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 (A_α-A_β) dimers, the α and β chains are both coded by genes in the *I-A* subregion; for I-A/E (E_α-E_β) dimers, in contrast, the α chain is coded in the *I-E* subregion, whereas the β chain (E_β, also called A_e) 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)[1] 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 ($H\text{-}2^k$) T cells, selection to SRC is marked in irradiated B10.AQR ($I\text{-}A^k I\text{-}B^k I\text{-}J^k I\text{-}E^k I\text{-}C^d$) (*kkkkd*) mice, not detectable in B10 (*bbbbb*) mice, and minimal in B10.A(3R) (*bbbkd*) 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)F_1 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 $I\text{-}A^k$- and $I\text{-}A/E^k$-restricted cells. Selection of the $I\text{-}A/E^k$-restricted subset requires that the filtration host expresses both chains of the $I\text{-}A/E^k$ ($E_\alpha{}^k$-$E_\beta{}^k$) molecule: these molecules

* Supported by grants CA-15822, AI-10961, and CA-09140 from the U. S. Public Health Service.

‡ Recipient of Medical Scientist Training Program grant NIHS-T32-GM07170.

[1] *Abbreviations used in this paper:* HRC, horse erythrocytes; PFC, plaque-forming cells; SRC, sheep erythrocytes; TDL, thoracic duct lymph.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/82/02/0548/09 $1.00
Volume 155 February 1982 548–556

can form in CBA (*kk*) (*cis*) and (4R × 3R)F_1 (*kb* × *bk*) (*trans*) mice but not in 4R or 3R mice. Exposure to SRC in 4R mice selects only the I-A^k-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 α and β 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_α chain is not synthesized and the E_β chain remains in the cytoplasm. The E_β chain is synthesized, however, in E_α^+ 3R (*bk*) mice, a finding that suggests that the E_α chain plays a crucial role in controlling the cell surface expression of the E_β chain. The second point to be stressed is that unlike the E_β chain, the E_α 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_α 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_α^+) strains can complement with the B10 strain for the response of B10.A(5R) (*bk*) T cells to GLϕ; 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_α chains by selecting CBA T cells to SRC in F_1 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*^{u}) strain, any E_α^+ (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 F_1 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 ~10^8 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 (2×10^6-2.5×10^6) 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*	*H-2* haplotype of selection hosts								SRC added during selection	PFC/spleen with CBA B cells‡			Ratio: total anti-SRC PFC/ total anti-HRC PFC
		K	*I*					*S*	*D*		Anti-SRC		Anti-HRC	
			A	*B*	*J*	*E*	*C*				IgM	IgG	IgM + IgG	
I.	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	−	8,830 (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	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	+	8,040 (1.08)	45,160 (1.05)	46,980 (1.12)	1.14
	B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	+	5,800 (1.31)	37,250 (1.11)	27,940 (1.29)	1.55
	B10.M	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*f*	+	12,750 (1.28)	48,020 (1.17)	45,620 (1.15)	1.34
	B10.RIII	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	+	9,720 (1.22)	49,140 (1.20)	33,860 (1.17)	1.74
	B10.P	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	+	16,540 (1.30)	44,480 (1.29)	48,760 (1.20)	1.26
II.	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	−	13,690 (1.15)	30,990 (1.03)	51,770 (1.16)	0.86
	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	+	730 (1.11)	0	41,080 (1.30)	0.02
	B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	+	6,190 (1.13)	15,910 (1.17)	35,440 (1.12)	0.62
	B10.A(3R)	*b*	*b*	*b*	*b*	*k*	*d*	*d*	*d*	+	10,560 (1.22)	36,090 (1.04)	69,690 (1.17)	0.67
	B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	+	4,590 (1.17)	32,990 (1.12)	42,790 (1.28)	0.87
	B10.S(7R)	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*	+	11,230 (1.13)	33,590 (1.15)	54,240 (1.08)	0.82
	B10.T(6R)	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*d*	+	15,210 (1.08)	30,130 (1.37)	43,050 (1.41)	1.05
	B10.RIII	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	+	7,880 (1.22)	30,000 (1.10)	33,520 (1.22)	1.11
	B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	+	9,500 (1.47)	37,250 (1.20)	60,720 (1.04)	0.79
III.	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	−	6,020 (1.11)	23,330 (1.20)	33,780 (1.12)	0.87
	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	+	100 (1.52)	470 (2.07)	36,230 (1.03)	0.03
	B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	+	1,430 (1.12)	10,310 (1.20)	33,900 (1.12)	0.34
	B10.PL	*u*	*u*	*u*	*u*	*u*	*u*	*u*	*u*	+	2,750 (1.29)	26,430 (1.22)	24,150 (1.07)	1.22
	B10.SM	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	+	5,490 (1.38)	26,990 (1.06)	30,110 (1.20)	1.09
	B10.WB	*j*	*j*	*j*	*j*	*j*	*j*	*j*	*j*	+	1,010 (1.27)	10,440 (1.30)	9,500 (1.35)	1.27

* 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 ~18 h later, and TDL were collected between 18 and 40 h after T cell injection. The lymph-borne T cells were used as helper cells in a dose 2.1×10^6–2.5×10^6 viable cells, the dose being constant within each experiment.

‡ Lymph-borne T helper cells + B cells (5×10^6 viable anti-Thy-1.2-treated spleen) and a mixture of SRC and HRC (0.1 ml of 1% 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 multipled 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, 800 to 2,750 for experiment II, and 980 to 3,200 for experiment III. T cells alone gave <300 PFC/spleen.

TABLE II

Negative Selection of CBA T Cells to SRC in Irradiated F_1 Hybrids Raised between B10.A(4R) and Various H-2-different Strains: Complementation with Strains Expressing Ia.7 Determinants

Experiment	Irradiated hosts used for selection of CBA T cells*	Expression of Ia.7 determinants by selection host	SRC added during selection	PFC/spleen with CBA B cell‡			Ratio: total anti-SRC PFC/ total anti-HRC PFC
				Anti-SRC		Anti-HRC	
				IgM	IgG	IgM + IgG	
I.	B10.BR	+	−	16,320 (1.28)§	30,260 (1.21)	21,440 (1.16)	2.23
	B10.BR	+	+	0	180 (1.10)	19,380 (1.13)	<0.01
	B10.A(4R)	−	+	9,480 (1.23)	15,540 (1.14)	27,770 (1.18)	0.90
	B10.A(4R) × B10	−	+	7,890 (1.05)	16,420 (1.06)	24,930 (1.03)	0.97
	B10.A(4R) × B10.S	−	+	8,120 (1.14)	16,890 (1.33)	28,000 (1.19)	0.91
	B10.A(4R) × B10.M	−	+	6,930 (1.14)	18,290 (1.05)	20,750 (1.10)	1.21
	B10.A(4R) × B10.T(6R)	−	+	8,330 (1.12)	19,370 (1.32)	29,970 (1.13)	0.97
	B10.A(4R) × B10.A(3R)	+	+	0	0	20,550 (1.08)	<0.01
	B10.A(4R) × B10.RIII	+	+	50 (1.24)	0	28,530 (1.15)	<0.01
	B10.A(4R) × B10.D2	+	+	0	1,180 (1.43)	21,000 (1.14)	0.05
II.	B10.BR	+	−	9,260 (1.24)	36,470 (1.18)	60,780 (1.15)	0.76
	B10.BR	+	+	950 (1.35)	1,690 (1.31)	52,620 (1.08)	0.06
	B10.A(4R)	−	+	9,040 (1.09)	15,080 (1.15)	35,430 (1.13)	0.69
	B10.A(4R) × B10.P	+	+	2,010 (1.10)	1,450 (1.37)	57,680 (1.06)	0.07
	B10.A(4R) × B10.WB	+	+	3,080 (1.16)	1,430 (1.53)	70,460 (1.24)	0.07
	B10.A(4R) × B10.PL	+	+	3,490 (1.15)	11,270 (1.19)	55,260 (1.07)	0.27

* As for Table I.

‡ 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.

tested, selection was either not detectable or very minimal, especially in terms of IgG responses; IgM responses were mildly reduced on occasions but this was not a regular finding. As reported previously (10), anti-SRC T helper responses were consistently reduced by ~40% with selection in 4R mice. Selection in 3R mice was moderate to minimal in some experiments and not detectable in others (10).[2]

Selection of CBA T Cells to SRC in F_1 Hybrids between 4R and Various H-2-different Strains. Table II shows the results of two representative experiments in which CBA T cells were selected to SRC in F_1 hybrids raised between 4R mice and the above-mentioned parental strains. A summary of these data, together with the data from several other experiments, is shown in Table III. For simplicity the data in Table III are shown in terms of the SRC/HRC PFC ratio observed after selection in each of the various hybrids tested, relative to the control ratio found with selection in homozygous 4R mice.

In the case of F_1 hybrids between 4R and each of four Ia.7$^-$ strains, i.e., B10(*I-A^b I-E^b*) (*bb*), B10.S (*ss*), B10.M (*ff*), and B10.T(6R) (*qq*), selection of CBA T cells to SRC was no more extensive in these mice than in homozygous 4R mice. Markedly different results were observed with F_1 hybrids between 4R and each of four *I-E*-incompatible Ia.7$^+$ strains, i.e., B10.RIII (*rr*), B10.D2 (*dd*), B10.P (*pp*), and B10.WB (*jj*). With each of these hybrids, selection to SRC was as extensive as that seen in (4R × 3R)F_1 mice or in B10.BR mice. Studies with a fifth Ia.7$^+$ strain, B10.PL (*uu*) gave

[2] 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_α^k chain per se. However, in view of recent evidence that the E_β^k and E_β^b chains are structurally quite similar (15), a more likely possibility is that T cells restricted by (E_α^k-E_β^k) dimers have slight cross-reactivity for (E_α^k-E_β^b) dimers.

TABLE III

Summary of Experiments on Negative Selection of CBA T Cells to SRC in Irradiated F_1 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	1.72 (1.10–2.58)‡
B10.A(4R)	−	11	1.00 Control
B10.BR	+	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 (0–0.13)
B10.A(4R) × B10.RIII	+	3	0.03 (0–0.10)
B10.A(4R) × B10.D2	+	2	0.03 (0–0.05)
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.

$$\ddagger \text{ Value shown} = \frac{\left(\frac{\text{total anti-SRC PFC}}{\text{total anti-HRC PFC}} \text{ for selection through test host}\right)}{\left(\frac{\text{total anti-SRC PFC}}{\text{total anti-HRC PFC}} \text{ for selection through B10.A(4R) host}\right)}.$$

a different finding. In three separate experiments, the degree of selection observed in (4R × B10.PL)F_1 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 (*bbbbb*) mice. The results shown in Table IV support this prediction.

Discussion

The main finding in this paper is that F_1 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) (*bk*) T cells to GLϕ. 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

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*	*I*-region of selection host					Expression of Ia.7 determinants by selection host	SRC added during selection	PFC/spleen with B10.A(4R) B cells‡			Ratio: total anti-SRC PFC/ total anti-HRC PFC
								Anti-SRC		Anti-HRC	
	A	*B*	*J*	*E*	*C*			IgM	IgG	IgM + IgG	
B10.A(4R)	*k*	*b*	*b*	*b*	*b*	−	−	18,390 (1.27)§	12,570 (1.27)	8,590 (1.22)	3.65
B10.A(4R)	*k*	*b*	*b*	*b*	*b*	−	+	1,190 (1.31)	720 (2.92)	11,580 (1.20)	0.11
B10.BR	*k*	*k*	*k*	*k*	*k*	+	+	1,250 (1.26)	250 (3.55)	19,160 (1.37)	0.10
B10	*b*	*b*	*b*	*b*	*b*	−	+	25,160 (1.35)	20,910 (1.43)	16,510 (1.08)	2.94
B10.D2	*d*	*d*	*d*	*d*	*d*	+	+	11,470 (1.14)	7,120 (1.47)	5,580 (1.36)	3.38

* 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).

§ As for Table I. Subtracted background values for B cells transferred without T cells ranged from 200 to 670 PFC/spleen. T cells alone gave <300 PFC/spleen.

molecules and not with I-A molecules: GLϕ associates with E_α^k-E_β^b dimers, whereas pigeon cytochrome c associates with E_α^k-E_β^k.

Collectively, these studies with three different antigens and two restricting haplotypes suggest that, with the exception of E_α^u (see below), E_α chains from all strains tested display no functional polymorphism. In view of the remarkable structural homology of E_α chains, this conclusion might come as no surprise. On this point it should be stressed that E_α chains do display small allelic variations: by tryptic peptide analyses using multiple amino acids as radiolabels, Cook et al. (13) have found that the E_α^k chain differs from $E_\alpha^{d,p,r}$ 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-2K^b vs. H-2K^{bml}) can radically alter T cell-restricting sites (19). The fact that E_α chain polymorphisms clearly do not affect T cell function might suggest that the contribution of the E_α chain to the E_α-E_β restricting site is relatively nonspecific. For example, the restricting site might be situated solely on the E_β chain, the E_α 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, F_1 hybrids between 4R and Ia.7$^+$ B10.PL (*I-E^u*) 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)F_1 stimulators with low concentrations of antigen were much less than with other Ia.7$^+$-heterozygous stimulators. Interestingly, (4R × B10.PL)F_1 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 E_α chains. The first possibility is that the E_α^u chain is structurally different from other E_α chains, the restricting sites on E_α^k-E_β^k molecules being only partly represented on E_α^u-E_β^k dimers. According to this notion, E_α^k-E_β^k-restricted T cells might have a degree of cross-

reactive specificity for E_α^u-E_β^k dimers, and thus show significant but incomplete selection to antigen presented by these dimers. Conversely, E_α^k-E_β^k-restricted T cells might consist of two distinct subgroups, one restricted by a private determinant unique to E_α^k-E_β^k and the other by a public determinant shared by E_α^u-E_β^k.

The alternative explanation is that the E_α^u chain is structurally similar to other E_α chains but, for obscure reasons, shows *cis* preference in terms of E_β 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_β^d allele and any E_α^+ allele, with the exception of E_α^u. These workers were able to raise high titered anti-Ia.23 antibody after immunization with cells bearing $E_\alpha^{k,p,d,r}$-E_β^d dimers; comparable immunization with cells of the E_α^u-E_β^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_α^u-E_β^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_α^u chains do indeed display strong *cis* preference when associating with various E_β 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 F_1 hybrids between B10.PL (E_α^u) and strains of $I\text{-}A^{k,b,s}$ ($E_\beta^{k,b,s}$) genotype do express *trans*-associated E_α^u-$E_\beta^{k,b,s}$ dimers, but only at a low level; this level is approximately one-eighth of that found with other E_α^+ chain combinations, e.g., E_α^k-$E_\beta^{k,b,s}$. The cell surface expression of the *cis* molecule (E_α^u-E_β^u), 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_α 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_α^+ CBA T cells, negative selection of E_α^- 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_α^- strains.

Summary

Previous studies on negative selection of T cells to sheep erythrocytes in irradiated mice showed that CBA ($I\text{-}A^k$,$I\text{-}E^k$) (*kk*) T cells comprise two subgroups of cells restricted by I-A (A_α-A_β) and I-A/E (E_α-E_β) molecules. Selection of the I-A/E-restricted subset requires that the donor T cells and the selection host share both *I-A* (E_β) and *I-E* (E_α) gene products; only the I-A-restricted cells undergo selection in B10.A(4R) (*kb*) mice. This paper demonstrates that negative selection of the I-A/E-restricted subgroup of CBA T cells can occur in F_1 hybrids between B10.A(4R) and various Ia.7$^+$ (E_α^+) *I-E*-incompatible strains; selection does not occur in hybrids between B10.A(4R) and Ia.7$^-$ (E_α^-) strains. These data suggest that, despite the fact that E_α chains display detectable structural allelic variations, these chains are func-

tionally nonpolymorphic. This conclusion applies to $E_\alpha^{k,d,p,r,j}$ chains. With F_1 hybrids between B10.A(4R) and another Ia.7$^+$ strain, B10.PL (*H-2^u*), in contrast, only intermediate selection is observed. This finding is consistent with recent evidence that cell surface expression of E_α^u-E_β dimers displays strong *cis* preference.

In contrast to E_α^+ CBA T cells, E_α^- B10.A(4R) (*kb*) 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 (*bb*) mice. These data imply that Ia-restricted T cells in E_α^- strains are probably restricted solely by I-A molecules.

Received for publication 11 June 1981.

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