EXTENDED REPORT

T cell reactivity against the SmD1$_{83–119}$ C terminal peptide in patients with systemic lupus erythematosus

G Riemekasten, C Weiss, S Schneider, A Thiel, A Bruns, F Schumann, S Bläss, G-R Burmester, F Hiepe

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See end of article for authors' affiliations

Correspondence to: Dr G Riemekasten, Department of Rheumatology and Clinical Immunology, Charité University Hospital, Schumannstr 20/21, D-10117 Berlin, Germany; gabriela.riemekasten@charite.de

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Background: The SmD1$_{83–119}$ peptide is a major target of the B cell response in patients with systemic lupus erythematosus (SLE).
Objective: To investigate the T cell response directed against this peptide, its disease specificity, and possible impact on SLE pathogenesis.
Methods: Peripheral blood mononuclear cells derived from 28 patients with SLE and 29 healthy and disease controls were stimulated by the SmD1$_{83–119}$ and the recombinant (r)SmD1 protein, and [^{3}H]thymidine incorporation was measured. Patients with SLE were simultaneously tested for autoantibodies, disease activity, clinical symptoms, and medical treatments.
Results: T cell reactivity against the SmD1$_{83–119}$ peptide was detected in 11/28 (39%) patients with SLE and against the rSmD1 protein in 10/28 (36%) patients. In contrast, only 2/29 (7%) controls exhibited SmD1 reactivity. An analysis of proliferation kinetics showed that SmD1 reactive T cells are activated in vivo, as additionally confirmed by cytometric analysis. Addition of mammalian dsDNA to rSmD1 enhanced the rSmD1-specific T cell response. SmD1$_{83–119}$-specific T cell reactivity was significantly more common in patients with cardiac and pulmonary symptoms. No correlation between T and B cell responses and disease activity was seen.
Conclusion: SmD1$_{83–119}$ is a major T cell epitope of SmD1, commonly recognised by T cells from patients with SLE and much less commonly found by healthy or disease controls. This strong T cell reactivity as well as the high frequency and specificity of anti-SmD1$_{83–119}$ antibodies in SLE suggest a possible role in SLE pathogenesis, at least in a subset of patients.

High affinity antibodies directed against the Sm antigens of the small nuclear ribonucleoproteins (snRNPs) are a hallmark of the immune response in some patients with systemic lupus erythematosus (SLE)[1] and seem to be associated with severe clinical symptoms and with poor prognosis.[2] The Sm-specific antibody response is polyclonal, has a high titre,[3] and its occurrence is associated with HLA-DR2 haplotypes.[4 5] These observations suggest that Sm-specific autoimmune responses are driven by autoreactive T cells.[5 6] Nevertheless, identifying and quantifying autoreactive T cells has been difficult owing to the low frequency of autoantigen-specific T cells[7] and the hyporesponsiveness to mitogen and minimal interleukin 2 secretion that develops before SLE onset.[8] However, reports have emerged demonstrating that patients with SLE have increased numbers of activated and affinity maturated T cells.[9] Furthermore, the frequency of T cells with mutations significantly correlated with disease duration and preceded flares of disease.[10]

Sm and snRNP reactive T cell clones have been generated from peripheral blood mononuclear cells (PBMC) derived from patients with SLE[11] using a panel of purified human fusion proteins such as 70 kDa RNP, B and D proteins. However, further characterisation of T cell epitopes of these snRNP proteins or analyses of the specificity of such autoantigen reactive T cell clones for SLE were not determined.

Anti-Sm antibodies are predominantly directed against the SmD1 protein of the snRNP complex.[12] Recently, we identified the C-terminal peptide of the SmD1 protein aa 83–119 as a major target for the autoantibody response in patients with SLE[13] with a sensitivity of 70% and an SLE specificity of 94%. Anti-dsDNA and anti-SmD1$_{83–119}$ antibody levels were correlated, suggesting functional linkages. In the (NZB×NZW)F1 lupus mice, a complex of SmD1 and dsDNA rather than one of either individual component appears to activate T and B cells,[14] probably by recognition of different epitopes.

This study aimed at analysing T cell reactivity directed against the SmD1$_{83–119}$ peptide and the full length SmD1 protein in patients with well characterised SLE and controls. Furthermore, the influence of dsDNA on the SmD1-specific T cell response was analysed. T cell reactivity was compared with antibody levels of anti-SmD1$_{83–119,}$ and anti-dsDNA, and with clinical characteristics, drugs, age, and disease duration.

PATIENTS AND METHODS

Patients

Blood samples were obtained from 34 patients with SLE, 12 patients with rheumatoid arthritis (RA), four patients with systemic sclerosis, and 13 healthy donors and analysed for T cell reactivity after informed consent. Only one of the patients with SLE was receiving a prednisone dose higher than 15 mg/day. Patients fulfilled the American College of Rheumatology classification criteria for SLE,[15] RA,[16] and systemic sclerosis.[17] Furthermore, serum samples simultaneously obtained from these patients were analysed for the SmD1$_{83–119}$ and anti-dsDNA antibody reactivity by enzyme linked immunosorbent assay (ELISA). Medical records of all patients

Abbreviations: ANA, antinuclear antibodies; AU, arbitrary units; BSA, bovine serum albumin; CFSE, carboxyfluorescein diacetate succinimidyl ester; CG, cell growth medium; ELISA, enzyme linked immunosorbent assay; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PHA, phytohaemagglutinin; RA, rheumatoid arthritis; SI, stimulation index; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index

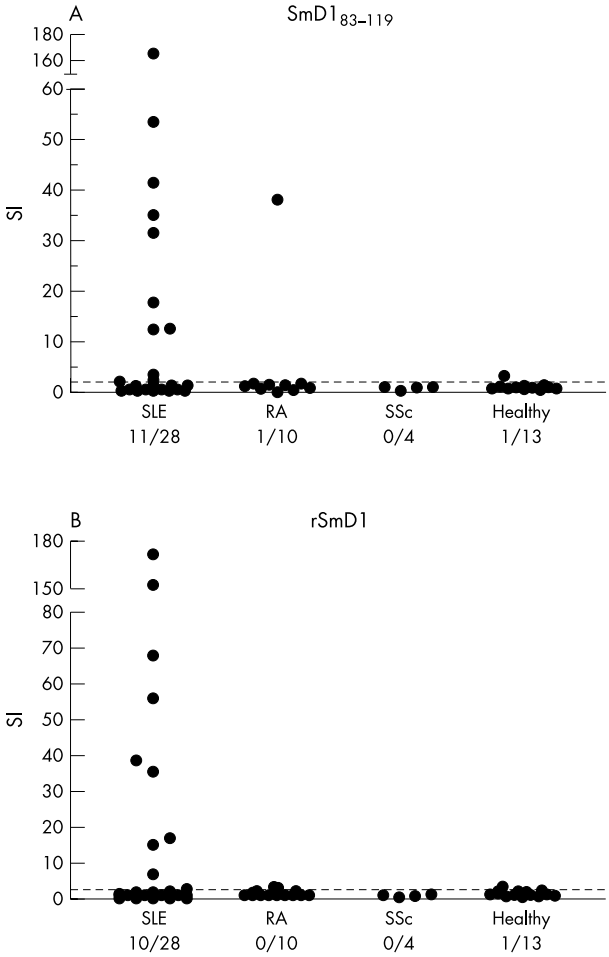

Figure 1 Maximal stimulation of PBMC derived from 28 patients with SLE induced by (A) the $SmD1_{83–119}$ peptide and (B) the full length SmD1 protein measured by [^{3}H]thymidine incorporation after three or seven days. Data are shown as median stimulation index (SI), calculated by dividing the mean counts per minute (cpm) of T cell cultures with antigen by the mean cpm of T cell cultures without antigen (medium). All SI≥2 (dashed line) were defined as positive.

with SLE were reviewed to determine disease duration, previous or present immunosuppressive treatments and clinical symptoms. Central nervous system involvement was determined as defined for the SLE Disease Activity Index (SLEDAI).[18] Patients were HLA haplotyped when possible.

Antigens

The $SmD1_{83–119}$ peptide (VEPKVKKREAVAGRGRGRGRGRGR GRGRGRGGPRR) was synthesised as free peptide according to the protocol described by Atherton[19] and purified by reverse phase chromatography (C18 Vydac column). Recombinant full length SmD1 protein was expressed as an *E coli* fusion protein, and purified as previously described.[13] Furthermore, mixtures of rSmD1 or $SmD1_{83–119}$ peptide and mammalian dsDNA (calf thymus DNA, Serva, Heidelberg, Germany) were additionally used as antigens for T cell stimulation to analyse potential synergistic effects of one of either SmD1 antigen plus DNA. Based on the molecular weights of dsDNA and $SmD1_{83–119}$ peptide a ratio of 10:1 was chosen.

Lymphocyte proliferation

To measure the spontaneous T cell proliferation, PBMC of heparinised blood derived from patients with SLE and controls were isolated by centrifugation on a Ficoll cushion and seeded at 2×10^6 cells/ml in cell growth medium (CG, Vitromex, Vilshofen, Germany). CG without serum supplementation is the preferred method for analysis of T cell proliferation because alloresponses or autostimulatory effects—both commonly seen in cultures supplemented with autologous serum or fetal calf serum—are avoided. B cells and natural killer cells do not proliferate to a significant degree as shown previously by FACS analyses.[20] PBMC were plated in triplicate to each well of a 96 well plate at 2×10^5 cells/well with medium (CG), penicillin (100 U/ml), streptomycin (100 µg/ml) or serial dilutions (0.5–5 µg/ml) of the appropriate antigen (calf thymus dsDNA, rSmD1, $SmD1_{83–119}$ peptide) at 250 µl final volume. If the number of cells allowed for further studies, mixtures of rSmD1/dsDNA and $SmD1_{83–119}$/dsDNA were used. Phytohaemagglutinin (PHA; Boehringer Mannheim, FRG) was used as the high positive control (1 µg/ml). All cells were cultured at 37°C and 5% CO_2. For the determination of lymphocyte proliferation, cells were pulsed with 1 µCi [^{3}H]methylthymidine (Amersham) for 16 hours. Plates were counted in the presence of microscint 0 in a Topcount (all Packard, Dreieich, Germany). DNA-incorporated radioactivity was determined by scintillation counting as a measure of proliferation. [^{3}H]Thymidine uptake was determined after 44–48 hours and six days of culture. Furthermore, proliferation of PBMC derived from 16 patients was studied in a kinetic analysis for 3–12 days. Stimulation indices (SIs) were determined as the quotient of probe and medium proliferation. A response with an SI≥2 was defined as positive, and responders significantly differed from non-responders ($p<0.001$, Whitney U test).

Labelling of PBMC with carboxyfluorescein diacetate succinimidyl ester (CFSE)

Ammonium-heparinised venous blood samples from five patients with SLE were analysed for [^{3}H]thymidine incorporation by CFSE labelling. Two of these patients showed a T cell response in the former assay. Labelling of PBMC with CFSE was performed as described earlier.[21 22] Briefly, PBMC were prepared by Ficoll-Paque PLUS (Amersham Pharmacia Biotech AB, Sweden) separation, washed twice in phosphate buffered saline (PBS) and resuspended in PBS at a final concentration of 2×10^7/ml. A 0.5 µM staining solution of CFSE (Molecular Probes, Eugene, OR) in PBS was prepared from a 5 mM stock solution stored at –80°C in dimethyl sulphoxide (Sigma, Germany). The cell suspension was added to the staining solution at a 1:1 ratio and labelled for four minutes at room temperature Subsequently, labelled cells were washed twice with PBS containing 0.5% bovine serum albumin (BSA; Sigma, Germany).

Cytometric analysis of $SmD1_{83–119}$ reactive T helper cell proliferation

For cytometric analysis of $SmD1_{83–119}$ reactive T helper cell proliferation, 2×10^6 CFSE labelled PBMC were cultured in RPMI 1640 (GIBCO BRL, Grand Island, USA) supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.3 mg/ml glutamine and 10% fetal calf serum (all PAA, Linz, Austria) for six days with 5 µg of $SmD1_{83–119}$ peptide in 1 ml of culture medium at 37°C and 5% CO_2. These culture conditions had been established previously to analyse antigen-specific T cells.[21 22] A second sample was cultured without $SmD1_{83–119}$ peptide. After six days cells were harvested and washed once with ice cold PBS-BSA. Anti-CD4-peridinin chlorophyll protein antibodies were used for cytometric analysis (Becton Dickinson Immunocytometry Systems, San Jose, CA). All stainings were performed for 10 minutes on ice in the dark in the presence of 1 mg/ml Beriglobin (Chiron Behring GmbH and Co, Marburg, Germany) to block unspecific binding of antibodies to Fc receptors. After washing, cells were resuspended in 250 µl PBS-BSA and analysed on a FACSCalibur flow cytometer (Becton Dickinson). Dead cells were excluded

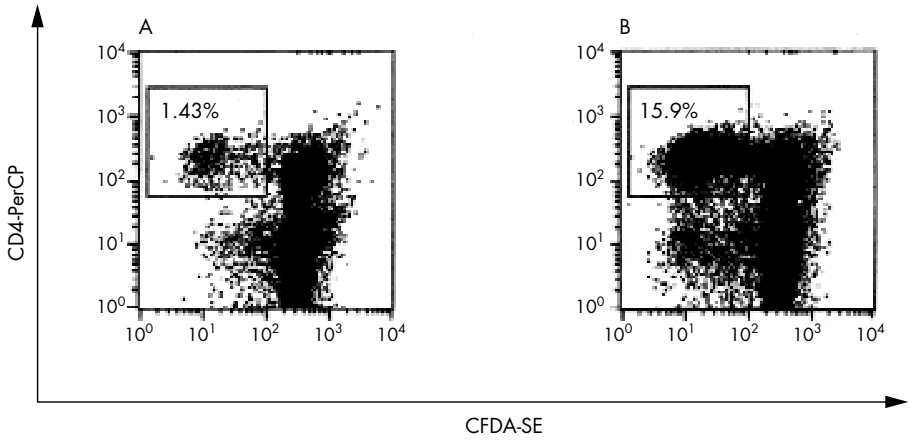

Figure 2 Proliferation of CD4 positive T cells derived from a patient with SLE with an SmD1$_{83–119}$-specific T cell reactivity by thymidine assay. PBMC were labelled with CFSE and cultured (A) without or (B) with the SmD1$_{83–119}$ peptide for six days. Sequential halving of CFSE fluorescence intensity is indicative of cell division. Anti-CD4 phenotypic-specific antibodies were used to analyse SmD1$_{83–119}$ specific T helper cells by flow cytometry.

Table 1 Stimulation indices of T cells from 16 patients with SLE who were tested for their reactivity against the SmD1$_{83–119}$ peptide, the rSmD1 as well as with mixtures of these antigens with mammalian (calf thymus) dsDNA

Antigens for T cell reactivity			
SmD1$_{83–119}$	SmD1$_{83–119}$/dsDNA	rSmD1	rSmD1/dsDNA
0.47	0.1	0.55	0.1
0.44	0.1	0.44	0.1
31.6	62.6	172	43.6
165	88	153	108
1.4	0.96	1.7	3.3
41.4	1.7	0.99	2.2
0.76	0.1	56	180
2.2	1.7	0.75	1.5
0.61	0.4	1	0.6
0.8	0.8	0.7	0.95
0.28	0.29	0.3	0.44
0.4	3.6	1.3	65
0.2	160	0.82	76
0.6	2.7	2.5	1
0.6	1.5	0.72	2.2
0.8	0.2	1.3	0.9

T cell reactivity was detected by [^{3}H] thymidine incorporation. Stimulation indices were calculated by dividing the mean counts per minute (cpm) of T cell cultures with antigen by the mean cpm of T cell cultures without antigen (medium) or with dsDNA (medium plus DNA, 0.05–5 µg/ml) for DNA/protein mixtures.

with 1 µg/ml propidium iodide (Sigma, Germany). Data were collected and analysed using Cellquest Research Softwear (Becton Dickinson). As sequential halving of CFSE fluorescence intensity is due to cell division,[22] proliferating T helper cells were determined by measuring the CFSE intensity decrease in CD4 positive lymphocytes.

Anti-SmD1$_{83–119}$ peptide antibodies

Anti-SmD1$_{83–119}$ peptide antibodies were detected by ELISA as described.[13] A standard curve was established for each assay using a highly reactive serum from one specific patient with SLE. Reactivity of this serum at a dilution of 1:100 was defined as 10 000 arbitrary units (AU). The upper limit of the normal range was defined as the mean value plus 2SD of the reactivities in 105 normal human sera (dilution 1:100). Thus, for the SmD1$_{83–119}$ peptide, the cut off was 223 units. Each serum was assayed in duplicate.

Anti-dsDNA antibodies

Anti-dsDNA antibodies were detected by ELISA as described previously.[23] In brief, methylated bovine serum albumin (BSA)-precoated and calf thymus dsDNA-coated microtitre plates were incubated with sera in duplicates for the detection of IgG and IgM anti-dsDNA autoantibodies.

Statistics

A χ^2 test was used to analyse clinical associations in patients with and without a T cell reactivity against the SmD1$_{83–119}$ peptide. Forty clinical findings of SLE were determined in 34 patients with SLE who were analysed for a T cell response against the SmD1$_{83–119}$ peptide. In addition to SI≥2 for defining T cell reactivity, the Whitney U test was used for the analyses of responders on SmD1$_{83–119.}$

RESULTS

T cell reactivity against the SmD1$_{83–119}$ peptide and the full length SmD1 protein

T cells derived from 6/34 patients with SLE and 2/12 patients with RA did not react with the high positive control (PHA) and were therefore considered to be anergic. T cells from all healthy donors showed a proliferative response to PHA. In those patients with SLE who were positive for PHA (n=28), both the SmD1$_{83–119}$ peptide and the full length recombinant SmD1 protein could induce T cell proliferation in some patients (figs 1A and B). The median SI was 1.3 for both antigens. A significant stimulation with an SI≥2 was induced in 11/28 (39%) patients with SLE by the SmD1$_{83–119}$ peptide and in 10/28 (32%) patients with SLE by the full length SmD1 protein. SI≥10 or higher were obtained in 8/28 patients with SLE by stimulation with the SmD1$_{83–119}$ peptide and in the same number with the rSmD1 protein. One of 10 patients with RA who was antinuclear antibody (ANA) positive (1/640, speckled pattern, SI=45), and 1/13 healthy donors (SI=3), but no patient with scleroderma (n=4) showed a T cell reactivity against the SmD1$_{83–119}$ peptide. For the full length SmD1 protein, T cell reactivity was found nearly exclusively in patients with SLE. Only one healthy donor who also reacted with the SmD1$_{83–119}$ peptide showed a weak proliferation (SI=3). All other patients remained negative for an rSmD1-induced proliferation.

Of the 11 patients with SLE with a proliferation response against the SmD1$_{83–119}$ peptide, nine were also reactive against the full length SmD1 protein. On the other hand, one patient showed a T cell response against the rSmD1 protein (SI>10), but not against the SmD1$_{83–119}$ peptide.

Table 2 Associations approaching or reaching statistical significance between clinical findings in patients with a T cell response on the $SmD1_{83–119}$ peptide compared with those in patients without T cell response on this autoantigen that was measured by thymidine incorporation. Data were obtained by analysis of 40 clinical findings at any time during the course of disease in all 34 patients with SLE investigated

Clinical features	Patients (No (%))		p Values
	With T cell response	Without T cell response	
Discoid lupus	6/10 (60)	2/22(9)	0.041
Sicca syndrome	9/10 (90)	10/22(45)	0.02
Nephritis	9/11 (82)	10/21(48)	0.066
Arrhythmia	3/8 (38)	1/22(5)	0.021
Myocarditis	6/10 (60)	2/22(9)	0.005
Pericardial effusion	6/9 (67)	4/22(18)	0.01
Depressions	5/9 (56)	1/22(5)	0.027
Pleural effusion	7/9 (78)	3/20(15)	0.001
Pneumonia	7/9 (78)	1/20(5)	< 0.001
Pathol. lung function	5/8 (63)	2/19(11)	0.011
Lung fibrosis	5/9 (56)	1/21(5)	0.005

Influence of mammalian dsDNA on T cell proliferation response

To analyse the influence of nucleic acids for critical epitope recognition, T cells derived from 16 patients with SLE were additionally stimulated by a mixture of dsDNA and the full length SmD1 protein as well as the $SmD1_{83–119}$ peptide, respectively (table 1). Six of these 16 patients had a proliferation response induced by either the rSmD1 protein or the $SmD1_{83–119}$ peptide. When both the rSmD1 and the mammalian dsDNA were used as antigen, the number of patients with a proliferative response was increased to eight of the patients with SLE. Four of these were reactive against the $SmD1_{83–119}$ peptide or the full length SmD1 protein, respectively, but four further PBMC showed a proliferative response that could only be induced by the mixtures and not by the SmD1 protein/peptide antigens alone. This suggests that dsDNA influences peptide recognition, probably by generation of further epitopes by the formation of protein/DNA complexes. Nevertheless, in some patients the response decreased when dsDNA was added, suggesting different epitope recognition.

Only five of these eight patients with SLE with a T cell response to the rSmD1/dsDNA mixture elicited a positive proliferation induced by the $SmD1_{83–119}$ peptide/dsDNA. Four of these were also reactive with the rSmD1/dsDNA, while the other showed a proliferative response induced by rSmD1 alone (table 1).

The median SIs of PBMC derived from these 16 patients were 0.8 induced by the $SmD1_{83–119}$ peptide, 1.0 by the rSmD1 protein alone, 1.5 and 2.2 by the mixtures of $SmD1_{83–119}$/dsDNA and rSmD1/dsDNA, respectively. When all these antigens were used the number of patients with a proliferative response increased to 10/16 (63%) patients. Only T cells derived from 2/13 controls (one healthy donor and one patient with ANA positive RA) showed a weak reactivity against the rSmD1/dsDNA mixture (SI=2.1). These two patients also elicited a T cell reactivity against the rSmD1 or the $SmD1_{83–119}$ peptide as described earlier.

Mammalian dsDNA alone did not induce any T cell responsiveness in any of the patients investigated.

Cytometric analysis of SmD1 reactive T helper cell proliferation

To characterise further the SmD1-specific T cell response, five patients were additionally analysed using CFSE labelling of PBMC and subsequent flow cytometric analysis. Two of those patients were $SmD1_{83–119}$ reactive in the [^{3}H]thymidine assay with SI=12 and SI=35, respectively. One of those patients displayed a high proportion of $SmD1_{83–119}$-specific CD4 positive T cells, comprising 16% of all CD4 proliferating cells (fig 2) compared with only 1.4% in the medium.

The other patient with a proliferation response by the [^{3}H]thymidine uptake did not show any proliferative response with the CFSE technique. Three patients did not show any T cell response in either the [^{3}H]thymidine assay or the cytometric analysis using the CFSE technique.

Kinetics of T cell proliferation against the $SmD1_{83–119}$ peptide in patients with SLE

Of the 11 patients with a positive proliferative response on the $SmD1_{83–119}$ peptide, the highest stimulation of T cells induced by the $SmD1_{83–119}$ peptide was on day 3 for six patients and on day 7 for five patients (data not shown). Seven of the 11 patients with SLE with a proliferative response of T cells on the $SmD1_{83–119}$ peptide were investigated for up to 12 days. For these patients, the median SI was 12.5 on day 3, 0.9 on day 7, 1.4 on day 9, and 6.1 on day 12. Furthermore, nine patients without a proliferation response on the $SmD1_{83–119}$ peptide or the rSmD1 were tested for a longer period. Two of these patients with SLE showed cell proliferation on days 9 and 12, respectively.

Characterisation of patients with $SmD1_{83–119}$-specific T cells

The median disease duration in patients with $SmD1_{83–119}$-specific T cells was 11 years (data not shown), and 10 years in patients without $SmD1_{83–119}$-specific T cells. The median age in patients with a positive proliferative response to $SmD1_{83–119}$ peptide was 45 years (data not shown) and 44 years in patients without a T cell reactivity.

Furthermore, T cell reactivity was associated with involvement of several types of organ (table 2). The frequency of myocarditis and pericarditis was strongly increased in patients with $SmD1_{83–119}$-specific T cell response (p=0.005 and p=0.01, respectively). In addition, arrhythmia was seen more often in patients with SLE with an $SmD1_{83–119}$-specific T cell proliferation (p=0.021). Furthermore, pneumonia and pleuritis were significantly more prevalent in patients with a positive T cell response (p<0.001 and p=0.001, respectively). Lung fibrosis, pathological radiography of the chest, and pathological lung function were also more prevalent in patients with SLE with a positive T cell reactivity.

Strong T cell reactivity was also seen in six patients who received cytotoxic or other immunosuppressive drugs and in two patients with a steroid dose >10 mg/day. Six patients with reactivity against the $SmD1_{83–119}$ peptide had undergone cyclophosphamide treatment some years ago (data not shown).

Association between autoantibody levels, disease activity, and $SmD1_{83–119}$-specific T cell proliferation

No correlation between anti-$SmD1_{83–119}$-specific antibody levels and T cell proliferation was seen (fig 3A). Patients with or without $SmD1_{83–119}$-specific T cells had $SmD1_{83–119}$-specific autoantibodies. The median anti-$SmD1_{83–119}$ antibody level was 290 AU in patients with a positive T cell response and 620 AU in patients without a T cell response. When the anti-dsDNA response in comparison with T cell proliferation that was induced by the $SmD1_{83–119}$ peptide was analysed, patients with a positive T cell response on the $SmD1_{83–119}$ peptide showed a median anti-dsDNA level of 2.8 compared with 1.8 in patients with SLE without a proliferation response on the $SmD1_{83–119}$ peptide (fig 3B). However, the differences between antibody levels in patients with or without proliferation response were not significant.

Furthermore, there was no association between the disease activity of SLE as detected by the SLEDAI score and T cell proliferation induced by the $SmD1_{83–119}$ peptide or the full length

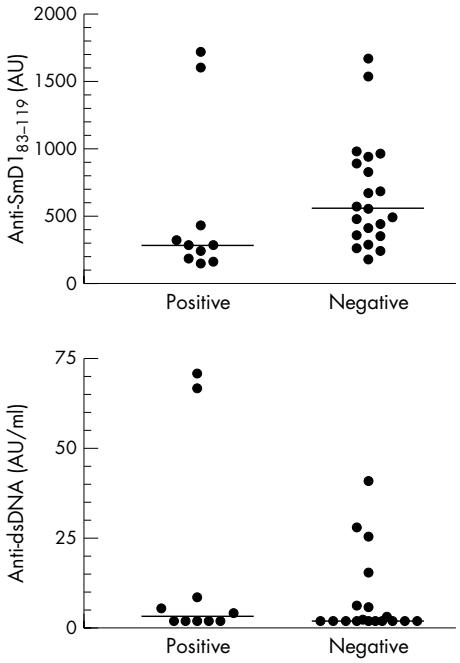

Figure 3 Comparison of anti-SmD1$_{83–119}$ and anti-dsDNA autoantibody levels in 32 patients with SLE with the simultaneously analysed T cell reactivity against the SmD1$_{83–119}$ peptide. T cell reactivity was measured by [^{3}H]thymidine incorporation and determined as positive for an SI≥2. Levels of antibodies were detected as arbitrary units (AU) by ELISA. Antibodies were found in both patients with or without a proliferation response of T cells.

SmD1 protein, respectively. The median SLEDAI of patients with a positive response induced by the SmD1$_{83–119}$ peptide was 6 compared with a median SLEDAI of 4 in the SLE group without a positive proliferation response (data not shown).

DISCUSSION

In this study the specific T cell response against the SmD1 C-terminal peptide aa 83–119 and the full length SmD1 protein was analysed in patients with SLE and a control group. The comparison of the proliferative response induced by the full length SmD1 protein and the SmD1$_{83–119}$ peptide confirms the predominant role of the C-terminus of the protein as has been described previously for the antibody response. Therefore, the SmD1$_{83–119}$ peptide is both a major B and T cell epitope in SLE. The addition of dsDNA to rSmD1 even enhanced the T cell responsiveness. The kinetic data showing responses after only some days of culture suggest that the SmD1-specific T cells are in vivo preactivated in many patients with SLE and, therefore, further emphasise the potential importance of SmD1-specific autoreactivity. Although no associations were found between the presence of SmD1$_{83–119}$-specific T cells and SmD1$_{83–119}$-specific antibodies or disease activity, a significant correlation with several organ manifestations of SLE was seen and may therefore be indicative of defined subgroups of patients with SLE. High T cell reactivity was also found in patients receiving immunosuppressive treatments, including cytotoxic drugs and prednisone doses up to 15 mg/day.

The role of T cells in the pathogenesis of SLE is impressively documented by animal studies.[24 25] In human SLE, data on specific T cellular targets are still rare. However, recently performed studies on T cell response in patients with SLE have clearly confirmed the results obtained by animal studies. Thus, antigen-specific T cells specifically occurring in patients with SLE were detected using nucleosomes.[25–27] Recently, Holyst *et al* used a panel of highly purified, soluble, recombinant human fusion proteins of the snRNP 70 kDa, B, and D polypeptides to produce T cell clones derived from six patients with SLE by leucopheresis.[11] These authors noted that individual patients showed different patterns of T cell responsiveness to the snRNP polypeptides which paralleled their serological reactivity of these antigens. Furthermore, these T cell clones expressed a T helper cell phenotype. However, T cell response was not analysed in greater detail and likewise data on disease specificity, associations with clinical findings, etc, characterisation are not available. Thus, to our best knowledge the present study is the first to analyse Sm-specific T cells in patients with SLE and controls.

Anti-Sm antibodies are highly specific for SLE.[1] Because anti-U1RNP sera are also reactive to a variable extent with B and B′ proteins,[28] the D1 protein appears to be the most important Sm antigen. Therefore, the SmD1 protein is a crucial component to investigate its molecular structure and immunodominant epitopes.

Despite the heterogeneity of human lupus, possible epitope spreading, or affinity maturation during disease course, we identified an epitope of the SmD1 protein that is recognised by some of the 28 patients with SLE studied. Analysing the specificity of this SmD1$_{83–119}$ induced T cell response, T cell reactivity was specifically determined in patients with SLE and was only rarely found in the control groups. One patient with ANA positive and DR4 positive RA showed a T cell response against the SmD1$_{83–119}$ peptide that may indicate further development into SLE. Furthermore, there is a high homology between the SmD1 C-terminus and the EBNA-2 protein of the Epstein-Barr virus, which might explain the low T cell reactivity in one healthy donor.[29]

Furthermore, this was the first study to compare quantitatively the SmD1-specific B and T cell responses in well characterised patients. Although the association of SmD1$_{83–119}$-specific autoantibodies with disease activity has often been described, this does not apply to T cell reactivity. An SmD1$_{83–119}$ peptide derived T cell response was found, although the disease was in long term remission and serum anti-dsDNA or SmD1$_{83–119}$ reactivities were not raised. On the other hand, despite an IgG anti-SmD1$_{83–119}$ reactivity there was no T cell responsiveness against the SmD1$_{83–119}$ peptide in most patients with SLE. Nevertheless, the association between disease activity and the levels of anti-SmD1$_{83–119}$ peptide antibodies was confirmed as previously described.[13] However, the patient with the highest SI (165) also had the highest disease activity (SLEDAI 18) and high levels of anti-SmD1$_{83–119}$ antibodies. The missing association between antibody levels and T cell reactivity induced by the corresponding antigen was described earlier by others using nucleosome specific T cells.[26 27] With respect to the correlation of the anti-SmD1$_{83–119}$ response with disease activity it may be assumed that antibodies or plasma cells persist for longer than T cells as has been suggested previously.[27 30] Furthermore, B and T cells may occur in compartments other than peripheral blood or might have different kinetics of activation during the disease course as suggested previously.[26 31] Thus, T cells precede B cell response, and T cell proliferation is often undetectable at the peak of antibody production.[31 32] In animal models of SLE, T cell responsiveness to several antigens is expanded very early in life, but the ability of specific T cells to help in the production of pathogenic autoantibodies appears several months later, along with the appearance of autoantibodies in the serum of animals.[25] Presumably, T cell activation is not always necessary for B cell activation.[33 34] The kinetics of T cell activation might explain why a response against the SmD1$_{83–119}$ peptide or the rSmD1 protein is missing at some times in patients with SLE who have previously shown a response (data not shown).

In our animal studies with (NZB×NZW)F1 mice we showed a strong proliferation of SmD1$_{83–119}$ reactive T cells at age 8 weeks, long before the occurrence of clinical symptoms.[14] For this animal model we suggested that the SmD1$_{83–119}$ peptide may give T cell help for the dsDNA-specific B cells and that a

complex of the $SmD1_{83–119}$ peptide with a nucleic acid such as dsDNA is presented to the immune system as a hapten-carrier complex.[14] Furthermore, it was speculated by us and others[35] that interaction of nucleic acids with proteins such as the rSmD1 might interfere with epitope formation and might result in the generation of critical T cell epitopes. Therefore, we additionally analysed the T cell response on a mixture of calf thymus dsDNA with the recombinant SmD1 protein. The number of patients with a T cell proliferation was enhanced by these antigens. Nevertheless, further analyses are necessary to understand the interaction of both the SmD1 protein and dsDNA, the interaction of B and T cells, their epitopes, activating factors, their cytokine release, and their role in the pathogenesis of SLE.

The proliferation kinetic studies demonstrate that the $SmD1_{83–119}$-specific T cell proliferation is "primed" in these patients, indicating that these cells have been fully activated in vivo and do not require time for in vitro expression of the high affinity interleukin 2 receptor and high level major histocompatibility complex class II.

Are there any associations between clinical findings in human SLE and the proliferation response on the $SmD1_{83–119}$ peptide? Previously, nucleosomal peptide epitopes were identified for nephritis-inducing T helper cells in a murine model of SLE.[36] Therefore, clinical findings of patients with SLE were analysed and compared with the T cell response.

Patients with SLE with a positive proliferation response significantly more often had cardiac and pulmonary symptoms. Furthermore, sicca syndrome, discoid lupus, and depression were found more often in patients with a positive T cell response. Despite a strong association between nephritis and the B cell response in our SLE cohort of 167 patients with SLE ($p<0.001$, unpublished observation), no significant association between nephritis and the $SmD1_{83–119}$-specific T cell response was found here. However, 82% of the patients with a T cell response had nephritis compared with only 48% of the patients who were negative for T cell proliferation. In this initial study the number of patients was limited and the prevalence of nephritis was high in both groups. Therefore, further investigations with more patients will be necessary to verify these data.

Furthermore, the association between T cell response and clinical symptoms such as sicca syndrome or myocarditis in patients with SLE probably suggests a role of cellular mechanisms in the pathogenesis of SLE that might be different from autoantibody mediated processes. Jeruc *et al* investigated tubulointerstitial infiltrates derived from kidney biopsy specimens of 190 patients with SLE. The inflammatory infiltrates were found to be composed of T cells, macrophages, and B cells. Their findings suggest that tubulointerstitial deposits do not have a major role in the pathogenesis of tubulointerstitial lesions.[37] These data were supported by characterisation of mononuclear cell subsets in renal cellular interstitial infiltrates obtained from patients with different diseases which did not show any specific pattern of infiltrate for a particular disease.[38] Nevertheless, the role of SLE-specific autoantibodies for glomerulonephritis is well established.

Interestingly, T cell stimulation induced by the $SmD1_{83–119}$ peptides was very high compared with the reactivity previously published by Bruns *et al* using nucleosomes as T cell antigen.[27] For both studies, T cell reactivity was determined by almost identical methods with only minor modifications. Thus, Bruns *et al* detected SIs up to 6 using nucleosomes in the identical proliferation assay, whereas the $SmD1_{83–110}$ peptide showed SIs up to 165.[27] The high proliferative response to the $SmD1_{83–119}$ peptide probably suggests the importance of the $SmD1_{83–119}$ peptide as a further major T cell antigen in some patients with SLE. Moreover, this T cell response was also seen in patients given immunosuppressive treatment, including cytotoxic drugs, further suggesting the high potency of this T cell epitope shared by the $SmD1_{83–119}$ peptide. Therefore, the 37 amino acid long $SmD1_{83–119}$ peptide is an appropriate candidate for further epitope mapping with smaller peptides to fine tune the T cell reactivity.

In conclusion, the $SmD1_{83–119}$ C-terminal peptide shares a T cell epitope that was specifically recognised by some patients with SLE. The high frequency of T cells that are reactive to the $SmD1_{83–119}$ peptide as well as the frequency of $SmD1_{83–119}$-specific B cells further suggest the important role of the SmD1 C-terminus as the main target structure of the SmD1 protein. Further analyses of these $SmD1_{83–119}$-specific T cells might give insight into the pathogenesis of SLE and open the way to T cell based treatments.

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Authors' affiliations

G Riemekasten, C Weiss, S Schneider, A Thiel, A Bruns, F Schumann, S Bläss, G-R Burmester, F Hiepe, Department of Rheumatology and Clinical Immunology, Charité University Hospital, Schumannstr 20/21, D-10117 Berlin, Germany

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