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A Receptor Presentation Hypothesis for T Cell Help That Recruits Autoreactive B Cells[1]

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To uncover mechanisms that drive spontaneous expansions of autoreactive B cells in systemic lupus erythematosus, we analyzed somatic mutations in variable region genes expressed by a panel of (NZB × SWR)F_1 hybridomas representing a large, spontaneously arising clone with specificity for chromatin. A single mutation within the Jκ intron that was shared by all members of the lineage indicated that the clone emanated from a single mutated precursor cell and led to the prediction that a somatic mutation producing a functionally decisive amino acid change in the coding region would also be universally shared. Upon cloning and sequencing the corresponding germline V_H gene, we found that two replacement somatic mutations in FR1 and CDR2 were indeed shared by all seven clone members. Surprisingly, neither mutation influenced Ab binding to chromatin; however, one of them produced a nonconservative amino acid replacement in a mutationally "cold" region of FR1 and created an immunodominant epitope for class II MHC-restricted T cells. The epitope was restricted by IA^q (SWR), and the SWR MHC locus is associated with systemic lupus erythematosus in (NZB × SWR)F_1 mice. These, and related findings, provoke the hypothesis that autoreactive B cells may be recruited by a "receptor presentation" mechanism involving cognate interactions between T cells and somatically generated V region peptides that are self-presented by B cells. ***The Journal of Immunology*****, 2001, 166: 1562–1571.**

A cardinal feature of systemic lupus erythematosus (SLE)[3] is the unregulated synthesis of autoreactive Abs with biologically significant affinities for self-Ags that include nuclear structures (1, 2). Several strains of mice spontaneously develop SLE-like disease at a high frequency and, accordingly, have been subjects of investigation into mechanisms underlying disease genesis. A significant insight was realized with the discovery that autoreactive Abs in MRL-Mp-*lpr/lpr* mice are frequently derived from large clones of B lymphocytes that appear to be products of an intense clonal selection pressure normally associated with T cell-dependent immunity to conventional foreign Ag (3, 4). Several laboratories have confirmed and extended this basic finding to other autoimmune-prone strains, including F_1 mice derived in crosses of the NZB strain to the SWR (SNF_1) or NZW (BW) strains (5–9). The idiosyncratic specificities of autoantibodies obtained from individual diseased mice and SLE patients provides additional support for a clonal origin of autoreactive Abs in SLE (10, 11). Even in mice that carry transgenes encoding autoreactive B cell receptors (BCRs), recruiting autoreactive B cells appears to be a rare event on a per cell basis, as indicated by specific clonal expansions and secondary V region gene rearrangements expressed in expanded B cell lineages (12, 13). These observations indicate that having an autospecific BCR is insufficient to ensure B cell participation in SLE.

The telltale presence of somatic mutations within autoreactive Abs and the clonal relatedness of B cells producing them strongly suggest that cognate and MHC-restricted T cell help plays a pivotal role in the generation of autoantibodies. This is supported by genetic studies showing an association between MHC-proximal chromosomal intervals and SLE (14, 15). It is also supported by inhibition of disease in mice treated with Abs intended to ablate T cells or disrupt T cell-B cell interactions in several of the autoimmune-prone strains (16–19). And it is most conclusively demonstrated from studies involving mixed bone marrow chimeras that revealed an MHC compatibility requirement between B and T cells for autoantibody synthesis in *lpr/lpr* mice (20). Although noncognate T cell-B cell interactions may also play a role (21, 22) due in part to intrinsic defects in, and hyperactivity of, B cells from autoimmune prone strains, it appears that MHC-restricted interactions with T cells are essential to the generation and expansion of large autoreactive clones of B cells.

Elucidating the nature of T cell help for spontaneously autoreactive B cells has proved to be an enigmatic problem. Studies from one group have provided evidence that peptides from nucleosomal proteins are the Ags that mediate T cell help to autoreactive B cells in SNF_1 mice (23, 24). An unexpected finding was that the T cell responses were not restricted by particular class II MHC presenting molecules. In vitro responses to nucleosomal Ags that were analyzed for an $\alpha\beta$ T cell clone also did not appear to require a specific Vβ gene. In contrast, studies from a second group have suggested that autoantibody V regions contain immunogenic epitopes for T helper cells in BW mice (25, 26). In some cases, V region peptides modulated kinetics and severity of disease. An unusual finding of this work was that numerous T cell epitopes were often present within the ~120 aa of sequence that define the Ab V region domain (27). A complicating element to interpretations of these and related studies is the phenomenon of determinant spreading, which is common to both systemic and organ-specific autoimmune diseases (28). Determinant spreading has confounded efforts to define the earliest T cell epitopes that initiate recruitment of autoreactive B cells.

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[3] Abbreviations used in this paper: SLE, systemic lupus erythematosus; SNF_1, (NZB × SWR)F_1; BW, (NZB × NZW)F_1; BCR, B cell receptor.

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Because somatic mutations within Ab V region genes provide a genealogical record of clonal expansion in vivo (29, 30) we reasoned that the oldest mutations in spontaneous autoreactive lineages of B cells might reveal important information about selection pressures that act at or near the point of recruitment into the disease process. To our surprise, we found that the earliest mutations marking the initial growth of a large spontaneous autoreactive lineage with specificity for a chromatin Ag did not influence Ab specificity or affinity for chromatin. Instead, one of these mutations created an immunodominant epitope for class II MHC-restricted T cells. These and related observations lead us to propose that autoreactive B cells are recruited by cognate interactions with T helper cells that are specific for somatically generated V region peptides that are self-presented by class II MHC molecules on the surface of the B cell.

Materials and Methods

Mice, cell lines, and peptides

SNF_1 mice were bred at the National Jewish Biological Resource Center from NZB and SWR strains purchased from The Jackson Laboratory (Bar Harbor, ME). Chromatin-specific B cell hybridomas, derived from a spontaneously autoimmune SNF_1 female mouse, have been previously described (8). A variant of BW5147 lacking the α- and β-chains of the TCR was used for T cell hybridoma formation (31). HPLC-purified peptides used in this study and their locations within the expressed 7183 V_H gene of the autoreactive lineage are listed in Table II.

T cell hybridomas

Young SNF_1 mice were immunized s.c. at the base of the tail with 50 μl containing 10 μg of peptide or 50 μg mAb, purified as described below and emulsified in CFA. In some experiments mice were sacrificed 7 days following immunization, and draining lymph node cells were cultured with Ag followed by IL-2 before polyethylene glycol (PEG)-induced cell fusion to the BW5147 thymoma as described (32). For secondary response T cell hybridomas, SNF_1 mice immunized 4 wk previously were given a booster injection of Ag in IFA under otherwise identical conditions. Three days later, draining lymph node cells were taken for culture and subsequent fusion. T cell hybridomas were screened for cytokine responses to SNF_1 splenic APC cultured with the mAb or peptide immunogens in an HT-2 survival assay, monitored with MTT as described (33). In most assays, 10^5 T cells were cocultured overnight with 3.5×10^5 APC and Ag at a final concentration of 833 nM. For MHC restriction studies, NZB and SWR splenocytes were used as APC, and in some experiments I-A^q restriction was verified on SNF_1 APC in MHC blocking assays with Abs 14-4-4S (I-E^d), MKD6 (I-A^d), and $3F_12$ (I-A^q) (32, 34–36).

V region use by the T cell hybridomas was initially determined through staining analyses performed with a panel of biotinylated mAbs directed against various TCR Vβ regions. Of 41 hybridomas examined, 36 expressed Vβ2. cDNA was prepared from 17 V$\beta2^+$ hybridoma cells using Trizol reagent (Life Technologies, Grand Island, NY) and a specific Cβ primer: 5′-CCTCTGGCCACTTGTCCTCCTCTG-3′. The cDNA was amplified and sequenced using standard procedures (37) and the primers listed below: Vβ2 (amplification) 5′-TGTACTCATGGCTTCTGTGGCTAC-3′; Vβ2 (sequencing) 5′-GTGAACCTACGCTGCATCTTGAAG-3′; Cβ (amplification) 5′-CCTCTGGCCACTTGTCCTCCTCTG-3′; Cβ (sequencing) 5′-TAGCTATAATTGCTCTCCTTGTAG-3′ (38). Vα use was determined by PCR performed with a panel of primers specific for 14 Vα families: Va1-Vα8, and Vα10-Vα15 as described (39). In some cases where a primer pair was known to amplify multiple members of a given Vα family, sequencing was performed to identify the specific member (40).

Purification of Abs

Culture supernatants from transfected KL-2.13 cells were passed through protein G columns, and adherent mAb were eluted with 0.1 N acetic acid. Following dialysis against PBS, crude mAb were treated with DNase (1 μg/ml; Worthington Biochemical, Lakewood, NJ) in the presence of 2 mM $MgCl_2$ at 37°C for 90 min. This material was then passed through a Sepharose column conjugated with affinity-purified goat anti-mouse IgG (heavy and light chain specific). NaCl (1.5 M, buffered with 10 mM sodium phosphate, pH 7.2) was then passed through the column to dissociate any histones associated with the bound mAb (41), which was subsequently eluted with 0.1 M glycine, 0.5 M NaCl, pH 2.5, and dialyzed extensively against PBS. Ab purity was monitored by SDS-PAGE. We found it necessary to carry out these elaborate purification steps because, with protein G chromatography alone, mouse chromatin was found to copurify with the mAb, as evidenced by proteins with mobilities coincidental to those of histones upon SDS-PAGE. Because DNA is present in culture supernatants, the DNA requirement for binding is not evident if culture supernatants are the subject of testing, even if highly purified histones free of all DNA are used in binding assays. Following this elaborate purification procedure, recoveries of mAb were only between 1 and 2 mg/L of culture supernatant.

Binding assays

For ELISA, 96-well microtiter plates (Falcon 3912; BD Labware, Franklin Lakes, NJ) were coated with 1) total histones (5 μg/ml); 2) total histones (5 μg/ml) together with 5 μg/ml dsDNA; 3) H2A and H2B (2 μg/ml); 4) H2A and H2B (2 μg/ml) together with dsDNA (5 μg/ml); 5) poly L-lysine (0.1%) followed by dsDNA (S1 nuclease treated, 5 μg/ml); or 6) bovine gamma globulin (20 μg/ml). (Mixtures 2, 3, and 4 were incubated together at 37°C before coating.) Coated wells were washed and blocked with PBS, supplemented with BSA (2 mg/ml) and gelatin (1 mg/ml), and then incubated with test Abs diluted in the PBS/BSA/gelatin buffer at various concentrations. Positive controls for DNA and H2A/H2B binding were included (mAb 1D12 and 2B1, respectively) (42). The secondary incubation step was performed with alkaline phosphatase-conjugated rat anti-mouse IgG2b (clone R12-3; PharMingen, San Diego, CA) or goat anti-mouse IgG (affinity purified, γ-chain specific; Sigma, St. Louis, MO) in the case of the controls, 1D12 and 2B1. Plates were developed with phosphatase substrate (*p*-nitrophenyl phosphate, disodium; Sigma 104) under standard conditions for periods not exceeding 1 h, and the OD was read at 410 nm.

To achieve the highest level of precision in relative avidity determinations, we performed radioimmunoassays, which offer an extended linear relationship between Ab bound and signal detected. For both the competitive and the direct binding experiments, Falcon 3912 plates were coated with chromatin isolated from murine thymocytes as described (42). Following a blocking step with PBS/BSA/gelatin, wells were incubated in quadruplicate with test Abs at various concentrations in PBS/BSA/gelatin buffer. In the case of competition experiments, various concentrations of chromatin were mixed with Abs at a fixed concentration of 1 μg/ml, which, in the absence of competitor, resulted in ~90% of maximum binding to chromatin-coated wells. After washing, ^{125}I-labeled rat anti-mouse IgG2b (10 ng) was incubated in the wells for the final step. Bound radioactivity was measured in a Beckman Gamma 5500B counter.

V_H gene cloning

The rearranged V_H-D-J_H gene expressed by hybridoma SN5-18 was cloned into phage λ gt10 from *Eco*RI-digested DNA that was enriched for fragments ranging in size from 2.8 to 3.5 kb by an electroelution procedure as described (43). Recombinant phage (5×10^5) were screened with a J_H intron probe (44) and a cloned 135-bp PCR-derived probe spanning codons 22–66 in the SN5-18 V_H gene. After two rounds of additional cloning, the recombinant insert was sequenced and shown to be identical with the previously acquired sequence from amplified SN5-18 cDNA (8). Sequencing here and elsewhere was performed using ^{32}P end-labeled primers in the dideoxy chain termination procedure. In all cases, both strands were sequenced.

NZB DNA was digested separately with *Bam*HI, *Bgl*II, *Bcl*I, and *Eco*RI and probed in a Southern blot with the 135-bp V_H probe. Hybridization and washes were performed at 47°C in a solution consisting of 50% formamide, 2× SSC, 0.2% SDS, 10% dextran sulfate, and 50 μg/ml ssDNA. *Eco*RI-digested NZB DNA that had been enriched for fragments of 10 kb by electroelution as described (43) were cloned into EMBL3 at an insert-to-arm ratio of 2:1 so that fewer phage were needed for screening. Approximately 90,000 recombinant phage were screened with the V_H probe.

A germline V_H gene tentatively corresponding to the expressed copies in the anti-H4 hybridoma lineage was obtained by direct sequencing of a PCR product amplified from genomic DNA. For amplification, a V_H7183 consensus primer located at position −72 in the leader intron (5′-AGTTT TCCAACCAGTATTCTCTGT-3′) was used in concert with an FR3 primer covering codons 83–91(antisense, 5′-GTGTCCTCAGACCTCA GACTGCTCAT-3′) primer.

Site-directed mutagenesis and expression

The unanimously shared V_H mutations among members of the anti-H2A/H2B lineage were reverted to germline sequence as a group in a cassette replacement procedure as described (45) using the Muta-Gene phagemid kit (Bio-Rad, Richmond, CA). Briefly, primers matching germline sequence in V_HFR1 codons 9–17 (5′-AGGCTTAGTGCAGCCTGGAAG TTC-3′) and FR3 codons 88–95 (antisense, 5′-GTAATACGTGGCTGT GTCCTCAGA-3′) were used to amplify the corresponding segment from

the germline V_H gene. Double stranded product was boiled and annealed to a single stranded plasmid carrying the SN5-18 gene on a 1.6-kb *Eco*RI-*Hin*dIII fragment, followed by extension, ligation and transformation, and screening. Individual reversions at the codon 28 and 57 positions were performed in a conventional single primer site-directed mutagenesis procedure using primers: 5′-TCTGGATTCACTTTCAGTGAC-3′ for codon 28 and 5′-TTATGATGGTAGTAGCACCACTATCTGGACTC-3′ for codon 57. All products were confirmed by sequencing both strands of DNA using ^{32}P end-labeled primers in a dideoxy chain termination procedure.

*Eco*RI *Hin*dIII fragments (1.6 kb) of DNA carrying the rearranged SN5-18 V_H-D-J_H gene or copies reverted to germline sequence at codons 28, 57, or both were cloned into an IgG2b expression vector (46). The vector contains a bacterial gene for guanine phosphoribosyltransferase that confers resistance to mycophenolic acid and a BALB/c IgG2b constant region gene with the intronic H chain enhancer and downstream termination and polyadenylation signals for Ig expression. Expression plasmids were linearized with *Eco*RI and used in electroporation-mediated transfections of a cell line (KL-2.13) that produces a κ-chain with an identical variable region sequence to that of SN5-18 (47). Mycophenolic acid-resistant clones growing in 96-well microtiter dishes were screened for expression of IgG2b, cloned twice by limiting dilution, and expanded to liter volumes for purification of mAb used in immunization and binding assays described above.

Results

A large autoreactive clone derived from a mutated precursor B cell

Our study focused on a group of seven B cell hybridomas generated in an earlier study from splenocytes of a 6-month-old spontaneously autoimmune, female SNF_1 mouse. The hybridomas secrete autoantibodies reactive with a complex of histone 2A/2B (H2A/H2B) and DNA and are representatives of a common B cell clone, as determined previously by results of V region gene sequencing analyses (8). The large size of the clone is inferred from the large number of hybridoma representatives that were generated in a cell fusion procedure with an efficiency of only $\sim 3 \times 10^{-5}$. Earlier genealogical analysis, based on 93 somatic mutations located within the V_κ coding regions and J_H and J_κ containing introns, revealed two important characteristics of this clone. First, the clone appeared relatively simple from a mutation/maturation standpoint because of a near absence of branch points in the genealogical tree and a modest 1% nucleotide mutation frequency. Second, all members were derived from a common B cell precursor that had acquired somatic mutation(s), as deduced by the unanimous sharing of a single point nucleotide replacement at position 324 in the κ intronic region (Fig. 1). Moreover, at the point of clonal selection defining this genealogical tree, somatic mutagenesis had apparently just recently initiated in the precursor B cell because only one nucleotide substitution was unanimously shared in over 1350 bases of sequence where mutations could be defined with certainty.

We deduced that a postmutational selection event had resulted in massive clonal expansion that overshadowed any proliferation occurring before the J_κ somatic mutation at position 324. Because somatic mutagenesis is normally induced several days following B cell activation (48, 49), we concluded that the mutant precursor cell had acquired an advantage over its siblings that presumably expressed unmutated or alternatively mutated versions of the same rearranged variable region genes. This led to the hypothesis of the current study that an unseen somatic mutation(s) had produced a structural alteration somewhere in the V region that imparted a decisive advantage to the precursor B cell.

Universally shared somatic mutations in V_H genes define a precursor B cell

In our earlier study, the only location where somatic mutations could not be identified for lack of a germline sequence was within the V_H coding region. Thus, we predicted that a somatic mutation(s) producing a functionally important amino acid alteration(s) would be present in the rearranged V_H genes expressed by the hybridomas. The strength of this prediction is that all of the hybridomas should contain the mutation(s), despite sharing only one mutation in ~1350 bases of visible sequence.

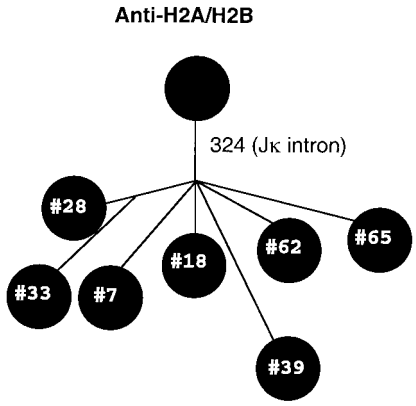

FIGURE 1. Members of anti-H2A/H2B lineage all derived from a mutated precursor. Genealogical relationships deduced from 93 somatic mutations identified using known germline sequences for V_κ coding region and J_κ and J_H intronic DNA. Lineage was constructed by minimizing the number of independent mutations and reversions (to germline sequence) needed to account for all of the mutation data. H and L refer to heavy and light chain genes, respectively. Somatic mutation at position 324 in the J_κ intron is shared by all members of the lineage indicating their derivation from a common mutated precursor cell.

To identify and clone the germline V_H gene expressed by the anti-H2A/H2B hybridomas, we first generated a highly specific V_H gene probe (see *Materials and Methods*) and performed a Southern blot analysis with hybridoma DNA. The probe generated a single hybridization signal at 3.2 kb, which upon subsequent analysis proved to define a DNA fragment carrying the gene that was rearranged and expressed by members of the hybridoma lineage. Our previous sequencing analyses of J_H intronic regions of NZB and SWR strains revealed that the hybridomas expressed an NZB heavy chain allele (8). When used against NZB genomic DNA under relatively stringent conditions, the probe generated single hybridization signals with each of the four restriction enzymes tested (Fig. 2). This, together with the single-copy signal intensity,

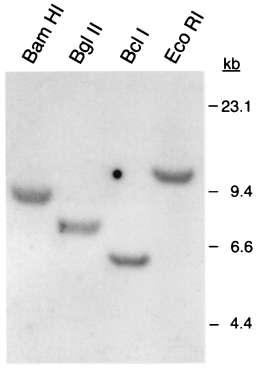

FIGURE 2. Southern hybridization defines V_H gene. NZB genomic DNA was digested with the enzymes indicated and subjected to a Southern hybridization procedure using a 135-bp V_H probe (codons 22–66) derived from the heavy chain gene expressed by hybridoma SN5-18. Single bands in each case indicate that the probe likely detects a single germline V_H gene.

suggested that the probe had identified the single sought-after germline V_H gene.

A recombinant EMBL3 phage library of NZB DNA was screened for hybridization with the V_H probe. Three positive clones were obtained that, upon two additional cycles of cloning, proved to carry independent isolates of the same V_H gene, as disclosed by V_H and upstream sequences, insert orientation, and presence of distinct secondary inserts of NZB DNA. Thrice independent isolation, together with the hybridization results and near sequence identity (98.9%) with a V_H consensus sequence from the hybridoma panel, provided convincing evidence that the three recombinant phage harbored the germline V_H gene expressed by members of the anti-H2A/H2B lineage. This interpretation has been confirmed by independent PCR cloning and sequencing studies (data not shown).

When the sequence of the cloned germline V_H gene was aligned with respect to expressed hybridoma sequences, three somatic mutations that were universally shared by members of the lineage were exposed (Fig. 3). One, at codon 58, was silent but our prediction was realized by two others that produced a nonconservative Thr-to-Ile replacement at codon 28 in FR1 and a Ser-to-Asn replacement at codon 57 in CDR2.

Autoantibody binding to histones and chromatin unaffected by shared mutations

One unusual feature of this genealogy was the unanimous sharing of somatic mutations by clone members that retain a low overall frequency of mutation (~1%) within their V region genes. Hybridoma and single cell sampling studies of splenic B cells in normal mice responding to foreign Ags seldom produce this type of pattern (50, 51). Following deliberate immunization, a nucleotide substitution frequency of ~1% is observed at ~2 wk postimmunization. But clonally related B cells generally share few mutations at this time, and their mutational relationships are described by genealogical "bushes" rather than trees, presumably because clonal proliferation in the absence of somatic mutation occurs for several days following B cell activation. Despite this, we conservatively speculated that one or both of the replacement mutations would improve Ab affinity for chromatin, largely because of established correlations between memory cell propagation and improvements to BCR ligand binding conferred by somatic mutations in both normal and autoimmune settings.

To test this idea, we isolated the rearranged heavy chain gene from one of the hybridomas (SN5-18) on a 3.2-kb *Eco*RI fragment in recombinant EMBL3 and cloned it in the context of an IgG2b

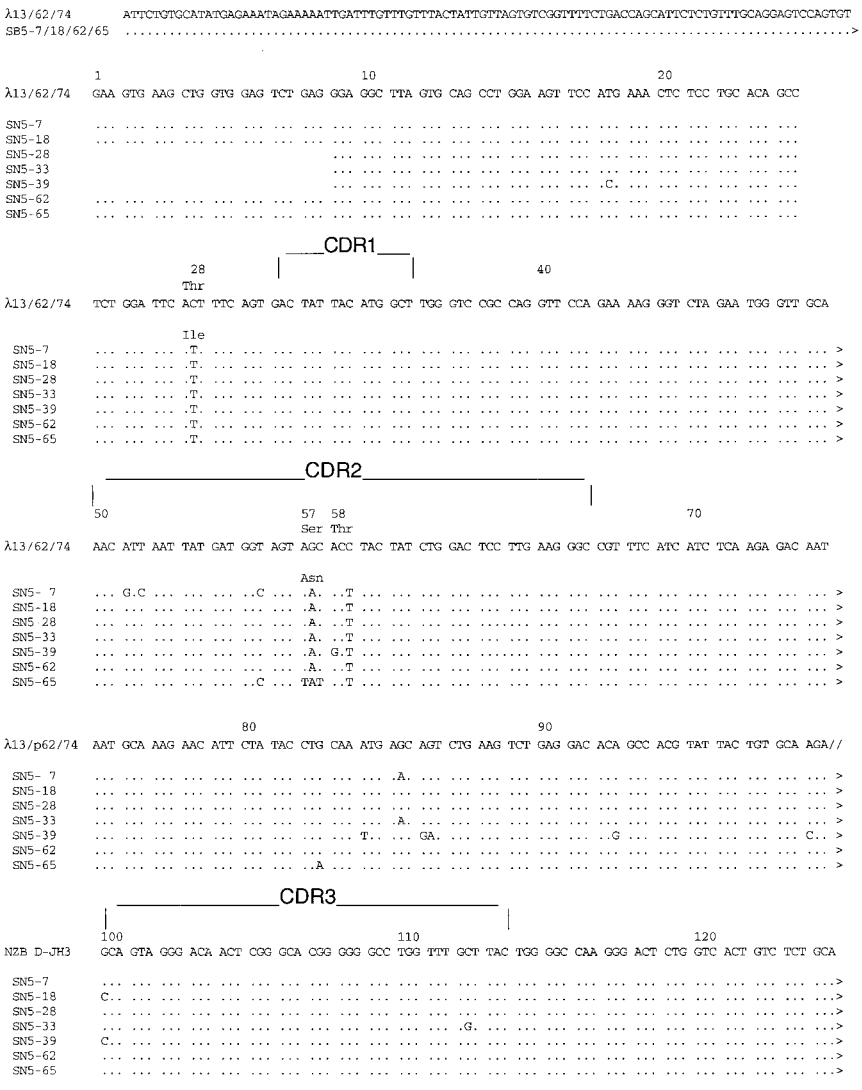

FIGURE 3. Unanimously shared somatic mutations in V_H genes of an autoreactive lineage. Sequences of expressed hybridoma V_H genes are aligned beneath that of the corresponding germline V_H 7183 gene isolated from NZB DNA by genomic cloning in phage λ. Dots indicate identities with the germline sequence. Unanimously shared mutations are at codons 28, 57, and 58. The hybridomas were produced from an unmanipulated SNF_1 mouse with high titers of autoantibodies to chromatin. All seven hybridomas produce Abs that bind a complex of histones 2A/2B and DNA.

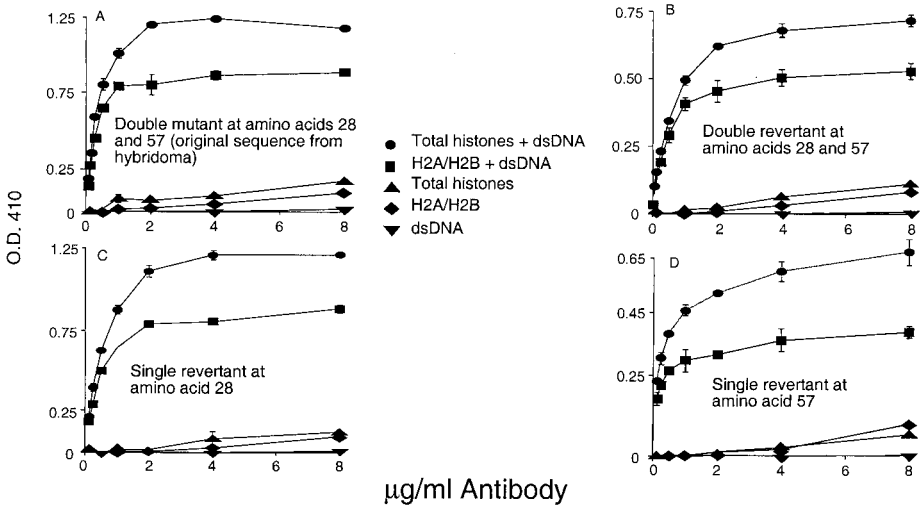

FIGURE 4. Conservation of autoantigenic specificity in germline revertants. Somatic mutations at codons 28 and 57 that were unanimously shared by members of the autoreactive anti-H2A/H2B lineage were reverted germline sequence, singly or in combination in SN5-18. Purified Abs were tested by ELISA for binding to various autoantigens, including histones and DNA. Each panel represents an independent experiment. SE values are indicated.

expression vector. SN5-18 was chosen because its κ-chain carries no somatic mutations and the only mutations located in its V_H are those that are unanimously shared by members of the lineage. A cell line (KL-2.13) producing an exact version of the SN5-18 κ V region (including J_κ and junctional residues) was available from previous studies (47) and served as the subject for transfections of SN5-18 heavy chain genes in which one, both, or neither of the codon 28 and 57 mutations had been reverted back to their corresponding germline sequences by site-directed mutagenesis. To our surprise, when purified Abs from culture supernatants of transfected cells were tested in a variety of binding assays against histones, DNA, and chromatin, we found no differences among the mAb. Representative results are shown in Figs. 4 and 5, where it can be seen that the DNA requirement for binding is preserved in all three revertants, and the Abs bind with indistinguishable avidities, even to whole chromatin, as assessed by direct binding and competition radioimmunoassays. These results indicate that the unanimously shared mutations in this clone did not impart an advantage in vivo by conferring improvements in receptor avidity for chromatin.

An immunodominant class II MHC-restricted epitope created by the universally shared FR1 mutation

The fact that the unanimously shared mutations did not affect binding avidity for chromatin implied that neither increased signaling via the BCR nor increased presentation of chromatin peptides was responsible for the selective expansion of the mutant precursor cell to this lineage. However, because B cells can process and self-present peptides from their Ab V regions (32, 52, 53), we considered the possibility that the unanimously shared somatic mutation(s) might have created an antigenic epitope(s) for T cell help. To test this idea, we undertook an extensive T cell hybridoma production study to determine which parts of the autoreactive Ab might be immunogenic for class II MHC-restricted T cells. Previous analyses in nonautoimmune-prone mice had taught us that more facile lymph node proliferation assays are not consistently reliable in detecting mutant V region peptide-specific T cells that are presumably rare in the repertoire due to tolerance of germline-encoded V region sequences (32, 54). Table I summarizes the results of the T cell hybridoma study. In these experiments, young, prediseased mice, most often male, were immunized once or twice with either purified transfectant-generated mAb that contained both or neither of the codon 28 and 57 mutations or with synthetic peptide correlates of 23 aa in length that incorporate the mutant codon 28 or 57 residues in the central position (Table II). In all fusions, T cell hybridomas were screened for cytokine responses to SNF_1 splenic APC cultured with the Ag used for immunization in an HT-2 survival (MTT) assay (33). The results conclusively demonstrate that the FR1 codon 28 mutation created an immunodominant epitope for T cells. Representative responses for two hybridomas reactive with SN5-18 are shown in Fig. 6.

The hybridoma assays revealed only one other subdominant V region epitope in CDR-3, which was only apparent when mice were immunized with the revertant mAb containing germline-encoded residues at positions 28 and 57. No T cell hybridomas specific for the mutant CDR-2 region were generated following immunization with

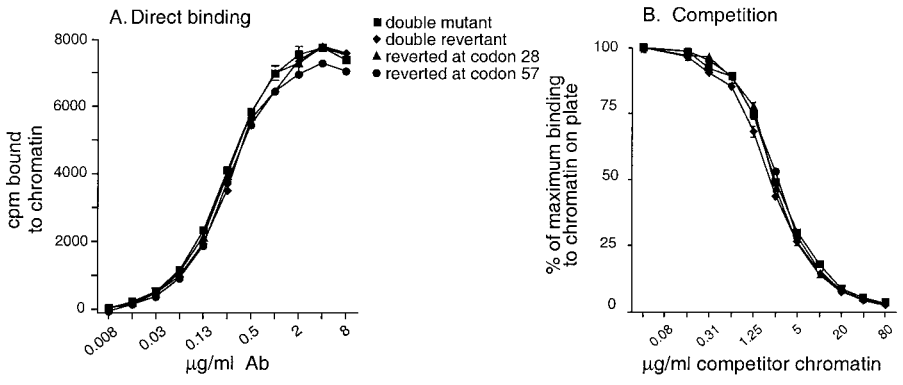

FIGURE 5. No effect of unanimously shared somatic mutations on mAb binding to mouse chromatin. Relative avidities of natural double mutant mAb SN5-18 and revertants, in which one or both unanimously shared somatic mutations were eliminated, were compared in direct binding and competition assays. *A*, Serial 2-fold dilutions of mAb were incubated in mouse chromatin-coated wells followed by secondary addition of an ^{125}I-labeled rat anti-mouse κ mAb. *B*, One hundred nanograms of each mAb was incubated in chromatin-coated wells in the presence of various concentrations of soluble mouse chromatin. SE values are indicated.

Table I. *Tests of immunogenicity by hybridoma production*

Immunogen	Mice	No. of Immuniza-tions	Hybridomas Screened	MHC Restriction	Test Ags: mAb SN5-18	mAb SN5-18R[a]	Control IgG2b[b]	Mutant FR1[c]	Germline FR1[c]	Mutant CDR-2[c]	CDR3[d]
Mutant FR1 (peptide)	One (F, 9 wk)	One	70	21/21 SWR	21/21	0/21	ND	21/21	7/21 (weak)[e]	ND	ND
	One (F, 21 wk)	Two	60	3/3 SWR	3/3	0/3	ND	3/3	0/3	ND	ND
Germline FR (peptide)	Two (F, 12 wk)	Two	65	ND	0/14	0/14	ND	3/14[f]	14/14	ND	ND
Mutant CDR2 (peptide)	Two (M, 10 wk)	One	34	ND	0/5	0/5	ND	ND		5/5	ND
mAb SN5-18 (natural mutant)	Two (M, 7 wk)	One	144	22/22 SWR	22/22	0/22	0/22	22/22	3/22 (weak)[g]	0/22	ND
	Two (M, 11 wk)	Two	109	17/17 SWR	17/17	14/17	14/17	3/17	0/17	0/17	0/17
mAb SN5-18R (double revertant)	Two (M, 14 wk)	One	147	15/15 SWR		15/15	15/15	ND	ND	ND	ND
	One (M, 14 wk)	One	147	7/7 NZB	ND	7/7	0/7	ND	ND	ND	7/7[h]
	Two (M, 13 wk)	Two	77	10/10 SWR	10/10	10/10	10/10	0/10	0/10	0 /10	0/10

[a] Codons 28 and 57 reverted back to germline sequence.
[b] Product of a J558 V_H gene expressed in the context of a BALB/c γ2b constant gene.
[c] Peptides of 23 aa centered on the residue of interest.
[d] Three overlapping peptides that span CDR3 were tested individually.
[e] Response against mutant FR1 peptide averaged 1875 U IL-2 and 40 U against germline FR1 peptide.
[f] Response against germline FR1 peptide averaged 2130 U IL-2 and 640 U against mutant FR1 peptide.
[g] Response against mutant FR1 peptide averaged 2560 U IL-2 and 40 U against germline FR1 peptide.
[h] All seven hybridomas responded to peptides CDR3-1 and CDR3-2 representing V_H-D junction as shown in Table II.

either the whole Ab or the corresponding peptide. A few of the more robust FR1-elicited T cell hybridomas cross-reacted with APC fed with the germline version of the FR1 peptide, presumably due to presentation of nonphysiologically high levels of peptide or trace contaminant peptides generated during synthesis. Similarly, immunization with the germline FR1 peptide led to the generation of hybridomas responsive to peptide-treated APC but not to APC fed with whole mAb.

An allotypic response to an undefined IgG2b epitope was also evident and dominated the overall T cell response in mice immunized with the double revertant mAb. This is due to the origin of the IgG2b constant gene in the expression construct, which was derived from the BALB/c strain (46). The allotypic response served as a useful positive control for appropriate immunization, in vitro stimulation, cell fusion, and culture in the case of mice immunized with the double revertant, which was otherwise poorly immunogenic. In contrast, T cell hybridomas responsive to the allotypic determinant were not observed when fusions were performed during the primary immune response to the mutant SN5-18 mAb, but they dominated fusions performed during the secondary immune response. Their gradual domination suggests that a more stable trimolecular complex between TCR, MHC, and peptide is formed in the case of the allotypic peptide than in the case of the mutant FR1 peptide (55). In contrast, dominance of the primary response by T cells that respond to the mutant FR1 peptide suggests that the responsive T cell precursor frequency is relatively high.

To test this idea, we analyzed TCR Vα and Vβ use by the panel of hybridomas responsive to the mutant FR1 peptide (37, 39). Although most of them expressed Vβ2 (36/41), this Vβ was paired with Vα genes derived from five different families. Additional sequence diversity was evident upon sequencing Vβ CDR3, which together with Vα use, revealed a total of at least 14 T cell clonotypes (Fig. 7). Identical independent clonotypes were observed in only one case consisting of four hybridomas from the same fusion, indicating that actual diversity is likely to be significantly greater.

Splenic APC from NZB and SWR mice were used to assess MHC restriction. All of the FR1-responsive T cell hybridomas were restricted by the SWR haplotype, which lacks a functional I-E molecule. I-A^q restriction was confirmed in several cases with blocking Abs. An association between the SWR MHC locus and autoimmune disease in the SNF_1 model was demonstrated previously in backcross studies (14).

A second autoreactive clone with similar characteristics

A second group of anti-histone 4-specific B cell hybridomas was also obtained from the same animal that produced the anti-H2A/H2B set. Previous sequencing analyses indicated that its six members also defined a single clone. But compared with the anti-H2A/H2B genealogical tree, that of the anti-H4 clone was more complex, as defined by a greater number of branch points and a 3-fold higher mutation frequency.

Nevertheless, all of its members were also derived from a common mutated precursor, as defined by a universally shared somatic mutation in the J_H intronic region, indicating a strong postmutational selection event had also acted upon the precursor B cell to this lineage. Like the anti-H2A/H2B clone, the anti-H4 clone also expresses a V_H gene that belongs to the 7183 family. We do not have a definitive germline sequence for the 7183 V_H gene used by this clone, but a tentative sequence has been obtained from a PCR product derived from genomic DNA that was amplified with consensus primers located in the V_H leader intron and in FR3. The candidate germline V_H gene is 97% identical in sequence to a previously cloned germline gene, both of which differ at codons 10 and 31 from V_H genes expressed by all members of the anti-H4 lineage, suggesting that the hybridomas may universally share somatic mutations at those positions (Fig. 8). It is also intriguing that four of the anti-H4 members also possess an Ile codon at position 28. If the Ile change is due to a somatic mutation, it is unlikely to be the consequence of an intrinsic bias in the mutation mechanism because the germline triplet sequences covering the position of the tentative

Table II. *Synthetic peptides used in this study*

Name	Residues	Sequence
Mutant FR1	17–39	SMKLSCTASGFIFSDYYMAWVRQ
Germline FR1	17–39	SMKLSCTASGFTFSDYYMAWVRQ
Mutant CDR2	46–68	EWVANINYDGSNTYYLDSLKGRF
CDR3 1	91–106	TATYYCARPVGTTRAR
CDR3 2	96–111	CARPVGTTRARGAWFA
CDR3 3	100–116	VGTTRARGAWFAYWGQ

nucleotide replacement are not preferred targets of the mutation mechanism (56). The average mutability index for the three triplets that encompass the nucleotide is 1.1, where 1.0 is the average for all triplets and 3.7 is the highest among all triplets. Furthermore, FR1 is empirically less mutable than predicted on the basis of triplet sequence composition alone (57). Whether the Ile residue is derived by mutation can only be determined with certainty by a direct cloning of the germline V_H gene.

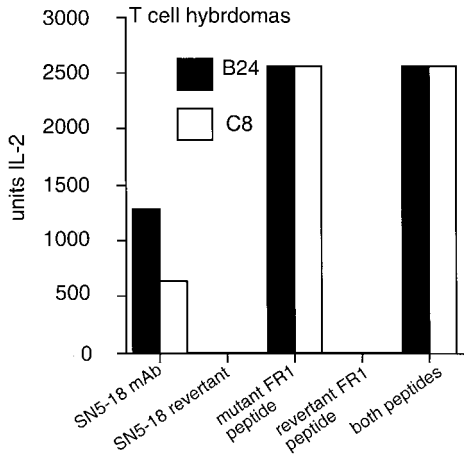

FIGURE 7. Representative responses by T cell hybridomas. Hybridomas B24 and C8 were generated following two immunizations with the SN5-18 mAb. SNF_1 splenocytes were used as APC. All Ags were tested at a concentration of 833 nM.

Discussion

Our results are consistent with the idea that chromatin-specific B cells are spontaneously recruited into autoreactive responses by T cell help directed to somatically generated V region peptides that are self-presented by B cells. This interpretation is in agreement with results of several independent avenues of investigation, indicating a requirement for T cell help in driving spontaneous autoantibody synthesis, and from results of a graft-vs-host model of SLE in which alloreactive T cell help elicits production of autoantibodies directed to self nuclear Ags (58, 59). T cell help appears to be a rate-limiting factor to autoantibody development in SLE, and our results support the idea that somatically altered V region peptides may provide the initial avenue for such deviant help.

Defining the early events in the genesis of SLE and other autoimmune diseases has proved to be a formidable challenge. Genetic analyses have implicated several loci in SLE (60, 61). Our somatic genetic studies complement this approach by defining physiological processes during which autoimmune deviation may occur. It is doubtful that the simple acquisition of a V region somatic mutation producing a T cell epitope is sufficient to result in unregulated B cell clonal expansion. If this occurred, autoimmunity would be the norm, not the exception, regardless of genetic considerations. Instead, our results draw attention to the importance of T cell help, which appears to be the object of a breakdown in tolerance in autoimmune-prone mice. In autoimmune-prone mice, T cells that are specific for class II MHC-presented mutant V region peptides may gain access to, and help B cells in a way that is likely precluded under normal physiological circumstances by tolerance or cellular segregation.

On the surface, our results appear to be consistent with those of Singh and colleagues, who report that T cells in BWF_1 mice are responsive to synthetic peptides designed after V region sequences in autoreactive mAb (25, 26). However, these authors observe promiscuous responses to peptides representing many parts of the Ab V region, and the reactive T cells appear to be abundant even in prediseased animals. In contrast, we do not observe promiscuous responses to many portions of the autoantibody V region. Our T cell hybridomas appear to be directed exclusively to somatically generated epitopes. In addition, we did not observe significant proliferative responses by splenocytes to purified peptides representing parts of the SN5-18 V_H region (L.J.W., unpublished data). A possible explanation for this apparent discrepancy is that the mAb V regions studied by Singh and colleagues are extensively mutated and that the observed T cell responses are indeed directed to many mutated segments of the V region. (Somatic mutations were not defined in this system.) Another possibility is that some of the T cell responses to peptides are not physiologically significant, possibly because of dose considerations or potential contaminants in the synthetic product. It is also possible that there is a breakdown in T cell tolerance of unmutated Ab diversity in BWF_1 mice. However, thus far we have seen no evidence of this in SNF_1 mice.

Because the precursor cell to the anti-H2A/H2B lineage carried only four mutations in 1722 bases of sequence, we infer that a critical selection event occurred shortly after the induction of somatic mutagenesis. Similarly, only one definitive somatic mutation in ~750 bases of available sequence was shared by all representatives of the large anti-H4 clone. The presence of a T cell epitope in a V region with so few mutations suggests that it is a target of selection because somatic mutations that create T cell epitopes in nonautoreactive Ab V genes are infrequent. From our previous analyses of anti-arsonate V regions, only two such epitopes were found in Abs that carried a total of 34 replacement mutations (32).

Hybrid	Vβ	Vα	D-Jβ	End of Vβ		(N/P)Dβ(N/P)				Beginning of Jβ			
Q21	2	13.1	D1/J1.1	S	A	D	G	G	G	T	E	V	F
B24	2	11	D1/J1.1	-	-	E	-	-	S	-	-	-	-
Q79	2	4.9	D1/J1.1	-	-	-	-	-	-	-	-	-	-
Q86	2	13.1	D1/J1.1	-	-	-	-	-	-	-	-	-	-
D80	2	11	D1/J1.1	-	-	-	-	V		-	-	-	-
C8	2	13.1	D2/J2.3	-	-	T	-	-		A	-	T	L
E22	2	22.1	D2/J2.3	-	-	G	-	-		A	-	T	L
Q81	2	5	D2/J2.4	-	-	G	-	L		D	T	L	Y
E6	2	11	D2/J2.5	-	-	A	-	-	Q	D	T	Q	Y
D79	2	5.2	D2/J2.5	-	-	A	-	-	A	D	T	Q	Y
Q73	2	13	D2/J2.7	-	-	-	-	V		E	Q	Y	-
E14	2	13.1	D2/J2.7	-	-	N	-	V		E	Q	Y	-

FIGURE 6. Diversity of TCRs that bind the mutant FR1 peptide in the context of I-A^q. CDR3 sequences are only shown for those hybridomas that used the Vβ2 gene segment. Others using Vβ8 and 14 were not sequenced.

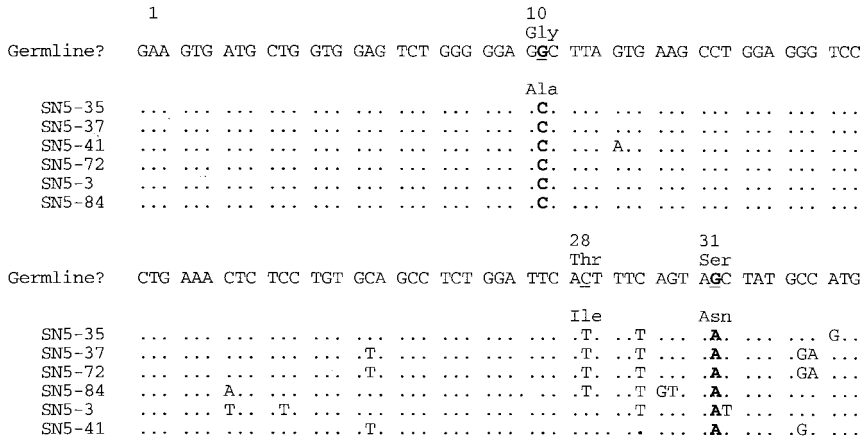

FIGURE 8. Potential unanimously shared somatic mutations in V_H genes expressed by members of anti-H4 lineage. Sequences of first 34 codons of V_H genes expressed by anti-H4 lineage members are aligned beneath the putative germline V_H sequence obtained several independent times by PCR-based cloning. Note also the Thr to Ile mutation at position 28 in four of the lineage members.

Alternative interpretations of the data are possible. Chromatin might not be the actual immunogen driving B cells in vivo, in which case the Ile_{28} mutation might enhance BCR binding to an unseen immunogen. However, the SN5-18 Ab binds with high avidity to mouse chromatin, which copurifies with this mAb on protein G columns, thereby necessitating elaborate purification steps (see *Materials and Methods*). Furthermore, H2A/H2B/DNA is one of the most consistent and early targets of autoantibodies in SLE (62–64). Finally, a National Center for Biotechnology Information BLAST search (65) with the SN5-18 sequence revealed 22 Abs encoded by the same or closely related V_H gene (94–98% sequence identity). None of these possessed the Ile_{28} codon, despite that fact that 17 reacted with chromatin autoantigens. Importantly, none of the 22 Abs was from mice carrying an *I-A*^{q} allele. These observations argue that the Ile_{28} mutation was not selected on the basis of conferring improved binding properties to the BCR for an unknown Ag.

It is also unlikely that light chain receptor editing singled out the anti-H2A/H2B precursor from its siblings for clonal expansion (66, 67). The expressed V_κ gene is assembled to the $J_\kappa 1$ gene segment, and the frequency of somatic mutations in the J_κ intron of the expressed locus is at least as high as it is in the J_H intron of the expressed heavy chain locus (39/2877 = 1.3% for J_κ and 21/2877 = 0.7% for J_H). Similarly, in the anti-H4 clone, which also expresses a V_κ-$J_\kappa 1$ rearrangement, the mutation load is similar in the Jκ intron (87/2400 = 3.6%) and the J_H intron (95/2400 = 3.9%).

A third possibility is that a hidden CDR3 mutation converted a nonautoreactive BCR to an autoreactive BCR in the precursor cell to the anti-H2A/H2B lineage. There is good evidence that autoantigens are a driving force in autoreactive B cell clonal expansions (68). Some evidence also suggests that autoreactive clones of B cells evolve by mutation and selection for mutant BCR with altered specificities and improved affinities for autoantigens (9, 12, 69). However, an important exception was reported by Jacobson et al. (70), who found no significant correlation between mutations and autoantibody affinity in a B cell lineage producing rheumatoid factor Abs. Regardless, it is clear that possessing an autoreactive BCR is insufficient to guarantee entry into an autoimmune process. In transgenic MRL-*lpr/lpr* mice that express autoreactive BCR with specificity for DNA, autoantibody production occurs clonally (12, 13) with the same kinetics as in nontransgenic MRL-*lpr/lpr* mice (71). Moreover, global B cell tolerance mechanisms appear to be intact in MRL-*lpr/lpr* mice (72, 73). If any one of these alternative mechanisms were solely responsible for clonal breakthrough, then one must invoke a series of coincidences to explain 1) a universally shared, mutationally created T cell epitope; 2) restricted by I-A^q; 3) created by a nonconservative amino acid replacement in a V_H framework region; 4) due to a nucleotide substitution in a mutationally "cold" region of the V_H; 5) which is apparently shared by members of a second B cell lineage with a different antigenic specificity but from the same animal. Nevertheless, we recognize that our results are retrospective in nature and, accordingly, more direct experimentation will be required to confirm or refute the "receptor presentation" idea.

Collectively, the results of this study lead us to the hypothesize that during early stages of their growth, autoreactive B cells can receive critical T cell help specified by somatically generated BCR peptides that are presented by class II MHC molecules on the B cell surface. The receptor presentation hypothesis is consistent with a wide body of evidence indicating a requirement for T cell help in autoantibody synthesis and with the clonal nature of autoreactive B cells, even in engineered mice that carry transgenes specifying autoreactive BCR. The hypothesis predicts that receiving T cell help by the receptor presentation avenue is a rate-limiting step in recruiting B cells into an autoimmune response, and that decisive flaws in tolerance influenced by genetic elements occur at a precarious point in B cell differentiation when neo antigenic epitopes may arise in V regions via somatic mutagenesis. Our results predict that it should be possible to isolate T cells or hybridomas that react with spontaneous autologous autoantibodies from diseased mice. However, testing this prediction may not be trivial because autoantibodies and the mutations they carry will likely differ from one autoimmune animal to the next.

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References

1. Pisetsky, D. S. 1986. Systemic lupus erythematosus. *Med. Clin. N. Am. 70:337.*
2. Tan, E. M. 1988. Antinuclear antibodies: diagnostic markers and clues to the basis of systemic autoimmunity. *Pediatr. Infect. Dis. J. 7:S3.*
3. Shlomchik, M., M. Mascelli, H. Shan, M. Z. Radic, D. Pisetsky, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med. 171:265.*
4. Shlomchik, M. J., A. H. Aucoin, D. S. Pisetsky, and M. G. Weigert. 1987. Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc. Natl. Acad. Sci. USA 84:9150.*
5. Bloom, D. D., E. W. St. Clair, D. S. Pisetsky, and S. H. Clarke. 1994. The anti-La response of a single MRL/Mp-*lpr/lpr* mouse: specificity for DNA and V_H gene usage. *Eur. J. Immunol. 24:1332.*

6. Losman, M. J., T. M. Fasy, K. E. Novick, and M. Monestier. 1992. Monoclonal autoantibodies to subnucleosomes from a MRL/Mp$(-)^{+/+}$ mouse: oligoclonality of the antibody response and recognition of a determinant composed of histones H2A, H2B, and DNA. *J. Immunol. 148:1561.*
7. Marion, T. N., D. M. Tillman, and N. T. Jou. 1990. Interclonal and intraclonal diversity among anti-DNA antibodies from an (NZB × NZW)F_1 mouse. *J. Immunol. 145:2322.*
8. Portanova, J. P., G. Creadon, X. Zhang, D. S. Smith, B. L. Kotzin, and L. J. Wysocki. 1995. An early post-mutational selection event directs expansion of autoreactive B cells in murine lupus. *Mol. Immunol. 32:117.*
9. Retter, M. W., P. L. Cohen, R. A. Eisenberg, and S. H. Clarke. 1996. Both Sm and DNA are selecting antigens in the anti-Sm B cell response in autoimmune MRL/*lpr* mice. *J. Immunol. 156:1296.*
10. Hardin, J. A. 1986. The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. *Arthritis Rheum. 29:457.*
11. Radic, M. Z., and M. Weigert. 1994. Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu. Rev. Immunol. 12:487.*
12. Brard, F., M. Shannon, E. L. Prak, S. Litwin, and M. Weigert. 1999. Somatic mutation and light chain rearrangement generate autoimmunity in anti-single-stranded DNA transgenic MRL/*lpr* mice. *J. Exp. Med. 190:691.*
13. Roark, J. H., C. L. Kuntz, K. A. Nguyen, L. Mandik, M. Cattermole, and J. Erikson. 1995. B cell selection and allelic exclusion of an anti-DNA Ig transgene in MRL-*lpr/lpr* mice. *J. Immunol. 154:4444.*
14. Ghatak, S., K. Sainis, F. L. Owen, and S. K. Datta. 1987. T-cell-receptor β- and I-A β-chain genes of normal SWR mice are linked with the development of lupus nephritis in NZB × SWR crosses. *Proc. Natl. Acad. Sci. USA 84:6850.*
15. Kotzin, B. L., and E. Palmer. 1987. The contribution of NZW genes to lupus-like disease in (NZB × NZW)F_1 mice. *J. Exp. Med. 165:1237.*
16. Connolly, K., J. R. Roubinian, and D. Wofsy. 1992. Development of murine lupus in CD4-depleted NZB/NZW mice: sustained inhibition of residual $CD4^+$ T cells is required to suppress autoimmunity. *J. Immunol. 149:3083.*
17. Early, G. S., W. Zhao, and C. M. Burns. 1996. Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black × New Zealand white mice: response correlates with the absence of an anti-antibody response. *J. Immunol. 157:3159.*
18. Hang, L., A. N. Theofilopoulos, R. S. Balderas, S. J. Francis, and F. J. Dixon. 1984. The effect of thymectomy on lupus-prone mice. *J. Immunol. 132:1809.*
19. Kalled, S. L., A. H. Cutler, S. K. Datta, and D. W. Thomas. 1998. Anti-CD40 ligand antibody treatment of SNF_1 mice with established nephritis: preservation of kidney function. *J. Immunol. 160:2158.*
20. Sobel, E. S., V. N. Kakkanaiah, M. Kakkanaiah, R. L. Cheek, P. L. Cohen, and R. A. Eisenberg. 1994. T-B collaboration for autoantibody production in *lpr* mice is cognate and MHC-restricted. *J. Immunol. 152:6011.*
21. Peng, S. L., M. P. Madaio, D. P. Hughes, I. N. Crispe, M. J. Owen, L. Wen, A. C. Hayday, and J. Craft. 1996. Murine lupus in the absence of α β T cells. *J. Immunol. 156:4041.*
22. Peng, S. L., J. M. McNiff, M. P. Madaio, J. Ma, M. J. Owen, R. A. Flavell, A. C. Hayday, and J. Craft. 1997. α β T cell regulation and CD40 ligand dependence in murine systemic autoimmunity. *J. Immunol. 158:2464.*
23. Kaliyaperumal, A., C. Mohan, W. Wu, and S. K. Datta. 1996. Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J. Exp. Med. 183:2459.*
24. Shi, Y., A. Kaliyaperumal, L. Lu, S. Southwood, A. Sette, M. A. Michaels, and S. K. Datta. 1998. Promiscuous presentation and recognition of nucleosomal autoepitopes in lupus: role of autoimmune T cell receptor α chain. *J. Exp. Med. 187:367.*
25. Singh, R. R., F. M. Ebling, E. E. Sercarz, and B. H. Hahn. 1995. Immune tolerance to autoantibody-derived peptides delays development of autoimmunity in murine lupus. *J. Clin. Invest. 96:2990.*
26. Singh, R. R., V. Kumar, F. M. Ebling, S. Southwood, A. Sette, E. E. Sercarz, and B. H. Hahn. 1995. T cell determinants from autoantibodies to DNA can upregulate autoimmunity in murine systemic lupus erythematosus. *J. Exp. Med. 181: 2017.*
27. Singh, R. R., and B. H. Hahn. 1998. Reciprocal T-B determinant spreading develops spontaneously in murine lupus: implications for pathogenesis. *Immunol. Rev. 164:201.*
28. Mamula, M. J. 1998. Epitope spreading: the role of self peptides and autoantigen processing by B lymphocytes. *Immunol. Rev. 164:231.*
29. Clarke, S. H., K. Huppi, D. Ruezinsky, L. Staudt, W. Gerhard, and M. Weigert. 1985. Inter- and intraclonal diversity in the antibody response to influenza hemagglutinin. *J. Exp. Med. 161:687.*
30. McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA 81:3180.*
31. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D. P. Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. *J. Immunol. 143:1822.*
32. Eyerman, M. C., X. Zhang, and L. J. Wysocki. 1996. T cell recognition and tolerance of antibody diversity. *J. Immunol. 157:1037.*
33. Heeg, K., J. Reimann, D. Kabelitz, C. Hardt, and H. Wagner. 1985. A rapid colorimetric assay for the determination of IL-2-producing helper T cell frequencies. *J. Immunol. Methods 77:237.*
34. Epstein, S. L., K. Ozato, J. A. Bluestone, and D. H. Sachs. 1981. Idiotypes of anti-Ia antibodies. I. Expression of the 14-4-4S idiotype in humoral immune responses. *J. Exp. Med. 154:397.*
35. Watts, T. H., A. A. Brian, J. W. Kappler, P. Marrack, and H. M. McConnell. 1984. Antigen presentation by supported planar membranes containing affinity-purified I-Ad. *Proc. Natl. Acad. Sci. USA 81:7564.*
36. Wei, B. Y., K. Schreiber, J. M. Buerstedde, M. Bell, A. Nilson, C. Huntoon, C. Chase, and D. J. McKean. 1994. Substitution of class II α chain polymorphic residues defines location of A α k serologic epitopes and alters association between α β and Ii polypeptides. *Int. Immunol. 6:297.*
37. Pullen, A. M., W. Potts, E. K. Wakeland, J. Kappler, and P. Marrack. 1990. Surprisingly uneven distribution of the T cell receptor V β repertoire in wild mice. *J. Exp. Med. 171:49.*
38. Gascoigne, N. R., Y. Chien, D. M. Becker, J. Kavaler, and M. M. Davis. 1984. Genomic organization and sequence of T-cell receptor β-chain constant- and joining-region genes. *Nature 310:387.*
39. Yague, J., M. Blackman, W. Born, P. Marrack, J. Kappler, and E. Palmer. 1988. The structure of V α and J α segments in the mouse. *Nucleic Acids Res. 16: 11355.*
40. Arden, B., S. P. Clark, D. Kabelitz, and T. W. Mak. 1995. Mouse T-cell receptor variable gene segment families. *Immunogenetics 42:501.*
41. Bornkamm, G. W., P. Nobis, and J. Sonnenbichler. 1972. Concerning the specificity of histone and non-histone dissociation from calf thymus chromatin by salt. *Biochim. Biophys. Acta 278:258.*
42. Kotzin, B. L., J. A. Lafferty, J. P. Portanova, R. L. Rubin, and E. M. Tan. 1984. Monoclonal anti-histone autoantibodies derived from murine models of lupus. *J. Immunol. 133:2554.*
43. Wysocki, L. J., T. Gridley, S. Huang, A. G. Grandea III, and M. L. Gefter. 1987. Single germline V_H and V κ genes encode predominating antibody variable regions elicited in strain A mice by immunization with *p*-azophenylarsonate. *J. Exp. Med. 166:1.*
44. Siekevitz, M., S. Y. Huang, and M. L. Gefter. 1983. The genetic basis of antibody production: a single heavy chain variable region gene encodes all molecules bearing the dominant anti-arsonate idiotype in the strain A mouse. *Eur. J. Immunol. 13:123.*
45. Near, R. I. 1992. Gene conversion of immunoglobulin variable regions in mutagenesis cassettes by replacement PCR mutagenesis. *Biotechniques 12:88.*
46. Sompuram, S. R., and J. Sharon. 1993. Verification of a model of a F(ab) complex with phenylarsonate by oligonucleotide-directed mutagenesis. *J. Immunol. 150:1822.*
47. Wysocki, L. J., G. Creadon, K. R. Lehmann, and J. C. Cambier. 1992. B-cell proliferation initiated by Ia cross-linking and sustained by interleukins leads to class switching but not somatic mutation in vitro. *Immunology 75:116.*
48. Berek, C., G. M. Griffiths, and C. Milstein. 1985. Molecular events during maturation of the immune response to oxazolone. *Nature 316:412.*
49. Wysocki, L., T. Manser, and M. L. Gefter. 1986. Somatic evolution of variable region structures during an immune response. *Proc. Natl. Acad. Sci. USA 83: 1847.*
50. Liu, A. H., P. K. Jena, and L. J. Wysocki. 1996. Tracing the development of single memory-lineage B cells in a highly defined immune response. *J. Exp. Med. 183:2053.*
51. Manser, T. 1989. Evolution of antibody structure during the immune response: the differentiative potential of a single B lymphocyte. *J. Exp. Med. 170:1211.*
52. Rudensky, A. Y., and V. L. Yurin. 1989. Immunoglobulin-specific T-B cell interaction. I. Presentation of self immunoglobulin determinants by B lymphocytes. *Eur. J. Immunol. 19:1677.*
53. Weiss, S., and B. Bogen. 1989. B-lymphoma cells process and present their endogenous immunoglobulin to major histocompatibility complex-restricted T cells. *Proc. Natl. Acad. Sci. USA 86:282.*
54. Eyerman, M. C., and L. Wysocki. 1994. T cell recognition of somatically-generated Ab diversity. *J. Immunol. 152:1569.*
55. Savage, P. A., J. J. Boniface, and M. M. Davis. 1999. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity 10:485.*
56. Smith, D. S., G. Creadon, P. K. Jena, J. P. Portanova, B. L. Kotzin, and L. J. Wysocki. 1996. Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. *J. Immunol. 156:2642.*
57. Shapiro, G. S., K. Aviszus, D. Ikle, and L. J. Wysocki. 1999. Predicting regional mutability in antibody V genes based solely on di- and trinucleotide sequence composition. *J. Immunol. 163:259.*
58. Kimura, M., F. M. van Rappard-van der Veen, and E. Gleichmann. 1986. Requirement of H-2-subregion differences for graft-versus-host autoimmunity in mice: superiority of the differences at class-II H-2 antigens (I-A/I-E). *Clin. Exp. Immunol. 65:542.*
59. Portanova, J. P., F. M. Ebling, W. S. Hammond, B. H. Hahn, and B. L. Kotzin. 1988. Allogeneic MHC antigen requirements for lupus-like autoantibody production and nephritis in murine graft-vs-host disease. *J. Immunol. 141:3370.*
60. Morel, L., and E. K. Wakeland. 1998. Susceptibility to lupus nephritis in the NZB/W model system. *Curr. Opin. Immunol. 10:718.*
61. Vyse, T. J., and B. L. Kotzin. 1998. Genetic susceptibility to systemic lupus erythematosus. *Annu. Rev. Immunol. 16:261.*
62. Losman, M. J., T. M. Fasy, K. E. Novick, and M. Monestier. 1993. Relationships among antinuclear antibodies from autoimmune MRL mice reacting with histone H2A-H2B dimers and DNA. *Int. Immunol. 5:513.*

63. Mohan, C., E. Alas, L. Morel, P. Yang, and E. K. Wakeland. 1998. Genetic dissection of SLE pathogenesis: Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J. Clin. Invest. 101: 1362.*
64. Burlingame, R. W., and R. L. Rubin. 1996. Autoantibody to the nucleosome subunit (H2A-H2B)-DNA is an early and ubiquitous feature of lupus-like conditions. *Mol. Biol. Rep. 23:159.*
65. Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res. 25:3389.*
66. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med. 177:999.*
67. Tiegs, S. L., D. M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med. 177:1009.*
68. Shan, H., M. J. Shlomchik, A. Marshak-Rothstein, D. S. Pisetsky, S. Litwin, and M. G. Weigert. 1994. The mechanism of autoantibody production in an autoimmune MRL/*lpr* mouse. *J. Immunol. 153:5104.*
69. Radic, M. Z., J. Mackle, J. Erikson, C. Mol, W. F. Anderson, and M. Weigert. 1993. Residues that mediate DNA binding of autoimmune antibodies. *J. Immunol. 150:4966.*
70. Jacobson, B. A., J. Sharon, H. Shan, M. Shlomchik, M. G. Weigert, and A. Marshak-Rothstein. 1994. An isotype switched and somatically mutated rheumatoid factor clone isolated from a MRL-*lpr/lpr* mouse exhibits limited intraclonal affinity maturation. *J. Immunol. 152:4489.*
71. Mandik-Nayak, L., S. J. Seo, C. Sokol, K. M. Potts, A. Bui, and J. Erikson. 1999. MRL-*lpr/lpr* mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Exp. Med. 189:1799.*
72. Kench, J. A., D. M. Russell, and D. Nemazee. 1998. Efficient peripheral clonal elimination of B lymphocytes in MRL/*lpr* mice bearing autoantibody transgenes. *J. Exp. Med. 188:909.*
73. Rubio, C. F., J. Kench, D. M. Russell, R. Yawger, and D. Nemazee. 1996. Analysis of central B cell tolerance in autoimmune-prone MRL/*lpr* mice bearing autoantibody transgenes. *J. Immunol. 157:65.*