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T Cell Epitope Mapping of the Smith Antigen Reveals That Highly Conserved Smith Antigen Motifs Are the Dominant Target of T Cell Immunity in Systemic Lupus Erythematosus

Beth L. Talken,*† Kim R. Schäfermeyer,*† Craig W. Bailey,*† David R. Lee,‡ and Robert W. Hoffman2*†

B cell and T cell immunity to the Smith Ag (Sm) is a characteristic feature of systemic lupus erythematosus (SLE). We have shown that T cell immunity against Sm can be detected in SLE patients, and that T and B cell immunity against Sm are linked in vivo. TCR usage by Sm-reactive T cells is highly restricted and characteristic of an Ag-driven immune response. Sm is a well-characterized complex Ag consisting of proteins B1, B2, D1, D2, D3, E, F, and G. A unique feature of all Sm proteins is the presence of homologous motifs, Sm motif 1 and Sm motif 2. We used limiting dilution cloning and synthetic peptide Ags to characterize the human T cell immune response against Sm in seven SLE patients. We sought to determine the precise antigenic peptides recognized, the common features of antigenic structure recognized, and the evolution of the T cell response against Sm. We found there was a highly restricted set of Sm self-peptides recognized by T cells, with three epitopes on Sm-B and two epitopes on Sm-D. We found that T cell immunity against Sm-B and Sm-D was encoded within the highly conserved Sm motif 1 and Sm motif 2, and that immunity against these epitopes appeared stable. The present study supports the concept that T cell immunity to Sm is an Ag-driven immune response directed against a highly restricted set of self-peptides, encoded within Sm motif 1 and Sm motif 2, that is shared among all Sm proteins.

Immunity against the Smith Ag (Sm) is a characteristic feature of systemic lupus erythematosus (SLE). We have shown that T cell immunity against Sm can be detected in PBMC of SLE patients and that T and B cell immunity against Sm are linked in vivo (1). Previously, we reported that Sm-reactive T cells have a typical T phenotype, expressing the cell surface markers CD4, CD45RO, and TCR αβ. Also, Sm-reactive T cells produce cytokines that are important in B cell help and differentiation, including IFN-γ, IL-2, and IL-4 (1). Finally, we have previously shown for Sm-B (2) and recently shown for Sm-D (3) that TCR usage by Sm-reactive T cell is highly restricted and characteristic of an Ag-driven immune response.

B cell immunity to Sm is a characteristic feature of SLE, with autoantibodies directed against Sm present in 20–30% of patients with SLE (4). The presence of anti-Sm Abs is one of the criteria proposed by the American College of Rheumatology for disease classification of SLE and is widely used in clinical practice (5). Anti-Sm Abs are frequently of the IgG isotype, which is characteristic of a T cell-dependent response (6). B cell epitope-mapping studies of Sm and other autoantigens have suggested that in autoimmune immunity the immune response may be directed toward self-Ags that encode domains with important biological functions (7). Other studies implicate molecular mimicry against microbial agents, such as EBV, in the initiation of the anti-Sm immune response (8). A unified model of disease pathogenesis may need to encompass all these possibilities as contributory.

Sm is a well-characterized complex Ag that consists of proteins B1, B2, D1, D2, D3, E, F, and G. The Sm complex is highly conserved throughout evolution and is expressed in all tissues. Sm is part of the spliceosomal complex, whose biologic function is the splicing of precursor mRNA to mature mRNA (9). The Sm proteins serve an essential role in spliceosomal complex formation (10). A unique feature of Sm proteins is the presence of highly conserved motifs, called Sm motif 1 and Sm motif 2 (see Fig. 1), that are hypothesized to be involved in protein-protein interactions (11). Detailed structural knowledge of this complex Ag provides the opportunity to seek insight into T and B cell immune responses against Sm.

Currently, there exist several alternative hypotheses on the pathogenesis of SLE. These hypotheses include loss of Ag-specific tolerance with Ag-driven immunity against unmodified self-Ags, Ag-driven immunity against self-Ags that have been modified (including apoptotic modification), immunity against self-Ags triggered through molecular mimicry by microbial agents, and generalized (nondirected) immune activation. Furthermore, multiple mechanisms could be operative. For example, molecular mimicry could require exposure of cryptic self-epitopes to elicit an autoimmune response. The work in the present study was designed to examine the hypothesis that SLE is in part a T cell-dependent, Ag-driven immune process in which T cell immunity is directed against a restricted set of self-peptides contained with the Sm Ag.

The goals of the present study were to characterize the T cell immune response against the Sm, including the precise Ag peptides recognized, the common features of antigenic structure recognized, and the evolution of the T cell response against Sm. We found that there was a highly restricted set of Sm self-peptides...
recognized by T cells, with three epitopes on Sm-B and two epitopes on Sm-D. T cell immunity against both Sm-B and Sm-D was encoded within the highly conserved Sm motif 1 and Sm motif 2. Finally, T cell immunity against these T cell epitopes appeared stable over time.

Materials and Methods

Patients

The seven patients (P1–7) selected for study met the classification criteria of SLE proposed by the American College of Rheumatology (5). Six patients were female. The University of Missouri institutional review board approved all studies involving human subjects. Sera were characterized for the presence of autoantibodies reactive with small nuclear ribonucleoprotein (snRNP) polypeptides Sm-B and Sm-D using immunoblotting and ELISA (12). All patients had autoantibodies against snRNP Ags, including Sm-B (P1–7) and Sm-D (P1 and P3). HLA typing was performed using serologic and DNA-based typing methods as described previously (13). In brief, this was done using a combination of subfamily-specific PCR and direct DNA sequencing of PCR amplified products (14, 15). The HLA-DR genotypes of the patients were: P1, HLA-DRB1*0101, 1302, DRB3*0301; P2, HLA-DRB1*0401, 1301, DRB3*0101, DRB4*0101; P3, HLA-DRB1*0401, 1301, DRB3*0101, DRB4*0101; P4, HLA-DRB1*0101, 0401, DRB4*0101; P5, HLA-DRB1*0302, 1302, DRB3*0301, P6, HLA-DRB1*0301, 0401, DRB4*0101; and P7, HLA-DRB1*0101, DRB3*0301.

One patient, P1, was analyzed with T cell cloning performed at several time points over 2 yr. Clinically, this patient had longstanding disease of 20-yr duration with an SLE disease activity index of 0–6 in the first year of the study and 4–6 in the second year of the study (16). The patient took 400 mg/day hydroxychloroquine throughout the study and took 5–15 mg/day prednisone during the second year of the study.

Recombinant Ags

Full-length cDNA for snRNP peptides Sm-B3 and Sm-D1 were subcloned into the pMAL-c2 plasmid vector (New England Biolabs, Beverly, MA) and were the gift of S. Hoch (La Jolla Institute for Experimental Medicine, La Jolla, CA) (1). When expressed, these constructs resulted in a fusion protein consisting of an nsnRNP peptide linked to a fragment of the maltose binding protein. Ags were analyzed by PAGE and immunoblotting for purity and antigenic activity. The protein concentration of each Ag was determined; the Ags were aliquoted and stored at −80°C before use.

Peptide design and synthesis

Peptides were synthesized using F-moc chemistry on an Applied Biosystems model 432A peptide synthesizer (Foster City, CA) or were purchased (Research Genetics, Huntsville, AL) (2). Peptides were analyzed for purity and antigenic activity. The protein concentration of each Ag was determined; the Ags were aliquoted and stored at −80°C before use.

Generation of human T cell clones

The T cell clones used in these studies were generated as described previously (17). In brief, cells were obtained by apheresis and then subjected to density gradient centrifugation using Histopaque (Sigma, St. Louis, MO). Patient PBMC were used immediately or were cryopreserved for use as APC to restimulate clones. Cells (5 × 10^6) were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) with 20 μg/ml gentamicin, 2 mM L-glutamine, 15% AB-positive human serum, and pooled autologous peptides at a final concentration of 1 μg/ml. As Ags, a series of synthetic peptides, 25 residues in length, was used. Each peptide spanned the previous peptide in the series beginning at residue 13. The entire series of peptides were designed to span either Sm-B2 (including the sequence for the shorter splice variant Sm-B1, which lacks nine residues present on the carboxyl terminus of Sm-B2), or Sm-D1. The Sm-B2 Ag consisted of a pool of 19 peptides, whereas the Sm-D1 Ag consisted of a pool of nine peptides. Cells in a final volume of 5 ml were placed in a 25-cm² flask and incubated in 5% CO₂ at 37°C. Cells were restimulated with 5 × 10^9 autologous APC irradiated with 30 Gy and Ag in fresh medium on day 7. On day 14, T cell blasts were separated by centrifugation on a density gradient (Percoll; Pharmacia Fine Chemical, Piscatway, NJ) and cloned by limiting dilution in the presence of pooled peptides, fresh irradiated autologous APC and 10 U/ml IL-2 (R&D Systems, Minneapolis, MN) as described previously (1, 2, 17).

Proliferation assay

T cells (2 × 10^4) in complete medium were cultured for 48 h in 96-well flat-bottom tissue culture plates and then pulsed for 18 h with 1 μCi/well Tdr. Cells were harvested, and Tdr incorporation was detected by liquid scintillation counting.

Homologous cell lines and Ab blocking of Ag-induced proliferation

To determine the HLA restriction element(s) used by T cell clones, Ag-induced proliferation was performed in the presence of blocking concentrations of mAbs specific for HLA-DR, HLA-DQ, HLA-DP, or HLA class I framework determinants or for clones derived from P1 using a series of well-characterized homologous lymphoblastoid cell lines of known HLA genotypes (American Society for Histocompatibility and Immunogenetics, Lenexa, KS) as APC (17, 18).

Searches for similarity with known DNA and protein sequences

Searches for similarities between T cell epitopes identified in the present study and all DNA and protein sequences in the GenBank database were performed using BLAST (National Center for Biotechnology Information, Bethesda, MD). BLAST is an alignment search tool that allows rapid comparison of a query sequence to both DNA and protein databases. BLAST scores have a well-defined statistical interpretation, and BLAST uses a heuristic algorithm that is able to detect relationships between the query sequence and those in the databases that may share only isolated regions of similarity.

Table 1. Epitopes recognized by human T cell clones specific for Sm-B

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sm-B-E1 (residues 16–33)</th>
<th>Sm-B-E2 (residues 64–81)</th>
<th>Sm-B-E3 (residues 136–153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>17</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>P2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P3</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>P4</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>P5</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>P6</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>P7</td>
<td>0</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

FIGURE 1. The amino acid sequence of the human Sm-B2 protein is shown. The regions that contain the T cell epitopes Sm-B-E1, Sm-B-E2, and Sm-B-E3 are indicated. Also indicated are the regions encoding Sm motif 1 and Sm motif 2.
Results

Sm-B-specific human T cell clones were generated from SLE patients

A total of 54 Sm-B reactive human T cell clones were generated from seven patients (P1–7). These are shown in Table I. Thirty clones were generated from P1, one from P2, three from P3, seven from P4, four from P5, one from P6, and eight from P7.

Three T cell epitopes were recognized by SLE patient T cell clones on the Sm-B protein

An iterative process was used to define the regions of the Sm-B2 protein recognized by human T cell clones. Clones were initially analyzed for proliferation against pools of 19 peptides, which together span Sm-B2. Clones were subsequently tested against smaller pools of peptides and eventually against individual peptides, 15 residues in length. Three regions were identified by Sm-specific T cell clones on the Sm-B protein. These epitopes are shown in Fig. 1 and are labeled Sm-B-E1, Sm-B-E2, and Sm-B-E3. Sm-B-E1 spans residues 16–33, Sm-B-E2 spans residues 64–81, and Sm-B-E3 spans residues 136–153 of the full-length Sm-B2 protein. T cell clones were successfully generated from seven of seven patients. Clones were identified from five patients that reacted with Sm-B-E1 (P1–5). Additionally, clones were identified from six patients that reacted with Sm-B-E2 (P1 and P3–7), and finally clones were identified from three patients that reacted with Sm-B-E3 (P1, P4–P7; Table I).

Patient P1 selected for longitudinal cloning and analysis

One patient (P1) was selected for longitudinal cloning and analysis to further characterize the Sm-B-E1 epitope, which was recognized by the majority of patients. Clones generated at different points in the patient’s illness were examined to determine the stability of T cell epitopes recognized over time. As illustrated by representative clones in Fig. 2, a series of peptides, 15 residues in length (overlapping the previous peptide in the series by 12 residues), was used to more finely map the T cell epitope recognized by the panel of T cell clones. Only two peptides were significantly stimulatory, and these shared the core 12 residues, CILQDGRIFIGT. In longitudinal studies of P1, reactivity with the T cell epitope Sm-B-E1 remained unchanged over the 2 yr of study (data not shown).

Minimal T cell epitope identified from Sm-B using overlapping peptides and truncated peptides

To determine the minimal region within the Sm-B-E1 epitope required for T cell recognition, truncated variants of the CILQDGRIFIGT peptide were synthesized and tested. Four representative Sm-B-reactive clones are described in Fig. 3. Stimulation of the clones was observed only when the truncated peptides contained the core sequence ILQDGRIFI (contained in the full-length Sm-B protein at positions 20–28).

MHC restriction element used in T cell recognition of minimal peptide determined

The MHC restriction element used by T cell clones from P1 was determined using Ag-induced proliferation in the presence of blocking concentrations of mAbs specific for HLA-DR, HLA-DQ, HLA-DP, or HLA class I determinants or using a series of well-characterized homozygous lymphoblastoid cell lines of known HLA genotypes as APC. All clones tested from P1 recognized Ag only in the context of HLA-DRB1*1302/B3*0301 (data not shown). Because the restriction elements HLA-DRB1*1302/B3*0301 exist in strong linkage disequilibrium among all cell lines available at the time of these studies, it could not be determined whether DRB1*1302 or DRB3*0301 was the HLA restriction element used by the clones reported here.
and Sm-D-E2. Sm-D-E1 spans residues 35–53, and Sm-B-E2.

Two T cell epitopes were recognized by SLE patient T cell clones on the Sm-D protein. Clones were generated from P1, three from P2, and 16 from P3. From three of seven patients. These are shown in Table II. Nine peptides, which together span Sm-D1. Clones were subsequently tested against smaller pools of peptides and eventually against individual peptides, 15 residues in length. Two regions were identified by Sm-specific T cell clones on the Sm-D protein. These epitopes are illustrated in Fig. 5 and are labeled Sm-D-E1 and Sm-D-E2 (P1–3; Table II).

**Patient P1 selected for longitudinal cloning and analysis**

P1 was again analyzed in detail to define the regions within the Sm-D protein recognized by the majority of T cell clones. Longitudinal studies were also performed to delineate the stability of T cell epitopes recognized. The T cell epitopes identified in P1 were Sm-D-E1 and Sm-D-E2 (Fig. 5 and Table II). These were the regions identified repeatedly by cloning over 2 yr of study (data not shown). These appeared similar to results obtained in cross-sectional studies of two additional patients from whom Sm-D-reactive clones could be generated. Only for P3 there was a clear preponderance of reactivity to Sm-D-E1 (15 clones) compared with Sm-D-E2 (one clone; Table II).

**Minimal T cell epitope identified from Sm-D using overlapping peptides and truncated peptides**

To determine the minimal region of the Sm-D-E1 and Sm-D-E2 epitopes required for T cell recognition, truncated peptides were designed and synthesized. Sm-D-E1 reactive clones are described in Figs. 6A and 7A. Sm-D-E2-reactive clones are described in Figs. 6B and 7B. As illustrated in Fig. 6, a series of peptides, 15 residues in length, was used to define more precisely the T cell stimulatory peptide contained within Sm-D-E1 and Sm-D-E2. For the Sm-D-E1 epitope the core peptide HLKAVKMTLK was identified (Figs. 6A and 7A), and for Sm-D-E2 the core peptide SIRGNIRY was identified (Figs. 6B and 7B). As was observed for Sm-B, reactivity with Sm-D epitopes was similar among clones generated from P1 at different time points.

**MHC restriction element used in T cell recognition of minimal peptide determined**

The MHC restriction element used by T cell clones from P1 was determined using Ag-induced proliferation in the presence of blocking concentrations of mAbs specific for HLA-DR, HLA-DQ, HLA-DP, or HLA class I determinants or using a series of well-characterized homozygous lymphoblastoid cell lines of known HLA genotypes as APC. As was found for Sm-B-reactive clones, all Sm-D Ag-reactive clones recognized Sm-D peptides only in the context of HLA-DRB1*1302/B3*0301 (data not shown).

**Alanine substitution defines essential contact residues on Sm-B peptides**

To define residues critical for MHC binding or T cell contact, peptides with sequential alanine substitutions of the core peptide sequence ILQDGRIF were synthesized and tested for their ability to induce proliferation of T cell clones. A representative experiment is described in Fig. 4. It was found that alanine substitution of residues at positions 1, 2, 4–6, and 9 abolished the stimulatory capacity of the peptide. Alanine substitution of the native residue at position 8 also markedly reduced the stimulatory capacity of the peptide. Alanine substitution of the native residue at position 5 also markedly reduced the stimulatory capacity of the peptide. Alanine substitution of the native residue at position 5 also markedly reduced the stimulatory capacity of the peptide.

**Sequence similarity identified between Sm-B-E1 T cell epitope and hypothetical Mycobacterium protein**

The core sequences identified within the Sm-B-E1, Sm-B-E2, Sm-B-E3, Sm-D-E1, and Sm-D-E2 epitopes were compared with known protein and DNA sequences in the GenBank database using BLAST. The epitope ILQDGRIF within Sm-B-E1 shared an identical seven-residue segment with a hypothetical protein Rv1061 (LQDGRIF) (19). The Sm-B-E2-E3, Sm-D-E1, and Sm-D-E1-E2 epitopes did not have similarity with other proteins, except for related snRNP.

**Sm-D-specific human T cell clones were generated from SLE patients**

A total of 28 Sm-D reactive clones were successfully generated from three of seven patients. These are shown in Table II. Nine clones were generated from P1, three from P2, and 16 from P3. Two T cell epitopes were recognized by SLE patient T cell clones on the Sm-D protein.

The same iterative process used to characterize Sm-B was used to define the regions of the Sm-D1 protein recognized by human T cell clones. Clones were initially analyzed for proliferation against pools of nine peptides, which together span Sm-D1. Clones were subsequently tested against smaller pools of peptides and eventually against individual peptides, 15 residues in length. Two regions were identified by Sm-specific T cell clones on the Sm-D protein. These epitopes are illustrated in Fig. 5 and are labeled Sm-D-E1 and Sm-D-E2. Sm-D-E1 spans residues 35–53, and Sm-D-E2 spans residues 53–67 of the Sm-D1 protein. There were individual clones generated from the three Sm-D-reactive patients that recognized Sm-D-E1 (P1–3) and others that recognized Sm-D-E2 (P1–3; Table II).

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Discussion

The present study was designed to examine the hypothesis that at least in part SLE is a T cell-dependent, Ag-driven immune process in which T cell immunity is directed against a restricted set of self-peptides contained within the Sm Ag. T cell epitope-mapping studies of the Sm Ag revealed that there were a limited number of T cell epitopes. As summarized in Figs. 1 and 5 and Tables I and II, among the seven SLE patients studied there were three T cell epitopes on Sm-B and two T cell epitopes on Sm-D. Using truncated peptides for more precise delineation of the T cell stimulatory region, a core sequence ILQDGRIFI was identified within the epitope Sm-B-E1 (Figs. 2 and 3). For the epitope Sm-D-E1, the core sequence HLKAVKMTLK was identified (Figs. 6A and 7A), and within Sm-D-E2 the core sequence SIRGNNIRY was identified (Figs. 6B and 7B). These findings are consistent with recent studies of TCR CDR3 use among Sm-B- and Sm-D-reactive clones, where limited clonal diversity of CDR3 was found (2, 3). In previous studies it was suggested that a limited number of immunodominant peptides could be the basis for highly selective TCR usage (2, 3). TCR CDR3 selectivity was marked, and in some cases identical CDR3 were used by T cell clones derived from different patients recognizing the Sm Ag (2, 3). The present study provides direct evidence that T cell recognition of Sm is targeted to a highly restricted set of self-peptides.

Analysis of the structural features of the epitopes recognized on the Sm Ag revealed a number of interesting characteristics. The dominant T cell epitopes identified in this study are regions encoding the so-called Sm motifs, Sm motif 1 and Sm motif 2. These were first described by Hermann et al. and are 32 and 14 residues in length, respectively (11). The Sm motifs are believed to be involved in direct protein-protein interactions (10, 11, 20). Hermann et al. have demonstrated that these motifs share similarity among the different Sm core proteins B1, B2, D1, D2, D3, E, F, and G and are evolutionarily conserved. For example, the human D1 protein shares 78% homology with its putative homologue in rice and 99% homology with its homologue in the mouse. The reader is referred to Hermann et al. for a comprehensive discussion of this subject (11). The fact that the Sm motifs are encoded in all eight Sm proteins has been suggested to explain in part the immunologic cross-reactivity by monoclonal and polyclonal Abs between multiple Sm proteins (21, 22). Although we have no direct evidence demonstrating T cells cross-reactivity between Sm-B and Sm-D, the shared structural features of the Sm motif offers an intriguing structural explanation for T cell immunodominance directed against this highly restricted set of self-peptides. This will be a focus of future research.

Although additional studies of larger numbers of patients at various stages of disease are needed, another important finding of this study was that the T cell epitopes identified among a cross-sectional panel of seven patients and longitudinal analysis of one patient were similar. Future studies of patients with early disease will be of considerable interest to determine whether early T cell reactivity is directed against similar or different T cell epitopes.
The recognition of a limited set of self-peptides, as observed here for Sm, has been described for T cell recognition of other autoantigens, including such autoantigens as topoisomerase I in systemic sclerosis and myelin basic protein in multiple sclerosis (23, 24). It has been proposed that a fundamental feature of autoimmunity is T cell recognition of cryptic self-Ags (25). Consistent with this hypothesis, we now provide direct evidence that T cell recognition of the Sm self-Ag is highly restricted in SLE.

An alternative concept, which has been proposed to explain the loss of self-tolerance, is molecular mimicry (26). We investigated this possibility for Sm reactivity in SLE. Similarity searches were performed between the core T cell epitopes identified in the present study and the GenBank database. Interestingly, the Sm-B-E1 core sequence has a seven-residue segment identical with an M. tuberculosis hypothetical protein RV1061 (19). Consistent with a role for Mycobacterium in initiating autoimmunity, Mycobacterium bovis has been found to induce an anti-Sm immune response in NOD mice (27). However, in preliminary studies, the seven-residue Mycobacterium peptide LQDGRIF did not cross-stimulate clones reactive with the Sm-B-E1 sequence ILQDGRIF (nor did longer M. tuberculosis-derived peptides; data not shown). Further studies exploring cross-reactivity between Sm-B-E1 and Mycobacterium peptides are in progress.

In other work related to mimicry, Harley and James (8) found that a region of Sm-B, PPPGMRRP, that has similarity to an EBV protein is one of the early dominant B cell epitopes recognized by anti-Sm Abs. They also found that PPPGRPRP peptides could initiate SLE-like disease in animals (28). Although the PPPGMRRP B cell epitope is distant from the T cell epitopes identified in the present study, the T cell epitopes Sm-B-E1 and Sm-B-E3 contain sequences recognized by non-cross-reactive anti-Sm Abs that develop late in the evolution of anti-Sm-B Ab epitope spreading (29). Finally, it is known that T and B cell epitopes may be distinct.

Studies were performed to characterize the molecular interactions of Sm with MHC molecules on APC. Experiments using blocking mAbs against HLA framework determinants and cell lines homozygous for HLA demonstrated that all clones studied were restricted in Ag presentation by HLA-DRB1*1302/DRB3*0301. Analogue substitution studies (Figs. 4 and 8) demonstrated that key HLA binding sites or TCR contact residues for Sm-B-E1 were positions 1, 2, 4–6, and 9, and for Sm-D-E1 they were positions 2 and 5–9. These results are consistent with known HLA binding motifs for DRB1*1302/DRB3*0301 (30, 31). Future studies directly testing HLA binding and TCR interactions using site-directed mutagenesis of cloned TCR genes expressed in TCR-deficient Jurkat cell lines are in progress to more precisely define the molecular interactions among Sm self-reactive peptides, HLA, and the TCR (32, 33).

The present study provides direct evidence that SLE is at least in part a T cell-dependent, Ag-driven immune process in which T cell immunity is directed against a highly restricted set of self-peptides, encoded within Sm motif 1 and Sm motif 2, that are shared among all Sm proteins. We provide direct evidence that this mechanism is operative. This is consistent with a broader model of etiopathogenesis in SLE in which tolerance to self-Ags is lost, perhaps through molecular mimicry and/or exposure to cryptic self-epitopes, and autoimmunity develops as an Ag-driven immune process against highly conserved self-Ags.

In summary, we found that there was a highly restricted set of Sm self-peptides recognized by T cells, with three epitopes on Sm-B and two epitopes on Sm-D. T cell immunity against both Sm-B and Sm-D was encoded within the highly conserved Sm motif 1 and Sm motif 2. These T cell epitopes appeared to be stable over time. The findings of the present study support the concept that T cell immunity to Sm Ag is an Ag-driven immune response directed against a highly restricted set of self-peptides encoded within the functional domains of Sm motif 1 and Sm motif 2, which are found on all Sm proteins.

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