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*

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When T cells encounter antigen in vivo, the responding cells leave the recirculating lymphocyte pool, e.g., thoracic duct lymph (TDL)[1], and become temporarily sequestered in regions of antigen localization, particularly the spleen (1). During this stage of negative selection, which lasts for 1–2 d, the antigen-specific T cells proliferate extensively before reentering the circulation in expanded numbers (positive selection). A similar sequence of events occurs if purified T cells are exposed to antigen (sheep erythrocytes [SRC]) after adoptive transfer to irradiated mice (2). In this situation the SRC-specific donor T cells are not detectable in TDL of the host for 1–2 d posttransfer but thereafter enter the lymph in large numbers as activated T helper cells.

Previous studies from this laboratory have shown that selection of purified homozygous T cells to SRC in irradiated mice requires that the donor and host share H-2 determinants (2); H-2 identity is not required because effective selection occurs in H-2-heterozygous mice. The interpretation of these findings is that T cells do not respond to free antigen in vivo but to antigen associated with H-2 determinants of radioresistant cells of the host used for selection. In the case of positive selection, it has been shown that the antigen-presenting cells are a class of nylon wool-adherent, non-T and non-B macrophage-like cells that expresses both I-A and I-E Ia antigens (3). These characteristics conform closely with the features of cells presenting antigen to T cells in vitro.

To define which part of the *H-2* complex controls selection, we have examined the helper specificity of T cells after negative selection to SRC in irradiated mice of various H-2-recombinant strains. The results in this paper show that selection is controlled by the *I*-region of the *H-2* complex. To obtain near-complete selection of T cells of the $H\text{-}2^k$ haplotype requires that the donor and host are matched at both the *I-A* and *I-E* subregions. From this and other evidence we conclude that $H\text{-}2^k$ T cells comprise a mixture of cells that recognize antigen in association with $I\text{-}A^k$ and $I\text{-}A/E^k$ molecules.

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

Materials and Methods

Mice. CBA/J (CBA), B10.A, and [C57BL/6 (B6) × DBA/2]F_1 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R) and B10.A(3R) mice were kindly provided by Dr. P. Doherty and Dr. B. Knowles, respectively, of The Wistar Institute, Philadelphia, Pa. B10.AQR (fourth backcross generation) and B10.T(6R) mice were a gift of Dr. W. Elkins, University of Pennsylvania, Philadelphia, Pa. (CBA × B6)F_1 mice were obtained from Cumberland View Farms, Clinton, Tenn. F_1 hybrids between B10.A(4R) and B10.A(3R) were bred in our animal facilities.

T Cell Purification. The T cells used for negative selection were obtained from the spleen and pooled lymph nodes (inguinal, mesenteric, axillary, and cervical) of mice primed with SRC and horse erythrocytes (HRC) 5–16 wk previously (2). To remove most B cells and macrophages, the lymphoid cells were passed through nylon wool-columns before use (one passage for lymph node cells and two consecutive passages for spleen cells). The effluent T cells were >90% Thy-1.2 positive.

Negative Selection. The technique for inducing negative selection was similar to that described previously (2). Nylon wool-passed T cells were transferred i.v. 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 i.v. 2–4 h 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 18–40 h post-injection. Under these conditions, 90–98% of the lymph-borne cells are typical small T (Thy-1.2 positive) lymphocytes of donor strain origin (2). 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 (2), 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 in B10.AQR and B10.A(4R) Mice. It was shown previously that CBA ($H\text{-}2^k$) T cells underwent effective negative selection to SRC in irradiated B10.BR ($H\text{-}2^k$) mice but not in B10 ($H\text{-}2^b$) or B10.D2 ($H\text{-}2^d$) mice (2). To determine which part of the *H-2* complex controlled selection, CBA T cells plus SRC were recirculated through irradiated B10.AQR (K^q, $I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}J^k$, $I\text{-}E^k$, $I\text{-}C^d$, $I\text{-}S^d$, D^d) (*qkkkkddd*) and B10.A(4R) (*kkbbbbbb*) mice. B10.T(6R) (*qqqqqqqd*) and (B6 × DBA/2)F_1 ($H\text{-}2^b \times H\text{-}2^d$) mice were used as controls.

As shown in Table I, negative selection to SRC was minimal or absent when CBA T cells plus SRC were filtered through totally H-2-different irradiated B10.T(6R) or (B6 × DBA/2)F_1 mice. In both situations, adding SRC during selection failed to inhibit T helper function, i.e., IgM and IgG anti-SRC responses were as high as with T cells filtered in the absence of SRC. By contrast, anti-SRC responses were reduced by >90% when CBA T cells were exposed to SRC in B10.AQR mice; selection was specific because T cell help for HRC was unimpaired.

That selection was near-complete in B10.AQR mice and not detectable in either B10.T(6R) or (B6 × DBA/2)F_1 mice indicated that the genes controlling selection

TABLE I

Negative Selection of CBA T Cells to SRC in Irradiated H-2-Recombinant Mice: Role of the H-21 Region

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‡		
			Anti-SRC		Anti-HRC
			IgM	IgG	IgG
(B6 × DBA/2)F_1	*b b b b b b b b / d d d d d d d d*	–	6,940(1.20)§[1]	20,390(1.16)[3]	51,640(1.21)
(B6 × DBA/2)F_1	*b b b b b b b b / d d d d d d d d*	+	6,030(1.18)	17,120(1.21)	35,530(1.44)
B10.T(6R)	*q q q q q q q d*	+	8,950(1.10)	23,750(1.14)	41,620(1.24)
B10.AQR	*q k k k k d d d*	+	780(1.56)	1,310(1.44)	51,670(1.09)
B10.A(4R)	*k k b b b b b b*	+	4,000(1.15)[2]	12,090(1.14)[4]	51,340(1.24)

* 10^8 nylon wool-purified T cells from mice primed to SRC and HRC were transferred intravenously with or without 1 ml of 50% SRC to irradiated (900 rad 6 h before) mice of various strains. The recipients (two mice per group) were cannulated 15 h later and TDL were collected between 18 and 40 h postinjection. To measure T helper function, 2.5×10^6 lymph-borne T cells were transferred with B cells plus SRC and HRC into CBA mice given 700 rad 1 d before. Direct (IgM) and indirect (IgG) PFC to SRC and HRC were measured in the spleen on day 7.

‡ 5×10^6 viable anti-Thy-1.2-antibody-treated spleen cells from SRC- and HRC-primed CBA mice.

§ Geometric mean; four mice per group. Figure in parenthesis 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) were: 470(1.22) (IgM SRC), 330(1.19) (IgG SRC), 440(1.10) (IgG HRC). T cells alone gave <200 PFC/spleen. *P* values: 1 vs. 2: >0.05, 3 vs. 4: < 0.05.

mapped in the *I*-region of the *H-2* complex, i.e., in the *I-A, I-B, I-J,* or *I-E* subregion. Because the restrictions controlling T-macrophage interactions and T-B collaboration have been mapped to the *I-A* subregion (4–8), it was expected that selection of CBA T cells would be as effective in B10.A(4R) (*kkbbbbbb*) mice as in B10.AQR mice. This was not the case. As shown in Table I, anti-SRC T helper responses by CBA T cells were reduced by only ≃40% after selection in B10.A(4R) mice. Similar findings were observed in five separate experiments. In none of these experiments did the reduction in anti-SRC responses exceed 50% (range 15–50%).

Selection in B10.A(4R) Mice Monitored with B10.A(4R) vs. B10.BR B cells. The simplest explanation for the incomplete selection of CBA T cells to SRC in B10.A(4R) mice is that CBA T cells comprise two subgroups of I-region-restricted cells, one subgroup restricted by I-A determinants and the other by determinants encoded, at least in part, in the *I-B, I-J,* or *I-E* subregion. According to this view, selection of CBA T cells to SRC in B10.A(4R) mice would remove one of these two T cell subgroups, i.e., the I-A-restricted subgroup. The other subgroup would not undergo selection and on transfer would stimulate CBA B cells as the result of recognizing the I-B, -J, or -E determinants on these cells. The prediction thus follows that, in contrast to normal T cells, CBA T cells selected to SRC in B10.A(4R) mice would be unable to stimulate B10.A(4R) B cells, i.e., cells that express $I\text{-}A^k$ determinants but not $I\text{-}B^k$, $\text{-}J^k$, or $\text{-}E^k$ determinants.

This prediction is verified by the experiment shown in Table II in which (CBA × B6)F_1 and B10.A(4R) mice were used as selection hosts and T helper function was monitored with B10.BR and B10.A(4R) B cells. When CBA T cells plus SRC were filtered through irradiated (CBA × B6)F_1 mice, anti-SRC responses were reduced by >90% with both B cell populations. Selection in B10.A(4R) mice, by contrast, caused only a 40% reduction in the anti-SRC response with B10.BR B cells but complete unresponsiveness with B10.A(4R) B cells. Responses to HRC were unimpaired.

Role of the I-E Subregion in Controlling Selection. Of the five known subregions of the

TABLE II

Negative Selection of CBA T Cells to SRC in Irradiated B10.A(4R) Mice: T-Helper Function with B10.BR Versus B10.A(4R) B Cells

Irradiated hosts used for selection of CBA T cells*	SRC added during selection	IgG PFC/spleen in irradiated CBA mice			
		B10.BR B cells‡ (*kkkkkkkk*)		B10.A(4R) B cells‡ (*kkbbbbbb*)	
		Anti-SRC	Anti-HRC	Anti-SRC	Anti-HRC
(CBA × B6)F_1	−	31,650(1.16)§[1]	24,120(1.24)	10,500(1.10)	5,270(1.25)
(CBA × B6)F_1	+	2,670(1.32)	26,740(1.20)	0	6,540(1.11)
B10.A(4R)	+	19,180(1.29)[2]	31,690(1.20)	0	7,780(1.84)

* As for Table I.

‡ B cells were transferred in a dose of 5×10^6 viable cells for B10.BR and 6×10^6 for B10.A(4R). To limit a host-versus-graft reaction, the irradiated CBA recipients of the T and B cells were pretreated 3 d before with 40 μl of rabbit anti-mouse anti-lymphocyte serum.

§ As for Table I. Subtracted background values for B cells transferred without T cells were: B10.BR B cells 2,050(1.52) (SRC), 2,770(1.30) (HRC); B10.A(4R) B cells 1,260(1.70) (SRC), 1,320(1.45) (HRC). T cells alone gave < 200 PFC/spleen. *P* values: 1 vs. 2: >0.05.

TABLE III

Negative Selection of CBA T Cells to SRC in B10.A(4R), B10.A(3R), and (4R × 3R)F_1 Mice

Irradiated hosts used for selection of CBA T cells*	*H-2* haplotype of selection host	SRC added during selection	PFC/spleen with CBA B cells*		
			Anti-SRC		Anti-HRC
			IgM	IgG	IgG
B10.A	*k k k k k d d d*	−	3,340(1.18)§[1]	21,530(1.24)[4]	21,950(1.11)
B10.A	*k k k k k d d d*	+	100(1.69)	440(1.20)	21,470(1.16)
B10.A(4R)	*k k b b b b b b*	+	1,130(1.24)[2]	13,850(1.17)[5]	18,320(1.29)
B10.A(3R)	*b b b b k d d d*	+	2,380(1.13)[3]	19,350(1.09)[6]	22,290(1.33)
(4R × 3R)F_1	*k k b b b b b b* / *b b b b k d d d*	+	180(1.32)	830(1.81)	17,240(1.31)

* As for Table I. Subtracted background values for B cells transferred without T cells ranged from 170 to 880 PFC/spleen.

‡ As for Table I. *P* values: 1 vs. 2: <0.05, 1 vs. 3: >0.05, 4 vs. 5: >0.5, 4 vs. 6: >0.5.

H-2I region, only the *I-A* and *I-E* subregions are known to control the expression of the Ia molecules present on most B cells and some macrophages (Discussion). It seemed likely, therefore, that the extra subgroup of T cells revealed after selection in B10.A(4R) mice was restricted by determinants controlled by genes mapping in the *I-E* subregion rather than the *I-B* or *I-J* subregion. If the second subgroup of T cells were restricted by I-E determinants per se, T helper responses of CBA T cells for CBA B cells should be partly reduced after filtration with SRC through I-E-compatible, I-A-incompatible B10.A(3R) (*bbbbkddd*) mice.

To test this notion, selection of CBA T cells was studied in B10.A(4R), B10.A(3R) and (4R × 3R)F_1 mice; B10.A (*kkkkkddd*) mice were used as controls (Table III). As in previous experiments, selection to SRC in B10.A(4R) mice reduced the anti-SRC response appreciably (70% for IgM PFC, 35% for IgG PFC). With selection to SRC in B10.A(3R) mice there was only a slight reduction in the anti-SRC response (30% for IgM PFC, 20% for IgG PFC). A comparable small reduction was observed in one other experiment but was not seen in two further experiments (data not shown).

The crucial finding was that in marked contrast to selection in B10.A(4R) and B10.A(3R) mice, selection to SRC was near-complete in (4R × 3R)F_1 mice. In this situation, as with selection in B10.A mice, anti-SRC responses were reduced by >95%.

Consecutive Negative Selection to SRC in B10.A(4R) and B10.A(3R) Mice. The finding that selection of CBA T cells was near-complete in (4R × 3R)F_1 mice implied that selection of the second subgroup of T cells depended upon the filtration host expressing both I-A^k and I-E^k subregion determinants. If these restricting elements had to be present on the same antigen-presenting cells, the residual helper function of T cells filtered with SRC through B10.A(4R) mice should remain unchanged if these T cells were subsequently filtered with SRC though B10.A(3R) mice.

The experiment shown in Table IV confirms this prediction. Whereas CBA T cells underwent near-complete selection to SRC in (4R × 3R)F_1 mice, consecutive exposure of CBA T cells to SRC in B10.A(4R) and then B10.A(3R) mice reduced the anti-SRC response with CBA B cells by <50%, i.e., as with selection to SRC in B10.A(4R) alone.

Discussion

In attempting to interpret the above findings it is first necessary to consider the biochemistry of *I*-region gene products. Ia molecules present on B cells and a subpopulation of macrophage-like cells fall into at least two categories (9–12). Each set of molecules consists of two subunits, a small β chain and a larger α chain. For one class of molecules, both chains (A_α, A_β) are encoded by genes mapping in the *I-A* subregion. The α and β chains (E_α, E_β) of the second class of molecules, by contrast, are encoded by two separated genes (E_α, A_e) situated in the *I-E* and *I-A* subregions, respectively.

Because both sets of Ia molecules are structurally homologous, have similar tissue

TABLE IV

Consecutive Negative Selection of CBA T Cells to SRC in B10.A(4R) Mice and Then B10.A(3R) Mice

Irradiated hosts used for selection of CBA T cells*	*I* region of selection host *A B J E C*	SRC added during selection	PFC/spleen with CBA B cells*		
			Anti-SRC		Anti-HRC
			IgM	IgG	IgG
(4R × 3R)F_1	$\frac{k\ b\ b\ b\ b}{b\ b\ b\ k\ d}$	−	11,820(1.08)‡[1]	40,660(1.08)[3]	24,770(1.20)
(4R × 3R)F_1	$\frac{k\ b\ b\ \bar{b}\ b}{b\ b\ b\ k\ d}$	+	970(1.22)	1,710(1.29)	25,790(1.19)
4R	$\frac{k\ b\ b\ \bar{b}\ b}{}$	+	6,200(1.10)[2]	21,130(1.20)[4]	21,340(1.11)
4R then 3R	$\frac{\bar{k}\ b\ b\ b\ b}{\bar{b}\ b\ b\ k\ d}$	+ +	11,540(1.10)	26,170(1.05)	34,140(1.40)

* As for Table I. For consecutive negative selection, 10^8 nylon wool-purified CBA T cells plus 1 ml of 50% SRC were transferred to 10 irradiated B10.A(4R) mice. TDL pooled from these mice 18–40 h later yielded a total of 160 × 10^6 cells. These cells were then transferred in a dose of 80 × 10^6 cells plus SRC into two irradiated B10.A(3R) mice. These mice yielded a total of 33 × 10^6 cells of which >95% were resistant to lysis by CBA anti-B6 alloantiserum and complement. These twice-filtered T cells were then used as T helper cells. Subtracted background PFC values for B cells transferred without T cells were <650 PFC/spleen.

‡ *P* values: 1 vs. 2: <0.01, 3 vs. 4: <0.02.

distributions and serve as strong alloantigens (13), one would expect a corresponding similarity in their capacity to act as restricting elements. The literature on this point however is confusing. Studies designed to map T cell-restricting elements appear to implicate only the *I-A*-encoded set of molecules because T cells interact well with I-A-compatible, I-E-incompatible cells but not with I-A-incompatible, I-E-compatible cells (4–8). To invoke a role for I-A/E hybrid molecules one thus has to postulate that restriction by these molecules is controlled not by the E_α chain alone but by hybrid determinants created by the association of the E_α and E_β chains (or possibly by the E_β chain alone [*vide infra*]).

A corollary to this assumption is that conventional mapping studies cannot reveal the presence of T cells restricted by I-A/E hybrid molecules. The presence of these cells will always be masked by the accompanying I-A-restricted T cell subset. For example, the action of the I-A-restricted subgroup alone is sufficient to account for the effective interactions observed with I-A-compatible, I-E-incompatible cells. To demonstrate the existence of the I-A/E-restricted subset would necessitate prior removal of the I-A-restricted cells. The prediction here would be that, in contrast to normal T cells, T cells depleted of the I-A-restricted subset would interact only with cells which were matched at both the *I-A* and *I-E* subregions. A precedent for this notion comes from studies on the phenomenon of α-β *Ir* gene complementation, where responsiveness to certain antigens, e.g. poly(L-Glu,L-Lys,L-Phe) (GLϕ) is controlled by two complementing genes (α and β) situated in the *I-E* and *I-A* subregions, respectively (14, 15). Schwartz et al. (16–18) argue that this phenomenon reflects the fact that, in responder haplotypes, the antigen makes an immunogenic association only with the set of I-A/E hybrid molecules and not with the *I-A*-encoded molecules. The functional deletion of the I-A-restricted cells in this situation thus reveals a T cell subset which interacts only with cells matched at both the *I-A* and *I-E* subregions.

The simplest interpretation of the present data is that CBA T cells comprise a mixture of cells restricted by $I\text{-}A^k$ ($A_\beta{}^k$-$A_\alpha{}^k$) and $I\text{-}A^k/E^k$ ($E_\beta{}^k$-$E_\alpha{}^k$) molecules, respectively; restriction by these molecules applies both during selection to antigen (a manifestation of T-macrophage interaction [3]) and during collaboration with specific B cells (7). With selection of CBA (*I-A*^{k}, *I-E*^{k}) (*kk*) T cells to SRC in irradiated B10.A(4R) (*kb*) mice, the anti-SRC T helper function of the selected cells for B10.BR B cells was reduced by <50%; in marked contrast, help for B10.A(4R) B cells was abolished (Table II). Such findings imply that selection to SRC in B10.A(4R) mice affects only the $I\text{-}A^k$-restricted subset of T cells, i.e., cells which can interact with either CBA (or B10.BR) (*kk*) B cells or with B10.A(4R) (*kb*) B cells. The subset of $I\text{-}A^k/E^k$-restricted T cells fails to undergo selection to SRC in B10.A(4R) mice and, on transfer, these cells collaborate only with B cells which express $I\text{-}A^k/E^k$ molecules, i.e., CBA or B10.BR B cells but not B10.A(4R) B cells.

Data from three approaches suggested that the second subset of T cells was restricted by $I\text{-}A^k/E^k$ hybrid molecules per se rather that by determinants encoded solely in the *I-E* region (or in other regions telomeric to the *I-A* subregion). Firstly, in contrast to selection in B10.A(4R) mice, selection to SRC in I-A-incompatible, I-E-compatible B10.A(3R) mice had little if any effect on the helper responses for CBA B cells (Table III).[2] Secondly, the residual helper function of CBA T cells for CBA B

[2] That selection to SRC in B10.A(3R) mice did lead to a small (~20%) reduction in anti-SRC T helper responses in two experiments (though not in two others) might indicate the presence of an additional small subgroup of I-E-restricted T cells (19). We could find no evidence for the existence of such cells in a study involving positive selection of H-2 heterozygous T cells (20).

cells after selection to SRC in B10.A(4R) mice remained unchanged when the cells were subsequently selected to SRC in B10.A(3R) mice (Table IV). Thirdly, near-complete selection of T cell help for CBA B cells occurred with selection to SRC in (4R × 3R)F_1 mice, i.e., where I-A^k/E^k hybrid molecules can be created by *trans* chain association (E_β^k from 4R and E_α^k from 3R) (Tables III and IV).

The notion that I-A/E hybrid molecules act as restriction elements is in line with our previous findings on the capacity of anti-Ia^k antibodies to block positive selection of F_1 T cells to SRC (20). These studies showed that (CBA × B6)F_1 T cells contained at least two subgroups of Ia^k-restricted T cells, only one of which could be blocked by monoclonal anti-I-A^k antibody. By exclusion it was argued that the other T cell subgroup was probably restricted by I-A/E hybrid molecules. Strong support for this conclusion has come from recent studies showing that positive selection of the second T cell subset can be blocked with a monoclonal antibody specific for I-A/E hybrid molecules (J. Sprent and E. Lerner, unpublished data).

The argument that the specificity of I-A/E-restricted T cells is directed to unique hybrid determinants on the I-A/E molecules needs qualification. An alternative possibility is that the restriction is mediated entirely by the E_β chain. To sustain this notion one has to account for the apparent failure of the I-A/E-restricted T cells to undergo selection in B10.A(4R) mice, i.e., mice with the E_β^k allele. Here it is crucial to point out that the E_β chain is not expressed on the cell surface in B10.A(4R) mice. The studies of Jones et al. (10) have demonstrated that the cell surface expression of the E_β chain is controlled by the E_α chain. In strains in which the E_α chain is not detectable, e.g., B10.A(4R), the E_β chain remains in the cytoplasm and is therefore unavailable to mediate restriction. Apropos the relative contributions of the α and β chains to the restriction sites on the I-A/E molecule, it should be pointed out that, unlike the E_β chain, the E_α chain is relatively nonpolymorphic. Hence, once the E_α-E_β complex is expressed on the cell surface, it is quite conceivable that the E_β chain alone restricts T cell function. Excluding this possibility is clearly difficult. On this point it is worth mentioning that Kimoto and Fathman (21) have reported that *trans* association of the *I-A*-encoded A_α and A_β chains can create unique T cell-restricting elements. Because both of these chains are highly polymorphic, in this situation each chain must contribute to the specificity of the restriction sites on the molecule.

That the E_α chain is not expressed in certain haplocytes, e.g., B10.A(4R) and B10, implies that T cells from such strains lack a population of I-A/E-restricted T cells. If so, complete selection of T cells from these strains would require donor-host matching only in the *I-A* subregion and not in the *I-E* subregion. Support for this prediction has come from the finding that selection of the anti-SRC helper response of B10.A(4R) (*kb*) T cells for syngeneic B cells is as effective in I-E-incompatible B10.BR (*kk*) mice as in syngeneic B10.A(4R) mice (unpublished data).

In view of the evident complexity of the I region, it is conceivable that there are in fact a multiplicity of different Ia-restricted T cell subsets. Thus, in addition to subsets restricted by A_α-A_β and E_α-E_β molecules, there might also exist T cells restricted by other possible associations of these chains, i.e., A_α-E_β (18) and E_α-A_β. Similarly, one could envisage restriction by as yet undiscovered *I-A*- or *I-E*-encoded elements. There is also the possibility of restriction controlled by genes situated in other Ia subregions, i.e., in the *I-B*, *-J*, or *-C* subregions. A priori, this might seem unlikely because donor-host matching limited only to the *I-A* and *I-E* subregions [selection in (4R × 3R)F_1

mice] was sufficient to obtain near-complete selection of CBA T cells. Moreover, there is no direct evidence that the *I-B*, *-J*, or *-C* subregions contributes to the expression of the typical Ia molecules present on B cells and macrophages, i.e., the most likely molecules for mediating restriction. It is nevertheless possible that these subregions might encode nonpolymorphic chains capable of forming unique restriction elements by associating with one of the known polymorphic chains of the I-A subregion. Assessing these various possibilities will require more detailed information on the biochemistry of *I*-region gene products.

Summary

Previous studies have shown that negative selection of T cells to sheep erythrocytes (SRC) after adoptive transfer to irradiated mice requires a sharing of H-2 determinants between the donor T cells and the selection hosts. This paper examines which part of the *H-2* complex controls selection. The results show that, in the case of T cells of the $H\text{-}2^k$ haplotype, complete selection occurs with donor-host matching limited to the *I-A* through *I-E* subregions of the *H-2* complex. Selection to SRC was partial in I-A-compatible, I-E-incompatible hosts, minimal or not detectable in I-A-incompatible, I-E-compatible hosts, but near-complete in hosts matched at both the *I-A* and *I-E* subregions. Consecutive selection in hosts matched solely at (*a*) the *I-A* subregion and (*b*) the *I-E* subregion led to incomplete selection. From these and other findings it is argued that $H\text{-}2^k$ T cells comprise a mixture of T cells restricted by I-A and I-A/E hybrid molecules.

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