmD183-119 peptide (VEPKVKKREAVAGRGRGGR RGGRG-RRGGPR) was synthesized as free peptide according to the protocol described by Atherton [25] and purified by a reverse phase chromatography (C18 Vydac column).

Isolation of PBMCs and culture conditions. Approximately 40 ml of venous blood were collected using heparinized S-monovettes (Sarstedt, Nümbrecht, Germany) from SLE patients and healthy donors. PBMCs were isolated by Ficoll Paque™ PLUS (Amersham Pharmacia Biotech AB, Sweden) density centrifugation, washed twice in Phosphate Buffer Saline (PBS)/Bovine Serum Albumine (BSA) and split: One fraction was suspended in growth medium [Roswell Park Memorial Institute (RPMI) 1640 (all supplements 2 mM t-glutamine, PAA Laboratories, Coelbe, Germany), 5% heat inactivated human AB serum (PAA Laboratories, Coelbe, Germany), and cultured at 37 °C and 5% CO2]. The other fraction was resuspended in chilled freezing medium (70% RPMI 1640, 20% fetal bovine serum, 10% DiMethyl Sulfoxide (DMSO)) at the concentrations of approximately 10 × 106 cells/ml and immediately frozen to −70 °C.

At day 3, one portion of the cultured cells received 10 μg/ml of the SmD1-peptide, the other portion did not receive any. At day 7, both cultures received 5 U/ml of recombinant (r)IL-2 (Stratham Biotec AG, Hamburg, Germany). At day 11, the frozen cells were quickly thawed by adding growth medium at room temperature and washed in a high surplus of growth medium. These cells were γ-irradiated (30 Gy, 10 min) and added as autologous feeder cells to the respective cultures. In addition, 10 μg/ml of the SmD1-peptide was given only to the culture previously stimulated with the SmD183-119 peptide. At day 18, 5 U/ml of rIL-2 were added to the cultures. Some days later (median at day 22), supernatants of the culture pairs (with and without SmD1 stimulation) were harvested and cells were restimulated with PMA (10 ng/ml) and ionomycin (250 ng/ml) for 6 h. After 3 h, 10 μg/ml brefeldin A was added to the cells.

The cells were harvested, washed in PBS and fixed in 2% paraformaldehyde for 20 min at room temperature. After washing with PBS/BSA, the cells were stored in PBS/BSA/acid at 4 °C overnight. The variability of the used culture system was controlled using PBMCs from healthy donors, demonstrating reproducible results in parallel cultures of cells from the same donor (no outlier values, s.d. comprising 9–54% of the mean value depending on the produced cytokine).

Intracellular cytokine staining and flowcytometric analysis. The cells were first subjected to anti-CD3-Biotin and then to streptavidin-Peridinin-chlorophyll-protein complex (PerCP) (PharMingen), then divided into two groups. One group was stained with anti-IFN-γ-Fluorescein IsoThioCyante (FITC), anti-IL-4-Phycocerythin (PE) and anti-IL-10-Cy5, the other group with anti-TNF-α-FITC, anti-IL-2-PE and anti-IL-4-Cy5. 16 cultures derived from 5 SLE patients were also stained for Cluster of Differentiation (CD)25 (anti-CD25-FITC, PharMingen, San Diego, CA, USA). For intracellular and surface staining, the following monoclonal antibodies (all from PharMingen) were used according to the manufacturer's instructions: IFN-γ-FITC (clone N748A and clone 4SB3), TNF-α-FITC (clone 1D12), IL-2-PE (clone N7.4A), IL-4-PE (clone 4D9), CD3-Biotin (clone UCHT1), IL-4-Cy5 (clone 4D9, clone 4D9), IL-10-Cy5 (clone 3.19F1).

Intracellular staining was performed in PBS/BSA/acid containing 0.5% saponin. All cells were blocked with 10% polyclonal human IgG (Flebogamma, Grifols, Langen, Germany) in a total of 50–70 μl staining volume. The incubation time for surface staining was 10 min, and 15 min for intracellular staining. Samples were analysed by a 4-colour-analysis using Fluorescence Activated Cell Sorting (FACS). Calibror flow cytometer (Becton Dickinson) and FACS-Express software, version 2.0.

Serum anti-SmD183-119 peptide antibodies were detected by enzyme-linked immunosorbent assay (ELISA) as described [26]. Reactivity of a standard serum at a dilution of 1:100 was defined as 10 000 arbitrary units (AU). Supernatants were diluted 1:2, sera were analysed at dilution 1:100. Serum reactivity above 123 AU was defined as positive as described before [26]. Anti-dsDNA antibodies were detected by ELISA as described previously [27]. Serum were tested at dilution 1:100, supernatants at dilution 1:2. Serum levels above 6 μg/ml were defined as positive.

Statistics. Graphpad Prism 3.0 was used for statistical analysis. The frequencies of T-cells expressing cytokines showed a skewed distribution. Therefore, Mann–Whitney U-test was used to compare cytokine expression in different culture conditions. Linear regression was used to compare disease activity and autoantibody levels with cytokine expression or ratios of cytokine expression. Wilcoxon matched pair test as well as paired t-test was used to compare the frequency of CD25+ T-cells in cultures with and without SmD1 stimulation. P-values <0.05 were defined as significantly independent from the R2 values.

Results

SLE patients reveal higher frequencies of TNF-α-expressing memory T-cells and lower frequencies of IL-10-expressing T-cells compared with healthy donors. Comparing the cytokine memory of peripheral T-cells in SLE patients with healthy donors, frequencies of TNF-α-expressing memory T-cells were higher in cultures from SLE patients with
when SLE patients are compared with healthy donors. After reactive memory Th cells activated and expanded expressing TNF-α, T-cells and SLE cultures were analysed for IFN-γ. In 34 SLE cultures, the frequency of TNF-α+ T-cells was measured, 31 SLE cultures were analysed for IL-10-expressing T-cells and 37 SLE cultures were analysed for IFN-γ.

In both cultures, with and without SmD183–119, the frequency of TNF-α+ T-cells expressing IL-10 correlated with disease activity also in the frequency of IL-10-expressing T-cells during active disease were also supported by a 6-month follow-up investigation of one SLE patient suffering from a lupus flare reflected by an increase in SLEDAI from 4 to 9 (Fig. 3). In this patient, increased frequencies of IFN-γ-expressing T-cells (from 2.9 to 4.8%), of TNF-α+CD3+ T-cells (from 3.4 to 4.5%) and of IL-2+ T-cell (from 3.6 to 12%) were detected as well as increased frequencies of IL-10-expressing T-cells (from 0.4 to 1.7%). In contrast, the frequency of IL-10 expression slightly decreased when active disease was compared with inactive disease (from 0.55 to 0.34%, Fig. 3). Throughout the study, stable, but very low frequencies of cytokine-expressing T-cells. In 34 SLE cultures, the frequency of TNF-α+ T-cells was measured, 31 SLE cultures were analysed for IL-10-expressing T-cells and 37 SLE cultures were analysed for IFN-γ.

Proinflammatory Th cytokine memory correlates to disease activity in SLE patients. To analyse a possible role of memory T-cells expressing one of the investigated cytokine on disease activity, the frequencies of memory/effector T-cells positive for the different cytokines were compared with the disease activity in the corresponding SLE patient.

In both cultures, with and without SmD183–119, the frequency of TNF-α expressing T-cells correlated with disease activity classified according to SLEDAI (P = 0.027 for the medium control, P = 0.021 for the SmD183–119 culture). Patients with active disease showed an increased cytokine memory for TNF-α compared with inactive patients (Fig. 2A). Higher frequencies of IL-10 memory T-cells were associated with lower disease activity in both cultures (P = 0.039 for the medium control, P = 0.032 for the SmD1 culture, Fig. 2B). The frequencies of IFN-γ, IL-2, and IL-4 expressing memory Th cells did not correlate with disease activity. The correlation between disease activity and ratio of cells expressing TNF-α vs IL-4 was only present in cultures stimulated with SmD183–119 (P = 0.007, R² = 0.028, Fig. 2C). Furthermore, there were significant correlations between disease activity and increased ratio of IFN-γ vs IL-10-expressing Th cells (P = 0.024 for the SmD183–119 cultures, data not shown) and ratio of Th cells expressing IL-2 vs IL-10 (P = 0.007 for the SmD183–119 culture, Fig. 2D). The increased ratio of cells expressing IFN-γ vs IL-10 and IL-2 vs IL-10 correlated with disease activity also in the cultures stimulated without SmD183–119 peptide (P = 0.042 and P = 0.003, respectively). The increase in the frequencies of memory/effector T-cells expressing proinflammatory cytokines and the decrease in the frequency of IL-10-expressing T-cells during active disease were also supported by a 6-month follow-up investigation of one SLE patient suffering from a lupus flare reflected by an increase in SLEDAI from 4 to 9 (Fig. 3). In this patient, increased frequencies of IFN-γ+CD3+ T-cells (from 2.9 to 4.8%), of TNF-α+ (from 3.4 to 4.5%) and of IL-2+ T-cell (from 3.6 to 12%) were detected as well as increased frequencies of IL-4+ T-cells (from 0.4 to 1.7%). In contrast, the frequency of IL-10 expression slightly decreased when active disease was compared with inactive disease (from 0.55 to 0.34%, Fig. 3). Another lupus patient with long-term clinical remission showed stable, but very low frequencies of cytokine-expressing T-cells.

Activation and expansion of SmD183–119-reactive T-cells revealed different memory T-cells expressing different cytokines. Stimulation with the SmD183–119 peptide and

![Fig. 1. Frequency of cytokine-expressing memory T-cells in SLE patients vs healthy donors by systemic T-cell stimulation. SmD183–119-reactive memory Th cells activated and expanded ex vivo showed a shift towards more cells expressing TNF-α (A) and less cells expressing IL-10 (B), when SLE patients are compared with healthy donors. After ex vivo restimulation with SmD183–119 ratios of memory Th cells expressing TNF-α vs IL-10 (C) and of those expressing IFN-γ vs IL-10 (D) were higher in SLE patients compared with the ratios of healthy donors. In 34 SLE cultures, the frequency of TNF-α+ T-cells was measured, 31 SLE cultures were analysed for IL-10-expressing T-cells and 37 SLE cultures were analysed for IFN-γ.](https://academic.oup.com/rheumatology/article-abstract/46/2/238/2289284)
FIG. 2. Proinflammatory systemic Th cytokine memory correlates to disease activity in SLE patients. Cytokine memory of ex vivo activated and expanded Th cells expressing the proinflammatory cytokine TNF-α correlated to disease activity classified according to SLEDAI (A). In contrast, higher frequencies of memory Th cells expressing IL-10 were associated with lower disease activity (B). The cytokine memory was very similar in both cultures, but more intensified by enrichment of SmD1183–1199-specific T-cells. 34 cultures were measured for TNF-α and 31 for IL-10. There was a correlation between disease activity and ratio of cells expressing TNF-α vs IL-4 (C) and ratio of Th cells expressing IL-2 vs IL-10 (D) only present in cultures stimulated with SmD1183–1199.

FIG. 3. Follow-up investigation in a lupus patient shows activity-dependent differences in the systemic cytokine memory. Follow-up investigation of a patient with a lupus flare changed the cytokine memory towards proinflammatory cytokines. The figure shows the frequencies of T-cells in cultures of T-cells stimulated with SmD1183–1199 prior to the PMA/ionomycin stimulation. To measure the disease activity, SLEDAI was used.
expansion of SmD1\textsuperscript{83–119}-reactive T-cells revealed a heterogeneous cytokine response. No differences were detected comparing the frequency of memory T-cells in the SmD1\textsuperscript{83–119} culture with the frequency in the culture stimulated only with IL-2 (Table 1). However, all cultures stimulated with SmD1\textsuperscript{83–119} revealed increased frequencies of CD25+ activated T-cells when compared with the medium control (P = 0.008, Fig. 4).

Comparing the cytokine memory of CD3+ T-cells from SLE patients and healthy donors, 57% of the ex vivo stimulated cultures from healthy donors reacted to SmD1\textsuperscript{83–119} by IFN-\(\gamma\) expression, whereas only 40% of the cultures from SLE patients did. The T-cell memory of SmD1\textsuperscript{83–119} reactive Th cells was shifted towards IL-4 expressing cells in 59% of the SLE patients, but only in 33% of the healthy donors (data not shown). Four SLE patients had increased frequencies of Th cells expressing any of the cytokines investigated when cultured with SmD1\textsuperscript{83–119} were compared with medium controls. The results indicate that the balances of cytokine-expressing T-cells might be more important.

The increase in frequencies of SmD1\textsuperscript{83–119}-specific T-cells expressing IFN-\(\gamma\), TNF-\(\alpha\), and IL-10 correlate with clinical findings in SLE patients. The frequency of CD3+ memory Th cells in cultures with and without SmD1\textsuperscript{83–119} was assessed to analyse cytokine memory of Th cells after activation and expansion of SmD1\textsuperscript{83–119} reactive T-cells. Higher frequencies of T-cells expressing a certain cytokine in the SmD1\textsuperscript{83–119} expanded culture reflect the cytokine memory of SmD1\textsuperscript{83–119} specific T-cells. To analyse different SLE patients with different basal frequencies of CD3+ memory Th cells from SLE patients, 16 different cultures from five different patients were analysed.

The increase in frequencies of IFN-\(\gamma\)-expressing Th cells in the cultures after expansion of SmD1\textsuperscript{83–119} specific T-cells (in%) correlated with serum anti-Sm and anti-dsDNA antibody levels (P = 0.003 and P = 0.016, respectively, Fig. 5A and B). The higher the increase in SmD1\textsuperscript{83–119}-specific T-cells expressing IFN-\(\gamma\), the higher were the corresponding serum antibody concentrations. The increase in frequencies of memory Th cells expressing TNF-\(\alpha\) upon SmD1\textsuperscript{83–119} stimulation also correlated with serum anti-dsDNA antibody concentrations (P = 0.038, Fig. 5C). The increase in frequencies of SmD1\textsuperscript{83–119}-specific T-cells expressing IL-10 correlated with disease activity (P = 0.006, Fig. 5D), but not with serum antibody levels.

Increase in frequencies of SmD1\textsuperscript{83–119}-specific T-cells expressing IL-10 is associated with low antibody levels in culture supernatants. Comparing the cytokine memory in cultures stimulated with SmD1\textsuperscript{83–119} with medium controls, differences in frequencies of IL-10 expressing memory T-cells upon stimulation by the SmD1\textsuperscript{83–119} peptide ranged from +53% (in the case of increased frequencies of IL-10 expressing T-cells upon SmD1\textsuperscript{83–119} stimulation compared with the medium control).

### Table 1. Cytokine memory in cultures either stimulated with SmD1\textsuperscript{83–119} or only with IL-2

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<th>SLE</th>
<th>Control</th>
<th>SmD</th>
<th>HD</th>
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<tr>
<td>HD</td>
<td>2.6 (0.9;8.3)</td>
<td>2.9 (1.1;8.6)</td>
<td>3.4 (1.0;4.9)</td>
<td>3.0 (0.2;17.8)</td>
<td>0.9 (0.5;2.3)</td>
<td>1.1 (0.5;2.2)</td>
<td>0.8 (0.9;2.3)</td>
<td>0.8 (0.7;2.6)</td>
<td>1.1 (0.5;2.2)</td>
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<tr>
<td>SLE</td>
<td>5.5 (2.3;9)</td>
<td>4.7 (1.6;5.7)</td>
<td>5.3 (3.8;8.8)</td>
<td>5.7 (2.8;12.9)</td>
<td>5.1 (2.8;11.8)</td>
<td>0.5 (0.3–1.7)</td>
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<td>0.5 (0.3–1.7)</td>
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The frequency of CD3+ memory T-cells (%) in the culture stimulated with SmD1\textsuperscript{83–119} compared with the medium control. 16 different cultures from five different patients were analysed.

**Discussion**

The analysis of the T-cell memory for cytokine expression, both systemic and autoantigen specific, may help to understand the role of autoreactive T-cells in SLE. Among the autoantigens that are known to be specifically recognized by the T-cells in SLE, the SmD1\textsuperscript{83–119} peptide has been described as an important autoantigen in murine and human lupus [26, 28–30]. SmD1\textsuperscript{83–119}-reactive T-cells have been identified and were associated with cardiopulmonary pathology in SLE patients [30]. Furthermore, SmD1\textsuperscript{83–119}-reactive T-cells have been shown to provide T-cell help for pathogenic anti-dsDNA and anti-Sm antibodies in various murine lupus models [29] (manuscript in preparation). Modulation of SmD1\textsuperscript{83–119}-reactive T-cells by immunization or by induction of high-dose tolerance influences anti-dsDNA antibody levels and survival in murine lupus [11, 28].
According to the pivotal role of SmD183–119 in lupus, T-cells are already primed \textit{in vivo} for this ubiquitous autoantigen. The present cytokine memory before further \textit{ex vivo} stimulation seems strongly reflected here by the similarity of the cytokine memory in both cultures either stimulated with SmD183–119 or IL-2 alone. Therefore, associations between disease activity and T-cells expressing TNF-α and IL-10 might represent cytokine memory of autoreactive T-cells such as SmD183–119-reactive T-cells.

Furthermore, similarities between both cultures can also reflect ongoing stimulations with autoantigens and, therefore, permanent activation and enrichment of autoreactive T-cells as it is suggested by studies showing the presence of nucleosomes in B-cell cultures [31]. One limitation of the approach used in the present study is that the \textit{in vitro} enrichment of autoantigen specific T-cells might yield different cytokine producers than \textit{in vivo} encounter of autoantigen in the specific lupus microenvironment.
Nevertheless, the association between cytokine memory of SmD183–119-reactive T-cells and disease activity, serum and supernatant autoantibody levels (Figs 4 and 5) support the role of SmD183–119 as an important autoantigen and suggest an in vivo priming by SmD183–119 that may be representative for other autoantigens in SLE.

Here, we observed an increased cytokine memory for TNF-α in cultures from SLE patients compared with those from healthy donors. Also, the frequencies of TNF-α-expressing memory T-cells correlated with disease activity. A role for TNF-α+ T-cells in disease pathogenesis is also suggested by the frequencies of TNF-α+ T-cells in the SmD183–119 driven ex vivo expansion in lupus patients. The higher the frequencies of memory T-cells expressing TNF-α responding to the SmD183–119 peptide the higher were the anti-dsDNA antibody levels in the corresponding patient. Despite divergent results concerning the role of TNF-α in lupus models [32, 33], TNF-α blockade has been successfully used in lupus patients suffering from nephritis [13]. Our results show that low frequencies of autoreactive TNF-α+ memory T-cells correlate to clinical benefit in SLE. The association between levels of anti-SmD183–119 antibodies in serum and the frequencies of IFN-γ+ SmD183–119-specific memory T-cells further underlines the importance of proinflammatory cytokines in SLE.

The cytokine memory for IL-10 was impaired in SLE. Frequencies of IL-10 expressing Th memory cells were decreased in the SmD183–119-expanded Th cells of SLE patients compared with healthy donors. A reduced frequency of IL-10+ T-cells has been detected previously in SLE patients [34], but their functional role in lupus pathogenesis remains unclear. Here, we show that this reduction is not only symptomatic but also affects autoreactive Th cells. Higher frequencies of SmD183–119-specific memory Th cells expressing IL-10 were associated with low autoantibody levels in the supernatants. The data suggest an involvement of SmD183–119-reactive IL-10 memory T-cells in the regulation of autoantibodies in SLE.

IL-10 appears to be a key cytokine in the pathogenesis of SLE since IL-10 polymorphisms are among the strongest susceptibility genetic factors in SLE [35]. This cytokine inhibits activation and effector function of antigen-presenting cells [36]. Thus, IL-10 has the potential to limit and terminate inflammatory immune reaction 37 including those of lupus. A beneficial effect of IL-10 is supported by studies in an experimental murine lupus model, showing a protective role of IL-10 [10, 11].

Paradoxically, at first sight a pathogenic role of IL-10 has also been postulated for lupus, based on the observation that systemic anti-IL-10 treatment is beneficial in murine and human lupus [38, 39]. This seemingly contradictory effect of IL-10 can be reconciled if one considers the source and time point of IL-10 production. IL-10 is expressed by various cells and IL-10 produced by non-T-cells appears to predominate [34]. While IL-10 producing T-cells may be protective by silencing Antigen Presenting Cell (APC) and Th1 cells as shown in in vitro studies [10, 11], IL-10 derived from other cells may drive autoantibody production by B-cells. Furthermore, as shown in animal studies, the role of IL-10 is influenced by the stage of disease [10]. Late in disease development, an increasing frequency of IL-10-expressing T-cells directed to the autoantigens is required to suppress the ongoing autoimmune process. This possible defense mechanism is reflected here by the association between SmD183–119-specific IL-10+ T-cells and disease activity as well as by our ongoing experiments in animal models showing a regulatory function of IL-10 expressing autoreactive T-cells. During further progress of the disease, IL-10+ T-cells fail to suppress Th1 cells and APC efficiently, and consequently, IL-10 from Th cells and other cells augments differentiation of B-cells into plasma cells producing pathogenic autoreactive antibodies [10, 11]. This would explain both the previously described beneficial effect of anti-IL-10 on established and active disease and the protective effect of IL-10 on lupus aetiology. It is to be shown, whether therapy with IL-10 memory Th cells may be superior to anti-IL-10 therapy as suggested by other groups in SLE [38, 39].

G.R. and F.H. have a patent for the commercial use of the SmD183–119 peptide (United States Patent 5,945,105) as diagnostic marker and for therapeutic use.

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T-cell cytokine memory in SLE


