T CELLS IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Robert W. Hoffman

Division of Immunology and Rheumatology, University of Missouri, One Hospital Drive, Columbia, Missouri, Department of Veterans Affairs Medical Center, 800 Hospital Drive, Columbia, Missouri

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1. ABSTRACT

The role of T cells in the pathogenesis of systemic lupus erythematosus (SLE) is reviewed with a focus on autoantigen-specific T cells in SLE. The initial clue to a role for T cells in SLE was histopathologic studies demonstrating extensive infiltration of T cells at the sites of inflammation. Later studies, showing association between HLA polymorphisms and specific autoantibodies, directly implicated a role for T cells in autoantibody production. More recently, we and others have identified and characterized autoantigen-specific T cells in SLE. We review these studies on the role of autoantigen-specific T cells in SLE and present new findings on the molecular characterization of T cell immunity to Sm-B, Sm-D and U1-70kD small nuclear ribonucleoprotein (snRNP) autoantigens.

2. INTRODUCTION

Systemic lupus erythematosus (SLE) is arguably the prototypic multi-systemic autoimmune disease. The skin, joints, kidneys and the central nervous system are frequent targets of autoimmunity in SLE, although virtually any organ system can be affected by the disease. While the occurrence of B cell and T cell dysfunction is now established in SLE, the precise pathogenesis of the disease remains unknown. Several alternate hypotheses for the pathogenesis of SLE have been proposed. It is possible, and perhaps quite probable, that several of these proposed mechanisms may be operative simultaneously. These mechanisms include: loss of antigen-specific tolerance with antigen-driven immune response against self-antigens, enhanced B cell responsiveness, enhanced T cell responsiveness, immunity to cryptic self-antigens,
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3.1. Pathologic evidence for T cells in disease

Clinical-pathologic studies of SLE have demonstrated that the presence of lymphocyte infiltrates, including T and B lymphocytes, is often associated with histopathologic tissue injury (1,2). In addition, a broad range of functional abnormalities of T cells has been reported in SLE and these been recently reviewed by Tsokos (3). Whether any of these T cell abnormalities are primary or secondary events has been unclear; however, substantial new evidence is accumulating that supports the hypothesis that T cells play a central role in the pathogenesis of SLE.

3.2. Immunogenetic association of HLA with autoantibodies

The identification of association between the presence of specific autoantibodies and select HLA phenotypes or genotypes has provided valuable insight into the pathogenesis of SLE and other autoimmune disorders that are characterized by autoantibody production. Many studies have now shown an association between HLA polymorphisms and autoantibodies (4). These associations suggest a direct role for T helper cells in the induction of and/or augmentation of autoantibody production (5). The production of anti-native or double-stranded (ds)-DNA antibodies has been associated with HLA-DR2 and DR3; the production of anti-Sm antibodies has been associated with HLA-DR2, DR7, DQA1*0102, DQB1*0602 and DQ3; the production of anti-RNP antibodies has been associated with HLA-DR4 and genes frequently linked to DR4-bearing haplotypes, including DRw53 and DQB1*03 subtypes; and the production of antibodies against both the U1-70kD snRNP polypeptide and against U1-RNA has been associated with selected genotypes of HLA-DR4 and DR2, including HLA-DRB1*0401, *0407, *0408 and DRB1*1501 (2, 4, 6-16).

Based upon these observations that the production of autoantibodies is linked to HLA polymorphisms, we proposed a model where B cells take up apoptotically modified self-antigens (such as U1-70kD) via their high affinity immunoglobulin receptors, process the antigen and present it to T cells on their cell surface, bound to self HLA molecules (figure 1). In the setting of inflammation (perhaps during a systemic infection by virus or another microbe) tolerance is overcome and autoimmunity initiated. If down-regulatory dampening mechanisms cannot alter the balance, a self-perpetuating process of autoimmunity may ensue.

It has become clear in recent years that many otherwise healthy individuals have B cell and T cell repertoires against self-antigens (17,18). It is currently believed that it is through low level activation by self-antigens that the normal immune repertoire is physiologically maintained (18,19). In the setting of inflammation, however, the normal balance of control mechanisms may be overcome and then self-perpetuating pathologic auto-reactivity ensues.

3.3. Identification of autoantigen-specific T cells in SLE

T cells reactive with a number of autoantigens, thought to be important in the pathogenesis of SLE, have been reported; these include T cells reactive with dsDNA, nucleosomal histone proteins, non-histone chromosomal protein high molecular group (HMG), and with the Sm-B, Sm-D, U1-A and U1-70kD snRNPs (20-26). Studies on T cells reactive with each of these specificities are reviewed below (see Section 5).

4. B CELL-T CELL INTERACTIONS

4.1. Autoantibodies against DNA and Sm in SLE

Autoantibodies are a hallmark of SLE. Antinuclear antibodies occur at some point in virtually all patients with SLE. The presence of some of these antinuclear antibodies is highly specific for SLE. Antibodies to dsDNA or to the snRNP Sm antigen occurs in 20-30% of SLE patients. Because of the high specificity of these autoantibodies they have been included as two of the classification criteria for SLE in the proposed revised classification criteria for SLE by the American College of Rheumatology (27). Thus, they have substantial diagnostic significance for study purposes.

4.2. Autoantibodies against U1-70kD

Autoantibodies to another snRNP autoantigen, the U1-70kD polypeptide, can also be present in some SLE patients with immunity to Sm. It is detected in the absence of Sm reactivity in the majority of patients with mixed connective tissue disease (MCTD). Whether MCTD should be included among patients classified as SLE or...
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4.3. T cell help in autoantibody production

Many of the families of autoantibodies that react with dsDNA, Sm or U1-70kD are of the IgG subclass and are present in serum in large quantities, suggestive of a T cell dependent B cell response (32). Furthermore, the presence of these autoantibodies has been linked to distinctive HLA genotypes. This has provided indirect evidence of a role for T cells in autoantigen presentation and T cell – B cell collaboration in autoantibody production (for review see 4,31).

4.4. Immunogenetic studies of HLA and anti-snRNP antibodies

We have previously published a series of studies examining the associations between the presence of autoantibodies against snRNP polypeptides U1-70kD, Sm-B and Sm-D and HLA polymorphisms (31,33-36). We found that the presence of high titer antibodies against U1-70kD was associated with select genotypes of HLA-DR4 and an epitope shared with the HLA-DRB1*1501 genotype of HLA-DR2. In separate studies, we found that the presence of anti-Sm-D antibodies in children was associated with HLA-DR2. A number of investigators have reported similar association of anti-U1-1RN with HLA-DR4 (reviewed in 31). Other investigators have also reported association between anti-Sm antibodies and HLA-DR2 bearing haplotypes, although results have been conflicting as to whether the primary association between anti-Sm antibodies is with HLA-DR or DQ alleles. (See section 3.2 above).

5. ANTIGEN-SPECIFIC T CELLS IN SLE

5.1. DNA-histone reactive T cells

Rajogopalan, Zordan, Tsokos and Datta first described T cell lines specific for dsDNA that selectively augmented the production of pathogenic IgG anti-DNA autoantibodies (20). Datta and colleagues subsequently published a series of elegant studies further characterizing human T cells specific for native DNA. They reported that the majority of such cells are CD4+ and can provide T cell help to pathogenic anti-DNA autoantibody producing cells and that these T cells possess common recurrent CDR3 motifs, characteristic of antigen selection against a limited number of T cell epitopes. They have also described T cells reactive with nucleosomal histone proteins or non-histone chromosomal protein HMG (37).

5.2. Sm reactive T cells

Sm-reactive T cells were first reported by Hoffman et al. in 1993 (23). Individual T cell clones were described, which were reactive with either the Sm-B or Sm-D polypeptides. These cells possessed a T helper cell surface phenotype, CD3+, CD4+, CD45RO+, TCR alpha/beta+. T cell receptor (TCR) V-beta (BV) analysis revealed that the T cell lines were clonal and monoclonal antibody inhibition of proliferation revealed that the clones were restricted in antigen presentation by HLA-DR but not HLA-DQ or HLA-DP molecules (23).

5.3. U1-70kD reactive T cells

The existence of RNP-reactive T cells was first reported by O’Brien et al. using a U1-70kD fusion protein and PBMC from patients classified as having autoimmune rheumatologic disorders, such as SLE and MCTD (22). T cell clones from MCTD patients recognizing U1-70kD were first described by Hoffman et al. (24); Fenning et al. and Wolff-Vorbeck et al. also cloned and characterized T cell clones from normal blood donors that were reactive against U1-70kD (25,26).

5.4. U1-A reactive T cells

Okubo et al. first reported that PBMC cells from patients with MCTD or SLE, who possessed anti-U1-A autoantibodies, proliferated against a U1-A snRNP fusion protein; whereas they did not proliferate against topoisomerase I (none of the patients exhibited anti-topoisomerase I antibodies) (21). PBMC from normal blood donors without anti-U1-A antibodies did not proliferate either. Okubo et al. also demonstrated that CD4+ T cells were the subset proliferating and, using limiting dilution analysis, that such U1-A-reactive T cells were relatively frequent in the MCTD patient group (1 of 4,065-1 of 23,256). They mapped the T cell epitope on U1-A to a region spanning the C-terminal 83 residues of the protein. This T cell reactive region contains the highly conserved RNP motif within the second RNP-80 RNA-binding domain (38).

6. T CELL IMMUNITY TO Sm AND U1-70KD SMALL NUCLEAR RIBONUCLEOPROTEINS (snRNP)

6.1. SnRNP autoantigen structure

Extractable nuclear antigen (ENA) contains both the Sm and RNP antigens. The Sm and RNP antigens have been biochemically and molecularly characterized to be components of uridylic acid-rich RNA (U) snRNP complexes. The Sm antigen consists of the B1-2, D1-3, E, F and G snRNP polypeptides which are non-covalently associated with U1, U2, U4/U6 and U5 RNA (figure 2) (39,40). U snRNP are part of the spliceosome complex that normally functions in the splicing of precursor mRNA to mature mRNA. The so-called RNP or nuclear RNP (snRNP) antigen consists of 70kD, A and C snRNP polypeptides which are non-covalently associated with U1-RNA (39,40).

While these snRNP antigenic complexes normally reside and function within the cell, it has been found that some antigens, including U1-70kD, undergo cleavage during UV-induced apoptosis and migrate to vesicles or blebs at or near the cell surface (41,42). It has been postulated that apoptotic alterations in structure and/or intracellular location of antigens such as U1-70kD may be important in breaking immunological tolerance and the development of autoimmunity (43-45). This topic is reviewed in detail in the accompanying article in this issue by Dr. Eric Greideringer.
70kD protein has recently been identified (53).

As a measure of B cell reactivity, patients’ sera were characterized for the presence of anti-snRNP polypeptide reactivity against U1-70kD, Sm-B and Sm-D polypeptides using immunoblotting and ELISA. We then attempted to generate T cell clones from the same panel of patients using identical cloning conditions for the three antigens. We then correlated the findings of B cell reactivity (i.e., the presence of anti-U1-70kD, anti-Sm-B and/or anti-Sm-D antibody) with T cell reactivity, as characterized by the ability to clone cells, against individual snRNP polypeptides, U1-70kD, Sm-B and/or Sm-D. We found that there was a statistically highly significant correlation between B and T cell reactivity as measured by this approach and we concluded that B cell and T cell reactivity against snRNP polypeptides appeared to be linked in vivo (46).

Finally, the use of synthetic peptides overcomes many of the technical problems described above. Large quantities of peptides of high purity can be produced and utilized to generate T cell clones. Synthetic peptides can be used to further characterize clones generated with fusion protein or other native antigens. Potential disadvantages of peptides are the fact that they lack post-translational modifications and may theoretically reveal previously cryptic T cell epitopes (also arguably an advantage).

6.2. Cloning and characterization of snRNP-reactive T cells from SLE and MCTD patients

We have generated a large series of human T cell clones specific for individual snRNP polypeptides, including Sm-B, Sm-D and U1-70kD (46-51). In our initial studies, these clones were generated using biochemically purified snRNP antigens (23) and later using recombinant fusion proteins (46-51). Most recently, we have used overlapping synthetic peptides as an alternative approach to clone snRNP reactive T cells (46-51). Each of these antigens has its own potential advantages and disadvantages.

In our initial studies antigens were biochemically purified using immunoaffinity columns and preparative scale polyacrylamide gel electrophoresis with rabbit thymic extract or HeLa extracts as the source of antigen. These antigen sources have the potential advantage of having undergone post-translational modification. They have several disadvantages, however, including the potential for proteolytic cleavage, lipopolysaccharide (LPS) contamination and xenoreactivity to the known sequence variations in rabbit snRNPs polypeptides compared to human snRNP polypeptides (52).

Recombinant fusion proteins have the theoretical advantage that they can be produced in large quantities, and the theoretical disadvantages that being expressed in bacteria they are not subject to post-translational modification. Furthermore, they possess additional fusion protein sequences that, if not removed, can be the source of an anti-fusion protein immunologic responses. In reality, the U1-70kD and other snRNP can prove difficult to express at high levels. There appears to be a number of reasons for this difficulty in protein expression, for example the presence of a repressor sequence in the U1-70kD protein has recently been identified (53).

6.3. T cell – B cell responses are linked

We have examined the relationship between B cell reactivity and T cell reactivity against snRNP polypeptides ex vivo. As a measure of B cell reactivity, patients’ sera were characterized for the presence of anti-snRNP polypeptide reactivity against U1-70kD, Sm-B and Sm-D polypeptides using immunoblotting and ELISA. We then attempted to generate T cell clones from the same panel of patients using identical cloning conditions for the three antigens. We then correlated the findings of B cell reactivity (i.e., the presence of anti-U1-70kD, anti-Sm-B and/or anti-Sm-D antibody) with T cell reactivity, as characterized by the ability to clone cells, against individual snRNP polypeptides, U1-70kD, Sm-B and/or Sm-D. We found that there was a statistically highly significant correlation between B and T cell reactivity as measured by this approach and we concluded that B cell and T cell reactivity against snRNP polypeptides appeared to be linked in vivo (46).

6.4. Cytokines characterized that may assist B cell help and differentiation

One could logically hypothesize that snRNP reactive T cell clones might have a T helper-2 (Th2)-like phenotype. To examine this possibility, we utilized complete PCR with so-called mimic target DNA as internal controls. We examined T cell clones for the production of IL-2, IL-4, IL-6, IL-10, IL-13, IFN-gamma and TGF-beta. Findings by PCR were confirmed for select cytokines in ELISA. We found that cytokine production was heterogeneous among clones and that overall the clones had a Th2-like phenotype. We did observe, however, that the clones produced substantial quantities of cytokines that can assist in B cell help and differentiations, including IL-2, IL-4 and IFN-gamma (46 and unpublished).

6.5. HLA-DR molecules serve as MHC restriction elements for autoantigen presentation

The snRNP-reactive clones, specific for either U1-70kD, Sm-D or Sm-B, described by Hoffman and colleagues, have been characterized for the HLA molecules that serve as the MHC restriction element in autoantigen presentations to T cell clones. All clones studied utilized HLA-DR, not HLA-DQ or HLA-DP, for their restriction element. This was shown in monoclonal antibodies blocking studies using blocking antibodies against framework determinants on either HLA-DR, DQ or DP. Further studies were done to characterize the HLA-DR alleles utilized in antigen presentation by testing a panel of Epstein-Barr virus (EBV) transformed homozygous cell line of known HLA genotypes (46). The Sm-B and Sm-D polypeptide were restricted in one patient by HLA-
DR1*1302/DRB3*0301 (47). Additional studies are in progress.

6.6. Rare T cell phenotypes, and T cell precursor frequency and detection

While the majority of snRNP reactive T cell clones had a CD4+ phenotype, we have found unusual clones that possess a CD8+ phenotype. These CD8+ cells exhibited strong proliferation to antigen and robust expansion. Additional studies on these clones will be of interest in that few human CD8+ T cell clones specific for an autoantigen have been previously described in the literature (50).

How common are snRNP reactive T cells in peripheral blood? Answering this question has been technically difficult but precursor frequency appears to be in a range similar to or greater than that for tetanus toxoid reactive T cells among immunized individuals (i.e. approximately 1/10,000 or greater). Can snRNP reactive cells be detected in proliferation assays using peripheral blood mononuclear cells (PBMC)? Using pools of overlapping peptides or fusion proteins, we have been unable to convincingly and reproducibly detect such cells. In studies on the MRL/lpr lupus-prone mouse model Muller and colleagues have identified strong proliferation of spleen T cells to a peptide spanning residues 131-151 of the U1-70kD protein, suggesting that it might be feasible to detect snRNP reactive cells from PBMC in SLE or MCTD patients. On the other hand, it is already known that it may be difficult to detect proliferation of PBMC to antigens to which an individual has been immunized and can be shown to have protective immunity, (such as tetanus toxoid or influenza hemagglutinin). The reasons for these discrepancies may include such factors as: well to well variability in the number of precursor cells, disassociation between proliferative responses and cytokine production, poor signal to noise ratio in detection assays, high baseline proliferation response and cryptic responses to peptides that obscure specific responses to the autoantigenic peptide of relevance. We are continuing to pursue this very important topic.

6.7. Abnormal T cell receptor signal transduction

The laboratory of Tsokos and the laboratory of Kanmer have published seminal studies identifying and characterizing the molecular basis of abnormal early T cell receptor signal transduction in SLE. These studies have been recently reviewed (54,55). Clearly, their work established that this is one of the pathogenic mechanisms operative in SLE. Craft and co-workers have recently provided evidence for similar abnormalities in T cell activation in the MRL/lpr lupus-prone mouse model (56). We have performed a series of studies suggesting that there might be an antigen driven immune response directed against a limited number of T cell epitopes. We used an iterative process to define the minimal peptides recognized by snRNP specific human T cell clones, including testing of peptides serially truncated from the carboxyl and amino terminus. Using this approach, we have defined 3 epitopes on Sm-B and 2 epitopes on Sm-D. These epitopes are shown in figure 3. Interestingly, 2/3 epitopes on Sm-B and 2/2 epitopes on Sm-D are contained within the highly conserved Sm motifs. The so-called Sm motifs are regions conserved on all Sm proteins, B1, B2, D1, D2, D3, E, F and G. These Sm motifs are hypothesized to function in protein-protein interaction in spliceosome functioning. Sm is part of the larger spliceosomal complex, whose biologic function in the normal cells is in splicing precursor mRNA to mature mRNA (57-59).
Our laboratory is also now completing T cell epitope mapping of the U1-70kD snRNP polypeptide. In preliminary studies, we have mapped 5 T cell epitopes on the 70kD polypeptide, all of which reside in the first RNP-80 RNA binding domain. Interestingly, all of these epitopes are contained within the RNA binding domain that interacts with U1-RNA stem loop I (60).

**Figure 3.** The three T cell epitopes identified on the Sm-B snRNP polypeptide and the two T cell epitopes on Sm-D snRNP polypeptides are illustrated here.

As discussed in Section 6.10, common features of T cell epitopes are that they span Sm motifs or Sm proteins and the RNA bind domain on the 70kD polypeptide. Muller has recently reviewed epitope mapping studies for snRNP and other autoantigens and described the RNP motif as a recurring target of B cell autoimmunity. Many years ago Tan first brought such general features of autoantigens to our attention (61). He observed that B cell epitopes on autoantigens are frequently regions of key function or have enzymatic activity and suggested that this might be a fundamental feature of autoreactivity. The mechanism for these observations, however, remain unknown. While the basis for these observations on T cell epitopes also are currently unknown, one could speculate that regions of RNA-protein or protein-protein interactions may be processed and presented differently from other regions of a molecule (62-64). We are currently examining this hypothesis.

There has been a longstanding interest in the hypothesis that molecular mimicry between microbes and self-antigens underlies autoimmunity in general and SLE in particular. The definition of T cell epitopes on snRNP autoantigens provides a unique opportunity to investigate this further. Once the minimal T cell epitope was defined, we performed alanine scanning on these minimal peptides to identify those residues which are critical for MHC binding on the antigen presenting cell or critical as T cell receptor contact residues. This approach allows for homology searches of the native peptide T cell epitope against the GenBank Database (i.e. ILQDGRIF1) and for searches of motifs containing only critical residues (i.e. ILXDGRXXI; where X=all possible amino acids) against the Database, as described above. Applying this approach, we found that there was homology between the Sm-B epitope 1 with a hypothetical *Mycobacterium tuberculosis* protein (8/9 residues were identical). We are now experimentally pursuing this observation.
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7. CONCLUSION

In conclusion, we have reviewed the current evidence for the role of autoantigen-specific T cells in the pathogenesis of SLE. Substantial evidence supports the concept that T cells are important in assisting in autoantibody production; through this and possibly other direct mechanisms, T cells are critically important in the pathogenesis of SLE. We have reviewed published work on several different autoantigen-specific T cell systems, including anti-dsDNA-histone, Sm, U1-70kD and U1-A reactive. Finally, we have reviewed in detail our own published work and described new and ongoing work on T cell immunity to Sm and U1-70kD. We report findings for these systems including: 1) that T cell and B cell responses appear linked; 2) that T cells producing cytokines may assist in B cell help and differentiation; 3) that we have identified and characterized HLA-DR molecules serving as MHC restriction elements in Sm autoantigen presentation; 4) that TCR CDR3 usage among Sm-B, Sm-D and U1-70kD reactive T cell clones is highly restricted; 5) that we have identified 3 T cell epitopes on Sm-B and 2 epitopes on Sm-D; 6) that functional regions (Sm motifs or RNA binding domains) appear to be the targets of T cell reactivity.

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9. REFERENCES


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**Send correspondence to:** Dr. Robert W. Hoffman,
Division of Immunology and Rheumatology, University of Missouri, MA427 HSC, One Hospital Drive, Columbia, Missouri 65212, Tel: 573-884-8792, Fax: 573-884-0304, E-mail: Hoffmanr@health.missouri.edu