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Kamal D. Moudgil,1,2 Jay Wang, Valentine P. Yeung, and Eli E. Sercarz2

Hybrid F1 mice derived from inbred parental mouse strains are extensively used as animal models of human autoimmune diseases and transplantation. It is generally believed that with regard to immunologic studies, hybrid F1 mice behave in a consistent manner, equivalent to any other inbred mouse strain. In this study, we report that in comparison to inbred parental strains, individual hybrid F1 mice revealed a broad heterogeneity of proliferative response to the immunodominant determinants within hen eggwhite lysozyme (HEL). Of five parental strains tested, individual mice of three strains responding to only a few dominant HEL determinants (B6, BALB/c, and B10.PL) showed quite homogeneous patterns of response, whereas two mouse strains responsive to several determinants of HEL revealed either relative homogeneity (CBA/J mice) or heterogeneity (SJL mice) of response. However, in SJL mice, responses to major, dominant determinants of HEL were quite consistent. On the contrary, regardless of the consistency of response of parental strains, all three of F1 mice ([B6 × BALB/c]F1, [B6 × CBA/J]F1, and [SJL × B10. PL]F1) revealed significantly greater heterogeneity of response, which even involved the major, dominant determinants of HEL. We attribute the above heterogeneity of response to the competitive as well as aleatory nature of the interaction between various factors, including the coexistence of different MHC (parental as well as hybrid MHC) molecules, determinant capture, and the T cell repertoire. These results have important implications for studies on autoimmunity, infection, and vaccine design in human populations, where heterozygosity is the norm rather than the exception. The Journal of Immunology, 1998, 161: 6046–6053.

The dominant T cell response to a region within a protein Ag of a particular individual is dependent on several factors, among which are a) the availability of that determinant following processing of the native Ag by the APC (1–3); b) the ability of the potential determinant to bind to the appropriate MHC molecule at a reasonable affinity (4, 5); c) the presence of the requisite T cells bearing the appropriate TCR in the mature T cell repertoire of the host (3, 4, 6, 7); d) the efficient interaction between the member components of the trimolecular (TCR-peptide-MHC) complex (7–10); and e) the presence or absence of regulatory T cells that can effectively down-modulate the T cell response at the initial phase of induction (11).

Of these, a major hurdle for a particular determinant to be presented efficiently to the TCR is the competition among different regions within the native Ag for binding to ambient class II MHC molecules (1, 2, 12, 13), and among different MHC molecules for binding to the “prodeterminant” (1) bearing many determinants. Accordingly, the interplay of various factors outlined above and the final outcome of the immune response to determinants of an Ag is expected to be much more complex in a hybrid F1 individual carrying each of the parental MHC molecules as well as hybrid MHC molecules (14, 15) in comparison to the inbred parental strains. Nevertheless, it is generally believed that with regard to the immune response to a foreign/self Ag, F1 mice derived from established inbred parental mouse strains behave in a consistent, reproducible fashion, like a new inbred strain. Hybrid F1 mice have extensively been used in immunologic studies in animal models of human autoimmune diseases [e.g., systemic lupus erythematosus (SLE), experimental autoimmunencephalomyelitis (EAE), experimental autoimmune uveoretinitis (EAU), experimental autoimmune pinealitis (EAP), etc. (16, 17)] and in tissue/organ transplantation (18).

In this report, we describe the patterns of immunodominance of determinants within hen eggwhite lysozyme (HEL)3 in five well-defined, inbred parental strains and three different hybrid F1 strains derived from them. We observed that in comparison to parental strains, individual members of F1 strains ([B6 × BALB/c]F1, [B6 × CBA/J]F1, and [SJL × B10. PL]F1) responded to HEL in a significantly heterogeneous manner, which involved changes in responsiveness to the major, dominant determinants. We attribute this heterogeneity to a degree of chaotic behavior owing to the abundance of different MHC molecules and background gene products in the F1 animal. This study provides a novel, antigenic determinant-specific perspective on the heterogeneity of individual F1 mice. These results have important implications for human immunology.

1 This work was supported by grants from the National Institutes of Health (CA-24442, AI-11183, and AR-3683406) and the American Cancer Society (IM-626).
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3 Abbreviation used in this paper: HEL, hen eggwhite lysozyme; LNC, lymph node cells; SI, stimulation index.
Materials and Methods

Mice

BALB/c (H-2b), B10.PL (H-2b), C57BL/6 (= B6) (H-2b), SJL (H-2d), and CBA/J (H-2k) mice were either purchased from The Jackson Laboratory (Bar Harbor, ME) or bred in our animal facility. F1 mice, namely (B6 × BALB/c)F1, (B6 × CBA/J)F1, and (SJL × B10.PL)F1, were bred from the above-mentioned parental strains. Mice of either sex, 6–20 wk of age, were used.

HEL, and peptide synthesis and purification

HEL was purchased from Sigma (St. Louis, MO) and further purified as described (13). HEL peptides were obtained from the following sources: a) Overlapping 15-mer HEL peptides were obtained from Chiron Mimotopes (San Diego, CA). These peptides were synthesized using the "multimip" peptide synthesis technique (19). The terminal amino group of each peptide was acetylated, whereas the carboxyl-terminal lysine-proline residues transformed into diketopiperazine. b) Some peptides were synthesized in our laboratory using the rapid simultaneous solid-phase multiple-peptide synthesis methodology, the so-called "teabag" method (20, 21). c) Some peptides were obtained from Macromolecular Resources (Colorado State University, Fort Collins, CO) (21), whereas others were synthesized in the UCL peptide laboratory using a Multiple Peptide Synthesizer (Advanced Chem Tech, Louisville, KY) (7). The identity and purity of these peptides were determined by fast atom bombardment mass spectrometry.

Lymph node proliferation assay

Mice were immunized with 7 nmol/mouse of HEL or a HEL peptide in PBS, in 1:1 (v/v) emulsion with CFA (Difco Laboratories, Detroit, MI), in a hind foot pad. After 9 days, the draining lymph nodes were removed, cell suspensions washed twice with HBSS (Life Technologies, Grand Island, NY), and then cultured in a flat-bottom 96-well plate at a concentration of 5 × 10^5 cells/well in X-Vivo-10 serum-free medium (BioWhittaker, Walkersville, MD) using different concentrations (1.66–20 μM or higher, final concentration) of the Ag. Tuberculin purified protein derivative (Parke-Davis, Morris Plains, NJ) was used at a final concentration of 4 μg/well as a positive control. The cells were incubated with 1 μCi/well of [3H]thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 h of a 5-day culture. The cells were then harvested using a Micro Cell Harvester (Skatron Instruments, Sterling, VA), and the incorporation of radioactivity was assayed by liquid scintillation counting using the LKB 1205 Betaplate counter (LKB-Wallac, Gaithersburg, MD). For some repeat experiments, X-Vivo-10 medium supplemented with 5 × 10^-5 M 2-ME was used. The results of these experiments were comparable to those of earlier, similar experiments in which X-Vivo-10 medium without 2-ME supplement was used. The results were expressed as mean cpm of duplicate or triplicate cultures. For presentation of data, background values of cpm [cpm of lymph node cells (LNC) cultured in medium without Ag] were subtracted from the cpm obtained with LNC plus Ag (A cpm). Alternatively, the value of cpm with Ag was divided by the cpm with medium alone to obtain a stimulation index (SI), Ag doses of 10–20 μM covered the optimum concentration for proliferative response of all the HEL peptides tested. The final results from a group of animals immunized with the same peptide were expressed either as mean cpm (or A cpm) ± SD or mean SI ± SD. Based on the value of average SI obtained with the optimal concentration of the peptide, the response to each peptide was arbitrarily graded as follows (21): SI of <3; 3–3.9; 4–4.9; 5–10; 10–24.9; 25–49.9, 3+; and 50–100, 4+.

Grading of pattern responses to HEL of individual parental/hybrid F1 mice

The patterns of response to HEL of individual members of parental strains were analyzed, and mice with similar patterns of response were grouped together. For comparison with F1 mice, an average pattern of response of all members of a particular parental strain was derived from the average response (average SI) to each peptide of HEL. Classification of patterns of response to HEL of F1 mice into different groups was done as follows: A, preference toward one parent; B, preference toward the other parent; A* (or B*), preference toward one parent but loss of one or more determinants (e.g., A*, A-3, A*3); A+ (or B+), preference toward one parent along with gain of one or more new determinants (e.g., A+1, A+2); A*+ (or B*+), a basic pattern like parent A but with loss of response to one or more determinant while concurrently gaining a response to one or more new determinants (e.g., A*+1, B+1); AB, response to determinants of both parents, the response profile varying from a full spectrum comprised of all determinants responded to by the two parental strains to a partial spectrum represented by only certain parent determinants. For example, group A*+1B*+ represents a pattern of response consisting of determinants responded to by the two parents, but in comparison to parent A, there is a loss of two determinants but a gain of a new determinant, whereas in comparison to parent B, there is a gain of a new determinant; C, loss of response to all determinants of both parents, but a positive response to native HEL and purified protein derivative. To determine the representative "whole group pattern," the mean SI of all F1 mice in that group for each of the HEL peptides tested was calculated separately, and the average response profile was assigned the appropriate category.

Statistical analysis

As a measure of homogeneity of a population with respect to a particular trait, we used H to represent the sum of squares of the population proportions. The value of H was computed from the observed number of population traits (i.e., number of different patterns of response to HEL of a given mouse strain), and the observed proportion of members of that population with a particular trait pattern (i.e., the proportion of mice of that strain giving an identical pattern of responses). H has values from zero to one (0 ≤ H ≤ 1), with a large value of H indicating a more homogeneous population, and a small value indicating a more heterogeneous population. To compare the homogeneity of response to HEL of parental vs F1 mouse strains, we employed a nonparametric, two-sample permutation (randomization) test based on hypergeometric distribution. We first determined the number of mice in each category that gave a similar pattern of response. This information was then used to determine whether parental strains differ significantly from the respective F1 strains in regard to consistency of the patterns of response. The statistical significance of the final result was assessed from the p value.

Results

To determine the consequence(s) of the coexistence of two different MHC haplotypes in the same animal, we studied T cell responses to the immunodominant determinants within HEL in three different strains of F1 mice and their parental strains. Mice were immunized with HEL/CFA in their hind foot pads and, after 9 days, the draining LNC were tested in a proliferation assay using HEL peptides. In each case, the pattern of response of F1 mice was compared with that of the respective parental strains.

Response to HEL of the parental mouse strains

The response of individual mice of five of the parental strains to HEL is shown in Tables I and II. Interestingly, the pattern of responses of parental strains that responded to only a few (one to three) HEL determinants (e.g., B6, BALB/c, and B10.PL) were quite consistent. On the other hand, mouse strains that responded to multiple determinants of HEL revealed either relative homogeneity (CBA/J) or heterogeneity (SJL) of response. Nevertheless, in the SJL strain, responses to major, dominant determinants of HEL were quite consistent. The above conclusions regarding the consistency of response patterns were supported by the results of statistical analyses (see Tables I and II).

Immunodominance of T cell determinants of HEL in hybrid F1 mice

The T cell responses of HEL/CFA-immunized (B6 × BALB/c)F1 are given in Table III. Strikingly, although B6 and BALB/c parents showed a high consistency of responses to HEL (Table I), individual (B6 × BALB/c)F1 mice revealed six different types of patterns (Table III). Interestingly, despite this heterogeneity, a majority (20 of 24; 83%) of F1 mice responded to determinant 106–116, which represents the sole immunodominant determinant in BALB/c mice (“determinant-specific” bias). In 14 of 24 (58%) F1 mice, there was a loss of response to all the B6-specific determinants, 20–35, 30–53, and 74–96 (pattern B), whereas 6 of 24 (25%) of F1 mice had patterns (e.g., A*+1B*+1, and A*-2*B*2) including certain determinants from both parents. The overall pattern of the average response of the whole group of F1 mice (pattern
Statistical analysis of the data revealed that the value of H [actual value (values corresponding to 6)] was 0.735 (0.613-0.858) for B10.PL, and 0.175 (0.157-0.193) for SJL. The difference in the consistency of the pattern of responses of mice was found to be similar to that of BALC/c mice (“haplotype-specific” bias).

The response to HEL peptides of individual (B6 × CBA/J)F1 mice immunized with native HEL could be categorized into seven distinct groups (Table IV). Surprisingly, an entirely opposite pattern of response was observed in these F1 mice regarding two determinants of HEL, 20–35 and 30–53, which are responded to least three determinants of HEL that represent the immunodominant-specific bias”, while on the other hand, responses to at least three determinants of HEL that represent the immunodominant determinants for the SJL strain (namely 20–35, 74–96, and 116–129) were significantly decreased or almost lost in (SJL × B10.PL)F1 mice.[21]

In the case of (SJL × B10.PL)F1 mice, the pattern of response to HEL of individual F1 mice could be grouped into 11 different patterns (Table V). Nevertheless, on one hand, 32 of 35 (91%) F1 mice responded to HEL p30–53 (a B10.PL determinant) (“determinant-specific bias”), while on the other hand, responses to at least three determinants of HEL that represent the immunodominant determinants for the SJL strain (namely 20–35, 74–96, and 116–129) were significantly decreased or almost lost in (SJL × B10.PL)F1 mice!

The difference in the consistency of the pattern of responses of each of the above three F1 strains of mice compared with that of their respective parental strains was found to be statistically significant (see Tables III-V). Moreover, the observed heterogeneity of individual F1 mice was not attributable to their age, sex, or housing conditions (data not shown).

**Hybrid F1 mice fail to respond to certain HEL determinants despite possessing the appropriate T cell repertoire**

We have described above that in each of the three strains of F1 mice studied, many individual mice immunized with native HEL did not raise a proliferative response to certain dominant HEL determinants to which one or both parents responded strongly (Tables III-V). To determine whether these F1 mice possessed the requisite T cell repertoire potentially directed against a particular HEL determinant (but yet could not raise a response to that determinant after challenge with native HEL), we immunized F1 mice with a HEL peptide (instead of native HEL), and then tested the draining LNC in a proliferation assay using the same peptide for a recall response. Another batch of F1 mice was immunized with native HEL and then tested for recall response to HEL peptide. The results are given in Table VI. Strikingly, mice of each of three F1 strains tested raised a vigorous recall response to the HEL peptide tested following peptide immunization. On the contrary, each of these F1 strains failed to give a recall response to the same HEL peptide following immunization with native HEL. These results demonstrate that the failure of some F1 mice to respond to certain determinants of HEL was not owing to particular holes in the T cell repertoire; instead, it was attributable to failure of processing and/or presentation of that determinant from native HEL by F1 APC. Interestingly, in some but not other individuals of (B6 × BALB/c)F1 and (B6 × CBA/J)F1 mice immunized with HEL peptide 30–53, response to this peptide could also be recalled with native HEL. These mice represent those F1 members that had the potential to respond to this determinant upon challenge with native HEL.

**Loss of response to a determinant of HEL in an F1 strain is associated with MHC and not non-MHC genes**

To further define the mechanism of loss of response to HEL determinant 30–53 in a majority of (B6 × CBA/J)F1 mice, we tested

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### Table I. T cell responses to HEL of B6, BALB/c, and CBA/J inbred parental mice

<table>
<thead>
<tr>
<th>HEL Peptide/HEL</th>
<th>B6</th>
<th>BALB/c</th>
<th>CBA/J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>11–25</td>
<td>20</td>
<td>0</td>
<td>1–18</td>
</tr>
<tr>
<td>20–35</td>
<td>++ b</td>
<td>13</td>
<td>81.2</td>
</tr>
<tr>
<td>30–53</td>
<td>++ b</td>
<td>14</td>
<td>87.5</td>
</tr>
<tr>
<td>57–78</td>
<td>0</td>
<td>46–61</td>
<td>++ ++ ++</td>
</tr>
<tr>
<td>74–96</td>
<td>++ b</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>106–116</td>
<td>++ b</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HEL</td>
<td>++ ++ ++</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>

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### Table II. T cell responses to HEL of B10.PL and SJL inbred parental mice

<table>
<thead>
<tr>
<th>HEL Peptide/HEL</th>
<th>B10.PL</th>
<th>SJL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>8–19</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>21–35</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>30–53</td>
<td>++ b</td>
<td>20 (100)</td>
</tr>
<tr>
<td>46–61</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>74–96</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>97–110</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>116–129</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>HEL</td>
<td>++ b</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

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*a* The value of H [actual value (values corresponding to ± 2 SD)] was 0.735 (0.613-0.858) for B10.PL, and 0.175 (0.157-0.193) for SJL.

*b* Some mice in this group raised a response measuring from 1+ to 3+.
the response to HEL peptide 30–53 in [B10.A(4R) × CBA/J]F1 mice immunized with HEL. We reasoned that the [B6 × CBA/J]F1 and [B10.A(4R) × CBA/J]F1 mice have nearly identical non-MHC background genes but differ in their MHC composition: [H-2b (A, E)] and [H-2h (A, E)]. Thus, if responses to determinant 30–53 were lost readily in [B6 × CBA/J]F1 mice but not in [B10.A(4R) × CBA/J]F1 mice following challenge with native HEL, it could possibly be attributable to the presence of the A\* molecule (“determinant capture”) (13); during antigen processing, the unfolding HEL molecule is efficiently captured by the A\* molecule, preempting other MHC molecules from binding to this determinant.

Contrary to the above-mentioned loss of response to one or more of the HEL determinants in a subset of F1 mice, some groups of F1 mice challenged with native HEL responded to a new determinant to which neither parent strain responded. For example, a subset of [B6 × BALB/c]F1 mice responded to peptide 11–25 of HEL (Table III). To determine which of the parental strains was potentially capable of contributing the requisite T cell repertoire to F1 mice responding to a new determinant of HEL, we immunized B6 and BALB/c mice with peptide HEL 11–25. After 9 days, the draining LN were tested using the same peptide as well as native HEL. The results given in Fig. 2 show that BALB/c but not B6 mice responded to HEL peptide 11–25, suggesting that the BALB/c parent contributes to the responsiveness of [B6 × BALB/c]F1 mice to HEL p11–25.

**Discussion**

In this study, we have determined the influence of the coexistence of two different MHC haplotypes in inbred, hybrid F1 mice on the T cell response to determinants within a well-characterized Ag, HEL. Our results demonstrate that individual members of a particular F1 strain show a broad heterogeneity in their pattern of proliferative T cell response to determinants within HEL, much more pronounced than the heterogeneity in parental strains. We believe that this heterogeneity arises from the numerous unpredictable extra competitive events assured when additional MHC and non-MHC molecules are expressed in an individual. From 6 to 11 different patterns of response were observed in F1 mice, and,

### Table III.  
**T cell response to HEL of hybrid (B6 × BALB/c)F1 mice and their parental strains**

<table>
<thead>
<tr>
<th>HEL Peptide</th>
<th>B6 (A)</th>
<th>BALB/c (B)</th>
<th>Individual mice</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11–25</td>
<td>–</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>20–35</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>30–53</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>57–78</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>74–96</td>
<td>–</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>106–116</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HEL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The value of H [actual value (value corresponding to ± 2 SD)] was as follows: B6 = 0.679 (0.539-0.819); BALB/c = 1.000; and (B6 × BALB/c) F1 = 0.378 (0.278-0.478), respectively. Using the nonparametric test, the value of p for both B6 vs. (B6 × BALB/c)F1 and BALB/c vs. (B6 × BALB/c)F1 was statistically significant (p < 0.001).

### Table IV.  
**Proliferative response to determinants within HEL of (B6 × CBA/J)F1 hybrid mice and their parental strains**

<table>
<thead>
<tr>
<th>HEL Peptide</th>
<th>B6 (A)</th>
<th>CBA/J (B)</th>
<th>Individual mice</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–18</td>
<td>–</td>
<td>±</td>
<td>–</td>
</tr>
<tr>
<td>20–35</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30–53</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>46–61</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>74–96</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>116–129</td>
<td>–</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>HEL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The value of H [actual value (value corresponding to ± 2 SD)] was as follows: B6 = 0.679 (0.539-0.819); CBA/J = 0.445 (0.359-0.531); and (B6 × CBA/J)F1 = 0.247 (0.177-0.317), respectively. Using the nonparametric test, the value of p for both B6 vs. (B6 × CBA/J)F1 and CBA/J vs. (B6 × CBA/J)F1 was statistically significant (p < 0.001).
importantly, this heterogeneity involved major, dominant determinants. Individual mice in several F1 strains show a preference for responding to determinants that are unique to a particular parent (determinant-specific bias). Because the average response of the whole group of two of the three F1 strains did not strictly resemble either parent, we have termed the observed patterns to reflect a “determinant-specific” bias to discriminate it from a “haplotype-specific” bias in which the average response pattern of the whole group strictly resembles one of the parents as seen in the case of (B6 × BALB/c)F1 mice.

It is important to realize that an apparently homogeneous response (e.g., a similar pattern of T cell response to the native, whole autoantigen, autoantibody profile and isotype, organ-specific pathology, and clinical disease, etc.) can be observed in some autoimmune F1 situations, such as the (NZB × NZW)F1 model of lupus and peptide-induced EAE in (B10.PL × SJL)F1 mice. Our results suggest that the heterogeneity of response of individual F1 mice of the same strain would not have been evident were the readout based on the proliferative response to either the whole Ag or to only one of its major, dominant determinant(s), or upon induction of a particular disease by a peptide comprising one major, dominant determinant of an Ag. Under these conditions, it would appear, although superficially and incorrectly, that all mice of a particular F1 strain were producing an identical immunologic response. In this regard, using multiple dominant and subdominant determinants of HEL for analysis, our study has provided a novel as well as more accurate, determinant-specific perspective on the heterogeneity of individual F1 mice. Interestingly, the parental (haplotype-specific) bias observed in (B6 × BALB/c)F1 mice in this study has also been observed in studies on a) the T cell response to myelin basic protein in (SJL × B10.PL)F1 mice (22, 23), and b) cytotoxic T cell responses to H-Y Ag in diverse types of F1 mice (24, 25). The novel aspect of our study is the determinant-specific perspective of the heterogeneity of individual F1 mice.

There are at least five major factors that could contribute to the heterogeneity of T cell response to HEL of individual F1 mice as well as to their determinant-specific bias:

1) Alterations in the expression of a particular MHC molecule in F1 mice. It has been demonstrated that there is a direct relationship between the amount of Ia (MHC) Ag expressed on the APC and the magnitude of the T cell proliferative response to an Ag (26–29). Generally, a F1 mouse would be expected to express on its APC approximately half of the amount of a particular MHC class II molecule derived from one of its parents. Contrary to this expectation, there is experimental evidence to show that the expression of certain homozygous parental MHC molecules can be significantly altered in F1 mice (28–33). This decrease was attributable to either a significantly increased expression of hybrid MHC molecules of mixed haplotypes or to complex interactions between different MHC gene products.

2) In hybrid F1 mice, expression of “unique haplotype mixtures” can lead either to a gain or a loss of the T cell response to an antigenic determinant. It is now well established that in homozygous inbred strains of mice as well as in hybrid F1 mice, T cell responses to several Ags can be restricted by hybrid I-A or I-E MHC molecules of infraisotypic (e.g., Aαβ, Aββ) or mixed-isotypic (e.g., Aββ, Eββ) origin (14, 22, 33–39). Moreover, owing to the preferential pairing of certain chains (e.g., Eβα with Eaαβαβ), some haplotypes have the advantage of quantitatively higher expression compared with other haplotypes in the same animal. Interestingly, it has been suggested that specific lymphokines or combination of lymphokines could induce the expression of haplotype-mismatched MHC molecules on certain cell types (40, 41). This could be one of the reasons why certain autoimmune diseases...
are triggered by viral or bacterial infections, which would provide the required cytokine milieu during inflammation/infection (42, 43). We suggest that alterations in the level of hybrid MHC molecules on F1 APC can contribute to the variations in T cell response of these mice in three ways: a) binding to, and presentation of, new antigenic determinant(s); b) decreasing the expression of one of the homozygous parental MHC molecules (28–33); and c) participation in positive/negative selection of the T cell repertoire, which would have a direct effect on the expressed phenotype of the F1 mice. Most pertinently, the proportion of chimeric molecules which would have a direct effect on the expressed phenotype of the participation in positive/negative selection of the T cell repertoire, one of the homozygous parental MHC molecules (28–33); and c) of new antigenic determinant(s); b) decreasing the expression of response of these mice in three ways: a) binding to, and presentation of, new antigenic determinant(s); b) decreasing the expression of one of the homozygous parental MHC molecules (28–33); and c) participation in positive/negative selection of the T cell repertoire, which would have a direct effect on the expressed phenotype of the F1 mice. Most pertinently, the proportion of chimeric molecules might be very low (about 1%), and, nevertheless, the determinant so restricted may be dominant (39), and the level of the chimeric molecule may differ among individuals.

3) Determinant capture. During Ag processing, the unfolding HEL molecule is efficiently captured by a particular MHC class II molecule, preempting other MHC molecules from binding to the same determinant(s) or to other nearby determinants on the same molecule. When there are several MHC molecules, a set of competitions is set up among the different MHC competing for the available sites and, depending on the initially most available determinant and its affinity for the ambient MHC, a hierarchy of response will emerge. The increased expression of a hybrid MHC molecule might be an additional factor in this equation. Most importantly, determinant capture not only can result in display of a new dominant determinant, but regularly leads to abrogation of the response to certain other (cryptic) determinants. Of particular importance to the heterogeneity of response among individuals, as more class II molecules join into the competitive milieu and more exo- and endopeptidases become available, greater levels of unpredictability result. As a direct consequence of determinant capture, any individual differences in endopeptidase cleavage(s) will lead to new opportunities for competition among MHC molecules, with an unpredictable outcome.

4) Variations in the level of proteases within hybrid F1 APC. It is not known how heterogeneous the qualitative and/or quantitative expression of various proteases is within APC from inbred mouse strains of different MHC haplotypes or background genes or from hybrid F1 mice. Furthermore, differential requirements of certain proteases for generation of different determinants from a native Ag (44–46) or vulnerability of certain determinants to proteolytic destruction (47, 48) could contribute to heterogeneity of the response of F1 mice.
5) Alterations in the T cell repertoire in F1 mice. Actually, the thymic development of the repertoire is an aleatory process, even with a single class II MHC molecule. With the various points mentioned above, an even greater level of unpredictability can be expected. Furthermore, development of the T cell repertoire (49, 50) of a F1 individual will be significantly influenced by the spectrum of self-determinants displayed in the thymus, which in turn depends on the nature and relative proportion of self-peptides contributed by the two parents and on the quantity and quality (type) of MHC molecules displayed on thymic APC. The latter can lead to a) the loss of a subset of the T cell repertoire as a consequence either of tolerance induction through negative selection (6, 37, 51) or of deletion of a particular TCR Vβ specificity through minor lymphocyte stimulating (Mls)Ags (52, 53); b) the failure of positive selection (28, 29, 54); and c) a gain of diversity in the T cell repertoire (7, 12, 33, 37, 38, 54–56). In addition, environmental agents/Ags can have a significant effect on the T cell repertoire (21, 57).

We have discussed above five important components of the immune system that can contribute to the heterogeneity of response of F1 mice. It is important to realize that each of these components per se is influenced by common elements of competitiveness and the aleatory nature of the event. Because of the unpredictability of each of the five components mentioned above, and the obvious interdependence of most of these elements, e.g., of determinant capture on the position of endopeptidase attack, an even higher level of unpredictability characterizes responsiveness in the F1 animal. Therefore, given the scrambling of the type (haplotype) and level of MHC expressed on APC surface, and the varying constellation of TCR specificities and Ag-processing enzymes in different individuals, it is clearly most difficult to predict the pattern of response to a multideterminant Ag in individual F1 mice.

The results of our study have important implications in understanding the susceptibility or resistance of different members of genetically heterogeneous human populations, or even members of the same family, to a viral/bacterial infection or to a particular autoimmune disease. An efficient and appropriate response to a key determinant of an infectious agent might be protective and life saving. For example, in murine leishmanial infection, a Th1 response to a key parasitic Ag is protective, whereas a Th2 response is pathogenic (58). On the contrary, if susceptibility to an autoimmune disease is determined by a Th2 cell response to a particular determinant within an autoantigen, then only those individuals in whom this determinant is efficiently displayed to the appropriate T cells might contract this disease, whereas others may well be resistant to the same disease (59). For example, mixed (hybrid) MHC molecules in a heterozygous individual could be instrumental in presentation of novel or disease-inducing autoantigenic determinants to T cells (22, 33, 35), e.g., lupus causation in (NZB × NZWF)1 mice (33). On the other hand, determinant/peptide capture (12, 13) or deletion of a particular TCR Vβ specificity by an MHC molecule (52) have been implicated in protection from diabetes. Furthermore, our results described above and those of others (32, 60, 61) suggest that a particular susceptibility gene or locus, in combination with another allele, might significantly change the susceptibility pattern of the individual, either enhancing susceptibility or affording protection from disease. For example, heterozygous individuals with HLA-DR3/DR4 are highly susceptible to diabetes, whereas those with HLA-DR2/DR3 or HLA-DR2/DR4 are less likely to develop diabetes (60). However, our results point to the inherent uncertainty that a particular combination of haplotypes will actually be protective in every instance.

Our findings also have direct application to vaccination with protein Ags. These results suggest that even in a human population sharing a MHC allele capable of response to a key antigenic determinant of a vaccine, a great diversity would be evident among individuals, and some would not be protected; inexplicably, certain individuals would not respond to this determinant within the protein. What is clear is that the response of one or both parents is only minimally predictive of the response of the offspring. In the case of the heterozygous human population, where segregation occurs to complicate the genetic picture, prediction of a vaccine’s protective response is hazardous, despite the known presence of the MHC molecule(s).

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