Brief Definitive Report

INHIBITION OF T CELL ACTIVATION IN VIVO WITH MIXTURES OF MONOCLONAL ANTIBODIES SPECIFIC FOR I-A AND I-A/E MOLECULES*

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Recently it was found that broad spectrum anti-Ia^k (A.TH anti-A.TL) antiserum could block the activation of Ia^k-restricted F_1 T helper cells in vivo (1). Thus, (CBA × B6)F_1 (*H-2*^{k} × *H-2*^{b}) T cells exposed to sheep erythrocytes (SRC) for 5 d in irradiated F_1 mice in the presence of anti-Ia^k antibody provided high levels of help for B10 (*I-A*^{b}, *I-B*^{b}, *I-J*^{b}, *I-E*^{b}, *I-C*^{b}) (*bbbbb*) or F_1 B cells, but not for B10.BR (*kkkkk*) or B10.A(4R) (*kbbbb*) B cells. Surprisingly, monoclonal anti-I-A^k antibody failed to inhibit the generation of help for B10.BR or B10 B cells, but effectively blocked help for B10.A (4R) B cells. This finding implied that Ia^k-restricted T cells fall into two subgroups, one set of cells being restricted by I-A^k determinants, and the other restricted by determinants mapping to the right of the *I-A* subregion. In view of the known biochemistry of Ia molecules (2–4), it was argued that this second T cell subgroup was probably restricted by determinants on I-A/E molecules. The latter molecules each consist of an *I-E*-encoded α chain (E_α) associated with an *I-A*-encoded β chain (E_β, also termed A_e); structurally (2–4) and functionally (5), I-A/E (E_β-E_α) molecules resemble the set of I-A (A_β-A_α) molecules, the α and β chains of which are both encoded in the *I-A* subregion.

The notion that Ia^k-restricted T cells comprise a mixture of I-A^k- and I-A^k/E^k-restricted cells implies that the blocking effects of A.TH anti-A.TL antiserum (which is known to include activity for both I-A^k and I-A^k/E^k determinants) could be mimicked by a mixture of monoclonal anti-I-A^k and anti-I-A^k/E^k antibodies. This paper investigates the blocking effects of several monoclonal antibodies, including the hybridoma Y-17, which detects a public specificity on the I-A^k/E^k molecule (5). We show here that although Y-17 alone has no demonstrable effect on T helper cell induction, a mixture of Y-17 plus anti-I-A^k antibody (hybridoma 10-3.6) virtually abolishes the activation of help for Ia^k (B10.BR) B cells.

Materials and Methods

Positive Selection in the Presence of Anti-Ia Antibody. As described in detail elsewhere (1), 7×10^7–12×10^7 nylon wool-purified (CBA × B6)F_1 T cells plus 0.5 ml of 25% SRC were injected intravenously into (CBA × B6)F_1 mice exposed to 900 rad 2 d earlier. The T cell recipients (one mouse/group) were injected intraperitoneally with large doses of anti-Ia antibody; one half of the dose was given 2–4 h before the injection of T cells plus SRC and the other half was given

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TABLE I

Positive Selection of (CBA × B6)F_1 T Cells to SRC in Irradiated F_1 Mice in the Presence of Monoclonal Anti-I-A^k, Anti-I-A^b, and Anti-I-$A^{k,b}/E^k$ Antibodies

Irradiated hosts used for positive selection of (CBA × B6)F_1 T cells to SRC*	Monoclonal antibodies added during selection	SRC added during selection	B cells for measuring T helper function‡	Anti-SRC PFC/spleen in irradiated F_1 mice	
				IgM	IgG
(CBA × B6)F_1	None	+	B10.BR	4,930 (1.19)§	25,710 (1.28)
			B10.A(4R)	3,480 (1.24)	11,240 (1.33)
			B10	5,360 (1.34)	8,480 (1.22)
(CBA × B6)F_1	None	−	B10.BR	320 (1.13)	<100
			B10.A(4R)	<100	<100
			B10	650 (1.07)	<100
(CBA × B6)F_1	Anti-I-A^k (public) (10-3.6)	+	B10.BR	12,590 (1.33)	25,410 (1.07)
			B10.A(4R)	350 (1.21)	<100
			B10	8,400 (1.12)	13,840 (1.21)
(CBA × B6)F_1	Anti-I-A^k (private) (11-5.2)	+	B10.BR	11,420 (1.11)	23,570 (1.21)
			B10.A(4R)	280 (1.14)	<100
			B10	5,570 (1.12)	8,090
(CBA × B6)F_1	Anti-I-A^b (public) (BP107)	+	B10.BR	7,520 (1.27)	25,580 (1.29)
			B10.A(4R)	5,800 (1.22)	10,910 (1.14)
			B10	250 (1.07)	820 (1.77)
(CBA × B6)F_1	Anti-I-$A^{k,b}/E^k$ (public) (Y-17)	+	B10.BR	7,530 (1.36)	23,780 (1.11)
			B10.A(4R)	8,760 (1.04)	19,650 (1.20)
			B10	18,090 (1.10)	16,410 (1.57)
(CBA × B6)F_1	Anti-I-A^k (10-3.6) plus anti-I-$A^{k,b}/E^k$ (Y-17)	+	B10.BR	1,220 (1.24)	810 (1.57)
			B10.A(4R)	870 (1.03)	320 (2.00)
			B10	11,940 (1.17)	13,900 (1.31)

* 10^8 unprimed nylon wool-purified (CBA × B6)F_1 lymph node T cells plus 0.5 ml ml of 25% SRC were transferred intravenously into (CBA × B6)F_1 mice exposed to 900 rad 2 d earlier. Anti-Ia antibody (2 ml/mouse, 4 ml for a mixture of 10-3.6 and Y-17) was given in divided doses intraperitoneally; 1 ml was injected 4 h before T cells plus SRC and the remainder was injected 1 d later. T cells were recovered from spleen plus mesenteric lymph nodes of recipients on day 5 after T cell injection; T cell recoveries were comparable to those reported previously (1). Small doses (0.5×10^6) of T cells were transferred with B cells plus 0.1 ml of 5% SRC intravenously into mice given 700 rad 1 d earlier. PFC were measured on day 7.

‡ SRC-primed B cells (anti-Thy-1.2-treated spleen) were transferred in doses of 5×10^6 viable cells for B10.BR, 10^7 for B10.A(4R), and 12×10^6 for B10.

§ Geometric mean; three mice per group. Figures in parentheses refer to values 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 <100 to 1,600 PFC/spleen. T cells alone gave <200 PFC/spleen.

1 d later. T cells were recovered from the spleen plus mesenteric lymph nodes of the hosts 5 d after injection and washed twice. Small doses ($\simeq 6 \times 10^5$) of T cells plus SRC were transferred intravenously with B cells (anti-Thy-1.2-treated SRC-primed spleen) into irradiated (750 rad) F_1 mice. Direct (IgM) and indirect (IgG) plaque-forming cells (PFC) were measured in the spleen 1 wk later.

Monoclonal Anti-Ia Antibodies. Hybridoma 10-3.6 (IgG_{2a}) detects a public I-A specificity (Ia.17) expressed by *k*, *f*, *r*, and *s* haplotypes, but not by *b*, *d*, *p*, or *q* haplotypes (6). Hybridoma 11.5.2 (IgG_{2b}) detects a private I-A specificity (Ia.2) found only in mice of the *k* haplotype (6). These two hybridomas were kindly made available by the Herzenberg group, Stanford University School of Medicine, Stanford, Calif. Hybridoma BP107 (IgG_1) detects a public I-A specificity expressed by *b*, *d*, *p*, *q*, *u*, and *j* haplotypes, but not by *k*, *f*, *r*, or *s* haplotypes.[1] Hybridoma Y-17 (IgG_{2b}) detects a public specificity present on a variety of I-A/E molecules, including E_β^k-E_α^k and E_β^b-E_α^k (5); Y-17 does not have detectable reactivity for I-A molecules.

[1] Symington, F. W., and J. Sprent. 1981. A monoclonal antibody detecting an Ia specificity mapping in the I-A or I-E subregion. Immunogenetics. In press.

TABLE II

Positive Selection of (CBA × B6)F_1 T Cells to SRC in Irradiated F_1 Mice in the Presence of Mixture of Monoclonal Anti-I-A^k, Anti-I-A^b, and Anti-I-$A^{k,b}/E^k$ Antibodies

Irradiated hosts used for positive selection of (CBA × B6)F_1 T cells to SRC*	Monoclonal antibodies added during selection	B cells for measuring T helper function‡	Total (IgM + IgG) anti-SRC PFC/spleen in irradiated F_1 mice
(CBA × B6)F_1	None	B10.BR	57,180 (1.13)§
		B10.A(4R)	47,360 (1.05)
		B10	68,380 (1.06)
		(4R × B10)F_1	44,890 (1.03)
		(CBA × B6)F_1	42,810 (1.18)
(CBA × B6)F_1	Anti-I-A^k (10-3.6) plus anti-I-A^b (BP107)	B10.BR	53,960 (1.16)
		B10.A(4R)	1,480 (1.48)
		B10	6,920 (1.29)
		(4R × B10)F_1	4,750 (1.52)‖
		(CBA × B6)F_1	30,200 (1,12)
(CBA × B6)F_1	Anti-I-A^k (10-3.6) plus anti-I-A^b (BP107) plus anti-I-$A^{k,b}/E^k$ (Y-17)	B10.BR	2,210 (1.21)
		B10.A(4R)	1,390 (1.43)
		B10	2,450 (1.99)
		(4R × B10)F_1	400 (1.76)‖
		(CBA × B6)F_1	1,670 (1.21)

* Anti-Ia antibodies were mixed before injection and injected in a total dose of 2 ml for 10-3.6, 2 ml for BP107, and 1 ml for Y-17; as in Table I, the antibodies were injected in two divided doses.

‡ (4R × B10)F_1 and (CBA × B6)F_1 B cells were injected in doses of 7×10^6 and 5×10^6, respectively.

§ Substracted background values for B cells transferred without T cells ranged from 200 to 2,400 PFC/spleen. Total PFC are shown because numbers of IgM PFC were very variable in this experiment.

‖ The apparent difference here was not seen in other experiments.

Antibodies from all four hybridomas were obtained from ascites fluid of mice sustaining the growth of the tumors. Antibody titers ranged from 5×10^4 to 10^6.

Results

(CBA × B6)F_1 T cells activated to SRC in F_1 mice for 5 d in the absence of anti-Ia antibody collaborated well with B10.BR, B10.A(4R), and B10 B cells after secondary transfer to irradiated F_1 mice (Table I); minimal helper responses were obtained if SRC were omitted during positive selection. As reported previously (1), T cell selection in the presence of anti-I-A^k antibody from the hybridoma 10-3.6 blocked T helper responses for B10.A(4R) B cells, but not for B10.BR or B10 B cells (Table I). 10-3.6 detects a public I-A determinant present on I-$A^{k,f,r,s}$ strains but not on I-A^b (Materials and Methods). Table I shows that similar blocking effects occur with 11-5.2 antibody, a reagent that detects a private specificity unique to I-A^k. BP107, which reacts with a public specificity on I-A^b but not I-A^k, inhibited helper function for B10 B cells, but not for B10.BR or B10.A(4R) B cells.

Y-17 antibody detects public determinants present on I-A^k/E^k and I-A^b/E^k molecules, but not on I-A^k or I-A^b molecules. By itself, Y-17 antibody caused no reduction in the generation of help for B10.BR, B10.A(4R), or B10 B cells (Table I). In marked contrast, selection in the presence of a mixture of Y-17 and 10-3.6 (anti-I-A^k) antibody caused a profound reduction in help for both B10.BR and B10.A(4R) B cells; help for B10 B cells was not affected.

Table II shows that selection of F_1 T cells in the presence of a mixture of anti-I-A^k

(10-3.6) and anti-I-A^b (BP107) antibodies blocked help not only for B10.A(4R) and B10 B cells, but also for (4R × B10)F_1 B cells. Help for B10.BR and (CBA × B6)F_1 B cells was maintained or reduced only slightly (the small reduction in help for (CBA × B6)F_1 B cells in Table II was seen in two further experiments but not in two others). Significantly, the addition of anti-I-$A^{k,b}/E^k$ (Y-17) antibody to the mixture of anti-I-A^k and anti-I-A^b antibodies blocked help for all five B cell populations (Table II).

Discussion

The finding that a mixture of anti-I-A^k and anti-I-$A^{k,b}/E^k$ antibodies virtually abolished the generation of help for B10.BR (*I-A*^{k}, *I-E*^{k}) (*kk*) B cells implies that the restricting elements for Ia^k-specific T cells are situated largely, and perhaps entirely, on I-A and I-A/E molecules. Both the α and β chains of the I-A^k/E^k molecule appear to contribute to the T cell-restricting site because the positive selection of (B6 × A/J)F_1 T cells to SRC in irradiated B10.A (*kk*) mice does not generate help for B10.A(5R) (*bk*) B cells and vice versa (1).[2] Similarly, complete negative selection of homozygous Ia^k T cells requires donor-host matching at both the *I-A* and *I-E* subregions (7).

At face value it might seem surprising that, individually, neither anti-I-A^k nor anti-I-$A^{k,b}/E^k$ antibodies impaired the generation of help for B10.BR B cells; in the case of anti-I-A^k antibody, this finding applied even with limiting doses of T helper cells (1). It was suggested previously that the inhibition of the I-A^k-restricted T cells by anti-I-A^k antibody might increase the in vivo "space" available for expansion of the I-A^k/E^k-restricted cells. By the same token, injection of anti-I-$A^{k,b}/E^k$ antibody might potentiate the expansion of the I-A^k-restricted cells; these cells would then give high levels of help for both B10.BR and B10.A(4R) B cells and thereby conceal the inhibition of selection of the I-A^k/E^k-restricted cells. According to this view, anti-I-A^k/E^k antibody would only appear effective if the I-A^k-restricted cells were blocked (e.g., by simultaneous injection of anti-I-A^k antibody) or were absent from the T cells used for selection. In this respect one should mention that Y-17 antibody alone is very effective at blocking proliferative responses to clones of I-A/E-restricted T cells (8).

The evidence of Kimoto and Fathman (9) for the existence of T cell clones restricted by (4R × B10)F_1 cells, but not by B10.A(4R) or B10 cells, suggests that I-A (A_α-A_β) dimers can form unique restriction elements by *trans* chain association in heterozygotes. On this point, it is of interest that selection of (CBA × B6)F_1 T cells to SRC in the presence of a mixture of anti-I-A^k and anti-I-A^b antibodies markedly impaired help for (4R × B10)F_1 B cells (Table II). This finding might indicate that the two antibodies together have specificity for all four of the possible A_α-A_β dimers on (4R × B10)F_1 cells. Alternatively, SRC-specific T cells specific for the hybrid I-A molecules on (4R × B10)F_1 cells may not exist; this possibility is being investigated.

In addition to four A_α-A_β dimers, (CBA × B6)F_1 macrophages and B cells presumably express two sets of E_β-E_α dimers, viz., E_β^k-E_α^k and E_β^b-E_α^k; dimers of E_β^b-E_α^b and E_β^k-E_α^b probably do not exist because there is no known gene product of the *I-E*^{b} allele.[2] Because the Y-17 antibody has reactivity for both E_β^k-E_α^k and E_β^b-E_α^k determinants (5), a mixture of Y-17, anti-I-A^k, and anti-I-A^b antibodies might be expected to have specificity for all of the six postulated restriction elements on F_1

[2] Because the E_α chain is relatively nonpolymorphic, the possibility that the E_β subunit alone controls restriction has not been excluded. It should be mentioned that in strains that lack an E_α chain, e.g., B10.A(4R) and B10, the E_β chain remains in the cytoplasm (2–4).

cells. The fact that a cocktail of these three antibodies did indeed substantially reduce help for (CBA × B6)F_1 B cells (Table II) is consistent with this view.

Summary

(CBA × B6)F_1 (Ia^k × Ia^b) T cells were activated to sheep erythrocytes in irradiated F_1 mice in the presence of various monoclonal anti-Ia reagents and then tested for their capacity to collaborate with B cells from B10.BR (*I-A*^{k}, *I-E*^{k}) (*kk*), B10.A(4R) (*kb*), and B10 (*bb*) mice. Anti-I-A^k antibodies blocked the generation of help for B10.A(4R) B cells, but not for B10.BR or B10 B cells. An anti-I-A^b antibody blocked help for B10 B cells, but not for B10.BR or B10.A(4R) B cells. An antibody (Y-17) specific for I-A^k/E^k and I-A^b/E^k molecules, but not for I-A^k or I-A^b molecules, failed to impair the generation of help for B10.BR, B10.A(4R), or B10 B cells.

In marked contrast to injecting each antibody separately, a mixture of anti-I-A^k and anti-I-$A^{k,b}$/E^k (Y-17) antibodies virtually abolished the generation of help for B10.BR B cells. A mixture of anti-I-A^k and anti-I-A^b antibodies effectively blocked help for (4R × B10)F_1 B cells, i.e., cells expressing hybrid I-A molecules. These two antibodies only marginally impaired help for (CBA × B6)F_1 B cells. To block help for (CBA × B6)F_1 B cells required selection in the presence of a cocktail of anti-I-A^k, anti-I-A^b, and anti-I-$A^{k,b}$/E^k antibodies. The implications of these findings are discussed.

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