Published August 1, 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*

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Optimal stimulation of T cells by antigen in vitro requires the presence of macrophages (1). The precise function of these cells in antigen presentation is controversial. A number of workers contend that macrophages render antigen immunogenic by presenting T cells with a complex of antigen bound to major histocompatibility complex (MHC)[1] determinants (2–6). Certain of these groups (3, 6), though not others (5), conclude that T-cell activation depends upon macrophages and the responding T cells sharing MHC determinants. By contrast, other groups argue that at least for some antigens, the main role of macrophages in vitro is to release factors which promote cell viability (7, 8).

It is important to establish whether macrophages and MHC gene products play a role in T-cell activation in vivo. The approach adopted in the present paper to study this problem is based on the observation that T cells encountering specific antigen in vivo leave the circulation, e.g. thoracic duct lymph, for a period of 1–2 days (9–12). During this stage of negative selection the cells become selectively sequestered in regions where the antigen is concentrated, e.g. the spleen. Here the cells proliferate extensively before re-entering the circulation in expanded numbers after 4–5 days—the stage of positive selection.

This chain of events could either reflect T-cell stimulation by antigen per se or, alternatively, by antigen processed by macrophages or related cells. If the second possibility were correct and, in addition, if the macrophages presenting the antigen had to be MHC-compatible with the T cells, then activation of T cells to antigen should not occur in an MHC-different environment.

The present studies verify this prediction with the demonstration that purified T cells transferred with a particulate antigen (heterologous erythrocytes) into irradiated mice fail to undergo either negative or positive selection to the antigen unless the donor and host share *H-2* determinants.

Materials and Methods

Mice. CBA/J (CBA) (*H-2*^{k}), C57BL/6 (B6) (*H-2*^{b}), C57BL/10 (B10) (*H-2*^{b}), B10.D2 (*H-2*^{d}), BALB/c (*H-2*^{d}), C3H/He (H-2^k), and (B6 × DBA/2 (*H-2*^{d}))F$_1$ mice were obtained from The

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

[1] *Abbreviations used in this paper:* HRC, horse erythrocytes; LN, lymph nodes; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRC, sheep erythrocytes; TDL, thoracic duct lymphocytes.

Jackson Laboratory, Bar Harbor, Maine. (CBA × B6)F_1 mice were obtained from Cumberland View Farms, Clinton, Tenn.

Media. RPMI-1640 (Microbiological Associates, Walkerville, Md.) supplemented with 10% fetal calf serum was used.

Injections. All suspensions of lymphoid cells, sheep erythrocytes (SRC), and horse erythrocytes (HRC) were given intravenously.

Cells. Thoracic duct lymphocytes (TDL) were obtained as described elsewhere (13). Suspensions of lymph node (LN) cells were prepared by teasing with fine forceps through an 80-mesh stainless steel sieve in cold medium.

Irradiation. Mice were exposed to ^{137}Cs γ-irradiation at a dose rate of 106 rads/min (14).

Cell Identification with Alloantisera. CBA anti-B6, B6 anti-CBA, and CBA anti-DBA/2 *H-2*-alloantisera and anti-Thy 1.2 (AKR anti-C3H thymus) antiserum were prepared as described elsewhere (14). For cell identification after negative selection, TDL were treated with alloantisera and complement by a two-step procedure (14). Cytotoxic indices were established with respect to control samples incubated with antisera without complement or with normal mouse serum plus complement. Percent lysis with the control samples was invariably <5%.

T-Cell Purification. The T cells used for negative selection were obtained from pooled mesenteric, axillary, cervical, and inguinal LN. Most Thy 1.2-negative cells were removed by passing cells over nylon-wool columns (15). The effluent T cells were >90% Thy 1.2-positive.

Preparation of B Cells. Spleen cells from mice primed with both SRC and HRC 2–4 mo previously were treated with anti-Thy 1.2 antiserum and complement as described previously (14).

Measurement of T-B Collaboration. Unless stated otherwise, the helper function of CBA T cells after negative selection was studied by transferring the cells in small doses (2×10^6) into irradiated (700 rads) CBA mice together with SRC and HRC (0.1 ml of 5% solution of each) and 5×10^6 anti-Thy 1.2-serum-treated spleen cells from SRC- and HRC-primed CBA mice as a source of B cells. Direct (IgM) and indirect (IgG) plaque-forming cells (PFC) to both SRC and HRC were then measured in the spleens of the recipients 7 days later (14).

Priming with Heterologous Erythrocytes. Unless stated otherwise, the T and B cells were both prepared from mice primed intraperitoneally with 0.2 ml of a 25% solution of a mixture of SRC and HRC 2–4 mo previously.

Statistical Analysis. Geometric means and values used to derive upper and lower limits of the SE of the mean (these values represent the anti-log of SE of the $\log_{10}$ geometric mean) were calculated from the $\log_{10}$ of the PFC counts. *P* values were determined by Students' *t* test. In the comparison of the mean of any two groups of observations a significance level of 0.05 was chosen.

Results

Experimental Design. The general plan of the experiments was to inject purified CBA T cells into irradiated syngeneic or allogeneic mice together with SRC and study the helper function of the donor-derived T cells recovered from thoracic duct lymph of the recipients 1–2 days later. The T cells were obtained from LN of mice primed to both SRC and HRC 2–4 mo previously. Before transfer, the T cells were depleted of B cells (and presumably most macrophages) by nylon wool filtration. The effluent T cells (>90% Thy 1.2-positive) were transferred intravenously in a dose of 10^8 viable cells together with SRC (0.5 ml of 50% solution) into mice that had received 900 rads 1 day before. Thoracic duct fistulas were inserted in the recipients 20 h later and TDL were collected between 24 and 40 h postinjection. Testing with anti-Thy 1.2 antiserum and complement showed that the lymph-borne cells consisted almost entirely (≥97%) of T (Thy 1.2-positive) cells. In situations where the cells were filtered through *H-2*-incompatible hosts, testing with appropriate alloantisera showed that the lymph-borne cells were invariably > 90% of donor origin. Cell viability was 99–100% and the yield of cells (compared with the numbers initially injected) was in the order of 10–15%.

TABLE I

Helper Function of Primed CBA T Cells Negatively Selected to SRC in Irradiated CBA Mice. T Cells Harvested from Lymph at 1 Day Post-Transfer

T-Cell group	Helper cells* (2.5×10^6)	SRC Added during negative selection	B Cells‡	PFC/Spleen at 7 days in irradiated CBA mice			
				Anti-SRC		Anti-HRC	
				IgM	IgG	IgM	IgG
A	CBA T Cells → irradiated CBA	–	CBA	11,880(1.09)§	36,530(1.25)	6,020(1.17)	44,670(1.16)
B	CBA T Cells → irradiated CBA	+	CBA	0	0	5,730(1.44)	36,620(1.45)
	Group A + group B (2.5×10^6 of each)		CBA	11,460(1.18)	42,960(1.34)	13,580(1.12)	79,530(1.04)

* 10^8 Nylon-wool-passed LN T cells from CBA mice primed with both SRC and HRC 2–4 mo previously were transferred intravenously ± 0.5 ml of 50% SRC into CBA mice given 900 rads 1 day before. Mice were cannulated at 20 h postinjection and TDL collected between 24 and 40 h. To measure helper function, the lymph-borne T cells were transferred with B cells, SRC, and HRC into irradiated (700 rads) CBA mice.

‡ 5×10^6 Viable anti-Thy 1.2-serum-treated spleen cells from mice primed with SRC and HRC.

§ Geometric mean of four mice per group; figure in parenthesis refers to value by which mean is multiplied by or divided by 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; 1,850 (1.24) (IgM SRC), 2,080 (1.73) (IgG SRC), 1.140 (1.18) (IgM HRC), 1,670 (1.68) (IgG HRC). Values for T cells transferred without B cells were all < 100 PFC/spleen.

To study their helper function the lymph-borne cells were transferred in a dose of 2×10^6 cells into irradiated (700 rads) CBA mice together with SRC and HRC (0.1 ml of 5% solution of each) and syngeneic B cells (5×10^6 anti-Thy 1.2-serum-treated spleen cells from CBA mice primed with both SRC and HRC). Numbers of direct (IgM) and indirect (IgG) PFC to SRC and HRC were then measured in the spleen 7 days later.

Negative Selection in Syngeneic Mice. When CBA T cells were transferred into irradiated CBA mice and recovered from thoracic duct lymph of the recipients 1 day later, the cells provided effective help for both SRC and HRC (Table I). By contrast, CBA T cells filtered through irradiated CBA mice in the presence of SRC failed to stimulate either IgM or IgG anti-SRC PFC responses. This abolition of help for SRC was specific since good responses were observed against HRC. The phenomenon did not appear to be due to suppression since the injection of both TDL populations together gave high responses. (For simplicity, background values obtained when B cells were transferred without T cells have been subtracted from the data shown in Table I and in subsequent tables; these values are shown in the footnotes to the tables.)

The specific unresponsiveness of T cells taken from the central lymph at 1 day after cell transfer was followed by a period of hyper-reactivity where the lymph-borne cells gave markedly increased responses to the injected antigen. Thus, as illustrated in Table II, T cells collected from the lymph at 5 days post-transfer, i.e. during the stage of positive selection to antigen, gave high IgM and IgG anti-SRC responses with as few as 0.5×10^6 T cells. These responses were over 40-fold higher than with the same dose of cells taken from irradiated mice given T cells but not antigen 5 days before. Responses against HRC were low with both T-cell populations unless higher doses of helper cells (2.5×10^6) were used.

Negative Selection in Allogeneic Mice. The negative selection to SRC observed when CBA ($H\text{-}2^k$) T cells were filtered from blood to lymph for 1 day

TABLE II

Helper Function of Primed CBA T Cells Positively Selected to SRC in Irradiated CBA Mice. T Cells Harvested from Lymph at 5 Days Post-Transfer

Helper cells*	SRC Added during positive selection	Dose of helper cells	B cells‡	PFC/Spleen at 7 days in irradiated CBA mice			
				Anti-SRC		Anti-HRC	
				IgM	IgG	IgM	IgG
CBA T Cells → irradiated CBA		0.5×10^6	CBA	460 (1.40)§‖	1,120 (1.54)‖	220 (1.77)‖	2,350 (1.30)‖
		2.5×10^6	CBA	4,420 (1.26)	20,320 (1.38)	1,890 (1.26)	18,700 (1.16)
CBA T Cells → irradiated CBA	+	0.5×10^6	CBA	8,280 (1.15)	51,180 (1.18)	670 (1.21)	4,490 (1.22)
	+	2.5×10^6	CBA	16,470 (1.10)	65,860 (1.13)	1,980 (1.15)	24,480 (1.07)

* As for footnote to Table I except that mice were cannulated to obtain TDL as helper cells at 5 days after T-cell transfer.

‡ As for footnote to Table I.

§ As for footnote to Table I. Subtracted values of B cells transferred without T cells were: 1,760(1.04) (IgM SRC), 2,920 (1.43) (IgG SRC), 630(1.17) (IgM HRC), 4,020(1.22) (IgG HRC). Values for T cells transferred without B cells were < 300 PFC/spleen.

‖ Not significantly above background values of B cells transferred without T cells.

TABLE III

Negative Selection of Primed CBA T Cells to SRC in Semiallogeneic Hosts but not in Allogeneic Hosts. T Cells Harvested from Lymph at 1 Day Post-Transfer

Helper cells* (2×10^6)	SRC Added during negative selection	B Cells‡	PFC/Spleen at 7 days in irradiated CBA mice			
			Anti-SRC		Anti-HRC	
			IgM	IgG	IgM	IgG
CBA T Cells → irradiated B6	–	CBA	5,420(1.33)§	8,670(1.44)	2,950(1.27)	12,510(1.30)
CBA T Cells → irradiated B6	+	CBA	2,560(1.27)	8,740(1.35)	2,240(1.07)	18,700(1.25)
CBA T Cells → irradiated (CBA × B6)F_1	–	CBA	5,240(1.20)	7,550(1.07)	1,180(1.13)	9,520(1.27)
CBA T Cells → irradiated (CBA × B6)F_1	+	CBA	0	110(1.50)‖	2,210(1.40)	11,540(1.08)

*‡ As for footnote to Table I. After filtration, > 94% of the lymph-borne cells were resistant to lysis by CBA anti-B6 alloantiserum; > 97% were lysed with anti-Thy 1.2 antiserum.

§ As for footnote to Table I. Subtracted values of B cells transferred with T cells were: 440 (1.49) (IgM SRC), 130 (1.18) (IgG SRC), 220 (1.42) (IgM HRC), 680 (1.38) (IgG HRC). Values for T cells transferred without B cells were < 200 PFC/spleen.

‖ Not significantly above value of B cells transferred without T cells.

through CBA mice did not occur when the cells were filtered through allogeneic B6 (*H-2*^{b}) mice. Thus, as shown in Table III, acute recirculation of CBA T cells through irradiated B6 mice in the presence of SRC did not affect the helper function of the cells for SRC. This was observed in five separate experiments. In no experiment was the IgG anti-SRC response reduced significantly; the IgM response was reduced by approximately 50% in two experiments (significant in only one experiment – Table III) but was not reduced in three other experiments.

TABLE IV

Negative Selection of Primed (CBA × B6)F_1 T Cells to SRC in Irradiated (DBA/2 × B6)F_1 Mice. T Cells Harvested from Lymph at 1 Day Post-Transfer

T-Cell group	Helper cells* (2×10^6)	SRC Added during negative selection	B Cells‡	PFC/Spleen at 7 days in irradiated (CBA × B6)F_1 mice		
				Anti-SRC		Anti-HRC
				IgM	IgG	IgG
A	(CBA×B6)F_1 T Cells → irradiated (DBA/2 × B6)F_1	–	B6	4,520(1.23)§	19,740(1.15)	14,460(1.10)
B	(CBA × B6)F_1 T cells → irradiated (DBA/2 × B6)F_1	+	B6	0	1,640(1.07)‖	17,190(1.19)
	Group A + group B		B6	5,760(1.13)	26,370(1.20)	39,140(1.31)

*‡ As for footnote to Table I. After filtration, > 90% of the lymph-borne cells were lysed by B6 anti-CBA alloantiserum which had been absorbed with DBA/2 spleen (this antisera lysed 100% of (CBA × B6)F_1 LN but < 10% of (DBA/2 × B6)F_1 LN); > 98% of the cells were lysed by anti-Thy 1.2 antiserum.

§ As for footnote for Table I. Subtracted values of B cells transferred without T cells were: 990(1.34) (IgM SRC), 3,200(1.37) (IgG SRC), 1,100(1.19) (IgG HRC). Values for T cells transferred without B cells were <400 PFC/spleen.

‖ Not significantly above background of B cells transferred without T cells.

Three trivial explanations might account for the failure to observe negative selection to SRC in allogeneic intermediate hosts. The first possibility is that the concomitant induction of a graft-versus-host reaction inhibited selection. The fact that excellent selection to SRC occurred in semiallogeneic (CBA × B6)-F_1 mice (Table III) rules out this possibility.

A second explanation is that a host-versus-graft reaction impaired selection. This was investigated by filtering (CBA × B6)F_1 T cells through irradiated (DBA/2 (*H-2*^{d}) × B6)F_1 mice in the presence of SRC and studying help provided for B6 Bcells. As shown in Table IV, effective selection to SRC occurred in this situation where both a graft-versus-host reaction and a host-versus-graft reaction could occur.

The third possibility is that B6 mice are nonspecifically less effective than CBA mice at being able to present antigen in an immunogeneic form to T cells. If so, syngeneic T cells would not undergo selection to antigen in B6 mice. The data shown in Table V rules out this possibility. Here it can be seen that effective selection occurred when B6 T cells were filtered with SRC through irradiated B6 mice and tested for their capacity to help B6 B cells.

Role of H-2 Complex in Negative Selection. In the experiments considered above, it is apparent that negative selection to the injected antigen occurred whenever *H-2*-determinants were shared between the donor T cells and the host presenting antigen to the T cells. To determine whether the phenomenon was indeed *H-2*-linked, negative selection of CBA (*H-2*^{k}) T cells was studied in irradiated B10.Br (*H-2*^{k}), B10 (*H-2*^{b}), and B10.D2 (*H-2*^{d}) mice (these congenic resistant strains have identical genetic backgrounds and differ only at the *H-2* complex). As shown in Table VI, effective selection to SRC occurred in *H-2*-

TABLE V

Negative Selection of Primed B6 T Cells to SRC in Irradiated B6 Mice. T Cells Harvested from Lymph at 1 Day Post-Transfer

T Cell group	Helper cells* (2×10^6)	SRC Added during negative selection	B Cells‡	PFC/Spleen at 7 days in irradiated B6 mice		
				Anti-SRC		Anti-HRC
				IgM	IgG	IgG
A	B6 T Cells → irradiated B6	–	B6	9,790(1.22)§	12,910(1.21)	27,220(1.31)
B	B6 T Cells → irradiated B6	+	B6	950(1.22)‖	1,070(1.19)‖	24,220(1.19)
	Group A + group B		B6	14,690(1.18)	15,050(1.19)	52,960(1.13)

*‡ As for footnote to Table I except that B6 T and B cells were used.

§ As for footnote to Table I. Background values of B cells transferred without T cells were: 1,230(1.54) (IgM SRC), 1,770(1.49) (IgG SRC), 880(1.13) (IgG HRC).

‖ Not significantly above values of B cells transferred without T cells.

TABLE VI

Negative Selection of Primed CBA T Cells to SRC in Irradiated B10.Br Mice but not in B10 or B10.D2 Mice. T Cells Harvested from Lymph at 1 Day Post-Transfer

Helper cells* (2×10^6)	*H-2* Region of filtration host	SRC Added during negative selection	B Cells‡	PFC/spleen at 7 days in irradiated CBA mice		
				Anti-SRC		Anti-HRC
				IgM	IgG	IgG
CBA T Cells → irradiated B10.Br	$H\text{-}2^k$	–	CBA	8,930(1.28)§	45,300(1.11)	42,910(1.22)
CBA T Cells → irradiated B10.Br	$H\text{-}2^k$	+	CBA	650(1.37)‖	320(1.56)‖	47,130(1.19)
CBA T Cells → irradiated B10	$H\text{-}2^b$	+	CBA	7,840(1.20)	39,170(1.18)	37,070(1.15)
CBA T Cells → irradiated B10.D2	$H\text{-}2^d$	+	CBA	9,150(1.11)	48,100(1.11)	42,120(1.17)

*‡ As for footnote to Table I. With cells filtered through B10 and B10.D2 mice, > 90% of the cells were resistant to lysis by CBA anti-B6 and CBA anti-DBA/2 alloantisera, respectively. All lymph-borne T cells were > 96% Thy 1.2 positive.

§ As for footnote Table I. Subtracted values of B cells transferred without T cells were: 950(1.08) (IgM SRC), 4,600(1.27) (IgG SRC), 880(1.14) (IgG HRC). Values for T cells transferred without B cells were < 400 PFC/spleen.

‖ Not significantly above values of B cells transferred without T cells.

compatible B10.Br mice but not in *H-2*-incompatible B10 or B10.D2 mice.

Negative Selection with Unprimed T Cells. All of the experiments considered above employed T cells taken from mice primed with both SRC and HRC. The experiment illustrated in Table VII shows that similar results occurred when the T cells were taken from unprimed mice. Thus, it is evident that unprimed CBA T cells underwent negative selection to SRC in *H-2*-compatible C3H ($H\text{-}2^k$) mice but not in *H-2*-incompatible BALB/c ($H\text{-}2^d$) mice. (To ensure

good helper responses in this experiment, the T and B cells were transferred in higher doses (4.5×10^6 and 7×10^6, respectively) than in the preceding experiments with primed T cells.)

Positive Selection in Allogeneic Mice. If negative selection is a prerequisite for positive selection (which seems very likely), the failure to observe negative selection to SRC in *H-2*-different hosts should be associated with an inability to induce T helper cell induction in this situation, i.e., an inability to induce positive selection to SRC. A priori, this might be tested by transferring T cells plus SRC to irradiated allogeneic mice and studying the helper function of cells harvested from the recipients 5 days later. This would be an unsatisfactory approach because at this stage most of the cells in the lymphoid tissues and the central lymph would be alloaggressive blast cells stimulated by the host alloantigens (16). This problem can be avoided by using T cells which have been depleted of specific alloreactive lymphocytes, e.g. by prior acute recirculation (without SRC) through irradiated mice of the strain to be used for activation (17).

Accordingly, LN cells from SRC-primed CBA mice were injected intravenously without SRC into irradiated (CBA × B6)F_1 mice and recovered from thoracic duct lymph of the recipients 20–40 h later (footnote, Table VIII). Of the cells harvested from the recipients, 95% were of donor origin (were resistant to lysis by CBA anti-B6 alloantiserum and complement) and >99% were T (Thy 1.2-positive) cells. These cells were transferred intravenously in a dose of 2×10^7 cells with or without SRC (0.1 ml of 50% solution) into (CBA × B6)F_1 and B6 mice that had received 850 rads 1 day previously. The recipients were cannulated 5 days later and TDL collected overnight. The lymph-borne cells (>90% T cells of donor CBA origin) were transferred with CBA B cells and SRC into irradiated CBA mice to measure their helper function.

TABLE VII

Negative Selection of Unprimed CBA T Cells to SRC in Irradiated C3H Mice but not in BALB/c Mice. T Cells Harvested from Lymph at 1 Day Post-Transfer

Helper cells* (4.5×10^6)	SRC Added during negative selection	B Cells‡	PFC/Spleen at 7 days in irradiated CBA mice		
			Anti-SRC		Anti-HRC
			IgM	IgG	IgG
CBA T Cells → irradiated C3H	–	CBA	20,760(1.23)§	100,560(1.35)	48,700(1.17)
CBA T Cells → irradiated C3H	+	CBA	2,790(1.23)	9,750(1.10)‖	53,190(1.14)
CBA T Cells → irradiated BALB/c	+	CBA	24,200(1.04)	102,710(1.20)	58,350(1.17)

*‡ As for Table I except that the purified T cells were prepared from unprimed mice. The B cells (taken from primed mice) were transferred in a dose of 7×10^6 viable cells.

§ As for footnote to Table I. Subtracted values of B cells transferred without T cells were: 980(1.43) (IgM SRC), 7,120(2.25) (IgG SRC), 1,880(1.98) (IgG HRC). Values for T cells transferred without B cells were < 200 PFC/spleen.

‖ Not significantly above background value of B cells transferred without T cells.

TABLE VIII

Positive Selection of Primed CBA T Cells to SRC in Irradiated (CBA × B6)F_1 Mice but not in B6 Mice. T Cells Rendered Unresponsive to B6 Alloantigens by Prior Filtration through Irradiated (CBA × B6)F_1 Mice. T Cells Recovered from Lymph of Secondary Recipients at 5 Days Post-Transfer

T-Cell group	Helper cells* (prefiltered through irradiated (CBA × B6)F_1 mice)	SRC Added during positive selection	Dose of helper cells	B Cells‡	Anti-SRC PFC/spleen in irradiated CBA mice	
					IgM	IgG
A	CBA T Cells → irradiated (CBA × B6)F_1	+	1×10^5	CBA	5,970(1.59)§	35,340(1.08)
			8×10^5	CBA	6,030(1.09)	83,040(1.23)
B	CBA T Cells → irradiated B6	+	8×10^5	CBA	950(1.22)	4,200(1.16)
C	CBA T Cells → irradiated B6	−	8×10^5	CBA	950(1.21)	4,500(1.19)
	Group A + Group B		8×10^5 of each	CBA	6,240(1.29)	79,560(1.10)

* Unfractionated LN cells from SRC-primed CBA mice were transferred intravenously in a dose of 1.5×10^8 cells without SRC into (CBA × B6)F_1 mice given 900 rads 8 h previously. Recipients were cannulated 18 h later and TDL collected between 20 and 40 h postinjection. The lymph-borne CBA T cells (99% Thy 1.2-positive and 95% resistant to lysis by CBA anti-B6 alloantiserum) were transferred intravenously in a dose of 2×10^7 cells ± 0.1 ml of 50% SRC into (CBA × B6)F_1 or B6 mice given 850 rads 1 day before (two mice per group). These mice were cannulated 5 days later and TDL collected overnight; for each group the TDL were > 97% Thy 1.2 positive and >90% resistant to lysis by CBA anti-B6 alloantiserum. Helper function was measured by transferring the lymph-borne cells with 0.1 ml of 5% SRC plus SRC-primed B cells into irradiated CBA mice as described in footnote to Table I.

‡ As for footnote to Table I.

§ As for footnote to Table I. Subtracted values of B cells transferred without T cells were: 100(1.50) (IgM SRC), 690(1.55) (IgG SRC). All values shown in Table are significantly above background values of B cells transferred without T cells. Values for T cells transferred without B cells were < 100 PFC/spleen.

As shown in Table VIII, CBA T cells positively selected to SRC in irradiated (CBA × B6)F_1 mice (group A) gave high anti-SRC responses with cell doses as low as 10^5. CBA T cells selected to SRC in B6 mice (group B), by contrast, gave only low responses, even with doses as high as 8×10^5. Though significant, these responses were no higher than the responses given by equivalent numbers of CBA T cells harvested from B6 mice not given SRC (group C). The fact that T cells from group B did not inhibit the helper function of group A T cells suggests that the failure to observe positive selection to SRC in irradiated B6 mice was not the result of suppression.

Discussion

The present studies demonstrate that marked negative selection to SRC occurs when purified T cells are acutely recirculated in the presence of SRC through irradiated mice which share *H-2* determinants with the T cells. Thus, when the data shown in Tables I, III, and VI are pooled, anti-SRC helper responses by primed CBA (*H-2*^{k}) T cells were reduced by a mean of 96% (95%

IgM, 97% IgG) when the cells were filtered in the presence of SRC through irradiated *H-2*^{k} (CBA or B10.Br) mice or through (*H-2*^{k} × *H-2*^{b})F_1 ((CBA × B6)F_1) mice. The response to a third-party antigen (HRC) was not affected (1% reduction of IgG response). Negative selection to SRC did not occur, however, when CBA T cells were filtered through *H-2*-different mice, i.e. through *H-2*^{b} (B6 or B10) or *H-2*^{d} (B10.D2) mice. In this situation, the capacity of the filtered cells to evoke IgG anti-SRC responses was reduced by a mean of only 1% (data pooled from Tables III and VI and from three other unpublished experiments). IgM responses were reduced significantly in one experiment (Table III) but not in four others (the mean reduction of IgM responses in five experiments was 15%). Similar results were found with unprimed T cells (Table VII).

No evidence was found that the failure to induce negative selection to SRC in *H-2*-different hosts was due to such factors as the concomitant onset of a graft-versus-host or host-versus-graft reaction (Tables III and IV). It would seem reasonable to conclude, therefore, that the T cells were unable to recognize the antigen in an *H-2*-different environment, even though the antigen was injected in a massive dose, i.e. 0.5 ml of 50% solution of SRC/10^8 lymphocytes (approximately 25 erythrocytes/lymphocyte). The reciprocal failure to detect positive selection (clonal expansion) of T cells in *H-2*-different hosts (Table VIII) strengthens this viewpoint.

The simplest interpretation of the data is that T cells recognize antigen in vivo only when it is presented in association with a cell type which shares *H-2* determinants with the T cells. The identity of the cell(s) presenting antigen in vivo is not known. In the present system radioresistant macrophages (or related cells) of the transfer hosts are the logical choice since macrophages are known to be radioresistant (18) and, at least for certain antigens, these cells play an important role in antigen presentation in vitro (Introduction). Currently we are attempting to identify the antigen-presenting cell by determining whether negative selection can be induced in *H-2*-different hosts by supplementing the donor T cells with other cell types. Preliminary results suggest that addition of macrophage-enriched populations, e.g. peritoneal exudate cells, will indeed lead to selection in this situation. Addition of small B lymphocytes from thoracic duct lymph has not been successful.

Precisely which genes are responsible for selection to antigen in vivo has yet to be established. Preliminary studies with recombinant mice as filtration hosts suggest that the *I* region per se is important, although which subregion is involved has not been studied.

Can one conclude by extrapolation from the present data that T cells are unable to recognize free antigen in vivo? This would be consistent with the evidence that T helper cells (or T cells with the Ly 1^+ 2^- 3^- phenotype) fail to bind antigen in vitro (19–22). While the data are in line with this viewpoint, an alternative argument is that T cells do recognize free antigen in vivo but that in an *H-2*-different environment the antigen is rapidly degraded by the allogeneic macrophages and thereby rendered nonimmunogenic. A critical question therefore is whether selection to antigen would occur in a syngeneic environment after blockade of the reticuloendothelial system. This is currently being investigated. Predicting the outcome is difficult since it hinges on the central ques-

tion of whether T cells have receptors for free antigen or only for MHC-associated antigen (23–25).

Perhaps the most surprising aspect of the present data is that the restriction observed applied to a crude particulate antigen (heterologous erythrocytes). In this respect, *H-2* gene control of T-macrophage interactions in vitro is reported not to apply to particulate antigens (26). Indeed, certain groups have concluded that the immune response to heterologous erythrocytes does not require macrophages (7, 8). The answer here is possibly that many antigens which remain in a particulate form in vitro are rapidly degraded to a soluble form in vivo. Consequently, one might expect *H-2* gene control of T helper cell induction in vivo to apply to a wide range of antigens. This remains to be proved.

Finally, it should be emphasized that the B cells used in this study were always syngeneic with the T cells. Hence, it might be asked whether negative selection would be apparent in *H-2*-different hosts if allogeneic B cells of this strain were used to monitor selection. Here it should be emphasized that in our hands T cells from normal (nonchimeric) mice do not stimulate *H-2*-different B cells in vivo (17). This applies even with unprimed parental strain T cells activated to antigen (SRC) in irradiated F_1 mice (J. Sprent, unpublished data).

Summary

When purified CBA lymph node T cells were mixed with sheep erythrocytes (SRC) and filtered from blood to lymph through irradiated syngeneic mice for 1–2 days, the donor cells lost their capacity to stimulate anti-SRC responses by CBA B cells; the response to a third-party antigen (horse erythrocytes) was unaffected and active suppression was not involved. This process of specific negative selection to SRC also occurred when semiallogeneic mice were used as filtration hosts. By contrast, when allogeneic hosts were used the helper function of the donor cells was not reduced; this applied to both primed and unprimed T cells. Studied with congeneic resistant strains indicated that negative selection to SRC occurred only when the donor and host shared *H-2* determinants.

Studies with T cells depleted of alloreactive lymphocytes showed that negative selection to SRC in irradiated F_1 hybrid mice was followed by a stage of positive selection where the donor cells gave greatly increased responses to the injected antigen. Positive selection did not occur in *H-2*-different mice, however, and the helper function of the donor cells remained unchanged.

By these parameters it was concluded that homozygous T helper cells have no detectable capacity to recognize antigen in an *H-2*-different environment.

Stimulating discussion with D. B. Wilson and the typing skill of Miss Karen Nowell is gratefully acknowledged.

Received for publication 3 May 1978.

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