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T Cell Studies in a Peptide-Induced Model of Systemic Lupus Erythematosus

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We have previously reported that immunization with a peptide mimetope of dsDNA on a branched polylysine backbone (DWEYSVWLSN-MAP) induces a systemic lupus erythematosus-like syndrome in the nonautoimmune BALB/c mouse strain. To understand the mechanism underlying this breakdown in self tolerance, we examined the role of T cells in the response. Our results show that the anti-foreign and anti-self response induced by immunization is T cell dependent and is mediated by I-E^d-restricted CD4$^+$ T cells of the Th1 subset. In addition, generation of the critical T cell epitope requires processing by APCs and depends on the presence of both DWEYSVWLSN and the MAP backbone. The breakdown in self tolerance does not occur through cross-reactivity between the T cell epitope of DWEYSVWLSN-MAP and epitopes derived from nuclear Ags. In this induced-model of SLE, therefore, autoreactivity results from the activation of T cells specific for foreign Ag and of cross-reactive anti-foreign, anti-self B cells. Despite the fact that tissue injury is mediated by Ab, the critical initiating T cell response is Th1. ***The Journal of Immunology,*** **2001, 166: 1667–1674.**

Many studies have shown that both B and T cells play a central role in autoimmune disease. The serum response in systemic lupus erythematosus (SLE)[2] contains autoreactivities to a variety of nuclear Ags, of which anti-dsDNA reactivity is the most important prognostic factor (1). In murine lupus models, disruption of T cell activation (2–8) or an absence of T cells (9, 10) abrogates autoantibody production and glomerulonephritis. In addition, nephritogenic anti-dsDNA Abs are high affinity, somatically mutated IgG Abs, all of which are features of a T cell-dependent, Ag-driven immune response (11, 12). Sequence analyses of anti-dsDNA Abs, showing a greater than random ratio of replacement to silent mutations in complementarity-determining regions, suggests that DNA could be the selecting Ag for these Abs (13–15). Since uncomplexed mammalian DNA is poorly immunogenic (16), and T cells are unlikely to recognize dsDNA in the context of MHC, models have emerged in which DNA-associated proteins or anti-dsDNA Abs themselves provide the T cell epitope that drives anti-dsDNA production.

In an attempt to identify other potential antigenic stimuli that could induce anti-dsDNA Abs, we used a high affinity mouse monoclonal IgG2b pathogenic anti-dsDNA Ab, R4A, to screen a phage decapeptide display library. Sequence alignment of the selected peptides revealed a five-amino acid consensus sequence (DWEYS) (17). Immunization with a multimeric form of a decapeptide containing this five-amino acid sequence (DWEYSVWLSN-MAP) led to an SLE-like syndrome in BALB/c mice (18). To understand the basis for the loss of self-tolerance in BALB/c mice following immunization and to determine the validity of this system as a model for SLE, we have examined the role of T cells in the anti-foreign and anti-self response.

Materials and Methods

Mice

Eight- to 10-wk-old female wild-type and nude BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in the Albert Einstein College of Medicine (Bronx, NY) animal (specific pathogen-free) barrier facility or in the barrier facility of Rockefeller University (New York, NY).

Antigens

The peptides, DWEYSVWLSN, DWEYSVWLSN-MAP, DWEYSVWLS-MAP, DWEYSVWL-MAP, DWEYSVW-MAP, DWEYSV-MAP, DWEYS-MAP, VWLSN-MAP, DWEYSVWLSN-MAP (4), DWEYSAA AAA-MAP, $(DWEYSVWLSN)_3$, DWEYSEGL-MAP, $(DWEYSEGL)_3$, DWSSDVWLSN-MAP, and DWEYSVWLSM-MAP used in immunizations and T cell proliferation assays were purchased from Research Genetics (Huntsville, AL), and their purities and/or sequences were verified by either HPLC or amino acid analysis. Unless otherwise indicated, all MAP peptides are linked to an eight-branch polylysine backbone. Peptides soluble at neutral pH were dissolved in dH_2O, whereas those insoluble at neutral pH were dissolved in dH_2O with pH adjusted to 8.5 to produce 10 mg/ml stock solutions. MAP backbone (Anaspec, San Jose, CA), histones (Sigma, St. Louis, MO), and Smith Ag/ribonuclear proteins (Sm/RNPs) (Immunovision, Springdale, AR) were dissolved in dH_2O to produce 10 mg/ml stock solutions.

Immunizations for serum studies

Mice were immunized s.c. with 100 μg of DWEYSVWLSN-MAP, DWEYSVWLSN, $(DWEYSVWLSN)_3$, $(DWEYSEGL)_3$, or DWEYS-MAP in 200 μl of PBS/CFA H37 Ra (1/1; Difco, Detroit, MI) on day 0 and then boosted in IFA (Difco) on days 7 and 14. Control mice received either 100 μg of MAP backbone (MAP core) in adjuvant or adjuvant alone using the same protocol. Serum was obtained on days 0, 7, 14, 21, 35, and 49.

Peptide ELISA

Enzyme immunoassay/RIA high binding 96-well plates (Costar, Corning, NY) were coated with 30 μl of 15 μg/ml of the relevant peptide and incubated overnight at 4°C. Plates were blocked with 100 μl of 3% FCS (HyClone, Logan, UT) in PBS at 37°C for 1 h, then washed with PBS-0.05% Tween. Thirty microliters of diluted sera were added to the plates for 2 h at 37°C. Plates were then washed with PBS-Tween, and 30 μl of alkaline phosphatase-conjugated goat anti-mouse IgG or IgG1 (Southern Biotechnology Associates, Birmingham, AL) diluted 1/1000 in 3% FCS/

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[2] Abbreviations used in this paper: SLE, systemic lupus erythematosus; DC, dendritic cells; Sm/RNP, Smith Ag/ribonuclear protein.

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PBS was added to the plates for 1 h at 37°C, followed by 30 μl of *p*-nitrophenyl phosphate solution (Sigma). OD was monitored at 405 nm using a Titer-Tek Multiscan ELISA reader (Titertek, Huntsville, AL).

Double-stranded DNA ELISA

Calf-thymus DNA (Calbiochem-Novabiochem, La Jolla, CA) was dissolved in PBS, sonicated, and filtered through a 0.45-μm nitrocellulose Millex syringe filter (Millipore, Bedford, MA) to produce dsDNA in 10- to 20-kbp fragments. Thirty microliters of 100 μg/ml dsDNA was used to coat enzyme immunoassay/RIA high binding 96-well plates (Costar) overnight at 37°C. The remainder of the assay was performed as described for the peptide ELISA.

T cell purification

Wild-type BALB/c mice were immunized in the right front and hind footpads with 100 μg of DWEYSVWLSN-MAP in 100 μl of PBS/CFA H37 Ra (1/1) and in the left front and hind footpads with 100 μl of PBS:/FA H37 Ra (1/1) alone (50 μl was administered per footpad). One week later, cells from the left and right popliteal, axillary, and brachial lymph nodes were harvested separately in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 5% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM β-ME. Following RBC lysis, cells at 2×10^7 cells/ml were incubated at 4°C for 30 min with supernatants from the following American Type Culture Collection cell lines (Manassas, VA): TIB-120 (anti-I-$A^{b,d,q}$ and anti I-$E^{d,k}$), HB-198 (anti-mouse macrophage), RA3-6B2.1 (anti-B220), and either TIB-207 (anti-CD4) or TIB-105 (anti-CD8). Excess Ab was washed away, and the cells were incubated with sheep anti-rat IgG Dynabeads (Dynal, Oslo, Norway) in a 1/4 ratio, respectively, at 4°C for 30 min with rotation. CD4 or CD8 T cells (>96% purity as determined by FACS) were isolated by magnetic bead depletion (Dynal).

Preparation and Ag pulsing of dendritic cells (DC)

Bone marrow-derived DC were grown as previously described (19). Briefly, bone marrow was harvested from the femur and tibia of wild-type BALB/c mice on day 0. RBC lysis was achieved using ammonium chloride, and the resulting cells were depleted of T cells, B cells, and APCs by resuspension at 1×10^7 cells/ml in supernatants from the American Type Culture Collection cell lines TIB-120 (anti-I-$A^{b,d,q}$ and anti I-$E^{d,k}$), TIB-211 (anti-CD8), TIB-207 (anti-CD4), and TIB-146 (anti-B220) in the presence of rabbit complement (Pel-Freeze Biologicals, Rogers, AR) for 1 h at 37°C. The resulting cells were washed and placed in 24-well plates at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 5% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and GM-CSF containing supernatants from J558L cells (diluted 1/30). Cells were fed with fresh medium on days 2 and 4. On day 6 the nonadherent cells were collected and plated at 5×10^5 cells/ml in GM-CSF-containing medium in the presence of 0.5 ng/ml LPS (Sigma) and various concentrations of DWEYSVWLSN-MAP. The Ag-pulsed DC were collected on day 7, washed twice in RPMI, irradiated with 1500 rad (^{137}Cs), and resuspended in Click's medium (Irvine Scientific, Santa Ana, CA) supplemented with 0.75% heat-inactivated normal mouse serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME.

Lymph node T cell proliferation assays

Mice were immunized in the right fore and hind footpads with 100 μg of DWEYSVWLSN-MAP in 100 μl of PBS:CFA H37 Ra (1/1) and in the left fore and hind footpads with 100 μl of PBS:CFA H37 Ra (1/1) alone (50 μl/footpad). One week later, cells from the left and right popliteal, axillary, and brachial lymph nodes were harvested separately and plated at 3×10^5 cells/well in 96-well flat-bottom plates in Click's medium supplemented with 0.75% normal mouse serum (Taconic Farms, Germantown, NY), 8 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM β-ME. Cells were pulsed with several concentrations of the various peptides or purified protein derivative (Statens Serum Institute Copenhagen, Denmark) in triplicate for 72 h. Each well was pulsed with 0.5 μCi of [^{3}H]thymidine (sp. act., 20 Ci/mmol) for 8 h. Cells were harvested, and the incorporated radioactivity was counted using a Wallac Tomtec harvester 96 Mach III M and a 1450 liquid scintillation Microbeta counter (Turku, Finland).

Cytokine ELISAs

Lymph node T cell proliferation assays were performed as described above. After 72 h supernatants from each well were tested for the presence of IL-2, IL-4, and IFN-γ using the Endogen mouse cytokine ELISA kits (Woburn, MA). Cytokine concentrations were determined based on standard curves.

Determination of peptide processing and responding T cells

The basic protocol for DC-induced T cell proliferation was as follows. T cells were purified from lymph nodes of wild-type BALB/c mice that were immunized in their footpads with DWEYSVWLSN-MAP in CFA or CFA alone 1 wk earlier as described above. These purified T cells ($1–3 \times 10^5$) were then cocultured in triplicate for 72 h with $0.1–1 \times 10^5$ DC that were purified, pulsed, and irradiated as described above. T cell proliferation was assayed as described above. To determine which population of T cells proliferates in response to DWEYSVWLSN-MAP, CD4 and CD8 T cells were each purified and used separately in these assays. To determine whether DWEYSVWLSN-MAP requires uptake for presentation to cognate T cells, DC were pulsed with various concentrations of MAP peptide at 4 or 37°C for 3 h on day 6 of culture, washed twice, and plated at 6×10^5 cells/ml in GM-CSF medium in the presence of 0.5 ng/ml of LPS. To safeguard against nonspecific effects of the lower temperature on the Ag-presenting capabilities of DC, control DC were placed at 4°C for 3 h before receiving Ag and LPS during overnight culture. On day 7, DC were washed, irradiated, and cocultured with T cells purified from lymph nodes of wild-type BALB/c mice as described above. T cell proliferation was then assayed as described. To determine whether DWEYSVWLSN-MAP requires processing for presentation to cognate T cells, DC were pulsed with various concentrations of peptide and LPS as described above in the presence or the absence of 20 mM NH_4Cl on day 6 of culture. On day 7, DC were washed twice in RPMI, then fixed with 4% paraformaldehyde in PBS for 30 min at 4°C. Control DC were either not fixed following NH_4Cl treatment or were fixed before overnight Ag pulsing. DC were washed, irradiated, and cocultured with T cells purified from lymph nodes of wild-type BALB/c mice as described above. T cell proliferation was then assayed as described.

MHC restriction of in vivo primed T cells

To determine the MHC haplotype responsible for presenting DWEYSVWLSN-MAP, lymph node T cell proliferation assays were conducted as described above in the presence of 50 μg/ml of DWEYSVWLSN-MAP and blocking Abs to I-A^d and I-E^d. These Abs were purified from ascites produced by the inoculation of SCID mice with the cell lines MKD6 (American Type Culture Collection HB-3) and 1444S (American Type Culture Collection HB-32), which secrete IgG2a anti-mouse I-A^d and anti-mouse I-E^d Abs, respectively. The purities of the Igs were verified by SDS-PAGE, and their concentrations were measured by both absorbance at 280 nm and quantitative ELISA using an IgG2a standard (20).

Statistics

Comparisons between two groups were performed using Student's *t* test. Error bars represent SEM values. $p < 0.05$ was considered statistically significant. Unless otherwise indicated, *p* values represent comparisons made on day 49 at the highest dose of antigenic pulse or at the highest concentration of blocking Ab.

Results

The anti-foreign and anti-self response is T cell dependent

The autospecificities that are correlated with disease manifestations of SLE, specifically anti-dsDNA Abs, are high affinity, IgG, somatically mutated Abs produced by B cells in a T cell-dependent fashion (11, 12). Peptide-induced SLE is characterized by anti-peptide, anti-DNA cross-reactive Abs that also display somatic mutation (18, 21). To determine whether the anti-foreign and anti-self response exhibited in peptide-induced SLE reproduces the T cell dependence of spontaneous SLE, nude and wild-type BALB/c mice were immunized with DWEYSVWLSN-MAP. Fig. 1 indicates that athymic nude mice do not mount an Ab response against either the peptide or dsDNA, affirming that the mechanism underlying induction of autoimmunity following peptide immunization is T cell dependent.

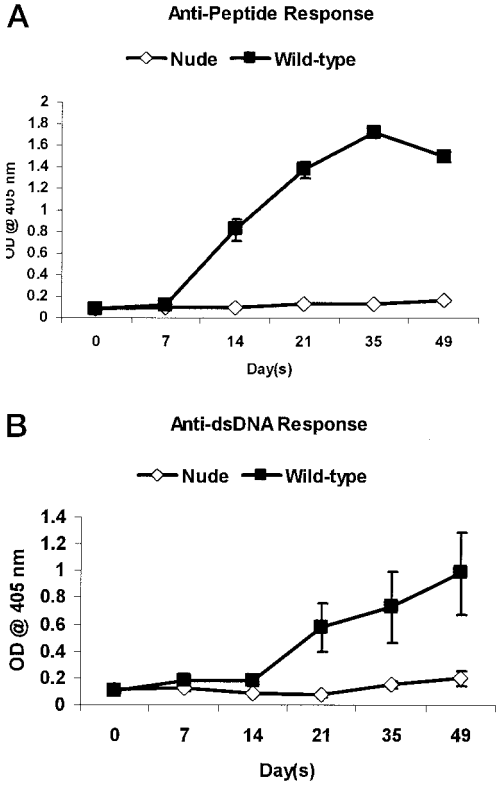

FIGURE 1. T cell dependence of anti-DWEYSVWLSN-MAP and anti-dsDNA response. Wild-type and nude BALB/c mice were immunized with DWEYSVWLSN-MAP in CFA on day 0 and then boosted in IFA on days 7 and 14. IgG anti-peptide (*A*) and IgG1 anti-dsDNA (*B*) reactivities assayed by ELISA in 1/500 diluted sera show that wild-type mice have significantly greater serum responses to peptide ($p = 4 \times 10^{-13}$) and to dsDNA ($p = 0.009$) than nude mice. Results are expressed as the mean absorbance at 405 nm of eight nude and five wild-type mice, each determined in duplicate, and are representative of two separate experiments each containing five to eight nude and wild-type mice.

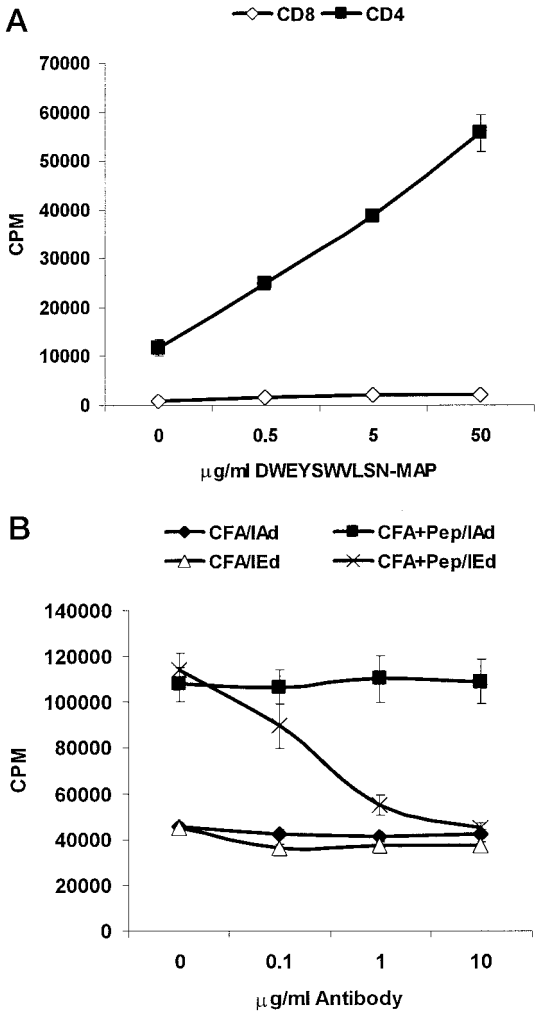

FIGURE 2. T cell restriction of DWEYSVWLSN-MAP. *A*, CD4 restriction. CD4 and CD8 T cells were purified from the lymph nodes of BALB/c mice immunized 1 wk earlier with DWEYSVWLSN-MAP. Part (1×10^5) of each T cell population was incubated in triplicate with 1×10^4 DC that had been pulsed with varying doses of peptide, washed, and irradiated as described in *Materials and Methods*. T cell proliferation was assayed as a function of incorporated radioactivity. Results are presented as the mean counts per minute for triplicate determinations of T cells and DC cultures and show dose-dependent CD4 T cell proliferation and no CD8 T cell proliferation in response to peptide ($p = 0.0004$). *B*, I-E^d restriction. Five mice were immunized with either CFA or CFA and DWEYSVWLSN-MAP. One week later their lymph node cells were harvested and plated in triplicate with 50 μg/ml of DWEYSVWLSN-MAP in the presence of increasing concentration of blocking Abs to I-Ed or I-Ad as described in *Materials and Methods*. The data show inhibition by anti-I-Ed Ab ($p = 5 \times 10^{-12}$) and are expressed as the mean counts per minute of five mice, each determined in triplicate.

The anti-peptide response is mediated by CD4 I-E^d-restricted Th1 T cells

To determine which T cell population was responsible for the anti-peptide response, CD4 and CD8 T cells were purified from the lymph nodes of mice immunized with DWEYSVWLSN-MAP. Fig. 2*A* shows that proliferation to peptide-pulsed DC was detectable only in the CD4 population, indicating that the peptide response is mediated by CD4 T cells. This is further supported by the observation that culturing lymph node cells from immunized mice with peptide results in the selective proliferation of CD4 and the death of CD8 cells. The ratio of CD4/CD8 increases from 1.7 to 5.0 during in vitro culture of primed lymph node cells with peptide ($p = 0.002$; data not shown).

It has been previously speculated that T cells that drive the propagation of anti-dsDNA-producing B cells are more likely to be I-E^d restricted because the preponderance of negative charges in the binding pocket of these class II molecules allows the binding of peptides derived from positively charged DNA-associated proteins (22). To determine the MHC restriction of the anti-peptide T cells, mice were immunized with either DWEYSVWLSN-MAP in CFA or CFA alone. Cells from the draining lymph nodes were harvested 1 wk later and pulsed with DWEYSVWLSN-MAP in the presence of various concentrations of Ab to either I-E^d or I-A^d. Fig. 2*B* shows that only Ab to I-E^d was able to block T cell proliferation in response to the peptide from maximal to background in a dose-dependent manner. Ab to I-A^d had no effect and served as an isotype-matched control for the I-E^d Ab.

Both the Th1 and Th2 T cell-derived cytokines have been implicated in mediating disease in SLE. To determine which cytokines are produced when T cells are activated in response to peptide immunization, we assayed for cytokine production in lymph node cells 1 wk following peptide immunization. As is apparent from Fig. 3, BALB/c mice up-regulate IL-2 and IFN-γ following peptide immunization, while IL-4 levels are unchanged, indicating that Th1 cells are the peptide-responsive T cells at the onset of the immune response.

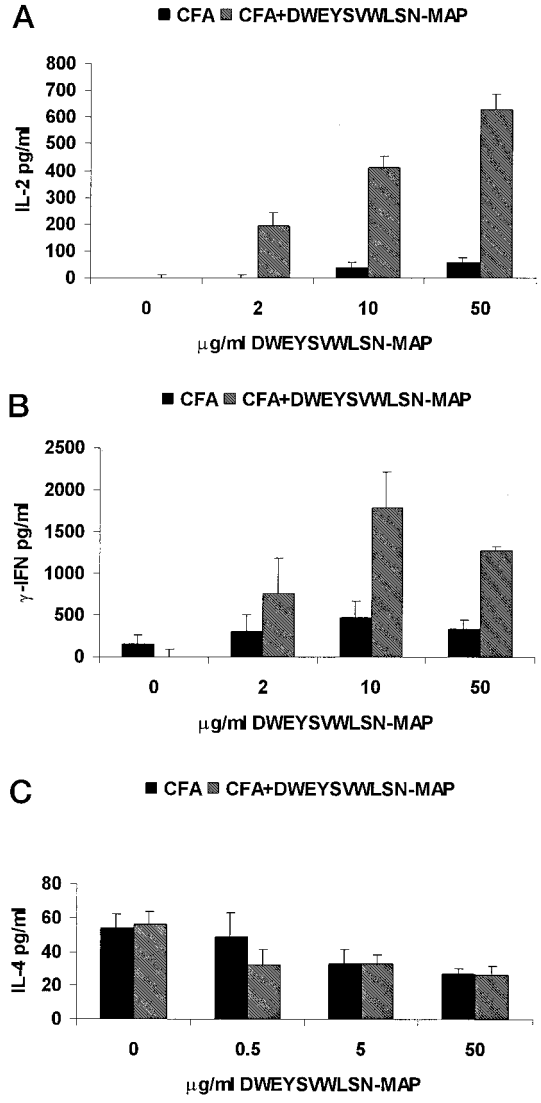

FIGURE 3. DWEYSVWLSN-MAP induces Th1 T cells. Cytokine ELISAs to assay for the production of IL-2 (*A*), IFN-γ (*B*), and IL-4 (*C*) by lymph node cells of mice immunized with either CFA or CFA and DWEYSVWLSN-MAP as described in *Materials and Methods*. Data are expressed as the mean cytokine concentration produced by proliferating T cells from five mice, each determined in triplicate. IL-2 ($p = 0.006$) and IFN-γ ($p = 0.0007$) production is peptide specific. IL-4 levels are not up-regulated by peptide pulsing. The results are representative of four separate experiments containing five mice in each group with similar results.

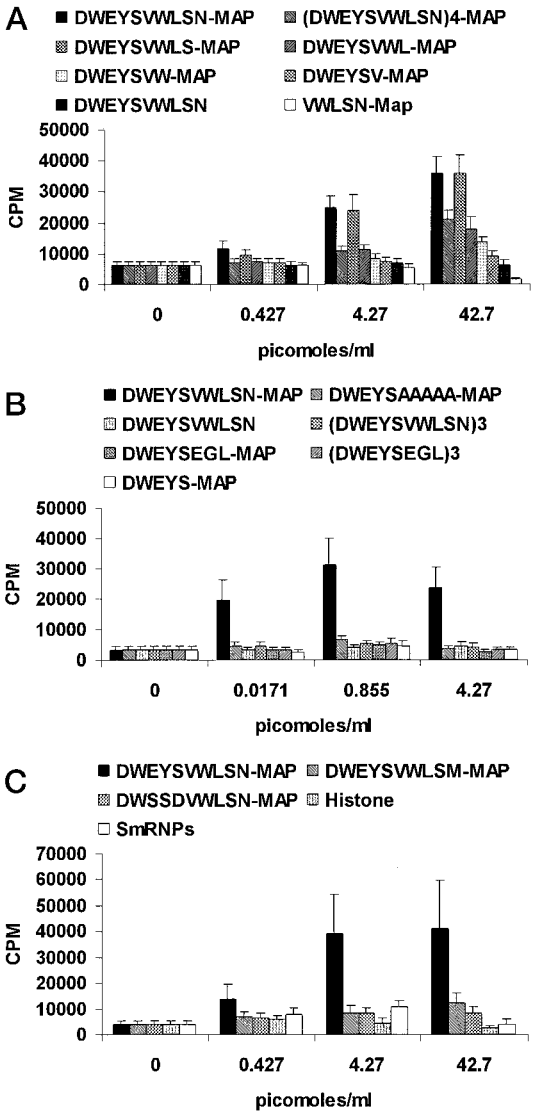

FIGURE 4. T cell proliferation of DWEYSVWLSN-MAP-immunized mice in response to various peptides. BALB/c mice were immunized with the eight-branched MAP peptide (DWEYSVWLSN-MAP) in CFA or with CFA alone. One week later their lymph node cells were pulsed in vitro with equimolar amounts of the original immunizing peptide or a panel of peptides with the sequences shown (*A–C*). Compared with the CFA control, which is not graphed, *p* values for proliferation in response to each peptide in *a* from *left* to *right* are 2.1×10^{-9}, 1.3×10^{-6}, 2.8×10^{-6}, 0.001, 0.002, 0.1, 0.2, and 0.4. The results are shown as the average counts per minute of five individual mice, each assayed in triplicate.

Both the peptide as well as the MAP backbone contribute to the T cell epitope in the anti-foreign and anti-self response

To understand how the MAP peptide-reactive T cells lead to subsequent autoimmunity, we sought to define the T cell epitope. Therefore, BALB/c mice were immunized with DWEYSVWLSN-MAP in CFA or CFA alone. One week later cells from the draining lymph nodes were harvested and pulsed in vitro with purified protein derivative, the immunizing peptide, or a panel of related peptides. T cell proliferation was assayed as a function of incorporated radioactivity. Fig. 4*A* shows that the immunizing peptide as well as the peptide lacking the asparagine at residue 10 were able to elicit comparable T cell proliferation, indicating that residue 10 is not a necessary component of the T cell epitope. Proliferation decreased with successively shorter peptides until the DWEYSV-MAP, where proliferation was no longer detectable. T cell proliferation also seems to require the presence of the MAP backbone, since monomeric peptides (DWEYSVWLSN and $(DWEYSVWLSN)_3$) induced no proliferation, and peptide linked to the four-branch MAP backbone showed less than maximal proliferation (Fig. 4, *A* and *B*). It is the actual sequence of the peptide and not its valency that is important, because peptides that had a nonconservative amino acid substitution from asparagine to methionine in the last position or that had alterations in the consensus sequence DWEYS (DWSSDVWLSN-MAP) or in the last five amino acids of the peptide (DWEYS-MAP, DWEYSAAAAA-MAP, and DWEY SEGL-MAP) did not induce T cell proliferation (Fig. 4, *B* and *C*). The results from these T cell studies confirmed the serologic data shown in Fig. 5 demonstrating that neither DWEYS-MAP, the monomeric peptide DWEYSVWLSN, nor the tandem repeat

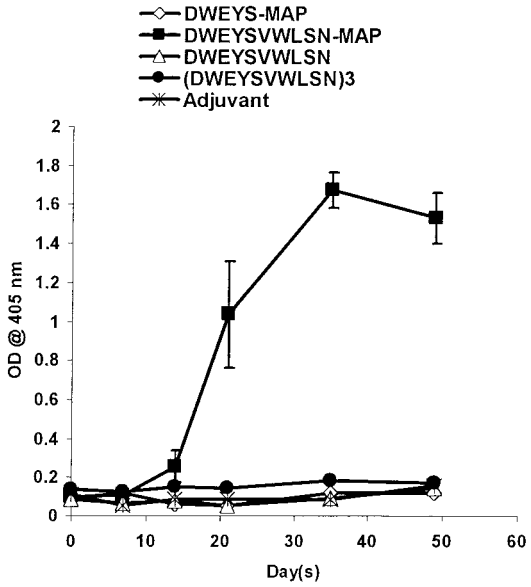

FIGURE 5. Serum response of mice immunized with various peptides. Mice were immunized with the peptides DWEYS-MAP, DWEYSVWLSN-MAP, DWEYSVWLSN, and $(DWEYSVWLSN)_3$ in CFA on day 0 and then boosted with IFA on days 7 and 14. Control mice were immunized with adjuvant alone using the same regimen. ELISA to assay for the presence of IgG Abs to the immunizing Ag was performed as described on 1/500 diluted sera. Results are presented as the mean absorbance at 405 nm of five mice in each group. All assays were performed in duplicate.

$(DWEYSVWLSN)_3$ could induce a serum response to the immunizing peptides, presumably because of their inability to generate a T cell epitope.

DWEYSEGL is another consensus (DWEYS)-containing peptide that was selected from the phage library (17). Interestingly, while immunization with a tandem repeat of this peptide was able to induce both T cell proliferation ($p = 0.008$; data not shown) and a serum response to the peptide (Fig. 6*A*), no anti-dsDNA response was detectable above background (Fig. 6*B*). In addition, the anti-$(DWEYSEGL)_3$ Abs generated did not cross-react with DWEYS VWLSN-MAP (Fig. 6*C*), DWEYS-MAP, or DWEYSVWLSN (data not shown), nor was there a common T cell epitope between $(DWEYSEGL)_3$ and DWEYSVWLSN-MAP (Fig. 4*B*). Taken together these results suggest that generation of the critical T cell epitope requires both the peptide and the MAP backbone and indicate the importance of the nonconsensus amino acids as well as the MAP backbone in generating the anti-dsDNA response and the anti-foreign response.

DWEYSVWLSN-MAP requires processing for presentation to T cells

We wanted to determine whether the MAP peptide had to be processed for presentation to cognate T cells. To this end, we tested whether NH_4Cl, which inhibits acidification and proteolysis within endocytic vacuoles (23), could block T cell proliferation to the peptide. Fig. 7*A* shows that DC pulsed with Ag in the presence of 20 mM NH_4Cl and then fixed were significantly less capable of inducing T cell proliferation than DC pulsed with Ag and then fixed ($p = 0.001$). In addition, DC fixed before Ag pulse were completely unable to induce T cell proliferation (data not shown). The results of these studies are further confirmed by experiments showing that DCs pulsed with Ag at 4°C, which prevents Ag internalization, were much less effective at inducing T cell proliferation than those pulsed at 37°C ($p = 0.03$; Fig. 7*B*). Overall, these results indicate that the peptide requires internalization and processing by APCs for the generation of a T cell epitope and subsequent proliferation.

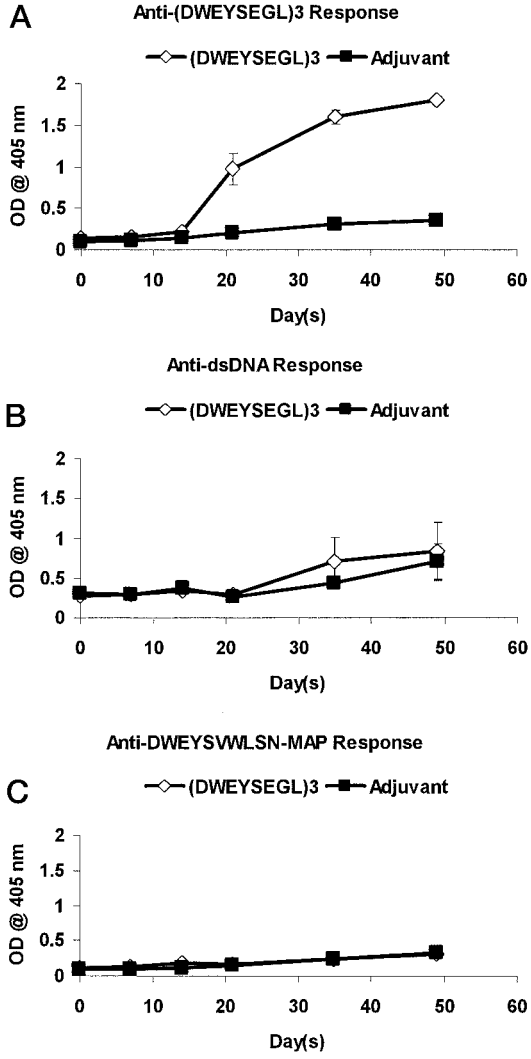

FIGURE 6. Serum reactivity following $(DWEYSEGL)_3$ immunization. Mice were immunized with $(DWEYSEGL)_3$ in CFA on day 0 and then boosted with IFA on days 7 and 14. Control mice were immunized with adjuvant alone using the same regimen. IgG anti-$(DWEYSEGL)_3$ (*A*), anti-dsDNA (*B*), and anti-DWEYSVWLSN-MAP (*C*) reactivities were assayed by ELISA in 1/500 diluted sera. Results are expressed as the mean absorbance at 405 nm of five mice in each group. All assays were performed in duplicate.

DWEYSVWLSN-MAP-specific T cells are not cross-reactive with autoantigens

We have previously observed that the serum response in this peptide-induced model of SLE includes a variety of Abs with specificities similar to those found in SLE, including anti-histone and anti-Sm/RNP (18, 21). Theories explaining the polyreactivity that is typical of the human disease include cross-reactivity of anti-foreign and anti-self Abs (24), somatic mutation of the anti-foreign response to generate autoreactivity (25, 26), and epitope spreading of the immune response to target self Ags (27–33). We have previously shown that cross-reactivity of the serum Abs accounts for much of the anti-self response in these mice (18). To determine whether cross-reactivity at the level of the T cell contributes to the

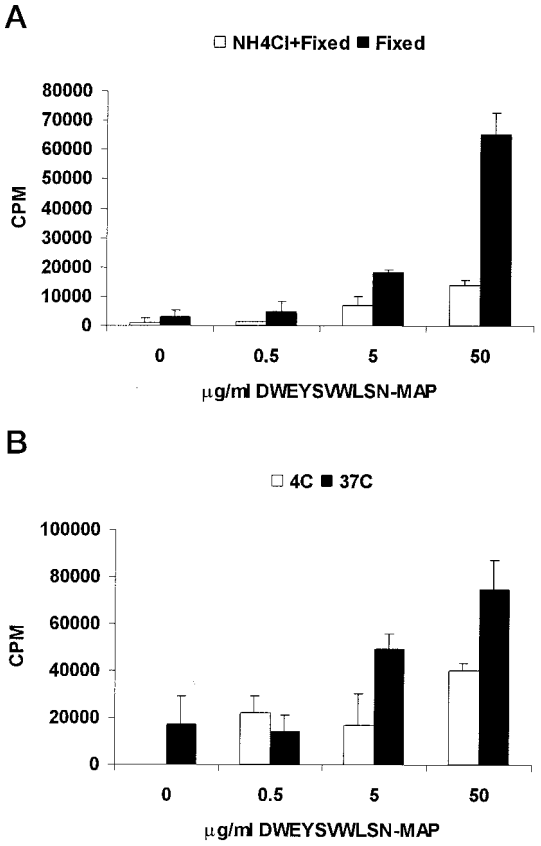

FIGURE 7. DWESYVWLSN-MAP requires Ag uptake and processing to induce T cell proliferation. *A*, T cells (3×10^5) purified from lymph nodes of mice immunized with DWEYSVWLSN-MAP in CFA or with CFA alone 1 wk earlier were cocultured in triplicate for 72 h with 1×10^5 fixed DC that had been pulsed with peptide in the presence or the absence of 20 mM NH_4Cl the previous day. Effective fixation was confirmed by trypan blue staining of DC. Control DC that had been incubated with Ag in the presence of NH_4Cl but not fixed were able to induce T cell proliferation comparable to DCs pulsed with Ag only, confirming that the effects of NH_4Cl are reversible and that the concentration of NH_4Cl used is not toxic to cells. *B*, T cells (2.5×10^5) purified from lymph nodes of mice immunized with DWEYSVWLSN-MAP in CFA or with CFA alone 1 wk earlier were cocultured in triplicate for 72 h with 1×10^4 DC that had been pulsed with peptide at either 4 or 37°C for 3 h the previous day. The lower temperature had no effect on cell viability as determined by trypan blue staining and did not cause nonspecific alterations in the Ag-presenting capabilities of DC, since control DC placed at 4°C for 3 h before Ag pulsing were just as capable of inducing T cell proliferation as those not incubated at the lower temperature ($p = 0.08$; data not shown). *A* and *B*, T cell proliferation was assayed as described above. Results are expressed as the mean stimulation index for triplicate determinations of T cells and DC cultures. Stimulation index is defined as the counts per minute observed in DC cultured with T cells derived from mice immunized with peptide in CFA divided by that observed in DC cultured with T cells derived from mice immunized with CFA alone. Results are representative of three separate experiments, all yielding similar results.

production of autoantibodies, we immunized mice with DWEYSVWLSN-MAP and pulsed lymph node cells with the immunizing peptide as well as histone and Sm/RNP. The results in Fig. 4*c* show that proliferation occurs only in response to the immunizing peptide, indicating that there is no cross-reactivity at the level of the T cell epitope in the initial T cell response.

Discussion

Many studies have shown that T cells play a central role in disease manifestations of SLE. Production of autoantibodies by B cells requires T cell help (34) and athymic MRL-*lpr* and NZB/NZW mice do not develop SLE (27, 35, 36). In addition, interference with T cell activation by blocking MHC class II TCR (2, 4), CD28-B7 (5, 6), or CD40-CD40 ligand interactions in several mouse models of SLE abrogates disease (7, 8). Although double-negative as well as $CD8^+$ T cells capable of providing the necessary signals for autoantibody production have been isolated from lupus patients, $CD4^+$ cells seem to be the primary cells responsible for initiating the autoimmune response. They provide the strongest help to anti-DNA B cells in coculture experiments (37–39), and anti-CD4 Ab prevents autoantibody production (3). Our studies indicate that both the peptide and the anti-self response in this Ag-induced model of SLE are T cell dependent and that the primary T cells responsible for the Ab production are $CD4^+$. This is similar to findings in both human SLE and the spontaneous mouse models of the disease.

Although the T cell requirement for production of pathogenic anti-dsDNA Abs is clear, there is much debate regarding the subset to which they belong. Th1 and Th2 cells differ in their secretion of cytokines and their effector function (40, 41). Th1 cells secrete IL-2, IFN-γ, and TNF and are responsible for cell-mediated immunity, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 and are a driving force for humoral immunity. The two subsets are able to regulate one another. Th1 cytokines along with IL-12 favor the development of Th1 cells and down-regulate Th2 cells, while IL-10 and IL-4 down-regulate Th1 responses. Because cytokines are such powerful modulators of immunity and inflammation, their role in SLE has been studied extensively. Although SLE is an Ab-mediated systemic disease, the prediction that it is a Th2-driven autoimmune disease has been questioned. It appears that both Th1 and Th2 cytokines can play an important role in disease pathogenesis. IL-2 (42–46), TNF-β (47–52), IL-4 (53–56), and TGF-β (57) have all been implicated in disease pathogenesis, although the exact roles of these cytokines remain ambiguous, with elevated levels causing disease remission in some models and exacerbation in other models. Elevated levels of IFN-γ, on the other hand, have been more consistently associated with SLE (58, 59). Lupus-prone mice given exogenous IFN-γ have accelerated disease, while their disease can be delayed by the administration of anti-IFN-γ Ab[60] or soluble IFN-γ receptor (60, 61) or by the deletion of either the IFN-γ gene or its receptor (62, 63). Decreasing IFN-γ levels seems to have its beneficial effects through a reduction in the level of MHC II on the surface of APCs (62), reduced T cell proliferation (64), and reduced production of NO (65). Alterations in IFN-γ levels have also been associated with differential processing and presentation of antigenic peptides on class II (66, 67) through the selective regulation of the cathepsins (68, 69). Consistent with the importance of IFN-γ in the disease manifestations of SLE is the finding that IL-12 is also elevated in autoimmune mouse models (70, 71). In this study the initial T cell response is characterized by the production of IFN-γ. The autoantibody response is primarily γ1, so it is possible that IL-4-producing T cells are recruited to the response over time.

Our results show that the T cell response is I-E^d restricted. It has been postulated that histones provide the T cell epitope for the anti-nucleosome anti-DNA response and that I-E^d molecules are especially able to present such positively charged peptides because of the extensive negative charge present in the key binding pockets of this class II molecule (22). It is of interest that the MAP-linked peptide (DWEYSVWLSN) used to induce anti-dsDNA Abs contains some of the features favorable for an interaction with I-E^d molecules, such as the aromatic residue tryptophan and the aliphatic residues valine and leucine, yet the peptide is extremely negatively charged, with an isoelectric point of 2.4. It is tempting

to speculate that lysines derived from the backbone provide the necessary positive charge for binding to I-E^d molecules. It has been shown that the MAP backbone can alter the MHC restriction of a malaria-derived peptide, possibly by generating new helper cell epitopes (72). Other studies, however, have shown that MAP peptides elicit similar T cell proliferation of MAP peptide-primed T cells as their monomeric counterparts, arguing against the creation of any neo-epitopes by MAP conjugation (73). Rather, it was postulated that the MAP backbone contributed to the breaking of B cell tolerance. The results presented in our study favor a role for the MAP backbone in generating the correct T cell epitope.

While we hypothesize a direct contribution of the MAP backbone to the T cell epitope as a likely explanation for our results, other possibilities should be considered. We have shown that DWEYSVWLSN-MAP requires uptake and processing by APC for presentation to cognate T cells. Therefore, it is also possible that the MAP backbone allows more effective internalization by APC, targets the peptide to the correct compartment for processing, or orients the peptide in such a way so as to allow generation of the correct T cell epitope.

We considered the possibility that cross-reactivity at the level of the TCR might occur between epitopes derived from DWEYSVWLSN-MAP and those derived from proteins that are targeted autoantigens in this model. We, therefore, immunized mice with DWEYSVWLSN-MAP and looked for T cell proliferation in response to histone and Sm/RNP. We found no cross-reactivity at the level of the peptide-specific T cells. These results make TCR cross-reactivity with nuclear Ags an unlikely mechanism for the initial breakdown in self-tolerance observed in BALB/c mice following peptide immunization; the autoantibody response to histone and Sm/RNP is not accompanied by a T cell proliferative response to these Ags. The possibility remains, however, that epitope spreading may occur later in the response and account for subsequent progression of the polyreactivity of the serum response. This observation is consistent with a study by Goodnow and colleagues (7) demonstrating that autoreactive B cells are less susceptible to tolerance induction that autoreactive T cells.

Overall, the data indicate that immunization of wild-type BALB/c mice with a peptide mimetope of dsDNA replicates the T cell dependence of SLE. The response is characterized by cells that produce IFN-γ, at least at the inception of the response. T cell proliferation and the generation of an anti-foreign and anti-self B cell response require the presence of both DWEYSVWLSN and the MAP backbone. The breakdown in self-tolerance does not occur through cross-reactivity at the level of the T cell with peptides from self Ags. Rather, T cells responding to a foreign Ag and not cross-reactive with peptides from self-Ag may activate cross-reactive B cells that recognize both foreign and self Ag. While there has been much focus on autoreactive T cells in SLE, these studies suggest that SLE may arise through activation of T cells to foreign Ag and molecular mimicry at the B cell level.

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