Promiscuous Presentation and Recognition of Nucleosomal Autoepitopes in Lupus: Role of Autoimmune T Cell Receptor α Chain

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Summary

T cells specific for nucleosomal autoepitopes are selectively expanded in lupus mice and these Th cells drive autoimmune B cells to produce pathogenic antinuclear antibodies. We transfected the TCR-α and -β chain genes of a representative, pathogenic autoantibody-inducing Th clone specific for the nucleosomal core histone peptide H471-94 into TCR-negative recipient cells. Although the autoimmune TCRs were originally derived from SNF1 (I-A<sup>d</sup>/q<sup>1</sup>) mice, the transfectants could recognize the nucleosomal autoepitope presented by APC-bearing I-A<sup>m</sup> molecules of all haplotypes tested, as well as human DR molecules. Competition assays indicated that the autoepitopes bound to the MHC class II groove. Most remarkably, MHC-unrestricted recognition of the nucleosomal peptide epitope was conferred by the lupus TCR-α chain even when it paired with a TCR-β chain of irrelevant specificity. Several other disease-relevant Th clones and splenic T cells of lupus mice had similar properties. The TCR-α chains of these murine lupus Th clones shared related motifs and charged residues in their CDRs, and similar motifs were apparent even in TCR-α chains of human lupus Th clones. The TCR-α chains of these murine lupus Th clones shared related motifs and charged residues in their CDRs, and similar motifs were apparent even in TCR-α chains of human lupus Th clones. The lupus TCR-α chains probably contact the nucleosomal peptide complexed with MHC with relatively high affinity/avidity to sustain TCR signaling, because CD4 coreceptor was not required for promiscuous recognition. Indeed, pathogenic autoantibody-inducing, CD4-negative, TCR-α-β<sup>+</sup> Th cells are expanded in systemic lupus erythematosus. These results have implications regarding thymic selection and peripheral expansion of nucleosome-specific T cells in lupus. They also suggest that universally tolerogenic epitopes could be designed for therapy of lupus patients with diverse HLA alleles. We propose to designate nucleosomes and other antigens bearing universal epitopes “Pantigens” (for promiscuous antigens).

In murine, as well as human systemic lupus erythematosus (SLE), the production of nephritogenic anticellular antibodies by autoimmune B cells is driven by cognate interactions with specific autoimmune Th cells (1-7). The pathogenic autoantibody-inducing Th cells of lupus have been cloned from (SWR × NZB)F1 (SNF1)<sup>1</sup> mice and also from patients with lupus nephritis. Representative Th clones from the SNF1 mice can precipitate glomerulonephritis upon transfer into preautoimmune animals, which establishes their relevance to disease (2). In SNF1 mice, the majority of these pathogenic Th clones are specific for nucleosomal peptides, which are processed and presented by the classical MHC II pathway (3, 6). Nucleosomes are routinely released from apoptotic cells and this event is not unique to lupus (8-10). However, the spontaneous expansion of nucleosome-specific T cells is a lupus-specific event that occurs very early in life (3, 6). These TTh cells are essential for sustaining the pathogenic autoantibody-producing B cells of lupus (4). Without such Th cell help, the potentially pathogenic B cells that arise even in normal subjects as an accompaniment of the immune response to common pathogens are destined to undergo apoptosis (11, 12). The presence of anionic residues in the junctional regions (CDR3) of the TCRs of these lupus Th cells suggested that they could be specific for peptides with cationic residues (2, 13, 14). Indeed, the Th clones of lupus were found to be specific for nucleosomal peptides containing multiple charged residues (3, 6).

Abbreviations used in this paper:HEL, hen egg lysozyme; SNF1, (SWR × NZB)F1.
To further investigate the structural basis for this autoimmune recognition event, we have cloned and expressed the TCR-α and β chain genes of the prototypic pathogenic autoantibody-inducing Th clone, 3A, which accelerates lupus nephritis in SNF1 mice. The TCR of this representative pathogenic Th clone is specific for a peptide spanning residues 71–94 of the nucleosomal core histone H4. H4 is in contact with DNA in the native nucleosome particle, thus allowing this epitope to be protected during autoantigen processing. In this study, we report that recognition of nucleosomal autoepitopes is MHC-dependent, but unrestricted. Remarkably, the TCR-α chain of the pathogenic Th clone is critical for this promiscuous recognition and nucleosomal peptide specificity, and this recognition response is CD4 coreceptor-independent.

Materials and Methods

Mice. BALB/c (H-2d), NZB (H-2b), NZW (H-2a), SWR (H-2e), C3H (H-2a), (BALB/c × SWR)F1 (H-2b), B6.C (H-2b, B10.M (H-2a), B10.S (H-2a), B10.RIII (H-2a), and B10.PL (H-2d) were from The Jackson Laboratory (Bar Harbor, ME). SNF1 mice (H-2d) were bred at Northwestern University animal facility.

Antigens. Hybridomas producing mAbs against Thy-1.2 (TIB99), CD3 (145-2C11), CD4 (GK1.5), I-A<sup>d</sup>, CD3 (145-2C11), CD8<sup>a</sup> (53-6.7), and nucleosomal peptide specificity, and this recognition response is CD4 coreceptor-independent. 

Human subjects previously described (7) were also used as APCs. 

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or biotinylated anti-Vβ8 (1:40) followed by staining with PE-conjugated streptavidin (GIBCO BRL) at 10 μg/ml. Stained cells were analyzed using a Becton Dickinson FACScan® flow cytometer. In all cases, dead cells were gated out using propidium iodide.

T Cell Stimulation and Inhibition Assay. Transfectants (10⁵), Th clones (10⁴), fresh splenic CD4⁺ T cells (5 x 10⁴), or short-term T cell lines (5 x 10⁵) were cultured either with irradiated APCs (5 x 10⁵) that had been preincubated with various concentrations of antigens and washed, or with APC and anti-CD3 mAb. The cultures were done in triplicate in flat-bottomed 96-well plates with or without ionomycin (10–100 ng/ml). For antibody blocking, anti-CD4 mAb (purified RM 4.5 or GK1.5) or mAb to MHC class II antigens were added to the cultures. After 24–36 h of incubation, IL-2 secretion was measured by harvesting 100 μl of culture supernatant from each well, which was added into a 96-well Nunc-Immuno Plate (MaxiSorb™, Dynatech Laboratories, Chantilly, VA) that had been coated with anti-IL-2 (1 μg/ml), and blocked with 10% horse serum in PBS. After incubation at 4°C overnight, the plate was washed and revealed with biotinylated anti-IL-2 (1 μg/ml) and horseradish peroxidase-conjugated streptavidin (1:2000) (Sigma Chemical Co.), followed by the substrate TMB (Sigma Chemical Co.) at room temperature for ~30 min, then stopped by using 1 N H₂SO₄ and read immediately at an OD. The results were analyzed using a Becton Dickinson FACScan® flow cytometer.

Results

Structural and Functional Properties of the Pathogenic Th Clones of Lupus. Approximately 15% of 268 T cell clones derived from T cells that are spontaneously activated in nephritic SNF1 mice have the ability to selectively augment the production of IgG anti-DNA and anti-nucleosome autoantibodies when cocultured with autologous B cells (1–3, 13). Similar Th cells are also found in patients with lupus nephritis (5, 7). Moreover, representative SNF1-derived clones (H/L-3A and L-1A) rapidly precipitate lupus nephritis upon transfer into young preautoimmune mice (2, 4). A compilation of such pathogenic Th clones is shown in Figure 1. The TCR gene usage of the clones previously published (2, 13) has been revised according to the most recent nomenclature (21). Each of the lupus Th clones expresses a single functional TCR-α and -β chain (2, 13). The nucleosome specificity of several additional hybridoma clones included in this panel was unmasked by using a suboptimal dose (50 ng/ml) of ionomycin that does not induce stimulation in the absence of antigen (Fig. 1). It is likely that the requirement for a suboptimal dose of ionomycin reflects a partially impaired antigen-specific signaling pathway in some of the Th hybridomas.

The TCR-α chain of a Pathogenic Th Clone Confers Nucleosome Specificity. The functionally rearranged TCR-α and -β chain genes from the prototypic lupus Th clone 3A (H/L-3A) were inserted into TCR-α and -β shuttle vectors and transfected in different combinations into the TCR loss mutant 4G4, as well as the EL4 T-cell lymphoma lines 3A. The nucleosome specificity, and its epitope has been mapped to the core histone H4 71–94 region. This peptide epitope can precipitate severe lupus nephritis in vivo by inducing similar Th cells, and T cells spontaneously primed to this epitope are prevalent in the spleens of very young SNF1 lupus-prone mice (6). Control transfections of different TCR-α and -β chain combinations were performed with HEL-specific 3A9 TCR-α chain and -β chain genes expressed by the Th hybridomas derived directly from splenic T cells; H/L hybridomas derived from cloned Th lines by fusion with TCR-negative BW5147 thymoma, after initial functional characterization. These H/L hybridomas retained their nucleosome specificity and pathogenic autoantibody-inducing ability. A proportion of the Th clones that were previously found to be unresponsive to nucleosomes (3) were restested in this study in the presence of suboptimal doses of ionomycin that unmasked the nucleosome specificity of some Th clones (H-15E3 and H-16G10). * help for IgG autoantibody production is expressed as fold increase in antibody production when Th clones were cocultured with syngeneic B cells as compared with control cultures containing B cells alone. ** The TCR-α and -β chain genes expressed by the Th clones were renamed according to WHO-IUIS nomenclature (21). Omital results from >10 experiments are shown.

| Th clone | Anti-CD3 | Anti-dsDNA | AVJ | BVJ | Nonspecific Response |
|----------|---------|-----------|-----|-----|---------------------|
| HEL-3A   | 15.0    | 32.5      | AV153141 | BV4531255 | 24.0                  |
| H-15E3   | 10.3    | 2.4       | AV175411 | BV4531251 | 14.4                  |
| H-16G10  | 13.0    | 13.3      | AV175417 | BV4531256 | 14.8                  |
| L-1A     | 22.8    | 8.6       | AV205517 | BV5532156 | 40.2                  |
| HEL-37   | 6.7     | 4.2       | AV359146 | BV5532156 | 7.4                   |
| HEL-3    | 11.0    | 2.6       | AV359147 | BV5532156 | 11.4                  |
| HEL-30   | 6.6     | 2.2       | AV359152 | BV5532154 | 4.8                   |
| L-5C7    | 26.1    | 7.6       | AV359155 | BV5532156 | 29.7                  |
| L-12C4   | 41.8    | 2.6       | AV359157 | BV5532156 | 34.0                  |
| H-19E6   | 44.5    | 3.6       | AV359158 | BV5532154 | 5.6                   |

Figure 1. Summary of pathogenic anti-DNA autoantibody-inducing Th clones derived from SNF1 mice with lupus nephritis (1–3, 13). L. cloned Th lines H, Th hybridomas derived directly from splenic T cells; H/L, hybridomas derived from cloned Th lines by fusion with TCR-negative BW5147 thymoma, after initial functional characterization. These H/L hybridomas retained their nucleosome specificity and pathogenic autoantibody-inducing ability. A proportion of the Th clones that were previously found to be unresponsive to nucleosomes (3) were restested in this study in the presence of suboptimal doses of ionomycin that unmasked the nucleosome specificity of some Th clones (H-15E3 and H-16G10). * help for IgG autoantibody production is expressed as fold increase in antibody production when Th clones were cocultured with syngeneic B cells as compared with control cultures containing B cells alone. ** The TCR-α and -β chain genes expressed by the Th clones were renamed according to WHO-IUIS nomenclature (21). Omital results from >10 experiments are shown.
TCR expression shuttle vectors (17). (H/L-3A), were amplified from genomic DNA and inserted into the expressed by the prototypic pathogenic autoantibody-inducing Th clone 3A in combination with the TCR-α chain of 3A9 or the EL4 TCR-β chain (unknown specificity) was sufficient to confer the MHC-dependent nucleosome specificity (Fig. 3). In contrast, the TCR-β chain of the Th clone 3A in combination with the TCR-α chain of EL4 or the HEL-specific clone 3A9 could not reconstitute nucleosome recognition (Fig. 3). Moreover, unlike the nucleosome-specific TCR-α of lupus clone 3A, the TCR-α chain of 3A9 required pairing with 3A9 TCR-β to reconstitute HEL specificity that was restricted to I-Ak (reference 16 and data not shown).

Nucleosome Presentation and Recognition by Lupus T Cells are Promiscuous. Another surprising finding was that the nucleosomal epitopes could be presented to the transfectants bearing colne 3A TCR by almost all MHC II haplotype APCs tested, including human DR molecules (Fig. 4). Again, the promiscuous recognition was dependent upon the TCR-α chain of the pathogenic lupus Th clone (Fig. 4, A and C), and pairing with its TCR-β chain did not restrict its promiscuity (Fig. 4, B and D). Moreover, the nucleosome-specific TCR did not mount an alloreactive response against the different APCs. The promiscuous recognition of nucleosomal antigen was also a property of three other lupus Th clones (H-16G10, H-15E3, and H-16B6) in this SN F1-derived panel (Fig. 1), that shared specificity for the H-14 nucleosome, however, two Th clones (H/L-9w7 and H-12C4) in the group were restricted to syngeneic MHC haplotypes (Fig. 5 and data not shown). Interestingly, the promiscuous Th clone H-16G10 expresses the same TCR-Vα family as clone 3A, but the others use members from various other TCR families (Fig. 1).

The universal presentation and promiscuous recognition of nucleosome epitopes were not just limited to the transfectants or the pathogenic Th clones. Freshly obtained CD4+ T cells or short-term T cell lines from SN F1 lupus mice also

Figure 2. (A) 3A TCR-α and -β chain gene constructs. Functionally rearranged segments of TCR genes AV13S3j41 and BV451D256, expressed by the prototypic pathogenic autoantibody-inducing Th clone 3A(H/L-3A), were amplified from genomic DNA and inserted into the TCR expression shuttle vectors (17). (B) Stable transfection of the 3Aα chain gene in 4G4 cells. Transcription of TCR-α chain gene is shown. Total RNA was prepared from each transfectant, converted to cDNA by reverse transcription, amplified by Taq polymerase using 3ACDR3SEN forward and Cα-EX1 reverse primers, then separated on a 1% agarose. Lane 1, H/L-3A (positive control); the TCR donor lung Th clone 3A; lane 2, 4G4 (TCR-negative recipient); lanes 3 and 5, examples of two separate clones of 4G4 transfected with 3Aα/3Aβ genes; lanes 4 and 6, two clones of 3Aα/3Aβ-transfected 4G4. Integration of 3Aα construct into genomic DNA of respective transfectants was detected by using the primers Vα13-5’-XhoI and Jα41-3’-NotI to amplify the specific AV13S3j41 fragment by PCR (data not shown). (B) Stable transfection of 3Aα chain gene in EL-4 cells. Transcription of TCR-α chain gene was detected by reverse transcription PCR. The method was specific because EL-4’s own endogenous TCR-α was not amplified. Lane 1, H/L-3A; lane 2, EL-4 (recipient); lanes 3–5, three separate clones of 3Aα gene-transfected EL-4; lanes 6 and 7: two examples of clones with 3Aα/3Aβ genes in EL-4; lanes 8 and 9, two clones of 3Aβ gene-transfected EL-4. Integration of 3Aα construct into genomic DNA of the transfectants was detected as above (data not shown). Stable transfection of the HEL-specific T cell-derived 3Aα-chain gene was detected by reverse transcription PCR using primers Vα3 and CαA (data not shown). (C) Expression of transfected 3Aα chain (Vβ4) and 3Aβ chain (Vβ8) in 4G4 and EL-4 cells, analyzed by flow cytometry after staining with anti-Vβ4-biotin or anti-Vβ8-biotin followed by PE-conjugated streptavidin. Lane 1, 3Aα/3Aβ in 4G4; lane 2, 4G4; lane 3, 3Aβ in EL-4; lane 4, EL-4; lane 5, 3Aα/3Aβ in 4G4; lane 6, 4G4; PCR of genomic DNA showed stable integration of the transfected TCR-β genes (data not shown). The mean fluorescence intensities of Vβ4 or Vβ8 staining of the transfectants were similar to the donor Th clone 3A or 3A9, respectively (data not shown).
had this property (Fig. 6). In lupus-prone mice, unlike normal strains, T cells are spontaneously primed in vivo against nucleosomal epitopes (3, 6). Therefore, the response of such nucleosome-primed T cells could be detected early, such as in a 24 h, IL-2 production assay, before alloreactive T cells could obscure the autoantigen-specific signal by mounting a primary mixed lymphocyte reaction. Promiscuous recognition of the nucleosomal H471–94 peptide became detectable after just one round of nucleosome stimulation of the splenic CD4+ T cell, which probably increased the frequency of such T cells in the bulk population (Fig. 6, A versus B).

Nucleosomal Peptide Epitopes Bind to Diverse MHC II Molecules. In competition assays with known conventional peptide antigens that bind to the class II groove, H471–94 and a series of truncated peptides spanning this region bound several murine I-A molecules and also human DR1 (Fig. 7). Markedly higher affinity for all the class II alleles tested was noted for the peptide spanning residues 76–90 of H4, located nearer to the COOH terminus of the parent
Figure 4. Promiscuous recognition of nucleosomal antigen presented by APCs of various MHC haplotypes by 4G4 transfected with 3A\textsubscript{a} 3A\textsubscript{b} (A) or 3A\textsubscript{a} 3A\textsubscript{b} (B) TCRs. In Figs. 4–6, the concentration of nucleosomes used to prepulse the APCs was 1 \( \mu \)g/ml and that of H 4 peptide was 1 \( \mu \)M. The APC haplotypes are indicated, H-2\textsuperscript{d/q} here designates (BALB/c × SWR)F1 APCs. Nucleosomal antigen presented by xenogeneic (human) MHC class II molecules is also recognized by 4G4 transfected with 3A\textsubscript{a} 3A\textsubscript{b} (C) or 3A\textsubscript{a} 3A\textsubscript{b} (D) TCRs. Transfectants were cultured with irradiated human HLA-DR2,4, w53 or DR1,3, w52 haplotype bearing EBV-B cell lines that had been prepulsed with antigen in the presence of anti–HLA-DR or control antibody (anti-DP, anti-DQ). Data are representative of more than five experiments. Mean IL-2 production of triplicate assays are shown; SD <10%.

Figure 5. Promiscuous recognition of nucleosomal antigen by other lupus Th clones from SNF1 mice: clones H-16G10 (A), H-15E3, and H-16B6 (data not shown) are promiscuous. However, recognition by Th clones H/L-9W7 (B) and H-12C4 (data not shown) are restricted to syngeneic MHC (H-2\textsuperscript{d/q} indicates SNF1 APCs). Data from representative experiments are presented in previous figures.
The TCRs of 3A and other lupus Th clones in the panel (Fig. 1), are mainly specific for H\textsubscript{471–94}. However, despite having a single pair of functional TCR-\(\alpha/\beta\), each of them also recognized H\textsubscript{416–39}, thus revealing a degenerate or cross-reactive specificity (6, 13) analogous to other TCR–antigen systems (23, 24). H\textsubscript{416–39} and its truncations also bound to the different class II alleles, particularly the peptides spanning position 22–39 in this region. Thus, this region of nucleosomal core histone may also be promiscuously recognized. However, another epitope for other pathogenic Th cells of lupus, which was previously localized in histone H2B\textsubscript{10–33} (6), did not show promiscuous binding (data not shown), suggesting that such a binding pattern is not found in every lupus-associated autoepitope.

Although the truncated H\textsubscript{476–90} bound to MHC with the highest affinity, it did not stimulate the 3A clone TCR as strongly as did the parental H\textsubscript{471–94} peptide (data not shown). And, unlike the case of the H\textsubscript{471–94} epitope (Fig. 6 B), reactivity to H\textsubscript{476–90} could not be detected in freshly obtained CD4\textsuperscript{+} T cells or short-term T cell lines from SNF1 mice (data not shown).

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**Shared Motif in the Nucleosome-specific TCR-\(\alpha\) Chains.** A comparison of the sequences of the various TCRs analyzed here revealed that the sequence motif of IR SNM E R (or conservative variations of it) was frequently present in the CDR 2 region (position 50–57) of the nucleosome-specific TCR V\(\alpha\) chains (Fig. 8 A). Furthermore, charged residues in the flanking region (position 57–62) of the CDR 2 were also prevalent. These features were not found in the HEL-specific TCR-\(\alpha\) chain.

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**CD4 Coreceptor Is Not Required for Promiscuous Recognition of Nucleosomes.** Although the anti-CD4 mAbs, GK1.5 and RM 4.5, which are known to inhibit antigen-specific responses (25), could not block the recognition of nucleosomal antigens by 4G4 and EL-4 transfectants expressing 3A TCR-\(\alpha\) chain (Fig. 9 and data not shown). Moreover, the promiscuous recognition of nucleosome antigens by these transfectants (Figs. 3 and 4) could not be blocked by the anti-CD4 mAb even at saturating concentrations (data not shown). The transfectants were surface CD4\textsuperscript{+} by flow cytometry (data not shown). In addition, 4G4 transfectants that expressed **Peptide**

| Peptide     | Sequence                  | DR1 (nM) | I-A\(\alpha\) (nM) | I-A\(\beta\) (nM) | I-A\(\beta\) (nM) |
|-------------|----------------------------|----------|-------------------|-------------------|-------------------|
| H\textsubscript{471–94} | TYYTMMKRVTYAMWVVANYLRQG | 76       | 2760              | 153               | 1284              |
| H\textsubscript{476–90} | VTYYTMMKRVTYAMWVVANYLRQG | 1.3     | 43               | 52               | 100               |
| H\textsubscript{471–94} | KTVTTMWWVYVKRQG | 25       | 1296              | 247               | 1402              |

**Figure 6.** Promiscuous recognition of nucleosomes by splenic CD4\textsuperscript{+} T cells of SNF1 mice. Freshly isolated splenic CD4\textsuperscript{+} T cells (A) or shortterm CD4\textsuperscript{+} T cell lines from SNF1 mice (B) were cultured with irradiated APCs of various MHC haplotypes that had been pulsed with antigen. Culture supernatants were assayed after 24 h for IL-2. For making short-term lines, splenic CD4\textsuperscript{+} T cells were stimulated once with nucleosome ex vivo in the presence of IL-2 and then rested for 10 d. Mean of triplicate assays are shown (SD <10%). Data are representative of three experiments.

**Figure 7.** Binding of histone H4\textsubscript{4–derived} truncated peptides to mouse and human MHC class II molecules in competition assays. The data is presented as 50% inhibitory concentration in nanomoles (nM). Peptide binding was ranked as follows: good binders (binding affinity <100 nM); intermediate binders (binding affinity = 100–1,000 nM); low binders (binding affinity = 1,000–10,000 nM); and negative binders (binding affinity >10,000 nM).
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Discussion

These studies show that certain nucleosomal peptides recognized by spontaneously arising, autoimmune T cells that cause lupus nephritis are degenerate MHC binders, and the corresponding T cells that recognize these peptides are MHC unrestricted although they were educated in the I-A/d thymus of SNF1 mice. The presentation of the nucleosomal H4 peptides (71–94 region) was clearly MHC dependent as shown by anti–class II antibody blocking. Either mAb to I-Ad or I-Aq could almost completely block nucleosome presentation by SNF1 (I-Ad/q) APCs, because the antibodies have cross-reactive specificity due to the highly homologous structure of I-Ad and I-Aq molecules (Fig. 3, reference 26, and data not shown). Presentation by class I (classical or nonclassical) is ruled out because transfectants derived from the mouse T cell lymphoma EL-4, which lacks class II but is capable of presenting antigen with class I molecules (27), did not produce IL-2 by themselves when exposed to the nucleosomal peptide (Fig. 3). Moreover, the nucleosomal peptides most likely bound the diverse set of class II molecules tested through engagement of the classical binding groove of MHC, because they efficiently competed with well-characterized peptides known to bind to the grooves. Furthermore, previous work has established that nucleosomes and their peptide epitopes do not behave as superantigens (3, 6, 13). Truncation of the H471–94 peptide increased its affinity for the class II alleles, and indeed its core region (amino acid position 78–89) does have a sequence motif (RKTVTAMDVVYA) that is homologous to other universal epitopes (20). However, truncation of

Figure 8. Amino acid sequences of CDRs of TCR-α (A) and -β (B), chains relevant to this study, compiled from references 2, 7, and 13, and aligned (21). All sequences are from mouse T cells except for DD2, which is a human lupus Th clone (7). Updated TCR-α chain sequence of HEL-specific clone 3A9 (52) was provided by Paul Allen (Washington University, St. Louis, MO). The EL-4 TCR-β chain (B) was sequenced in this study (available from EMBL/GenBank/DDB under accession number Bankit 13531 AF020206).
Our results demonstrate that promiscuous recognition of nucleosomal epitopes by single $\alpha$/\$ pairs of autoimmune TCRs is a property conferred by the TCR-$\alpha$ chain. Indeed, lupus susceptibility is not restricted to any particular MHC haplotype. Different MHC alleles are found in different lupus strains, and the same ones are also present in normal strains. Interestingly, degenerate MHC binding of certain myelin basic protein peptides and their recognition by T cell lines from multiple sclerosis patients have also been described. However, weakly cross-reactive binding to only DR molecules was reported, and TCRs responsible for the degenerate recognition were not analyzed (30).

TCR promiscuity was thought to occur in the context of DR and its murine homologue, the I-E molecules, assuming that the TCRs were recognizing the peptide epitopes along with the nonpolymorphic $\alpha$ chain in these MHC molecules (31, 32). However, this is not the case with the nucleosomal peptide-specific TCRs described here, or the universal peptide epitopes described in other systems (20, 33, 34), where the epitopes either bind or are recognized in the context of both human DR and murine I-A molecules, the latter being polymorphic in both $\alpha$ and $\beta$ chains. In general, promiscuous or cross-reactive recognition by a TCR is thought to be the basis for positive selection in the thymus and alloreactivity, i.e., TCRs can somehow “learn” from one MHC allele to interact with others (35–37). However, it is notable that although the lupus TCRs in this study recognized the histone peptide in the context of the different MHC alleles, they were not reactive to APCs bearing those alleles in the absence of the histone peptide. Indeed, promiscuous recognition of nucleosomal epitope was not only a feature of the pathogenic Th clones, but was also detectable in freshly obtained splenic T cell populations from the SNF1 mice. In lupus mice, nucleosome-primed T cells arise spontaneously and are prevalent in vivo from an early age (3, 6).

The CDR2 regions of these Th clones appear to have a related sequence motif and multiple negative residues, which in combination with other sequences of their TCR-$\alpha$ and $\beta$ chains could confer the property of promiscuous recognition of nucleosomal antigen. TCR-$\alpha$ chain CDR2 has been implicated in other systems for MHC-antigen recognition (15, 38–41). It is noteworthy that a pathogenic autoantibody-inducing Th clone recognizes certain myelin basic protein peptides and their recognition by T cell lines from multiple sclerosis patients have also been described. However, weakly cross-reactive binding to only DR molecules was reported, and TCRs responsible for the degenerate recognition were not analyzed (30).

Promiscuous recognition was a property conferred by the TCR-$\alpha$ chain. Indeed, lupus susceptibility is not restricted to any particular MHC haplotype. Different MHC alleles are found in different lupus strains, and the same ones are also present in normal strains. Interestingly, degenerate MHC binding of certain myelin basic protein peptides and their recognition by T cell lines from multiple sclerosis patients have also been described. However, weakly cross-reactive binding to only DR molecules was reported, and TCRs responsible for the degenerate recognition were not analyzed (30).

Figure 9. Effect of the purified anti-CD4 mAb, RM4.5, on the responses of lupus T cells to nucleosomal peptide H471–94. Solid symbols represent anti-CD4 mAb containing cultures and the corresponding cultures without anti-CD4 are designated by open symbols. The MHC haplotypes of different APCs used to present the peptide are shown at the top of each panel; H-2d represents syngeneic SNF1 APCs. Background values of IL-2 production in corresponding cultures (T cells out antigen have been subtracted from each data point. (Top row) Left panel shows 4G4 transfected with 3A/3b TCRs; middle shows Th clones H/L-3A (circles) and H-15E3 (squares), and right panel shows two short-term, CD4+ T cell lines from SNF1 spleen (square and circle). Responses of pathogenic autoantibody-inducing Th clones H/L-3A and H-15E3 to nucleosomal peptide presented by different allologeneic APCs are shown in second and third rows, respectively, and that of the short-term T cell lines are shown in the bottom row. The anti-CD4 mAb, RM 4.5, was used at 1 $\mu$g/ml, which is the saturating concentration (25), but similar results were obtained at a concentration of 10 $\mu$g/ml. Another anti-CD4 mAb (GK1.5), concentrated 10 and 5 times from culture supernatants by ammonium sulphate precipitations, gave similar results (data not shown). Mean of triplicate assays are shown above. SD <10%.

The 24-mer H4 peptide to 15-mer decreased T cell recognition, suggesting that residues outside the MHC-binding core region can either directly or indirectly affect TCR engagement of MHC-peptide complexes (28, 29). Another degenerate MHC-binding and promiscuous Th cell recognition epitope was mapped to residues 16–39 of nucleosomal histone H4. However, another T cell epitope in histone H2B (5) did not share those properties. These results suggest that nucleosomal autoantigens are frequently but not always associated with degenerate MHC binding and promiscuous recognition by lupus T cells.

Crystallographic data has recently illustrated how recognition of MHC-peptide complexes by monogamous TCR molecules (43, 44) is largely dependent on a single TCR.
pocket engaging a prominent side chain of the peptide antigen. It is thus possible to speculate that nucleosomal peptide antigens that can bind multiple MHC molecules (degenerate binders) and carry multiple positive charges can engage certain lupus TCR-\(\alpha\) chains that carry multiple binding pockets and reciprocally charged residues, generating an interaction of such strength as to overcome the usual apparent requirement for MHC restriction (promiscuous T cell recognition). This hypothesis is further supported by the lack of the requirement of CD4 coreceptor for signaling the promiscuous recognition response by the lupus TCR, even at the lowest stimulatory concentrations of the autoantigen. These results cannot be attributed to possible expression of TCR at high density in the transfectants, because other nucleosome-specific lupus Th clones and freshly prepared CD4\(^+\) T cell lines from the spleens of SNF1 mice also shared this property, whereas the HEL-specific TCR (3A9) transfectants were not promiscuous. Indeed, CD4\(^-\), TCR-\(\alpha\)/\(\beta\) T cells that help in the production of pathogenic antinuclear autoantibodies are markedly expanded in both human and murine SLE (1, 2, 5, 7). The beneficial effect of chronic anti-CD4 antibody administration to lupus mice in vivo (45) is apparently contradictory to the above observations. However, the therapeutic effect of anti-CD4 in vivo could be due to a combination of factors and not exclusively due to an inhibition of CD4\(^+\) T cells. The CD4 molecule is also expressed on the surface of hematopoietic stem cell precursors, which could affect the generation of autoantigen-presenting APCs and autoimmune B cells, not just T cells (46). Significantly, the CD4 molecule is also expressed on APCs such as dendritic cells, monocytes, and macrophages (47). Thus, anti-CD4 therapy could interfere with autoantigen presentation and other effector and inflammatory components of lupus. Moreover, chronic anti-CD4 antibody therapy generates downregulatory T cells in vivo (48). Indeed, for anti-CD4 therapy to be effective it has to be administered repeatedly and for the long term to lupus-prone mice, beginning early in their lives (45), suggesting that something more than just inhibition of CD4\(^+\) T helper cells is responsible for the therapeutic effect.

Crystallographic and modeling data also indicate that different TCR-peptide-MHC complexes will have distinct structural orientations and contact points (14, 23, 41, 44, 49–51). Therefore, analysis of the promiscuous lupus TCRs in contact with the H4 peptide complexed with different MHC molecules could reveal some unique features of this trimolecular interaction.

Since the lupus TCR-\(\alpha\) chains could confer nucleosomal peptide specificity as well as promiscuity in combination with TCR-\(\beta\) chains of unrelated specificities, the nucleosome-specific T cell repertoire is potentially vast. How it is kept in check in normal mice and why they are selected in the lupus thymus is pressing questions that will be addressed in transgenic mice we are developing with these TCR constructs. These studies also open up the possibility of designing "universally tolerogenic" epitopes for the therapy of patients with lupus, despite the diversity of their HLA alleles.

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