Mutant Mouse Lysozyme Carrying a Minimal T Cell Epitope of Hen Egg Lysozyme Evokes High Autoantibody Response

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Mutant Mouse Lysozyme Carrying a Minimal T Cell Epitope of Hen Egg Lysozyme Evokes High Autoantibody Response

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Self proteins including foreign T cell epitope induce autoantibodies. We evaluated the relationship between the size of foreign Ag introduced into self protein and the magnitude of autoantibody production. Mouse lysozyme (ML) was used as a model self protein, and we prepared three different ML derivatives carrying a T cell epitope of hen egg white lysozyme (HEL) 107–116, i.e., heterodimer of ML and HEL (ML-HEL), chimeric lysozyme that has residue 1–82 of ML and residue 83–130 of HEL in its sequence (chiMH), and mutant ML that has triple mutations rendering the most potent T cell epitope of HEL (sequence 107–116). Immunization of BALB/c mice with these three ML derivatives induced anti-ML autoantibody responses, whereas native ML induced no detectable response. In particular, mutML generated a 104 times higher autoantibody titer than did ML-HEL. Anti-HEL107–116 T cell-priming activities were almost similar among the ML derivatives. The heterodimerization of mutant ML and HEL led to significant reduction of the autoantibody response, whereas the mixture did not. These results show that size of the nonself region in modified self Ag has an important role in determining the magnitude of the autoantibody response, and that decrease in the foreign region in a modified self protein may cause high-titered autoantibody response. The Journal of Immunology, 2000, 165: 3606–3611.

B cells capture and internalize foreign Ags via specific surface Ig receptors, degrade Ags at endosomes and/or lysosomes with processing enzymes, and present antigenic peptide/MHC class II complexes to the cell surface (1). CD4+ T cells recognize these complexes through their Ag-specific TCRs and provide direct help to B cells followed by production of specific Abs (2, 3). T-dependent Ab response normally does not occur to self proteins, because self-reactive B and T cells are subjected to both elimination and functional silencing in their development (4–9). However, self-reactive B cells are continuously generated by somatic hypermutation of V region genes in secondary lymphoid organs after formation of the preimmune repertoire (10) and some functional self-reactive B cells are not rendered tolerant (11). The reason that these self-reactive B cells do not produce autoantibodies could be explained by the absence of T cell help resulted from induction of profound and sustained T cell tolerance (12, 13).

Autoimmune B cells are activated and produce autoantibodies if they are exposed to autoantigens along with foreign carriers, e.g., self proteins chemically coupled to foreign antigenic peptides or proteins (14–17), p53 tumor suppressor self protein coupled with SV40 large T Ag (18), ubiquitin inserted foreign T cell epitopes into its sequence (19, 20), and foreign proteins that resemble self proteins chemically coupled to foreign antigenic peptides or proteins (21, 22). Thus, collaboration of autoimune B cells with T cells specific for the foreign epitope is an essential mechanism triggering autoantibody response.

Suppression and enhancement of humoral immune responses to self Ags are important issues in medical practice, i.e., neutralizing Abs induced by some therapeutic protein drugs reduce drug potencies (23–25), and protective Abs to toxic autoantigens (14, 15) and cytokines (26, 27) can regulate the malignant activities. Although many qualitative studies have been done on autoantibody responses in modified autoantigens, little is known of quantitative factors influencing the autoantibody response. To control autoantibody responses artificially, it is important to elucidate the factors responsible for regulating autoantibody responses.

Mouse lysozyme (ML)2 is secreted by macrophages, monocytes, and polymorphonuclear leukocytes, and is widely distributed in body fluids and tissues (28, 29). We selected ML as a model self Ag and introduced a foreign T cell epitope of hen egg white lysozyme (HEL) into ML, using three different methods, the objective being to evaluate the effect of size of HEL regions in modified ML on titers of serum autoantibodies. Peptide 107–116 of HEL has been defined as a minimal immunodominant T cell determinant in BALB/c mice and is presented by I-Ek MHC class II molecules (30). We prepared a dimer lysozyme by coupling one ML with one HEL with an alkyl-linkage (ML-HEL), a chimera lysozyme by replacing peptide of 83–130 of ML with peptide of 82–129 of HEL (chiMH), and a mutant ML by replacing Ala114 with asparagine, His115 with arginine, and Gin117 with lysine, procedures that result in introducing the same sequence as those of peptide 107–116 of HEL (mutML). The three derivatives retained the folded conformation and antigenic B cell determinants of ML. We found that mutML, which has a minimum HEL region, induced the highest autoantibody response, which is about 104-fold higher than that of ML-HEL; the order of size of the HEL region is 1) ML-HEL, 2) mutML, 3) ML.

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Materials and Methods

Cloning, construction, and expression of ML

Total RNA was prepared from 3774.1 cell (31) (RCB0434; Riken Cell Bank, Tsukuba, Japan) by the guanidium isothiocyanate method, and then MML mRNA was reversely transcribed into cDNA, using the 3′-specific complementary primer. The cDNA fragment encoding ML was amplified by PCR (Expand High Fidelity PCR system; Boehringer Mannheim, Mannheim, Germany) and introduced into vector pKPI700 (32). Recombinant ML was expressed with yeast Saccharomyces cerevisiae AH22 (32) or Pichia pastoris GS115 (Invitrogen, San Diego, CA) (33), as described previously.

Construction and expression of chiMH

Site-directed mutagenesis of ML cDNA was done using the M13 phage vector pKPI700 and methods of Kunkel et al. (34). To construct the chiMH gene, the nucleotide sequence 244AGTGGCTCT 252 of ML was mutated to AGCGCGCCTG, yielding BssHII site (GCCGCCG). The BssHII site was also introduced into HEL cDNA as well as to ML cDNA. By recombination at the BssHII site of the mutated ML cDNA and HEL cDNA, the chiMH gene was constructed. chiMH was expressed with yeast S. cerevisiae AH22, as described above.

Construction and expression of mutML

Site-directed mutagenesis of ML cDNA was done as described above. The codon changes in positions 114 (GCA to AAC), 115 (CAC to AGA), and 117 (CAA to AAG) were introduced using one oligonucleotide. mutML codon changes in positions 114 (GCA to AAC), 115 (CAC to AGA), and 117 (CAA to AAG) were introduced using one oligonucleotide. mutML was expressed with yeast P. pastoris, as described above.

Purification of ML derivatives

Yeast culture supernatants were applied to a column (4 × 15 cm) of CM-Toyopearl 650 M, which was eluted with a gradient of 500 ml of 50 mM NaCl acetate buffer (pH 5) and 500 ml of the same buffer containing 0.5 M NaCl at 4°C. In the case of yeast P. pastoris, culture supernatants were dialyzed at 1/10 with distilled water and then applied to the column. The protein fraction was exhaustively dialyzed against distilled water and then lyophilized. ML, chiMH, and mutML were obtained at about 10, 0.3, and 10 mg, respectively, per one lysozyme were prepared using cation-exchange chromatography with a flow rate of 1 ml/min.

Preparation of ML-HEL heterodimer

One hundred milligrams of HEL (kindly donated by QP, Tokyo, Japan) were dissolved in 10 ml of PBS and stirred for 1 h at room temperature with 2.2 mg of succinimidyl 3′-(2-pyridyldithio) propionate (SPDP; Wako Chemical, Osaka, Japan) and dissolved in 50 ml of DMSO. Similarly, 80 mg of ML or mutML was reacted with 2.8 mg of N-(8-maleimidocaproylxy) succinimide (HMCS; Dojindo Laboratories, Kumamoto, Japan). SPDP-HEL and HMCS-ML, which were introduced one SPDP and HMCS, respectively, per one lysozyme were prepared using cation-exchange chromatography with the gradient of 50 mM acetate buffer (pH 5) and the same buffer containing 0.5 M NaCl. The numbers of SPDP and HMCS in the conjugates were determined by mass spectrometry (MALDI-TOF/TOF Voyager; PerSeptive Biosystems, Framingham, MA). To liberate the free thiol group from SPDP-HEL, 2 mg of SPDP-HEL was dissolved in 500 µl of PBS containing 10 mM EDTA, followed by reduction for 30 min at room temperature with a 10 molar excess of DTT. The mixture was subjected to gel filtration using a BioGel P-4 column (1.5 × 30 cm; Bio-Rad, Richmond, CA) to separate activated SPDP-HEL from released thiopyridine and DTT. Two milligrams of HMCS-ML were added to the solution of activated SPDP-HEL, followed by overnight stirring at room temperature. Formed heterodimers, ML-HEL, were purified on a cation-exchange HPLC column of CM-Toyopearl 650 S (250 × 4.6 mm) with the gradient of 50 mM acetate buffer (pH 5) containing 0.15 M NaCl and the same buffer containing 0.7 M NaCl. The purity and the dimer formation were checked by SDS-PAGE.

Preparation of peptides

To prepare peptides of ML derivatives and HEL, disulfide bonds were reduced with 2-ME and S-alkylated with N-(3-bromopropyl)-N,N,N′,N′-pentamethyl-1,3-propanediol(aminonimide bromide), as described (35). Two milligrams of N-(3-bromopropyl)-N,N,N′,N′-pentamethyl-1,3-propanediol (aminonimide bromide) lysozymes were dissolved in 25 mM Tris-HCl (pH 9) containing 4 M urea, and 2 µg of l-lysyl endopeptidase (EC 3.4.21.50; Wako, Osaka, Japan) was added to the solution. After 18-h incubation at 30°C, the resultant peptides were separated by reverse-phase HPLC on a column of YMC-Pack ODS-AM (250 × 4.6 mm) at a flow rate of 1 ml/min with a gradient of acetonitrile containing 0.1% HCl ranging from 1 to 40% over the 100 min, as described (36). Concentration and amino acid composition of peptides were measured using a Hitachi 835 amino acid analyzer after hydrolysis in 6 M HCl, under vacuum at 110°C for 20 h.

ELISA

In inhibition experiments, ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 50 µl of lysozyme dissolved in 0.1 M carbonate buffer (pH 9.6) at the concentration of 2 µg/ml. Plates were washed three times with PBS containing 0.05% Tween-20 (PBST), and residual binding sites were blocked overnight at 4°C with 100 µl of 2% nonfat dry milk in PBST (blocking buffer). After washing with PBST, 50 µl of peptides or antibodies that were preincubated with serial dilutions of competitors were added to each well, followed by incubation for 1 h at room temperature. After washing with PBST, 50 µl of alkaline phosphatase-conjugated goat anti-rabbit or mouse IgG (Zymed, San Francisco, CA) diluted 1/1000 in blocking buffer was added to each well and followed by incubation for 1 h. After washing with PBST, the final reaction was visualized by incubation with p-nitrophenyl phosphate (Wako) in 0.1 M carbonate buffer (pH 9.6) containing 1 mM MgCl₂. The absorbance was measured at 405 nm.

For detection of IgG Abs in mouse sera, plates were coated overnight at 4°C with 50 µl of proteins or peptides at 2 µg/ml. Residual binding sites were blocked, and 1/1000 dilution of serum in blocking buffer was added to the coated wells. After 1 h of incubation and washing with PBST, 50 µl of alkaline phosphatase-conjugated goat anti-mouse IgG (Zymed, San Francisco, CA) diluted 1/1000 in blocking buffer was added to each well, followed by incubation for 1 h at room temperature. A standard curve was generated using affinity-purified rabbit anti-ML and anti-HEL IgG, and data were expressed as mean + SE (µg/ml).

Immunization and bleeding

BALB/c of either sex mice 8–12 wk old were obtained from the center of Biomedical Research, Kyushu University (Fukuoka, Japan). Group of five mice were immunized by s.c. injection of 3.5 µg of HEL derivatives with CFA emulsion (Sigma, St. Louis, MO). Each mouse was bled on days 0, 9, 21, and 28 from orbital sinus into capillary tubes. Sera were isolated by centrifugation and stored at −20°C until use.

T cell proliferation assay

Cultures for lymph node T cell proliferation assay were set up, as described (37). Briefly, groups of two BALB/c mice were immunized s.c. into both hind foot pads with 0.35 nmol of ML derivatives emulsified in CFA. Nine days later, these mice were killed and popliteal lymph nodes were removed. Pooled lymph node cells were washed with HBSS and suspended in serum-free HL-1 medium (Hicor, Irvine, CA) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM L-glutamine, and 50 µM 2-ME, and 4 × 10^5 cells in 200 µl of medium were dispensed into wells of 96-well cell culture plates (Nunc). HEL or HEL 98–116, in 40 µl of saline were added to triplicate culture wells, and plates were incubated in a humidified atmosphere of 5% CO₂ in air and at 37°C for 4 days. The number of live cells was evaluated, using MTT (Sigma), as described (37). Data are presented as the mean MTT formazan formation of triplicate cultures with background values (medium alone) subtracted (ΔΔA570).

Results

Preparation and characterization of ML derivatives

Fig. 1 shows the three ML derivatives with HEL sequences that we prepared. These three derivatives contain the same amino acid sequence of HEL 107–116, which is a dominant T cell epitope of HEL in H-2d mice and is presented by I-Ed molecules. Using rabbit
anti-ML and anti-HEL polyclonal IgG, antigenicity of B cell epitopes in the derivatives was evaluated. ML-HEL inhibited both anti-ML and anti-HEL Ab responses, with the same potency as the respective monomer lysozymes (Fig. 2). Thus, ML-HEL retains almost intact B cell epitopes of both ML and HEL. chiMH was recognized by both Abs; however, the activities were about 1/500 of the native ML and HEL. Positions of 114, 115, 117 are replaced with the corresponding HEL residue, and ML that has HEL107–116 sequence were constructed (mutML).

Autoantibody-inducing capacity of ML derivatives

Groups of five BALB/c mice were immunized with ML, ML-HEL, chiMH, or mutML and bled on days 0, 9, 21, and 28. Reactivities of these antisera to ML and HEL were analyzed, using ELISA. Mice immunized with native ML did not develop anti-ML responses, while mice immunized with three ML derivatives evoked autoantibody responses (Fig. 3A). Anti-ML IgG titers differed for the three ML derivatives; mutML and chiMH elicited 10^4 and 2 x 10^2 times, respectively, higher autoantibody responses than ML-HEL on day 28. When mice were immunized with an equimolar mixture of ML and HEL, anti-ML IgG was not detected. We also confirmed that anti-ML IgG responses could be detected by conjugating ML either with OVA or pigeon cytochrome c (data not shown). mutML did not elicit autoantibodies in C57BL/6 (H-2b) and C3H/HeN (H-2k) mice in which HEL107–116 region is not an immunodominant determinant for T cells (38, 39) (data not shown). Thus, carrier regions derived from HEL in the derivatives are critical role for breaking B cell tolerance. In contrast to the anti-ML IgG response, mutML induced the lowest anti-HEL IgG response; mutML and chiMH elicited 2 x 10^2 and 60 times, respectively, a lower anti-HEL IgG response than ML-HEL on day 28 (Fig. 3B). Hence, increase in the HEL region in ML derivatives (Fig. 1) decreased the anti-ML Ab response. These results suggest that size of the foreign region in a self-nonself conjugate determines titer of the autoantibodies.

Ag specificity of autoantibodies

As sequence identity between ML and HEL is 57% (40), cross-reactive autoantibodies might be generated. To evaluate the Ag specificity of autoantibodies induced by ML derivatives, ELISA inhibition assay was done. The autoantibody response was inhibited by ML, whereas HEL was without effect under our experimental conditions (Fig. 4A). As autoantibodies induced by ML derivatives did not cross-react with HEL, the original antigenic determinants of ML were probably the target for autoantibodies.

To determine precise regions recognized by autoantibodies, ELISA plates were coated with peptide fragments of ML and epitope mapping was performed. The highest response was observed for ML146–69 (Fig. 4B), which is the original sequence of the autoantibody.
ML and apart from the mutated region. Therefore, original deter-
minants in ML are major epitopes of autoantibodies and cross-
reactivity between ML and HEL is not the main factor in the au-
tonibody response.

Induction of HEL-specific T cells in mice immunized with ML
derivatives

The different autoantibody titers in mice primed with ML deriva-
tives might relate to T cell help that is mainly derived from T cells
specific for HEL107–116. Similar and adequate presentation of
HEL107–116 was confirmed in each ML derivative in vitro, using
HEL107–116-specific T cell hybridoma and the B cell lymphoma
A20 (data not shown). To evaluate the T cell response magnitude
induced by ML derivatives, lymph node cells from BALB/c mice
immunized with ML derivatives were cultured and stimulated in
vitro with native HEL or HEL98–116. When mice were immunized
with ML-HEL, chiMH, or mutML, similar HEL-specific T cell
proliferative responses were observed for the three Ags, while ML
did not elicit any substantial anti-HEL T cell response (Fig. 5,
A and B). Control T cell proliferative responses to purified protein
derivative were fairly equivalent for these four Ags (data not
shown). These results suggest that the three ML derivatives pos-
sess a similar capacity to induce HEL-specific T cells that provide
help to autoreactive anti-ML B cells. Thus, the hierarchical auto-
antibody titer in ML derivatives, as illustrated in Fig. 3A, might not
be due to the quantitative difference in anti-HEL Th responses.

Diminished autoantibody response of mutML by conjugating
with HEL

Anti-ML autoantibody titer of ML derivatives is inversely corre-
lated with increasing amounts of HEL (Fig. 3A). If the HEL region
plays a critical role in diminishing autoantibody response, conjuga-
tion of mutML with HEL may reduce the highest autoantibody-
inducing capacity. We prepared a dimer of mutML and HEL
(mutML-HEL) and the autoantibody response was evaluated.
Immunication of mutML together with HEL did not suppress the
autoantibody response of mutML, whereas the autoantibody titer
of mutML–HEL was about 10^3 lower than of mutML (Fig. 6).
Therefore, the higher autoantibody response of mutML may not be

FIGURE 4. Specificity of anti-ML IgG induced by ML derivatives. A, Recognition in competitive ELISA of anti-native ML IgG (filled symbol)
and HEL IgG (open symbol) derived from mice primed with ML deriva-
tives. Serially diluted soluble ML and HEL were added to ELISA plates
precoated with native ML in the presence of pooled mice sera obtained on
day 28 after immunization with ML-HEL (circle), chiMH (triangle), and
mutML (square). Sera were used at a dilution of 1/100 for ML-HEL,
1/10,000 for chiMH, and 1/200,000 for mutML. B, Epitope mapping of
anti-ML autoantibodies. Sera were used at a dilution of 1/100 for each ML
derivative in this experiment. Data are expressed as mean values and SEs.

FIGURE 5. T cell-dependent proliferative response of lymph node cells
from mice immunized with ML derivatives. BALB/c mice were immu-
nized s.c. into both hind footpads with 5 μg/head of ML and ML deriva-
tives emulsified 1:1 in CFA, and 10 days later, draining lymph node cells
were cultured with serial dilutions of HEL (A) or HEL98–116 peptide (B).
After 4 days of incubation, the number of live cells was evaluated, using
MTT assay. Data are present as mean MTT formazan formation of tripli-
cate cultures ± SD with background values (medium alone) subtracted
(ΔA570).

FIGURE 6. Diminished autoantibody response of mutML by conjugat-
ing with HEL. Groups of five BALB/c mice were immunized with 0.35
nmol of mutML, mixture of mutML and HEL, or mutML-HEL het-
erodimer emulsified 1:1 in CFA on day 0. Each mouse was bled on day 28,
and anti-ML and anti-HEL IgG responses were measured using ELISA. A
standard curve was generated, using affinity-purified rabbit anti-ML and
anti-HEL IgG. Data are expressed as mean values and SEs.
the result of a strong effective Th induction capacity, but rather may be related to the minimal size of the HEL region.

Discussion
To prepare the heterodimer of ML and HEL, we selected SPDP and HMCS as heterobifunctional cross-linking reagents to form a stable linkage without affecting secondary and tertiary structures (41). Indeed, ML-HEL retained the individual B cell epitopes of both ML and HEL and induced anti-ML IgG responses at the level of 0.1 μg/ml (Fig. 3), whereas the mixtures of ML and HEL were without effect in autoantibody responses. Hence, covalent binding between self proteins and foreign proteins containing immunogenic T cell epitopes is critical to induce autoantibody responses.

To introduce an immunodominant T cell epitope of HEL (HEL107-116) into ML, the ML gene corresponding to amino acid residue 83–130 is replaced by exons of the HEL gene corresponding to amino acid residue HEL82–129. The chimera lysozyme (chiMH) had reduced yet substantial enzymatic activity (data not shown) and antigenicities (Fig. 2), suggesting that chiMH retains a native-like conformation of lysozyme. Interestingly, autoantibody-inducing capacity of chiMH was 10^2–10^3 times higher than ML-HEL (Fig. 3). Dalum et al. reported that introduction of an immunogenic peptide OVA325–336 into ubiquitin, named UbiOVA, resulted in strong autoantibody responses toward native ubiquitin (19). They compared the autoantibody responses between UbiOVA and ubiquitin-OVA conjugate and found that the antiguinin titer of UbiOVA was approximately 20 times higher than that of the conjugate (42). Their results are consistent with our finding, and a higher autoantibody response may be induced by a modified self protein, which is by packing an immunogenic T cell epitope rather than by linking a whole protein. The immunogenic sequence of HEL82–129 in chiMH is longer than the dominant core sequence of HEL107-116. We mutated amino acids of ML at Ala114, His115, and Gln117 into asparagine, arginine, and lysine, respectively, and the core immunodominant T cell epitope of HEL107-116 is introduced into ML (Fig. 1). The mutML retained a structure similar to that of the native ML (Fig. 2). The autoantibody titer of mutML was 10^4 times higher than ML-HEL and 50 times higher than chiMH (Fig. 3).

Why mutML shows such the highest autoantibody response? Schutz et al. suggested that competition between clonally expanded carrier-specific B cells and hapten-specific B cells is the reason for the depressed antihapten response (43). In our study, HEL-specific B cells (anticarrier) may compete with ML-specific B cells (antihapten) on Ag recognition and may depress autoantibody response, because the anti-ML IgG response inversely correlated with the anti-HEL IgG response (Fig. 3) and the conjugation of mutML with HEL attenuated anti-ML Ab responses and enhanced anti-HEL Ab responses (Fig. 6). Therefore, the low antigenicity of the HEL region for B cells is a critical factor for inducing the high autoantibody response. Since some autoreactive anti-ML B cells may be functionally inactivated by continuous exposure to soluble ML, the frequency to differentiate into Ab-secreting cells should be less than that of anti-HEL B cells. Once anti-HEL B cells expand, ML-HEL and mutML-HEL may be recognized and internalized mainly by HEL-specific B cells, the result being reduction of anti-ML IgG titer. mutML may have minimal B cell epitopes for HEL-specific B cells, and thus ML-specific B cells should be effectively activated without competing with HEL-specific B cells.

Goodnow et al. demonstrated that autoreactive B cells are rendered functional silencing (anergy) in double transgenic mice (6) and that these B cells do not produce anti-HEL Ab in the presence of T cell help if the cells continuously bind to HEL (6, 44). On the other hand, we demonstrated that the ML-specific B cells were activated by HEL-specific T cells and produced autoantibodies. The inconsistency may be explained by the distinct affinity of Ig receptors for self Ag, i.e., polyclonal ML-specific B cells in normal mice have various affinity for ML, while anergic HEL-specific B cells in double transgenic mice have high affinity for HEL (Kd = 2 × 10^4 M^-1) (6). B cells that bind self Ag with low affinity tend to escape tolerance (45).

In our experimental system, T cell help is provided from HEL-specific T cells. Th responses are the important factors for an Ab response. When we evaluated HEL-specific T cell responses in mice immunized with three ML derivatives, no differences were evident (Fig. 5). We also examined the possibility that new T cell epitopes might be generated by mutation in mutML. However, T cell proliferative responses of mutML-primed mice were only toward the region HEL107-116 and no response to any other peptide region of ML was detected (data not shown). Therefore, the high autoantibody titer of mutML is probably not due to the strong Th-inducing capacity.

Human recombinant proteins have been used in medical practice, e.g., erythropoietin, IFN, G-CSF. Amino acid substitution is a potent strategy to improve enzymatic activity and protein stability (46–49). In this respect, mutant proteins have the potential to function as potent drugs, for example tissue-type plasminogen activator (50). We found that a mutant self protein (mutML) induced a high-titered autoantibody response in BALB/c (H-2d) mice (Fig. 3), but not in C57BL/6 (H-2b) and C3H/HeN (H-2k) mice. This means that mutant self proteins have the potential to evoke vigorous autoantibody responses in particular individuals in whom HLA molecules present mutated peptide regions to Th cells specific for the regions. Thus, mutation of therapeutic proteins may induce neutralizing Abs and/or a cause of anaphylactic responses.

Mutant proteins may be used for production of neutralizing Abs against endogenous cytokines that show undesirable effects. Neutralizing autoantibodies are tested for their availability to inhibit cytokine-mediated diseases (26, 27). The mutant protein may serve as useful and effective inducer of neutral autoantibody to the internal malignant molecules.

In conclusion, we demonstrated that self proteins carrying a minimal foreign T cell epitope can activate autoreactive B cells, when the mutated region strongly binds to MHC molecules and is presented to T cells. Care must be taken to prepare safe therapeutic mutant self proteins.

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