ROLE OF THE H-2 COMPLEX IN INDUCTION OF T HELPER CELLS IN VIVO

III. Contribution of the I-E Subregion to Restriction Sites Recognized by I-A/E-restricted T Cells*

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Until recently, it has been tacitly assumed that T helper cells are restricted solely by I-A molecules (1-5). Increasing evidence suggests, however, that restriction can also be mediated by I-A/E molecules (6-10). Each set of Ia molecules consists of two chains, α and β, which are noncovalently associated (11-15). For I-A (Aα-Aβ) dimers, the α and β chains are both coded by genes in the I-A subregion; for I-A/E (Eα-Eβ) dimers, in contrast, the α chain is coded in the I-E subregion, whereas the β chain (Eβ, also called Aε) is coded in the I-A subregion.

Evidence that both I-A and I-A/E molecules can restrict T helper function has come from studies on selection of T cells to sheep erythrocytes (SRC) in vivo (8-10). Various approaches have shown that T cell selection to SRC in irradiated mice reflects a response to antigen presented in association with H-2I determinants on radioresistant macrophage-like cells (or possibly dendritic cells) of the selection host. After intravenous transfer, the SRC-reactive T cells initially undergo negative selection: the responding T cells disappear from thoracic duct lymph (TDL) for 1-2 d and become sequestered in the spleen. Negative selection is dependent upon a sharing of H-2I determinants between donor and host (16); without such sharing the donor T cells in TDL retain normal T helper function for SRC. In the case of CBA (H-2k) T cells, selection to SRC is marked in irradiated B10.AQR (I-AkI-BkI-JkI-EkI-Cdk) (kkkkk) mice, not detectable in B10 (bbbbb) mice, and minimal in B10.A(3R) (bbkd) mice (10). Partial selection occurs in B10.A(4R) mice, T helper responses here being consistently reduced by ~40%. Selection is maximal in (4R × 3R)F1 mice, but remains incomplete after consecutive filtration of T cells through 4R mice and then 3R mice. These findings apply to T helper function for CBA B cells. Different findings occur with 4R B cells: CBA T cells selected to SRC in 4R mice, while retaining the capacity to interact with CBA B cells, fail to provide help for 4R B cells (10).

The interpretation of these findings is that CBA T cells consist of a mixture of I-Ak- and I-A/Ek-restricted cells. Selection of the I-A/Ek-restricted subset requires that the filtration host expresses both chains of the I-A/Ek (Eα-Eβk) molecule: these molecules

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Abbreviations used in this paper: HRC, horse erythrocytes; PFC, plaque-forming cells; SRC, sheep erythrocytes; TDL, thoracic duct lymph.
can form in CBA (kk) (cis) and (4R × 3R)F1 (kb × bk) (trans) mice but not in 4R or 3R mice. Exposure to SRC in 4R mice selects only the I-Ak-restricted subset. The I-A/E\(^k\)-restricted cells ignore antigen in 4R mice and, on further transfer, provide help for CBA B cells, i.e., cells expressing I-A/E\(^k\) determinants. B cells that lack these determinants, e.g., 4R B cells, are not stimulated.

The notion that the \(\alpha\) and \(\beta\) chains of the I-A/E molecule both contribute to the specificity of the T cell restricting site requires comment. Two points should be emphasized. First, I-A/E molecules are not expressed in certain haplotypes, e.g., in 4R and B10 mice (both I-E\(^b\)) (11-15). Such strains lack Ia.7 determinants. In these strains, the \(E_\alpha\) chain is not synthesized and the \(E_\beta\) chain remains in the cytoplasm.

The \(E_\beta\) chain is synthesized, however, in \(E_\alpha^+ 3R (bk)\) mice, a finding that suggests that the \(E_\alpha\) chain plays a crucial role in controlling the cell surface expression of the \(E_\beta\) chain. The second point to be stressed is that unlike the \(E_\beta\) chain, the \(E_\alpha\) chain displays only a limited degree of polymorphism as assessed by tryptic peptide analysis (13, 15). This raises the key question of whether the \(E_\alpha\) chain shows any functional polymorphism.

Evidence against this notion has come from the finding of Schwartz et al. (17) that a variety of Ia.7\(^+\) (\(E_\alpha^+\)) strains can complement with the B10 strain for the response of B10.A(5R) (bk) T cells to GL4b; responses to this antigen are controlled by two complementing \(Ir\) genes, one mapping in the I-A subregion and the other in the I-E subregion. In this paper we have searched for functional polymorphism of \(E_\alpha\) chains by selecting CBA T cells to SRC in F1 hybrids raised between 4R mice and various I-E-incompatible strains. The results suggest that, with the notable exception of the B10.PL (H-2\(^b\)) strain, any \(E_\alpha^+\) (Ia.7\(^+\)) strain can complement with 4R to form the restriction site recognized by I-A/E\(^k\)-restricted T cells.

### Materials and Methods

**Mice.** CBA/J (CBA), C57BL/10 (B10), B10.S, B10.M, B10.RIII, B10.PL, B10.D2, B10.SM, and B10.WB mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R) mice were purchased from Clarence R. Reeder, Fort Detrick, Md. B10.P, B10.S(7R), and B10.T(6R) mice were generously provided by Dr. J. Stimpfling (McLaughlin Research Institute, Great Falls, Mont.), Dr. C. David (Mayo Clinic, Rochester, Minn.), and Dr. W. Silvers (University of Pennsylvania), respectively. All F1 hybrid mice were bred in our animal facilities.

**Negative Selection.** As described elsewhere (10), pooled spleen and lymph node (LN) cells from SRC-plus horse erythrocyte (HRC)-primed mice were depleted of most B cells and macrophages by passage over nylon-wool columns. The effluent T cells (>90% Thy-1-positive) were transferred intravenously in a dose of ~10\(^6\) viable cells with or without SRC into mice given 900 rad of irradiation 6-9 h before; SRC (1 ml of a 50% solution) were injected intravenously 2-4 hr before the injection of T cells. Thoracic duct cannulae were inserted in the recipients ~15 h after T cell injection and TDL were collected between 18 and 40 h post-injection. Under these conditions, 90–98% of the lymph-borne cells are typical small T (Thy-1-positive) lymphocytes of donor strain origin. In both syngeneic and allogeneic donor-host combinations, cell yields amounted to 10–20% of the numbers initially injected.

**Measurement of T Helper Function.** As described elsewhere (10), small doses (2 × 10\(^5\)-2.5 × 10\(^6\)) of the lymph-borne T cells were transferred with SRC- and HRC-primed B cells (spleen cells treated with anti-Thy-1.2 antibody and complement) plus SRC and HRC (0.1 ml of a 1% solution of each) into CBA mice given 700 rad 1 d before. Direct (IgM) and indirect (IgG) splenic plaque-forming cells (PFC) were measured on day 7.
Results

Experimental Design. The general approach was to transfer purified CBA T cells with or without SRC into heavily irradiated mice, harvest the donor T cells from TDL of the recipients 1–2 d later, and measure T helper function for SRC and HRC on adoptive transfer with CBA B cells. T cells and B cells (T cell-depleted spleen) were both taken from mice primed with a mixture of SRC and HRC; T-B collaboration was assayed in irradiated CBA mice.

Selection of CBA T Cells to SRC in H-2-different Homozygous Mice. Before studying selection in heterozygous mice, it was first necessary to show that selection would not occur in the relevant homozygous parental strains. The results in Table I show anti-SRC and anti-HRC responses of CBA T cells after acute recirculation in the presence of SRC through various H-2-different strains; to correct for the slight variability seen in the response to the control antigen (HRC) the data are also expressed in terms of the SRC/HRC PFC ratio.

Selection to SRC was near-complete in H-2-compatible B10.BR mice, i.e., anti-SRC responses were generally reduced by ≥95% relative to responses given by T cells filtered in the absence of antigen. In the case of the 10 I-A- and I-E-mismatched hosts

Table I
Failure of CBA T Cells to Undergo Negative Selection to SRC in Irradiated H-2-different Homozygous Mice

| Experiment | Irradiated hosts used for selection of CBA T cells* | SRC added during selection | PFC/spleen with CBA B cells† | Anti-SRC | Anti-HRC | SRC/HRC PFC ratio |
|------------|---------------------------------------------------|---------------------------|----------------------------|---------|---------|-----------------|
| I. B10.BR  | k k k k k k k k | 8,530 (1.29) | 44,090 (1.12) | 36,650 (1.15) | 1.48   |
| B10.BR     | k k k k k k k k | 600 (1.06)  | 2,410 (1.40)  | 53,590 (1.20) | 0.06   |
| B10        | k k k k k k k k | 8,040 (1.08) | 38,160 (1.05) | 96,980 (1.12) | 1.14   |
| B10S       | k k k k k k k k | 5,000 (1.31) | 37,250 (1.11) | 27,980 (1.26) | 1.55   |
| B10M       | k k k k k k k k | 12,750 (1.28) | 48,020 (1.17) | 45,620 (1.13) | 1.34   |
| B10.2H     | k k k k k k k k | 9,720 (1.22) | 49,140 (1.20) | 36,860 (1.17) | 1.47   |
| B10. P     | k k k k k k k k | 16,750 (1.30) | 44,480 (1.29) | 46,750 (1.20) | 1.26   |

* Nylon-wool-purified CBA T cells from SRC + HRC-primed mice were transferred intravenously into hosts (usually 2 mice/group) given 900 rad 4–6 h before; SRC (1 ml of 50% solution) were injected intravenously 2–4 h before T cell injection. The recipients were cannulated ~8 h later, and TDL were collected between 1 and 40 h after T cell injection. The lymph-borne T cells were used as helper cells in a dose 2.1 x 10^6 to 2.5 x 10^6 viable cells.

† Lymph-borne T helper cells + B cells (5 x 10^6 viable anti-Thy-1.2-treated spleen) and a mixture of SRC and HRC (0.1 ml of 1/10 solution of each) were transferred intravenously into irradiated (750 rad 1 d before) CBA mice. PFC in spleen were measured on day 7.

§ Geometric mean of 4 mice/group. Number in parentheses refers to value by which mean is multiplied or divided to give upper and lower limits, respectively, of SE. Background values given by B cells transferred without T cells have been subtracted; these values (PFC/spleen) ranged from 400 to 900 for experiment I, 900 to 2,750 for experiment II, and 980 to 3,050 for experiment III. T cells alone gave <300 PFC/spleen.
Table II

Negative Selection of CBA T Cells to SRC in Irradiated F1 Hybrids Raised between B10.A(4R) and Various H-2-different Strains: Complementation with Strains Expressing la.7 Determinants

| Experiment | Irradiated hosts used for selection of CBA T cells<sup>a</sup> | Expression of la.7 determinants by selection host | SRC added during selection | PFC/spleen with CBA B cell<sup>d</sup> | Ratio: total anti-SRC PFC/total anti-HRC PFC |
|------------|-------------------------------------------------------------|---------------------------------------------|--------------------------|----------------------------------------|---------------------------------|
|             |                                                             |                                             |                          | IgM                                  | IgG                             |
|             |                                                             |                                             |                          | IgM + IgG                             |                                 |
| I.          |                                                             |                                             |                          |                                       |                                 |
| B10.BR      | +                                                           | −                                           | 16,320 (1.28)<sup>f</sup> | 30,260 (1.21)                        | 21,440 (1.16)                  |
| B10.BR      | +                                                           | +                                           | 0                        | 80 (1.18)                            | 19,380 (1.13)                  |
| B10.A(4R)   | −                                                           | +                                           | 9,480 (1.23)             | 15,340 (1.14)                        | 27,770 (1.18)                  |
| B10.A(4R) × B10 | −                                                       | +                                           | 7,890 (1.03)             | 16,420 (1.06)                        | 24,930 (1.03)                  |
| B10.A(4R) × B10.S | −                                                       | +                                           | 8,120 (1.14)             | 16,840 (1.32)                        | 20,000 (1.19)                  |
| B10.A(4R) × B10.M | −                                                       | +                                           | 6,530 (1.14)             | 18,290 (1.05)                        | 20,700 (1.10)                  |
| B10.A(4R) × B10.T(6R) | −                                                      | +                                           | 8,350 (1.12)             | 19,370 (1.32)                        | 29,970 (1.13)                  |
| B10.A(4R) × B10.A(3R) | +                                                      | +                                           | 0                        | 0                                    | <0.01                          |
| B10.A(4R) × B10.RIII | +                                                      | +                                           | 56 (1.24)                | 0                                    | 28,530 (1.15)                  |
| B10.A(4R) × B10.D2  | +                                                      | +                                           | 0                        | 1,180 (1.45)                         | 21,000 (1.14)                  |
| II.         |                                                             |                                             |                          |                                       |                                 |
| B10.BR      | +                                                           | −                                           | 9,260 (1.30)             | 36,470 (1.10)                        | 60,780 (1.15)                  |
| B10.BR      | +                                                           | +                                           | 950 (1.35)               | 1,600 (1.31)                         | 52,620 (1.08)                  |
| B10.A(4R)   | −                                                           | +                                           | 9,040 (1.09)             | 15,080 (1.15)                        | 35,430 (1.13)                  |
| B10.A(4R) × B10.P | +                                                      | +                                           | 2,010 (1.10)             | 1,450 (1.37)                         | 57,680 (1.06)                  |
| B10.A(4R) × B10.WB | +                                                      | +                                           | 3,080 (1.16)             | 1,430 (1.53)                         | 70,560 (1.24)                  |
| B10.A(4R) × B10.PL | +                                                      | +                                           | 3,490 (1.15)             | 11,270 (1.30)                        | 55,290 (1.07)                  |

* As for Table I.

† As for Table I. Subtracted background values (PFC/spleen) for B cells transferred without T cells ranged from 1,150 to 1,440 for experiment I and from 870 to 2,550 for experiment II. T cells alone gave <300 PFC/spleen.

* As mentioned previously (10), the mild degree of selection of CBA (kk) T cells in 3R (bk) mice in some experiments might reflect the existence of a third subgroup of cells restricted by the E<sub>4</sub> chain per se. However, in view of recent evidence that the E<sub>4</sub> and E<sub>5</sub> chains are structurally quite similar (15), a more likely possibility is that T cells restricted by (E<sub>4</sub>-E<sub>5</sub>) dimers have slight cross-reactivity for (E<sub>4</sub>-E<sub>9</sub>) dimers.
552 ROLE OF I-E SUBREGION IN INDUCTION OF T HELPER CELLS

Table III
Summary of Experiments on Negative Selection of CBA T Cells to SRC in Irradiated F1 Hybrids between B10.A(4R) and Other Strains

| Irradiated hosts used for selection of CBA T cells to SRC | Expression of Ia.7 determinants by selection host | Number of experiments | Anti-SRC PFC:anti-HRC PFC ratio relative to selection through B10.A(4R) (range) |
|---------------------------------------------------------|--------------------------------------------------|-----------------------|--------------------------------------------------------------------------------|
| Unselected T cells*                                      | 11                                               | 11                   | 1.72 (1.10-2.56)$^+$                                                             |
| B10.A(4R)                                               | -                                                | 11                   | 1.00 Control                                                                      |
| B10.8R                                                  | +                                                | 8                    | 0.06 (0-0.13)                                                                     |
| B10.A(3R)                                               | +                                                | 4                    | 1.29 (1.09-1.70)                                                                  |
| B10.A(4R) × B10                                         | -                                                | 2                    | 1.06 (1.04-1.08)                                                                  |
| B10.A(4R) × B10.S                                       | -                                                | 3                    | 1.10 (0.96-1.33)                                                                  |
| B10.A(4R) × B10.M                                       | -                                                | 2                    | 1.33 (1.30-1.35)                                                                  |
| B10.A(4R) × B10.T(6R)                                   | -                                                | 2                    | 0.99 (0.89-1.08)                                                                  |
| B10.A(4R) × B10.A(3R)                                   | +                                                | 5                    | 0.07 (0.03-0.13)                                                                  |
| B10.A(4R) × B10.RII                                     | +                                                | 3                    | 0.03 (0-0.01)                                                                     |
| B10.A(4R) × B10.DII                                     | +                                                | 2                    | 0.03 (0-0.03)                                                                     |
| B10.A(4R) × B10.P                                       | +                                                | 5                    | 0.05 (0-0.11)                                                                     |
| B10.A(4R) × B10.WB                                      | +                                                | 3                    | 0.05 (0-0.11)                                                                     |
| B10.A(4R) × B10.PL                                      | +                                                | 3                    | 0.38 (0.32-0.42)                                                                  |

*T cells selected through irradiated mice (usually B10.BR) in the absence of SRC.

$^+$ Value shown = \(
\frac{\text{total anti-SRC PFC for selection through test host}}{\text{total anti-HRC PFC}}
\)

a different finding. In three separate experiments, the degree of selection observed in (4R × B10.PL)F1 mice was never complete but was appreciably greater than that seen in 4R mice.

Selection of 4R T Cells. Since 4R mice express only I-A molecules and not I-A/E-molecules, T cells from these mice might be expected to consist solely of I-A-restricted cells. If so, selection of 4R (kbbbb) T cells should be as effective in B10.BR (kkkkk) mice as in syngeneic hosts. Conversely, no selection would be expected in B10 (bbbbbb) mice. The results shown in Table IV support this prediction.

Discussion

The main finding in this paper is that F1 hybrids between 4R (kb) and four of five Ia.7+ I-E-incompatible strains are as effective as H-2-identical B10.BR(kk) mice at inducing negative selection of CBA T cells to SRC. This finding corroborates two studies in vitro on positive selection of T cells to antigens under dual Ir-gene control. First, as mentioned earlier, Schwartz et al. (17) have reported that Ia.7+ haplotypes k, d, p, and r can complement with B10 (bb) to induce proliferation of B10.A(5R) (bb) T cells to GL4. Second, Matis et al. (18), using T cell lines, have shown similarly that various Ia.7+ strains can complement with 4R to elicit responses of B10.A (kk) T cells to pigeon cytochrome c; the use of antigen-specific long-term T cell lines in this latter study avoided the problem encountered by Schwartz et al. (17) of high "background" counts due to concomitant alloreactivity. Schwartz and Matis argue that, in responder strains, the above two antigens make an immunogenic association only with I-A/E
molecules and not with I-A molecules: GLφ associates with Eαφ-Eββ dimers, whereas pigeon cytochrome c associates with Eαk-Eβκ.

Collectively, these studies with three different antigens and two restricting haplotypes suggest that, with the exception of Eαu (see below), Eα chains from all strains tested display no functional polymorphism. In view of the remarkable structural homology of Eα chains, this conclusion might come as no surprise. On this point it should be stressed that Eα chains do display small allelic variations: by tryptic peptide analyses using multiple amino acids as radiolabels, Cook et al. (13) have found that the Eαk chain differs from Eαd′p′r chains by one to three peptides. From a functional viewpoint, these differences cannot be dismissed as trivial on a priori grounds because, in the case of class I molecules, 1-2 amino acid substitutions (e.g., H-2Kβ vs. H-2Kβ1) can radically alter T cell-restricting sites (19). The fact that Eα chain polymorphisms clearly do not affect T cell function might suggest that the contribution of the Eα chain to the Eα-Eβ restricting site is relatively nonspecific. For example, the restricting site might be situated solely on the Eβ chain, the Eα chain serving merely as a scaffold. Without further information it is difficult to assess this possibility.

The finding that, in marked contrast to other hybrids, F1 hybrids between 4R and Ia.7 + B10.PL (I-Eu) failed to cause complete selection of CBA T cells is of some interest. Selection in these mice was clearly greater than in homozygous 4R mice but was far from maximal. Matis et al. (18) have made similar observations for the in vitro response of T cell lines to pigeon cytochrome c. In their hands, proliferative responses evoked by (4R × B10.PL)F1 stimulators with low concentrations of antigen were much less than with other Ia.7+ heterozygous stimulators. Interestingly, (4R × B10.PL)F1 cells could be induced to evoke high responses if the cells were exposed to high antigen concentrations.

Two explanations might account for these anomalous findings with B10.PL; both explanations rest on the assumption that the strong expression of Ia.7 determinants in this strain (20) signifies quantitatively normal expression of Eα chains. The first possibility is that the Eαu chain is structurally different from other Eα chains, the restricting sites on Eαk-Eβk molecules being only partly represented on Eαu-Eβk dimers. According to this notion, Eαk-Eβk-restricted T cells might have a degree of cross-

### Table IV

**Negative Selection of B10.A(4R) T Cells to SRC: Equivalent Degree of Selection in Irradiated B10.A(4R) and B10.BR Mice**

| Irradiated hosts used for selection of B10.A(4R) T cells | Region of selection host | Expression of Ia.7 determinants by selection host | SRC added during selection | PFC/spleen with B10.A(4R) B cells§ | Anti-SRC PFC/total anti-HRC PFC |
|--------------------------------------------------------|--------------------------|-----------------------------------------------|---------------------------|-----------------------------------|--------------------------------|
|                                                        | A B J F E C               |                                               |                           | IgM                               | Anti-HRC PFC/IgM + IgG               |
| B10.A(4R)                                              | k b b b b                | −                                             | −                         | 10,900 (1.27)§                    | 12,570 (1.27) §                     |
|                                                        |                           |                                               |                           | 8,590 (1.22)                       | 9,200 (1.28)                        |
|                                                        |                           |                                               |                           | 3.65                              | 3.65                              |
| B10.BR                                                 | k k k k k                | +                                             | +                         | 1,190 (1.31)                      | 720 (2.92)                         |
|                                                        |                           |                                               |                           | 11,580 (1.20)                     | 11,580 (1.20)                      |
|                                                        |                           |                                               |                           | 0.11                              | 0.11                              |
| B10                                                     | b b b b b                | −                                             | −                         | 1,250 (1.26)                      | 250 (3.55)                         |
|                                                        |                           |                                               |                           | 19,160 (1.37)                     | 19,160 (1.37)                      |
|                                                        |                           |                                               |                           | 0.10                              | 0.10                              |
| B10.B2                                                 | d d d d d                | +                                             | +                         | 25,160 (1.35)                     | 20,910 (1.43)                      |
|                                                        |                           |                                               |                           | 16,510 (1.08)                     | 16,510 (1.08)                      |
|                                                        |                           |                                               |                           | 2.94                              | 2.94                              |

* As for Table I. B10.A(4R) mice were used as T and B cell donors and as recipients for measuring T-B collaboration.

§ As for Table I (see above).

Subtracted background values for B cells transferred without T cells ranged from 200 to 670 PFC/spleen. T cells alone gave <300 PFC/spleen.
reactive specificity for $E_n^u$-$E_k^k$ dimers, and thus show significant but incomplete selection to antigen presented by these dimers. Conversely, $E_k^k$-$E_k^k$-restricted T cells might consist of two distinct subgroups, one restricted by a private determinant unique to $E_n^u$-$E_k^k$ and the other by a public determinant shared by $E_n^u$-$E_k^k$.

The alternative explanation is that the $E_u^u$ chain is structurally similar to other $E_n^u$ chains but, for obscure reasons, shows cis preference in terms of $E_k^k$ chain association. Evidence in favor of this possibility has come from the observations of Yokota et al. (21) on the expression of the Ia.23 specificity. This specificity is expressed on I-A/E molecules and requires the co-expression of the $E_k^d$ allele and any $E_n^+$ allele, with the exception of $E_n^u$. These workers were able to raise high titered anti-Ia.23 antibody after immunization with cells bearing $E_n^{k,b,d}$. Comparability immunization with cells of the $E_n^u$-$E_k^d$ haplotype, however, elicited no antibody, even when tested on the cells used for immunization. For this reason the authors concluded that cell surface expression of $E_n^u$-$E_k^d$ dimers in appropriate hybrid mice is either very low or absent. Direct support for this contention has come from recent studies of McNicholas et al. (22) using other strain combinations. These workers have shown that $E_n^u$ chains do indeed display strong cis preference when associating with various $E_k$ chains. From several different approaches, including fluorescence-activated cell sorter analysis and two-dimensional gel electrophoresis with monoclonal antibodies, McNicholas et al. have demonstrated that cells from F1 hybrids between B10.PL (E$^u$) and strains of $I-A^k,b^d,b^h$ (E$^k,b^d,b^h$) genotype do express trans-associated $E_n^u$-$E_k^{k,b,h}$ dimers, but only at a low level; this level is approximately one-eighth of that found with other $E_n^+$ chain combinations, e.g., $E_n^{k,b}$. The cell surface expression of the cis molecule ($E_n^u$-$E_k^k$), by contrast, is high.

A considerable body of evidence suggests that H-2-restricted T cell specificity is the result of confrontation with the appropriate H-2 determinants in the thymus during early T cell differentiation (23). A corollary to this theory is that the subset of I-A/E-restricted T cells would not form in mice that lack an $E_n$ chain: the Ia-restricted cells in these mice would be restricted solely by I-A molecules. Support for this notion comes from the finding that, unlike $E_n^+$ CBA T cells, negative selection of $E_n^-$ 4R T cells was virtually complete in hosts matched only in the $I-A$ (and $K$) subregion, i.e., in B10.BR mice (Table IV). This observation, together with studies on the capacity of monoclonal anti-Ia antibodies to block positive selection to antigen (8, 9), implies that I-A-encoded molecules are probably the sole I-region restricting elements in homozygous $E_n^-$ strains.

Summary

Previous studies on negative selection of T cells to sheep erythrocytes in irradiated mice showed that CBA (I-A$^k$,I-E$^k$) (kk) T cells comprise two subgroups of cells restricted by I-A (A$^+_n$-$A^k$) and I-A/E (E$^+_n$-$E_k^k$) molecules. Selection of the I-A/E-restricted subset requires that the donor T cells and the selection host share both I-A ($E_k^d$) and I-E ($E_n^+$) gene products; only the I-A-restricted cells undergo selection in B10.A(4R) (kk) mice. This paper demonstrates that negative selection of the I-A/E-restricted subgroup of CBA T cells can occur in F1 hybrids between B10.A(4R) and various Ia.$7^+$ (E$^+_n$) I-E-incompatible strains; selection does not occur in hybrids between B10.A(4R) and Ia.$7^-$ (E$^+_n$) strains. These data suggest that, despite the fact that $E_n$ chains display detectable structural allelic variations, these chains are func-
tionally nonpolymorphic. This conclusion applies to $E_{a}^{kd,p,r}$ chains. With $F_{1}$ hybrids between B10.A(4R) and another Ia.7+ strain, B10.PL (H-2m), in contrast, only intermediate selection is observed. This finding is consistent with recent evidence that cell surface expression of $E_{a}^{a}-E_{a}^{b}$ dimers displays strong cis preference.

In contrast to $E_{a}^{+}$ CBA T cells, $E_{a}^{-}$ B10.A(4R) (kk) T cells undergo complete negative selection in hosts matched only in the I-A (and H-2K) subregion, i.e., B10.BR (kk) mice; no selection occurs in B10 (kk) mice. These data imply that Ia-restricted T cells in $E_{a}^{-}$ strains are probably restricted solely by I-A molecules.

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References

1. Katz, D. H., and B. Benacerraf. 1975. The function and interrelationship of T cell receptors, Ir genes and other histocompatibility gene products. Transplant. Rev. 22:175.

2. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. J. Exp. Med. 142:460.

3. Yano, A., R. H. Schwartz, and W. E. Paul. 1977. Antigen presentation in the murine T-lymphocyte proliferative response. I. Requirement for genetic identity at the major histocompatibility complex. J. Exp. Med. 146:828.

4. Sprent, J. 1978. Restricted helper function of F1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the H-2 complex. J. Exp. Med. 147:1159.

5. Vadas, M. A., J. F. A. P. Miller, A. M. Whitelaw, and J. R. Gamble. 1977. Regulation by the H-2 gene complex of delayed-type hypersensitivity. Immunogenetics. 4:137.

6. Longo, D. L., and R. H. Schwartz. 1980. Gene complementation. Neither Ir-GL$^{a}$ gene need be present in the proliferative T cell to generate an immune response to poly(Glu$^{a}$-Lys$^{b}$-Phe$^{c}$)$^{n}$. J. Exp. Med. 151:1452.

7. Lerner, E. A., L. A. Matis, C. A. Janeway, Jr., P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. Monoclonal antibody against an Ir gene product? J. Exp. Med. 152:1085.

8. Sprent, J. 1980. Effects of blocking helper T cell induction in vivo with anti-Ia antibodies. Possible role of I-A/E hybrid molecules as restriction elements. J. Exp. Med. 152:996.

9. Sprent, J., E. A. Lerner, J. Bruce, and F. W. Symington. 1981. Inhibition of T cell activation in vivo with mixtures of monoclonal antibodies specific for I-A and I-A/E molecules. J. Exp. Med. 154:188.

10. Sprent, J., and B. Alpert. 1981. Role of the H-2 complex in induction of T helper cells in vivo. II. Negative selection of discrete subgroups of T cells restricted by I-A and I-A/E determinants. J. Exp. Med. 153:823.

11. David, C. S. 1976. Serologic and genetic aspects of murine Ia antigens. Transplant. Rev. 30:299.

12. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. J. Exp. Med. 148:925.

13. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1979. Structural studies on the murine Ia alloantigen. III. Tryptic peptide comparisons of allelic products of the I-E/C subregion. Mol. Immunol. 16:29.

14. Silver, J., and W. A. Russell. 1979. Structural polymorphism of I-E subregion antigens determined by a gene in the H-2K to I-B genetic interval. Nature (Lond.). 279:437.

15. McMillan, M., J. A. Frelinger, P. P. Jones, D. B. Murphy, H. O. McDevitt, and L. Hood. 1981. Structure of murine Ia antigens. Two-dimensional electrophoretic analyses and high-
pressure liquid chromatography tryptic peptide maps of products of the I-A and I-E subregions and of an associated invariant polypeptide. J. Exp. Med. 153:936.

16. Sprent, J. 1978. Role of the H-2 complex in induction of T helper cells in vivo. I. Antigen-specific selection of donor T cells to sheep erythrocytes in irradiated mice dependent upon sharing of H-2 determinants between donor and host. J. Exp. Med. 148:478.

17. Schwartz, R. H., C. Chen, and W. E. Paul. 1980. Gene complementation in the T lymphocyte proliferative response to poly(Glu35Lys38Phe8)n. Functional evidence for a restriction element coded for by both the I-A and I-E subregions. Eur. J. Immunol. 10:708.

18. Matis, L. A., P. P. Jones, D. B. Murphy, S. M. Hedrick, E. A. Lerner, C. A. Janeway, Jr., J. M. McNicholas, and R. H. Schwartz. 1982. Immune response gene function correlates with the expression of an Ia antigen. II. A quantitative deficiency in \text{A}_a:\text{E}_a complex expression causes a corresponding defect in antigen-presenting cell function. J. Exp. Med. 155:508.

19. Nairn, R., K. Yamaga, and S. G. Nathenson. 1980. Biochemistry of the gene products from murine MHC mutants. Ann. Rev. Genet. 14:241.

20. LaFuse, W. P., J. G. McCormick, P. S. Corser, and C. S. David. 1980. Gene complementation to generate Ia antigens (Ia.23) on hybrid molecules. Transplantation (Baltimore). 30:341.

21. Yokota, S., W. P. Lafuse, J. F. McCormick, and C. S. David. 1981. Detection of hybrid (combinatorial) Ia antigens using parent anti-Fi sera. J. Immunol. 126:371.

22. McNicholas, J. M., D. B. Murphy, L. A. Matis, R. H. Schwartz, E. A. Lerner, C. A. Janeway, Jr., and P. P. Jones. 1982. Immune response gene function correlates with the expression of an Ia antigen. I. Preferential association of certain \text{A}_a and \text{E}_a chains results in a quantitative deficiency in expression of \text{A}_a:\text{E}_a complex. J. Exp. Med. 155:490.

23. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells. Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv. Immunol. 27:52.