H-2 Effects on Cell-Cell Interactions in the Response to Single Non-H-2 Alloantigens

IV. Variation in the Proliferative Response to H-Y and H-3

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Abstract. Individual mice were tested for their proliferative T-cell response to H-Y- and H-3-incompatible stimulator cells in secondary mixed lymphocyte culture. Responders expressing the \( H-2^b \) haplotype were restricted in their response to stimulators presenting H-Y and H-3 in the context of \( H-2^b \). Lymphocytes from individual B10 females proliferated in response to H-Y presented with \( I-A^b \) and \( D^b \). The ratio of \( I-A^b/D^b \)-restricted responses varied between individual responders, indicating significant qualitative variation between genetically identical responders. The majority of the proliferative response in all tested mice was restricted to the entire \( H-2^b \) haplotype suggesting complementation of \( I-A^b \)- and \( D^b \)-region genes in presenting the H-Y antigen. Similar observations were made in the response of individual B10.LP mice to the H-3 antigen. H-3-specific, proliferating T cells were restricted to H-3 antigen presented with \( K^b A^b \) and \( D^b \) with significant variation between individuals in their preference for H-3 plus \( K^b A^b \) and \( D^b \). In contrast to the response to H-Y, the proliferative response to H-3 plus \( H-2^b \) could be accounted for by the summation of the proliferative responses to H-3 plus \( K^b A^b \) and \( D^b \). These observations demonstrate that the proliferative response to non-H-2 H antigens in the context of I-region determinants is not a sine qua non for the T-cell response to these antigens. Further, the individual qualitative and quantitative variation observed with individual genetically identical mice has strong implications for our knowledge of intrastrain variation in immune responsiveness and the characterization of inbred strains for immune responsiveness.

Introduction

The role of \( H-2K/D \) genes in restricting the effector function of cytotoxic T cells has been documented extensively for virus-specific (Zinkernagel and Doherty 1974), hapten-specific (Shearer 1974), and non-H-2 histocompatibility (H) antigen-specific
T cells (Bevan 1975, Gordon et al. 1975). Single non-H-2 H antigens are presented preferentially by different K and D alleles. The following preferences have been reported for the H-2b haplotype (1) D^b: H-Y (Gordon et al. 1975), (2) D^b: H-7.1 (Wettstein and Frelinger 1977), (3) K^b: H-4.2 (Wettstein and Frelinger 1980), and (4) K^b and D^b: H-3.1 (Wettstein and Frelinger 1980). The H antigen-specific nature of this preferential restriction is reminiscent of the role of I-region Ir genes in the presentation of soluble protein antigens to proliferating T cells (reviewed in Schwartz et al. 1978). This similarity prompted the suggestion that H-2K/D genes serve an Ir-gene function in the presentation of antigen to cytotoxic T cells (Wettstein and Frelinger 1980).

Although I-region Ir genes have been shown to regulate the T-cell response to single non-H-2 H antigens in vivo (Bailey and Hoste 1971, Wettstein and Haughton 1977) and in vitro (Hurme et al. 1978a), their role in regulating presentation of H antigens to proliferating T cells has not been documented as in the case of the Ir-gene-controlled proliferative response to soluble protein antigens. If I-region, H-antigen-specific Ir genes regulate the response to H antigens in an identical manner to Ir genes specific for soluble antigens, then they should regulate the presentation of H antigens to proliferating T cells that are restricted to the I region. Initial experiments demonstrated that the proliferative response in primary MLC for H-7.1 was primarily directed to H-7.1 presented with D^b (Wettstein and Frelinger 1977).

These studies have been extended with the response to H-Y and H-3. In vivo priming alone has not been a sufficient stimulus for amplifying a detectable primary MLC response to these antigens. Therefore, a secondary proliferation assay was perfected, which allowed the employment of stimulator cells expressing different H-2 haplotypes. Responder lymphocytes from individual donors were assayed separately in order to reveal possible quantitative and qualitative variation in responsiveness. The results of these assays are reported in this communication. The H-Y antigen was presented with both I-A^b and D^b. Female mice from the same strain differed in the magnitude of their response as well as in their preference for H-Y presented with either I-A^b or D^b. The proliferative response to H-3 was restricted to K^bA^b and D^b with similar variation in preference between individual responders. These observations demonstrate a previously undetected qualitative variation in the immune response of genetically identical mice.

Materials and Methods

Mice. The strains of mice employed in these experiments and the origin of their H-2 haplotypes are presented in Table 1. The majority of mice were produced in this laboratory with the following exceptions: C57BL/6J (B6) males and females were purchased from The Jackson Laboratory, Bar Harbor, Maine, and B10.D2 (R107) and B10.HTG males were generously provided by Dr. Barbara Knowles, The Wistar Institute. Two C57BL/10 (B10) substrains, Sn and Sg, were employed. Reciprocal circle skin grafting (Bailey and Kohn 1965) between full sibs and between the two substrains demonstrated that the two substrains had not diverged at detectable H loci. Likewise, cross-immunization followed by mixed lymphocyte culture (MLC) did not result in the generation of cytotoxic effectors.
Table 1. Mouse strains employed and their H-2 haplotypes

| Strain          | H-2 haplotype | Origin of H-2 regions | H-3 allele |
|-----------------|---------------|-----------------------|------------|
|                 |               | K | A | B | J | E | C | S | D |
| B10             | b             | b | b | b | b | b | b | b | a |
| B10.A           | a             | k | k | k | k | d | d | d | a |
| B10.D2/3        | d             | d | d | d | d | d | d | d | a |
| B10.A(1R)       | h1            | k | k | k | d | d | d | d | a |
| B10.A(2R)       | h2            | k | k | k | d | d | b | d | a |
| B10.A(4R)       | h4            | k | k | b | b | b | b | a | a |
| B10.A(5R)       | i5            | b | b | b | k | d | d | a | a |
| B10.A(18R)      | i18           | b | b | b | b | b | b | a | a |
| B10.D2(R107)    | i7            | d | d | d | d | d | d | d | a |
| B10.HTG         | g             | b | b | b | b | b | b | b | a |
| B6.C-H-2<sup>bml1</sup> | bml1 | b | b | b | b | b | b | b | a |
| B6.C-H-2<sup>bml1.3</sup> | bml3 | b | b | b | b | b | b | b | a |
| B6-H-2<sup>bml1.4</sup> | bml4 | b | b | b | b | b | b | b | a |
| B10.LP          | b             | b | b | b | b | b | b | b | b |

Primary and secondary mixed lymphocyte cultures. Primary MLCs were performed as described previously (Peck and Bach 1973, Wettstein and Fretinger 1977); Click’s medium (Click et al. 1972) supplemented with 5% fetal calf serum (FCS) was used in all primary cultures. Responder lymphocytes were spleen cells harvested 14 days after in vivo priming with a single i.p. injection of 20 x 10⁶ non-H-2-incompatible spleen cells. Stimulator cells were non-H-2-incompatible spleen cells which had received 2000 rad gamma irradiation. Responders and stimulators were suspended at 5 x 10⁶ cells/ml in Click’s medium plus 5% FCS and mixed in equal proportions in 25 cm² tissue culture flasks. The cultures were incubated in a humidified atmosphere of 95% air and 5% CO₂. Secondary MLCs were performed 14 days after primary culture. Responders were harvested from the primary MLCs and resuspended to 1 x 10⁶ cells/ml in Click’s medium plus 1% normal mouse serum (NMS). No attempt was made to enrich for blasts. Stimulators were prepared as described for primary cultures; the final suspension was 7.5 x 10⁶ cells/ml. One hundred microliters of responders and stimulators were delivered in quadruplicate to flat-bottomed wells of 96-well plates. The plates were incubated as above. The cells were pulsed at 24 h with 2 μCi [³H] thymidine/well and harvested onto glass filters at 48 h with a semi-automated cell harvester (Otto Hiller Co., Madison, Wisconsin). Samples were counted in a scintillation counter equipped with a Datalogger (The Computer Store, Bryn Mawr, Pennsylvania) for sequential data acquisition. The mean (±s.e.) uptake was determined and the specific cpm (experimental mean – syngeneic mean) computed only when the experimental mean (±s.e.) did not overlap with the syngeneic control mean (± s.e.); “zero” signifies no significant proliferation. The proliferation data are presented as the A cpm of specific response. Stimulation indices (allogeneic/syngeneic) were calculated for the secondary responses to the immunizing and boosting cell type.

Results

H-Y. Twenty-five B10 and seven virgin B6 females were primed in vivo with syngeneic male spleen cells; their spleens were removed 14 days later and suspended for culture. Spleen cells from individual females were cultured with syngeneic male spleen-cell stimulators for 14 days. The lymphocytes surviving 14 days of primary culture were mixed with spleen-cell stimulators from males expressing different H-2 haplotypes. For the sake of brevity, the results of secondary MLCs of ten individuals are presented in Table 2. The lack of response to B10.A female stimulators...
Table 2. The proliferative response of B10♂ to H-Y in secondary MLC

| Stimulator          | H-2          | Δ cpm [3H] Tdr uptake with B10♀ responder* |
|---------------------|--------------|------------------------------------------|
|                     | K A E S D    | 1(1)  | 2(1)  | 3(1)  | 4(1)  | 5(1)  |
| B10♂                | b b b b b    | 102.81 | 87.057 | 33.207 | 51.298 | 64.763 |
| B10.A♂              | k k d d d    | 3.290  | 1.263  | 0     | 0     | 0     |
| (B10 × B10.A)♂      | k k d d d    | 22.630 | 18.786 | 9.193  | 8.913  | 24.352 |
| B10.A(1R)♂          | k k d | b b b b | 46.459 | 34.601 | 0     | 4.015  | 22.158 |
| B10.A(4R)♂          | k k | b b b d | 28.753 | 29.140 | 0     | 0     | 24.422 |
| B10.A(5R)♂          | b b | d d d d | 39.674 | 23.988 | 22.063 | 28.026 | 3.979  |
| B10.A(18R)♂         | b b b b b d | 43.267 | 23.558 | 17.730 | 22.764 | 6.964  |
| B10.D2♂             | d d d d d    | 7.690  | 1.880  | 0     | 0     | 0     |
| B6.C-H-2bm1♂        | bml b b b b | 82.182 | 69.867 | 26.031 | 55.776 | 30.292 |
| B6.C-H-2bm13♂       | b b b b b b | 34.973 | 21.001 | 16.700 | 28.041 | 0     |
| B6-H-2bm14♂         | b b b b b | 37.504 | 20.409 | 16.597 | 23.629 | 7.747  |
| B10.A♀              | k k d d d    | 0     | 0     | 0     | 0     | 0     |

* B10♂ primed in vivo and boosted in vitro with B10♀ spleen cells.
† Experiment number.
‡ Not tested.
‖ Stimulation index for B10♂ versus B10♀.

demonstrated that there was no allogeneic H-2 response. All individuals responded to B10 male stimulators (H-2b); significant quantitative variation was observed even within the same experiment. Minimal responses were observed to H-Y presented with H-2a (B10.A) or H-2d (B10.D2), indicating that the proliferating cells were restricted to H-2b. A strong effect of H-2 heterozygosity was observed on the ability of male cells to stimulate H-Y-specific proliferation; (B10 × B10.A)F1 male spleen cells stimulated relatively low levels of proliferation in comparison with B10 male spleen cells.

In order to determine the region or subregion of H-2b required for optimal presentation of H-Y, a panel of males expressing H-2 haplotypes which were derived by (1) recombination between the H-2b and H-2a haplotypes and (2) mutation of the H-2b haplotype were used as stimulators in secondary MLC. The results in Table 2 demonstrate that individual B10 females varied significantly in their preference for H-Y presented in the context of different regions of the H-2b haplotype, specifically the KbA b and D b regions. As shown in Table 2, nos. 1 and 2 responded equally well to H-Y presented with either KbA b [B10.A(5R) and B10.A(18R)] or D b [B10.A(1R) and B10.A(4R)]. Numbers 3 and 4 responded primarily to H-Y presented with KbA b. However, nos. 5, 6, and 7 responded primarily to H-Y presented with D b. The response to the two D b mutants bml3 and bml4 correlated with the response to B10.A(5R) and B10.A(18R), demonstrating that both mutants adversely affected the ability of D b to present H-Y to D b-restricted, proliferating T cells. The slightly lower proliferation stimulated by B6.C-H-2bm1 male cells in comparison with B10 males may arise from minor differences between the B10 and B6 strains. The ratio of the KbA b-versus D b-restricted response is presented in Figure 1 for the 32 individual B10
and B6 females. The ratio of $K^bA^b/D^b$ ranged from zero to more than 6 for B10 females. No correlation was observed for subregion preference with experiment, age of responder, B10 substrain, parents, or cage origin. The only homogeneous group of mice were the seven B6 females which responded equally well to H-? presented with $K^bA^b$ and $D^b$. Of particular interest was the observation that the $K^bA^b$- and $D^b$-restricted response did not quantitatively account for the response of any mice to H-Y in the context of $H-2^b$. In all cases there appeared to be a response specific to H-Y plus $H-2^b$. In the most extreme cases, nos. 9 and 10, the only response observed was with the entire $H-2^b$ haplotype, suggesting the presence of at least two complementary $H-2^b$ genes required for the presentation of H-Y in these individual females. Similar complementation was observed with responder no. 8 which responded to H-Y presented in the context of complementary genes in $H-2^b$ and $H-2^i$, suggesting one complementary gene maps proximal to the $H-2^h4$ recombination site and a second maps between the $H-2^f5$ and $H-2^i8$ recombination sites.

$H-3$. Eighteen B10LP mice were primed with B10 spleen cells in vivo and boosted with B10 spleen cells in primary MLC. Surviving lymphocytes were harvested at 14 days of culture and were mixed in secondary culture with H-3-incompatible stimulators with different $H-2$ haplotypes. The results of these assays are presented in Table 3. Thirteen individuals responded to B10 stimulators. There was no anti-allogeneic $H-2$-response based on the minimal response to B10A. However, the proliferative response to H-3 was not as highly restricted as the H-Y response; a certain amount of unrestricted proliferation was observed with nos. 9, 10, 11 and 13. This cross-reaction was observed previously for H-3 in CML assays (Wettstein and Frelinger 1980). The ability of $H-2^b$ to present H-3 was inherited as a dominant trait, as demonstrated by the high stimulatory capacity of (B10 × B10A)F$_1$ cells in contrast to the results obtained in the H-Y-specific response in Table 2.
Fig. 1. Relative proliferative responses of individual females to H-Y presented with I-A^b and D^b.

Fig. 2. Relative proliferative responses of individual B10.LP mice to H-3 presented with K^b A^b and D^b.

Previously reported CML studies demonstrated that the H-3 antigen is presented with both K^b and D^b (Wettstein and Frelinger 1980). In the proliferative assays reported here individual mice varied in their preference for H-3 presented with different H-2 regions. Six of the thirteen responders proliferated equally to H-3 presented with either K^b A^b or D^b. However, 5 out of 13 responded preferentially to

Table 3. The proliferative response of B10.LP-H-3^b mice to H-3 in secondary MLC

| Stimulator  | H-2 | \( A \) | \( E \) | \( S \) | 1(1) | 2(1) | 3(1) | 4(1) | 5(1) |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| B10         | b   | b   | b   | b   | 21807 | 52810 | 84080 | 127340 | 65065 |
| (B10 \times B10.A)F_1 | k   | k   | d   | d   | 0    | 0    | 0    | 0    | 0    |
| B10.A(1R)   | k   | k   | d   | d   | 11042 | 36621 | 58364 | 112660 | 37088 |
| B10.A(2R)   | k   | k   | d   | d   | 11964 | 30794 | 58019 | 118695 | 32974 |
| B10.A(4R)   | k   | k   | d   | d   | 4759  | 16727 | 44005 | 89752  | 20567 |
| B10.A(5R)   | b   | b   | d   | d   | 10226 | 28799 | 47036 | 33680  | 29134 |
| B10.A(18R)  | b   | b   | b   | b   | 13132 | 32247 | 50459 | 37047  | 39701 |
| B10.D2      | b   | d   | d   | d   | 0    | 0    | 0    | 0    | 0    |
| B10.D2(R107) | b   | b   | b   | b   | 11422 | 35119 | 50927 | 106222 | 35786 |
| B6.C-H-2\(^{bm1}\) | bm | b   | b   | b   | NT   | NT   | NT   | NT   | NT   |
| B6-H-2\(^{bm1\dagger}\) | b   | b   | b   | b   | 14292 | 26619 | 49851 | 47483  | 39111 |
| Preference  | K^b A^b = D^b | K^b A^b = D^b | K^b A^b = D^b | D^b > K^b A^b | K^b A^b = D^b |

* Responders primed in vivo and boosted in vitro with B10 spleen cells.
\(^1\) Experiment number.
\(^\dagger\) Not tested.
H-3 presented with $D^b$. Only 2 out of 13 responded preferentially to H-3 presented with $K^b A^b$. The ratio of $K^b A^b$ to $D^b$ for each individual responder to H-3 is presented in Figure 2. The recombinants and mutants employed did not allow the identification of the gene in the K end of H-2$^b$ which is required for H-3 presentation for responders preferring $K^b A^b$. The low proliferation of responder no. 12 to B6.C-H-2$^{bm1}$ stimulators suggested that the $K^b$ allele presents the H-3 antigen to this responder. However, the H-2$^{bm1}$ mutation had no effect on the presentation of H-3 to responders 11 and 13. It may be possible that $K^b$, I-A$^b$, and $D^b$ present H-3. The H-2$^{bm14}$ mutation eliminated the presentation of H-3 to $D^b$-restricted proliferating T cells in that the proliferation to B6-H-2$^{bm14}$ correlated with the proliferation to B10.A(5R) and B10.A(18R). Unlike the proliferative response to H-Y presented in the context of H-2$^b$, the response to H-3 in the context of H-2$^b$ could be accounted for by the sum of the proliferative responses to H-3 plus $K^b A^b$ and $D^b$.

Discussion

The role of genes mapping in the K and D regions of the H-2 complex in presenting non-H-2 antigens to cytotoxic effector T cells has been extensively documented. Of particular interest is the observation that H-2K/D genes selectively present non-H-2 H antigens in an antigen-specific manner (Gordon et al. 1975, Wettstein and Frelinger 1980) in much the same way as I-region Ir genes present soluble protein antigens to proliferating T cells (Schwartz et al. 1978). The existence of proliferating T cells specific for non-H-2 H antigens presented in the context of Ia molecules (encoded by Ir genes) has been suggested (von Boehmer et al. 1978) but not

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\begin{array}{cccccccc}
6(1) & 7(1) & 8(2) & 9(2) & 10(2) & 11(3) & 12(3) & 13(3) \\
41091 & 78661 & 78750 & 21553 & 15113 & 75546 & 35611 & 33429 \\
22.3 & (26.3) & (9.8) & (4.9) & (2.7) & (21.0) & (3.0) & (4.9) \\
0 & 0 & 4690 & 8352 & 6414 & 0 & 0 & 0 \\
39466 & 81945 & 61931 & 18695 & 14059 & 62258 & 25657 & 17885 \\
33975 & 63902 & 48090 & 0 & 0 & 45126 & 0 & 11208 \\
33773 & 72518 & 54235 & 8463 & 4147 & NT & NT & NT \\
25910 & 57732 & 56699 & 5462 & 2800 & 53611 & 0 & 14232 \\
15858 & 37301 & 13747 & 16160 & 9579 & 28056 & 37025 & 16865 \\
16523 & 30052 & 10492 & 14397 & 7899 & 24652 & 28985 & 15272 \\
0 & 0 & 0 & 12815 & 11081 & 27406 & 0 & 8303 \\
17457 & 35639 & 13905 & 6460 & 0 & NT & NT & NT \\
32763 & 61627 & 71622 & 10887 & 0 & 54816 & 0 & 9201 \\
NT & NT & NT & NT & 0 & 78840 & 9074 & 33269 \\
19939 & 39961 & 8545 & 8549 & 8672 & 28105 & 39876 & 19269 \\
\end{array}
\]

$D^b > K^b A^b$, $D^b > K^b A^b$, $D^b > K^b A^b$, $K^b A^b > D^b$, $K^b A^b > D^b$, $K^b A^b = D^b$, $K^b A^b > D^b$, $K^b A^b = D^b$.
confirmed. The experiments presented in this communication were designed to confirm the presence of proliferating T cells specific for non-H-2 H antigens and to identify the H-2 region(s) required to be expressed in stimulators for optimal T-cell proliferation. Lymphocytes expressing the H-2\(^b\) haplotype were primed in vivo and boosted in vitro with H-Y- and H-3-incompatible cells. H-2\(^b\) lymphocytes proliferating in secondary MLC were H-2 restricted, responding to H-Y and H-3 in the context of H-2\(^b\). In the case of H-Y, the expression of at least D\(^b\) or K\(^b\) A\(^b\) in male stimulator cells was required for proliferation. Based on the observations of others (Gordon et al. 1975) that the K\(^b\) gene is not involved in presentation of H-Y, it is proposed that the gene required for presentation of H-Y in secondary MLC is in fact I-A\(^b\) in the K\(^b\) A\(^b\) region. Quite unexpectedly, individual mice varied in their preference for H-Y in the context of D\(^b\) and I-A\(^b\). However, the magnitude of the response to H-Y plus H-2\(^b\) could not be accounted for by the sum of the responses restricted to I-A\(^b\) and D\(^b\), suggesting the major part of the proliferative response was restricted to the entire H-2\(^b\) haplotype. This and the observation that a single mouse responded preferentially to H-Y presented with H-2\(^b\) and H-2\(^{118}\) and not H-2\(^{215}\) and H-2\(^{2b1}\) suggest that optimal presentation of H-Y requires expression of at least two complementary H-2 genes, one of which maps proximal to the H-2\(^{a4}\) recombination site and a second that maps distal to the H-2\(^{15}\) recombination site. In the case of H-3, the expression of at least K\(^b\) A\(^b\) or D\(^b\) in stimulators was required for proliferation. Individual mice vary in their preference for H-3 presented with K\(^b\) A\(^b\) and D\(^b\). The magnitude of the H-2\(^b\)-restricted response could be accounted for by the summation of the K\(^b\) A\(^b\)- and D\(^b\)-restricted proliferative responses. These observations have provided evidence for the existence of non-H-2 H antigen-specific, proliferating T cells which are restricted to both K/D genes and I genes; the identity of K/D genes restricting proliferating cells correlated with the previously reported preferential restriction of cytotoxic effector T cells specific for the particular H antigens.

The results reported in this paper are the first evidence for the existence of I-restricted, proliferating T cells specific for non-H-2 histocompatibility antigens. The role of I-region Ir genes in regulating the T-cell response to a variety of antigens, including soluble protein antigens (reviewed in Schwartz et al. 1978), and H alloantigens (Stimpfling and Reichert 1971, Hurme et al. 1978a, b) has been extensively documented. In at least the case of soluble protein antigens, Ir-gene regulation is accomplished at the level of antigen-presenting cells, specifically macrophages (Yano et al. 1978). That is, the ability of macrophages to present particular protein antigens to proliferating T cells is determined by their expression of the high response Ir genes mapping in the I-A and I-E subregions. Analysis of the T-cell response to non-H-2 H antigens is considerably more complex. The in vivo response to the H-Y antigen was originally shown to be controlled by a gene mapping in the I-A subregion (Stimpfling and Reichert 1971); a more recent study suggesting that a gene mapping in the I-B subregion regulates the in vivo response to H-Y (Hurme et al. 1978b) has most certainly confused the issue. Further, the capacity to generate H-Y-specific cytotoxic effectors in vitro is regulated by at least two genes in the H-2\(^b\) haplotype (Hurme et al. 1978a). The D\(^b\) allele is required for presentation of the H-Y antigen to cytotoxic effectors (Gordon et al. 1975) and is presumably required in stimulating cells for generation of cytotoxic effectors (Hurme et al. 1978a). An additional gene mapping in the I-A subregion is required...
for the generation of effectors (Hurme et al. 1978a). Therefore, both the $I-A^b$ and $D^b$ genes are required for generation of H-Y-specific effectors in $H-2^b$ responders. However, the cellular level of expression of these genes and their manner of regulation is unknown. If the putative $I-A$-subregion gene regulates the response to H-Y in a manner analogous to that of $Ir$ genes regulating the proliferative T-cell response to protein antigens, then it should be expected that the expression of an appropriate high responder allele at the $I-A$ subregion is a prerequisite for the proliferative response to H-Y.

The results reported in this communication demonstrate that $I$-region $Ir$ genes are indeed involved in the proliferative response through their regulation of the ability of H-Y-incompatible cells to stimulate secondary proliferation. However, the results with individual mice indicate that the expression of high responder, $I-A$-subregion $Ir$ alleles in stimulators is not a sine qua non for the optimal proliferative response in all mice of the same strain. Although lymphocytes from some female B10 mice proliferate in response to H-Y presented with $I-A^b$, other mice simply require the expression of the $D^b$ allele. Similar results have been reported for the H-7.1 antigen in which proliferating T cells in primary MLCs are primarily restricted to $D^b$ (Wettstein and Frelinger 1977). The results reported here for H-3 suggest that genes in the $K^bA^b$ and $D^b$ regions are sufficient for presentation of H-3 to proliferating T cells. The predominance of the $D^b$-restricted proliferative response to this antigen is consistent with the reported preference of H-3-specific cytotoxic effectors for the $D^b$ allele (Wettstein and Frelinger 1980). Experiments are in progress to determine the Ly phenotype of T cells that proliferate in response to H-Y and H-3. It is expected that Ly-1$^+2^-$ T cells respond to H antigens plus $I-A^b$ and Ly-1$^+2^+$ T cells respond to H antigens plus $K^b/D^b$.

An important observation was made regarding the stimulation of H-Y- and H-3-specific proliferating T cells by $H-2^a/H-2^b$ heterozygous male cells. H-Y-specific T cells were not effectively stimulated by $H-2$ heterozygous male cells, whereas H-3-specific T cells were stimulated as effectively by $H-2$ heterozygous cells as by $H-2^b$ homozygous cells. The difference between these two responses may lie in the dependence of the majority of the H-Y-specific response on interactions between complementary $H-2^b$ genes for optimal stimulation. The random assortment of $I-A$ $\alpha$ and $\beta$ subunits coupled with possible complexing with different D-region gene products could result in sufficient percentages of complexes which do not effectively present H-Y. This assortment in $H-2$ heterozygotes would suggest that there is a major contribution by proliferating T cells which recognize H-Y in the context of “parental” Ia molecules. Antigen-specific, proliferating T cells that recognize soluble protein antigens in the context of parental and hybrid Ia antigens have been reported (Kimoto and Fathman 1981). The question of the importance of this subunit assortment for the presentation of H-Y will be approached using appropriate $H-2$ heterozygous stimulators in future secondary MLCs, as well as the selection of T-cell lines that recognize H-Y on $H-2^b$ and $H-2^b/H-2^a$ stimulators.

A major advantage of mice as experimental models has been the ease of producing inbred strains which, within a strain, are uniform and predictable. However, the results presented in this communication demonstrate that, within certain limitations, presumably identical mice of a single strain vary both quantitatively and qualitatively in their ability to respond to a single non-H-2 H
antigen. The basis for this variation is not understood. The possibility that the substrains employed are undergoing extensive genetic drift appears to be ruled out by the fact that the B10 substrains used in this study are histocompatible. This conclusion is based on the fact that skin grafts transplanted between members of the same substrain and both substrains survive for more than 10 weeks (P. J. Wettstein, unpublished observations). Further evidence for their lack of divergence is the observation that cytotoxic effectors could not be generated after priming in vivo and boosting in vitro with cells from the opposing substrain. It would appear that the events giving rise to the observed variation occur prior to priming, i.e., during development and maturation of the immune system, or during and after priming, i.e., during expansion of discrete H antigen-specific T cells both in vivo and in vitro. Regarding prepriming variation, no correlation was observed between responsiveness and the date of experiment, the housing of the mice in particular cages, or the age of mice. The possibility that individual mice, even those housed in the same colony, may vary in their history of viral and bacterial infection must be considered. The only group of mice that responded with no variation in preference were B6 females purchased from The Jackson Laboratory; this lack of variation could be due either to their laboratory origin or to the fact that they are B6s.

Possible variation following priming must be considered as well. Since these mice can respond to H antigens presented with different single regions of H-2, any consideration of variation following priming must deal with the possibility of competition between different antigen-specific T cells. It would appear unlikely that limitations in “biological room” would account for variation in H-2-region preference in individual mice. Further, the apparently equivalent capacity of T cells restricted to I-A, K, and D, to proliferate would seem to rule out the possibility that particular T cells proliferate more rapidly than others. A likely explanation for postpriming variation is that initial antigen recognition is made by a single T cell or simultaneously by more than one T cell, after which that T cell(s) proliferates and suppresses the incipient response by other T cells. For example, if an H-Y-specific T cell restricted to D\textsuperscript{b} is the first to recognize H-Y on injected male cells, it will proliferate and suppress the incipient I-Ab-restricted response. If both D\textsuperscript{b}- and I-A\textsuperscript{p}-restricted T cells recognize H-Y initially, then both would proliferate.

Regardless of the origin of variation in the responsiveness of individual mice of the same strain, the observations presented here have strong implications for the use of inbred strains in immunology. The employment of pools of mice to characterize the responsiveness of inbred strains may obscure more interesting phenomena of individual variation. Further, the characterization of the responsiveness of inbred strains by typing small numbers of mice may be inaccurate.

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