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Identification of a Minimal T Cell Epitope Recognized by Antinucleosome Th Cells in the C-Terminal Region of Histone H4

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Autoreactive T cells responding to systemic autoantigens have been characterized in patients and mice with autoimmune diseases and in healthy individuals. Using peptides covering the whole sequence of histone H4, we characterized several epitopes recognized by lymph node Th cells from non-systemic lupus erythematosus-prone mice immunized with the same peptides, the H4 protein, or nucleosomes. Multiple T epitopes were identified after immunizing H-2d BALB/c mice with H4 peptides. They spanned residues 28–42, 30–47, 66–83, 72–89, and 85–102. Within the region 85–102, a minimal CD4+ T epitope containing residues 88–99 was characterized. Although Abs to peptide 88–99 recognized H4, this peptide does not contain a dominant B cell epitope recognized by anti-H4 Abs raised in BALB/c mice or Abs from NZB/NZW H-2d/z lupus mice. Th cells primed in vivo with H4 responded to H4, but not to peptide 88–99. However, this peptide was able to stimulate the proliferation and IL-2 secretion of Th cells generated after immunization with nucleosomes. H488–99 thus represents a cryptic epitope with regard to H4 and a supradominant epitope presented by nucleosome, a supramolecular complex that plays a key role in lupus. This study shows that in the normal repertoire of naive BALB/c mice, autoreactive Th cells specific for histones are not deleted. The reactivity of these Th cells seems to be relatively restricted and resembles that of Th clones generated from SNF1 ((SWR × NZB)F1; I-A\(^d\)) lupus mice described earlier.


Systemic lupus erythematosus (SLE) is characterized by the production of a variety of autoantibodies against cell surface, nuclear, and cytoplasmic Ags. The detection of nucleosome-specific Abs in the absence of detectable Abs to dsDNA or histones in the serum of patients with SLE and in lupus mice, as well as the finding that elevated levels of oligonucleosomes circulate in the plasma of SLE patients, support the view that nucleosome particles play a central role in the development of lupus. The finding that in lupus nephritis, nucleosomes probably released from apoptotic cells, nucleosome-specific Abs, and nucleosome-IgG complexes are present in the glomerular immune deposits (1–5) has also supported the assumption that nucleosomes are implicated in the pathogenesis of SLE. In contrast to other systemic autoimmune diseases, T lymphocytes in SLE do not appear to play a direct role in tissue damage. However, Th cells are clearly involved in the development of autoantibody production. Using a panel of 145 overlapping peptides covering the four core histones, H2A, H2B, H3, and H4, Kaliyaperumal et al. (6) identified three critical histone autoepitopes recognized by nephritogenic autoantibody-inducing Th cell clones derived from (SWR × NZB)F1 lupus mice. These epitopes were localized in residues 10–33 of H2B and 16–39 and 71–94 of H4. None of the clones generated from SNF1 mice reacted with free, individual histones. The three core histone peptides also triggered the pathogenic Th cells of SNF1 lupus mice in vivo to induce the development of severe lupus nephritis and stimulated the production of Th1-type cytokines (6). Other H3 and H2A peptides stimulated splenic T cells of prenephritic SNF1 mice, but with the exception of peptide 85–102 of H3, they did not activate pathogenic Th clones. It is noticeable that the N-terminal regions of H2B and H4 and the C-terminal residues of H3 contain dominant B cell epitopes targeted by autoantibodies from patients and mice with lupus (7, 8). The N-terminal region of H2B and C-terminal regions of H3 and H4 are also known to be highly accessible at the surface of free nucleosome (9). Additional experiments by Shi et al. (10) showed that the H4 peptides, but not peptide 10–33 of H2B, recognized by the SNF1 (I-A\(^d\))–derived lupus Th cells behave as universal epitopes and promiscuously bind various I-A MHC molecules as well as HLA-DR molecules. Recently, the same group showed that repetitive i.v. injections of peptides 10–33 of H2B, and 16–39 and 71–94 of H4 (and particularly the peptide 16–39 of H4) to 3-mo-old prenephritic SNF1 mice that already produced pathogenic Abs delayed the onset of severe lupus nephritis (11). Although a complete tolerogenic effect of these soluble peptides was not observed, certain pathogenic functions of T and B cells were significantly impaired in peptide-treated mice.

The presence of pathogenic autoantibody-inducing Th cells specific for chromatin subparticles or histones has also been demonstrated in human lupus (12–14). In these studies, histone H4 was again found to contain epitope(s) recognized by pathogenic autoantibody-inducing Th cells. Lu et al. (14) characterized several autoepitopes in the four core histones, and remarkably some of them overlap major autoepitopes identified in SNF1 mice. Two additional Th epitopes were identified in residues 34–48 of H2A and 49–63 of H4. Among histone subtypes, H4 is the most highly

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2 Abbreviations used in this paper: SLE, systemic lupus erythematosus; LNC, lymph node cell; RT, room temperature; SI, stimulation index; SNF1, (SWR × NZB)F1; TFA, trifluoroacetic acid.
conserved through evolution, but is also the site of many post-translational modifications (8). The observation that, within the nucleosome structure, H4 seems to play an important role at the T cell level in human and murine lupus is an intriguing feature. To further address this question, we studied the ability of H4 as well as that of overlapping peptides spanning the whole sequence of H4, to elicit Th and B cell responses in normal, non-SLE-prone mice. We also tested in vitro the capacity of H4 and H4 peptides to stimulate Th cells primed in vivo with purified nucleosomes and examined their cytokine profiles. The overall results indicate that in the normal repertoire of BALB/c H-2d mice, autoreactive Th cells specific for histones are not deleted and that in terms of specificity, the reactivity of these Th cells seems to be relatively restricted and resembles that of Th clones generated from SNF1 lupus mice.

Materials and Methods

Peptides, histones, and nucleosomes

Histone peptides (calf thymus sequence) were synthesized using classical Fmoc (N-[9-fluorenyl] methoxycarbonyl) solid-phase chemistry (15). Each peptide was purified by reversed-phase HPLC using a Perkin-Elmer (Roissy, France) preparative HPLC system on an aquapore ODS 20-μm column (10 × 100 mm). The elution was achieved by a linear gradient of aqueous 0.1% trifluoroacetic acid (TFA; solvent A) and 0.08% TFA in 80% acetonitrile-20% water (solvent B) at a flow rate of 6 ml/min with UV detection at 220 nm. The homogeneity of each peptide was checked by analytical HPLC on a nucleosil C18, 5-μm column (4.6 × 150 mm), using a linear gradient of 0.1% TFA in water and acetonitrile containing 0.08% TFA. The identity of purified peptides was assessed by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry using a protein TOF apparatus (Bruker Spectrospin, Bremen, Germany).

Histone H4 and (H3-H4)2 tetramer were prepared from calf thymus and purified, as described previously (16). There is no change in the primary structure of calf and mouse core histones. Some experiments were also performed with commercial histone preparations purchased from Roche (Mannheim, Germany) and Sigma (St. Louis, MO). The homogeneity of each histone fraction was checked by 18% SDS-PAGE. Nucleosomes were prepared from calf thymus, as described previously (17), and purified on a 5–29% (w/v) sucrose gradient. The nucleosome preparations were characterized by 1.5% agarose gel electrophoresis, and the content in histones was checked by 18% SDS-PAGE. An example of preparation is shown in Fig. 1.

Mice

Female BALB/c and NZB/NZW F1 (B/W) mice were purchased from Janvier (Le Genest St. Isle, France) and Harlan (Gannat, France), respectively. For T cell experiments using BALB/c mice, two 8–10-wk-old mice/Ag/experiment were injected s.c. in hind footpads and at the base of the tail with 100 μg of peptide or histone diluted in H2O and mixed (v/v) with CFA. Alternatively, BALB/c mice were immunized with 10 μg (expressed in terms of histone content) of mono- or tetranucleosome in sucrose in the presence of CFA (v/v). To study the B cell response to H4 and nucleosome, BALB/c mice (two to three mice/Ag/experiment) were immunized, as described above, or s.c. in the flanks. Booster injections were performed in IFA on a fortnightly basis, alternatively with bleedings. A prebleeding of each mouse was performed and used as control in each assay. B/W mice, 8–10 wk old at the beginning of the experiments, as well as nonimmunized control BALB/c mice of the same age, were bled regularly during 40 wk.

Lymphocyte proliferation assay and measurement of cytokine secretion

In the case of BALB/c mice immunized with the different Ags, the proliferation assay was essentially as described previously (18). Ten days after immunization of mice, inguinal, popliteal, and periaortic lymph nodes were removed and washed in RPMI 1640-Glutamax 1 (Life Technologies,
were collected after 24–48 h and tested in ELISA, as described below. 1/5,000). Enhanced chemiluminescent (ECL) reagents (Amersham Phar-

IgG Abs (diluted 1/10,000 in TBS-T) or goat anti-rabbit IgG Abs (diluted 0.05% Tween and 0.5% BSA (PBS-T-BSA) and either HRP-conjugated goat anti-mouse IgG diluted 1/15,000 or HRP-conjugated goat anti-rabbit IgG diluted 1/40,000. The cutoff points of each assay were determined previously (17), using mouse sera at different dilutions in PBS containing 0.05% Tween and 0.5% BSA (PBS-T-BSA) and either HRP-conjugated goat anti-mouse IgG diluted 1/15,000 or HRP-conjugated goat anti-rabbit IgG diluted 1/40,000. The cutoff points of each assay were determined using the sera from twelve 8-wk-old female BALB/c mice. Mouse sera were considered positive when the absorbance (A) was greater than the mean absorbance plus 2 SD. 

ELISA for cytokine detection

INF-γ, IL-4, IL-6, and IL-10 secretion was evaluated by sandwich ELISA using commercial Abs from Pharmingen and polyclonal antibodies (Falcon, Oxnard, CA; reference 3912). Standard curves performed with known concentrations of recombinant cytokines (Pharmingen, San Diego, CA). In the proliferation assay, 2.5 × 10^4 purified CD4^+ T cells were cultured with 5 × 10^5 mitomycin C-treated autologous spleen cells as APCs in the presence of peptide, Con A, or medium alone. As an additional control, treated APCs were cultured alone in the presence of the peptide. IL-2 secretion was evaluated after 24 h, and cell proliferation was measured after 72 h, as described above.

ELISA for cytokine detection

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ELISA and Western immunoblotting for Ab detection

For the test of mouse sera, polyclonal microtiter plates (Falcon) were coated overnight at 37°C in 1 μM histone peptide or 800 ng/ml purified histone diluted in 0.05 M carbonate buffer, pH 9.6. In each assay, sera were also tested in a noncoated well incubated with coating buffer alone as a control. Abs raised in rabbits against each of histone peptides tested in this study (17) were used to check that plates were satisfactorily coated with each peptide. The subsequent steps of the test were performed, as described previously (17), using mouse sera at different dilutions in PBS containing 0.05% Tween and 0.5% BSA (PBS-T-BSA) and either HRP-conjugated goat anti-mouse IgG diluted 1/15,000 or HRP-conjugated goat anti-rabbit IgG diluted 1/40,000. The cutoff points of each assay were determined using the sera from twelve 8-wk-old female BALB/c mice. Mouse sera were considered positive when the OD values were higher than the mean OD value + 2 SD, i.e., OD values ≥0.1 in the case of all H4 peptides except 1–29, 28–42, 30–47, and 42–59 (calculated cutoff = 0.2), and ≥0.2 in the case of histones except H3 (calculated cutoff = 0.1).

To more closely define the location of the T cell epitope(s) within the C-terminal region of H4, four 12- to 15-mer additional peptides were synthesized and tested as described above. They successively encompassed residues 81–92, 85–99, 88–99, and 88–101 (Table I). No proliferation and IL-2 secretion were observed when peptide 81–92, which overlaps the two positive peptides 72–89 and 85–99, was added to cultures containing T lymphocytes removed from control mice injected with CFA alone. However, proliferative responses with IL-2 secretion in varying magnitudes were reproducibly found with several peptides when they were added to cultures containing T lymphocytes removed from immunized mice (Fig. 2, A and B). The strongest responses were systematically measured with the C-terminal peptides 72–89 and 85–102, while relatively weak or no reactivity was found with the N-terminal peptides 1–29, 18–34, and 42–59 (Figs. 2 and 3A).

To further study the T cell response to H4, BALB/c mice were immunized with purified histone H4, and we tested the ability of LNC to proliferate ex vivo in the presence of the same protein or

Table I. Sequences of the 13 H4 peptides used in this study

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–29</td>
<td>SGRGKGGKgLGKGGGAKHRKVLRDNIQG</td>
</tr>
<tr>
<td>18–34</td>
<td>HKRVLDRDNIQGITKPA</td>
</tr>
<tr>
<td>28–42</td>
<td>GITKPAIRLARRGG</td>
</tr>
<tr>
<td>30–47</td>
<td>TKPAIRRLARRGGVVKRIS</td>
</tr>
<tr>
<td>42–59</td>
<td>GYRKRSGLIYETRGLK</td>
</tr>
<tr>
<td>57–74</td>
<td>VLKVFLVENIRDVAYTE</td>
</tr>
<tr>
<td>66–83</td>
<td>IRDVATYETHAKKRTVTA</td>
</tr>
<tr>
<td>72–89</td>
<td>YTEHAKKRTVTAAMDVYYA</td>
</tr>
<tr>
<td>85–102</td>
<td>DVVYALKQRGTLYGFG</td>
</tr>
<tr>
<td>81–92</td>
<td>VTAMDVYYALKR</td>
</tr>
<tr>
<td>85–99</td>
<td>DVVYALKQRGTLYG</td>
</tr>
<tr>
<td>88–99</td>
<td>YALKQRGRTLYG</td>
</tr>
<tr>
<td>88–101</td>
<td>YALKQRGRTLYGFG</td>
</tr>
</tbody>
</table>

aThe four last peptides were synthesized for analyzing the Th cell response against the C-terminal region of H4 in more detail.
H4 peptides. No proliferation and IL-2 secretion were observed with any of the 13 H4 peptides. However, H4-primed LNC responded well to H4: this response was specific and histone dose dependent (Fig. 2, C and D).

The H4 peptide 88–99 is recognized by nucleosome-primed T cells from BALB/c mice and corresponds to a dominant Th epitope

Although we found that ex vivo H4 peptides failed to stimulate T cell response primed in vivo with H4, we then examined whether these peptides may induce the proliferation of nucleosome-primed T cells. We first determined whether in nonautoimmune BALB/c mice, nucleosomes could elicit an efficient Th cell response. BALB/c mice were primed s.c. with purified calf thymus mononucleosomes in CFA. After 10 days, draining lymph nodes were removed and LNC were cultured in the presence of nucleosome and purified histones. As above, each suspension was tested for its ability to proliferate and produce IL-2 ex vivo. No proliferation and IL-2 secretion were observed when purified mono- or tetranucleosomes were added to cultures. However, a significant proliferative response with IL-2 secretion was reproducibly observed with H4 as well as with the (H3-H4)2 tetramer (Fig. 2, E and F). No or nonsignificant response was found with purified H2A, which presents at its N terminus a sequence of eight residues that are identical to those located in the N terminus of H4. These data thus show that H4 and the (H3-H4) tetramer, but not nucleosomes and isolated H2A, can be efficiently processed and presented by APCs from H-2d BALB/c mice and are recognized in this context by T cells primed in vivo with purified nucleosomes.

We next tested the 13 partially overlapping H4 peptides with nucleosome-primed T cells. It appeared that of 13 H4 peptides, peptide 88–99, and at a lower extent peptide 28–42, induced a significant and peptide dose-dependent IL-2 secretion (Fig. 4).

FIGURE 2. Identification of several Th cell epitopes in the C-terminal end of histone H4. The proliferative response and IL-2 secretion were measured ex vivo in the presence of variable amounts of Ag, as indicated. T cell proliferation and IL-2 secretion are expressed both in total cpm (right scale) and as SI (left scale) corresponding to cpm in cultures with peptide/cpm in cultures without peptide. These experiments are representative of at least three independent experiments with similar results. A and B, BALB/c mice (two mice/peptide) were immunized with the panel of nine H4 peptides, and LNC were recalled ex vivo with the same homologous peptides. The maximal response of T cells measured in the presence of Con A corresponded to average SI values of 5 (proliferation) and 25 (IL-2 secretion). The maximal SI in the three independent experiments (3 × 2 mice) were 4.3–6.5, 3.7–4.1, 4.1–9.2, and 4.4–6.3, respectively, for peptides 28–42, 66–83, 72–89, and 85–102 (proliferation). In the IL-2 secretion test, the maximal SI were 26.8–27.5, 15.1–32.2, 9–71.6, and 8.4–69.3 for the same peptides. C and D, BALB/c mice were immunized with H4, and LNC were recalled ex vivo with H4. No response was observed when H4 peptides were used to recall H4-primed LNC. E and F, BALB/c mice were immunized with nucleosomes, and LNC were recalled ex vivo with H4, (H3-H4)2 tetramers, and H2A. The same results were obtained when mono- and tetranucleosomes were tested.
Although cell proliferation was systematically weak with SI about 1.5, IL-2 production was repetitively found in independent experiments, indicating that with regard to nucleosome, the Th cell epitope in peptide 88–99 is dominant.

**Th cell response to H4 peptide 88–99**

The immune response against peptide 88–99 of H4, which constitutes a dominant Th cell epitope of nucleosome in normal BALB/c mice, was studied in more detail. We first confirmed that CD4$^+$ cells were effectively engaged in this response. LNC from mice primed with peptide 88–99 were separated using CD4-conjugated magnetic beads, and the purity of the CD4$^+$ and CD4$^-$ populations was assessed by immunofluorescence analysis. The purified cell population obtained by positive selection contained 92.9% CD4$^+$ cells, whereas the negative population contained 1.7% CD4$^-$ cells. Purified cells were cultured with mitomycin C-treated APCs from autologous spleen in the presence of peptide 88–99. CD4$^+$ cells showed a significant proliferation with IL-2 secretion (Fig. 5, A and B), whereas no response was observed with either CD4$^-$ cells or mitomycin-treated APCs incubated with peptide without CD4$^+$ T cells.

We tested the nature of cytokines secreted by LNC from mice primed with peptide 88–99 and recalled ex vivo with the same peptide (Fig. 5, C–F). Culture supernatants were collected after 24 and 48 h, and cytokine secretion was evaluated by ELISA. In addition to IL-2 secretion evaluated using the CTL-L cell line (results described above), a significant and peptide dose-dependent IFN-γ secretion was induced in response to peptide 88–99 added ex vivo to the LNC cultures (Fig. 5C). A basal production of IL-10 was found (Fig. 5F). However, no Ag dose-dependent IL-10, IL-4, or IL-6 production was observed, suggesting that the Th cell response to H4 peptide 88–99 is Th-1 dependent.

**Abs to peptide 88–99 react with H4, but this peptide does not contain a dominant B cell epitope recognized by anti-H4 Abs**

To determine whether the H4 peptides that contain Th cell epitopes also contain B cell epitopes, BALB/c mice (three mice/peptide) were repetitively injected with six nonconjugated peptides covering the N- and C-terminal regions, namely peptides 1–29, 72–89, 85–99, 85–102, 88–99, and 88–101. Antisera were tested in ELISA for their reactivity with homologous or heterologous H4 peptides. No reactivity was observed in the sera from mice injected with CFA alone. Four s.c. injections in the presence of Freund’s adjuvant were necessary to raise detectable anti-peptide IgG Abs. Sera from mice immunized with peptides 72–89, 85–102, and 88–99 gave a strong homologous anti-peptide response, whereas a relatively weak reaction was detected in the sera from mice injected with peptide 85–99 and no reactivity was found in the sera from mice that received peptides 1–29 and 88–101. No phenomenon of spreading could be observed between nonrelated H4 peptides, for example between N- and C-terminal peptides. As far as the peptide 88–99 was concerned, the same results were obtained.

**FIGURE 3.** Fine characterization of the Th cell epitopes of H4. BALB/c mice (two mice/peptide) were immunized with the panel of 13 H4 peptides, and LNC were recalled ex vivo with the same homologous peptides (A) or with H4 (B). Only IL-2 secretion is shown, and a single concentration of each Ag is represented. The average tritiated thymidine incorporation in the absence of peptide was 200 cpm.

**FIGURE 4.** Recognition of peptide 88–99 by nucleosome-primed LNC. BALB/c mice were immunized with nucleosomes, and LNC were recalled ex vivo with the panel of 13 H4 overlapping peptides. Only IL-2 secretion is shown. A, A single concentration of each peptide is represented. B, Reactivity of nucleosome-primed LNC in the presence of increasing amounts of peptide 88–99 and 66–83. IL-2 secretion is expressed both in total cpm (right scale) and as SI (left scale).
when mice were injected s.c. in the flanks or in footpads and the tail.

Peptide antisera were then tested in ELISA for their reactivity with histone H4. Antisera from mice immunized against unconjugated peptides 72–89, 85–99, and 85–102 (3/3 mice) and 88–99 (1/3 mice) strongly reacted with H4. The other sera were negative with H4. Similar results were found with histone H4 tested in Western immunoblotting. Antisera from mice immunized with peptides 72–89 (3/3 mice), 85–99 (1/3 mice), 85–102 (2/3 mice), and 88–99 (1/3 mice) possessed Abs reacting with blotted H4. The other sera were negative with H4. Similar results were found with histone H4 tested in Western immunoblotting. Antisera from mice immunized with peptides 72–89 (3/3 mice), 85–99 (1/3 mice), 85–102 (2/3 mice), and 88–99 (1/3 mice) possessed Abs reacting with blotted H4. Taken together, these results indicate that several sequences in the C-terminal region of H4 covered by peptides 72–89, 85–99, 85–102, and 88–99 are located at the surface of H4. The surface accessibility of the region 85–102 of H4 at the surface of the free nucleosome in solution was previously demonstrated (9).

The panel of peptides was also used to map B cell epitopes on H4 free or associated to nucleosome. No reactivity with H4 and H4 peptides was detected in ELISA with sera from three BALB/c mice that received five injections of nucleosome or H4-RNA complex in the presence of CFA/IFA or CFA alone. Antisera from two of three mice immunized with non-RNA complexed H4 reacted relatively weakly (OD value ≤0.4) with H4 in ELISA. The serum from one of these mice cross-reacted strongly with histones H2A, H2B, and H3; the second positive serum recognized H4 only and not the other core histones. These three sera were tested with the panel of 13 H4 peptides. Three peptides, namely peptides 1–29, 28–42, and 30–47, all located in the N terminus half of H4, were found positive with the serum, which also showed the initial highest reactivity with H4 (Fig. 6A).

**Peptide 88–99 does not contain a dominant B cell autoepitope**

We tested the sera from fourteen 36–38-wk-old H-2d/z B/W lupus mice. All sera except one showed a strong IgG reactivity (0.8, OD, 3) with dsDNA in ELISA. Most of them (12/14) also reacted with H2A, H2B, and H3, and 6 reacted with H4 in ELISA. In Western immunoblotting, 10/14 sera reacted with histone H4. The 14 sera were tested in ELISA with the panel of H4 peptides, and it was found that 7 reacted with peptide 1–29, 6 with peptide 28–42, 2 with peptide 30–47, and one with peptides 18–34, 42–59, and 88–101 (results shown in individual mice in Fig. 6B). No reaction was found with the other H4 peptides including peptide 88–99, and no reactivity with any of these peptides or histones was detected in the sera from four BALB/c mice of the same age collected in parallel (Fig. 6B). Thus, although this study has no statistical value, it is interesting to note that the reactivity of B/W mice resembles that of the H4-positive BALB/c mouse immunized against H4 (Fig. 6A) with apparently a dominant specificity for epitopes located in the N terminus of H4 and not in the C terminus.
epitope containing residues 88–99 was characterized. Th cells primed in vivo by at least two of these peptides (30–47 and 88–99) responded ex vivo to the whole histone H4. When we explored the immunogenicity of H4, we found that immunization of BALB/c mice with autologous histone H4 generated a T cell response that could be recalled by H4, but not by any of the partially overlapping H4 peptides tested. Intact nucleosomes failed to prime T cells that could be activated ex vivo by the same nucleosome. It has been similarly observed that nonautoimmune strains of mice failed to mount a T cell response to immunization with autologous purified small nuclear ribonucleoproteins (22, 23). However, CD4+ T cells generated after immunization of mice against nucleosomes proliferated and secreted IL-2 ex vivo in the presence of autologous H4, (H3-H4)2 tetramer, and particularly one of the 13 overlapping H4 peptides tested, namely peptide 88–99. According to the criteria of Sercz et al. (24), H4 peptide 88–99 represents a cryptic epitope with regard to H4 and an immunodominant epitope presented by nucleosome, which forms a supramolecular complex. We might call it a supradominant epitope. This result is important if we consider the apparent key role of the nucleosome in lupus. It is known that the initial form in which an Ag is delivered can influence the APC type involved in T cell priming (25). It is possible that using different cell surface receptors, H4 and H4 bound to nucleosomes are not delivered to the same APC subsets and therefore are not internalized, processed, and presented with the same efficacy. It is also likely that even though the same APCs are engaged, important differences exist regarding the processing of individual histones and histones bound to nucleosomes. Regions of histones in contact with DNA within the nucleosome are most probably protected from autoantigen processing and should be presented preferentially to Th cells.

The most prominent of our findings was the discovery in normal mice of a supradominant epitope (H488–99) recognized by nucleosome-specific T cells. At this stage, we do not know whether these T cells can help B cells from autoimmune mice. On the other hand, of course, we realize that our failure to detect epitopes recognized by H4-specific Th cells or additional epitopes recognized by nucleosome-specific T cells might be the result of the insensitivity of our method. Other studies with normal mice have identified Th cell epitopes of proteins that are often targeted by lupus Abs (23, 26, 27). For example, Reynolds et al. studied the T cell response to murine and human La in normal mice (26). No autologous T epitope could be identified when mice were immunized with murine La and the T cells recalled with peptides corresponding to the murine sequence. However, multiple xenogeneic T epitopes (containing one or a few amino acid exchanges between human and mouse) were identified in murine La after immunizing mice with the human recombinant protein. Deshmukh et al. (27) recently identified several Ro60 Th epitopes in non-SLE-prone mice. Three peptides spanning residues 121–140, 281–300, and 311–330 were able to recall the proliferative response in SJL/J mice after immunization of mice with the mouse rRo60 protein. It is not known whether Ro particle-specific T cells react with any of these peptides.

Peptides 28–42 and 88–99 recognized by nucleosome-specific T cells partially overlap the promiscuous epitopes 16–39 and 71–94 characterized in SNF1, H-2Kb mice and lupus patients (6, 14) (Fig. 7). In addition to these two peptides, the sequences that are the most strongly recognized by T cells generated against H4 peptides contain residues 30–47, 66–83, and 72–89. It is noticeable that these Th epitopes also overlap partially most of the H4 Th epitopes recognized by nucleosome-specific T cells from lupus patients (14) (Fig. 7). From these observations, we can conclude
first that in the normal repertoire of BALB/c H-2d mice, autoreactive Th cells specific for histones are not deleted, and second, that in terms of specificity, the reactivity of these Th cells is relatively restricted and resembles that of Th clones generated from SNF1 lupus mice previously described by Datta and collaborators.

The region 88–99 of H4 (YALKQGRTLYG) does not contain any potential posttranslational modification site, it is extremely conserved during evolution, and is present in a region known to be exposed at the surface of the nucleosome in solution (9). It contains epitopes recognized by rabbit Abs raised against H4 (8), but apparently not by Abs raised in BALB/c H-2d mice against H4 and Abs from nonimmunized NZB/NZW H-2d mice previously described by Datta and collaborators. In our study, no evidence of clinical disease, including proteinuria, alopecia, or loss of weight, was observed in any of the normal animals immunized with H4 peptides 72–89, 85–102, and 88–99 are immunogen in normal BALB/c mice (as well as in rabbits) (28) and that Abs induced against these C-terminal peptides react with the whole H4 protein. Immunization of prenephritic SNF1 mice with peptide 71–94 accelerated the development of severe glomerulonephritis and augmented the production of pathogenic antinuclear autoantibodies (6). In our study, no evidence of clinical disease, including proteinuria, alopecia, or loss of weight, was observed in any of the normal animals immunized with H4 peptides 72–89, 85–102, and 88–99 in the presence of Freund’s adjuvant. Interestingly, i.v. injection of H4 peptides 16–39 and 71–94 into young SNF1 mice delayed the onset of severe lupus nephritis (6). Such experiments will be conducted with peptide 88–99 for evaluating its possible biological properties in autoimmune mice.

The observation that certain histone peptides can be promiscuously presented and recognized in the context of diverse MHC alleles and are recognized by autoimmune Th cells from lupus mice and patients is of considerable importance for developing therapeutic strategies in humans despite their HLA diversities (29). To this regard, it is remarkable that after immunization with nucleosomes or histone peptides in Freund’s adjuvant that induces the costimulatory APC functions that are necessary for T cell priming and mimics a transient inflammation state, the specificity of Th cells from normal mice resembles that of Th clones generated from SNF1 lupus mice. This might indicate that in lupus, the multiple functional defects among the cells of the immune system and their signaling (30–33) do not directly affect the nature of nucleosomal peptides that are presented by MHC molecules and recognized by the TCRs. It is obviously also possible that still unknown discrete neo-determinants for T cells are created and contribute to the breakdown of peripheral tolerance to nucleosomal proteins. Such possible determinants may be located in any of the histones and, as suggested in several studies (27, 29, 34, 35), a deletorius T cell activation and a diversified T and B cell response could occur via an intramolecular spreading phenomenon.

**Acknowledgments**

We thank Drs. Mireille Viguier and Sylvie Fournel for critical reading of the manuscript and helpful discussions.

**References**


13. Voil, R., E. A. R. Roth, I. Girkontaita, H. Fehr, M. Herrmann, H. Lorenz, and J. R. Kalden. 1997. Histone-specific Th0 and Th1 clones derived from systemic **FIGURE 7.** Mapping of T epitopes in histone H4. Footnote 1: when H4 was used to prime BALB/c mice, none of the overlapping peptides was able to recall Th cells ex vivo. Footnote 2: data from Refs. 6 and 14.

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<th>H4 Peptides</th>
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<td>28 – 42</td>
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<tr>
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<tr>
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