RESTRICTED HELPER FUNCTION OF F_1 HYBRID T CELLS POSITIVELY SELECTED TO HETEROLOGOUS ERYTHROCYTES IN IRRADIATED PARENTAL STRAIN MICE

I. Failure to Collaborate with B Cells of the Opposite Parental Strain Not Associated with Active Suppression*

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Several studies have shown that F_1 hybrids derived from parental strains differing at the major histocompatibility complex (MHC)[1] contain two distinct subpopulations of T cells, each reactive to antigen presented in the context of one of the two parental strains. This has been demonstrated with respect to a variety of T-cell functions, e.g., measurement of proliferative responses to antigen-pulsed macrophages in vitro (1, 2), elicitation of delayed type hypersensitivity (DTH) on adoptive transfer (3), and expression of cell-mediated lympholysis (CML) directed against viruses (4), haptens (5), and minor histocompatibility determinants (6). With respect to two of these functions – T-cell proliferation and expression of DTH – the response involves T cells with the Ly 1^+ 2^- 3^- phenotype (7, and R. H. Schwartz, personal communication). This phenotype is also expressed by helper T cells involved in T-B collaboration (8).

Recent evidence suggests that macrophages play a critical role in presenting antigen in an immunogenic form to Ly 1^+ 2^- 3^- T cells. The precise contribution of macrophages in antigen presentation is not clear, although with certain antigens it has been observed that T-cell activation depends upon the responding T cells and macrophages sharing MHC determinants. In this respect, studies of Erb and Feldmann (9–11) suggest that, in vitro, mouse macrophages process antigen and complex it to small particles coded for by the *I-A* subregion of the *H-2* complex. These complexes stimulated T cells to express helper activity, but only when the T cells and the macrophages forming the complex were I-A-compatible. It is perhaps tempting to conclude from these data that all helper T cells, and perhaps Ly 1^+ 2^- 3^- cells in general, are unable to recognize antigen unless it has been processed by MHC-compatible macrophages. At present, this generalization seems premature because it fails to explain why certain antigens, e.g., particulate antigens such as heterologous erythrocytes or keyhole limpet hemocyanin cross-linked to Sepharose beads, are able to induce helper function in vitro either in the virtual absence of macrophages (12) or in the presence of MHC-incompatible macrophages (9).

The purpose of the studies presented in these two papers was to determine whether or

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[1] *Abbreviations used in this paper*: CML, cell-mediated lympholysis; DTH, delayed-type hypersensitivity; HRC, horse red blood cells; LN, lymph nodes; MHC, major histocompatibility complex; PE, peritoneal exudate; PFC, plaque-forming cells; SRC, sheep red blood cells; TDL, thoracic duct lymphocytes.

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not the reported dichotomy in the reactivity of F_1 hybrid T cells involved in such functions as CML and DTH also applies to helper T cells involved in T-B collaboration. This question was approached by activating purified F_1 T cells to heterologous erythrocytes in irradiated mice of one parental strain, and then harvesting the cells at a time (5 days) when the antigen-reactive precursors were presumed to have undergone clonal expansion (positive selection) after proliferating in response to the antigen in such organs as the spleen (13). The helper function of the activated T cells was then assessed in terms of their capacity to collaborate with parental strain B cells. The results show that despite the particulate nature of the antigen used, F_1 T cells exposed to sheep or horse erythrocytes in irradiated mice of one parental strain developed excellent helper activity for B cells derived from this strain, but gave only minimal help for B cells of the opposite strain. In contrast to the results of an analogous study by Skidmore and Katz (14), good collaboration was observed with F_1 B cells, and there was no evidence that suppressor cells accounted for the phenomenon.

Materials and Methods

Mice. For most experiments CBA/Cum (CBA) (*H-2*^{k}), C57BL/6 Cum (B6) (*H-2*^{b}), and (CBA × B6)F_1 hybrids were used. These mice were obtained from Cumberland View Farms, Clinton, Tenn. CBA/J mice obtained from The Jackson Laboratory, Bar Harbor, Maine were also used in some experiments. CBA/J and CBA/Cum failed to respond to each other in mixed lymphocyte culture, and were therefore assumed to be H-2- and *Mls*-identical (*Mls*^{d}). Results obtained with B cells derived from CBA/J compared with CBA/Cum mice were not discernibly different. DBA/2 (*H-2*^{d}) and (DBA/2 × B6)F_1 mice, also obtained from Cumberland View Farms, were used in one experiment.

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

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

Cells. Thoracic duct lymphocytes (TDL) were obtained as described elsewhere (15). Suspensions of spleen and lymph node (LN) cells were prepared by teasing the organs 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.

Cell Identification with Alloantisera. CBA anti-B6 and B6 anti-CBA *H-2* alloantisera and anti-thy 1.2 (AKR anti-C3H thymus) antiserum were prepared as described previously (16). These antisera, all of which were shown to be specific before use, were employed to establish the identity of the cells in the central lymph of irradiated B6 or CBA mice given (CBA × B6)F_1 T cells plus SRC 5 days before. In the experiment in which (DBA/2 × B6)F_1 T cells were activated in irradiated DBA/2 and B6 mice, DBA/2 anti-B6 and B6 anti-DBA/2 alloantisera were used; these sera were prepared as referred to above. 50 μl of the test lymphocytes (5×10^6/ml) were incubated at 4°C for 30 min with 25 μl of antisera in small plastic tubes and washed once by centrifugation. The cell pellet was resuspended in 50 μl of complement (1:5 dilution of guinea pig serum) for 30 min at 37°C. Cytotoxic indices were then calculated by dye exclusion with respect to controls incubated with normal mouse serum plus complement or with antiserum without complement. Percent lysis with the control samples was invariably <5%. The anti-thy 1.2 serum appeared to be specific for T cells, since it lysed 97–100% of LN cells filtered from blood to lymph through irradiated mice (16), 20–30% of spleen cells, and <5% of bone marrow cells.

Preparation of T Cells for Positive Selection. The T cells used for positive selection were obtained from pooled mesenteric, inguinal, axillary, and cervical LN of unprimed (CBA × B6)F_1 or (DBA × B6)F_1 mice. LN cells were depleted of most thy 1.2-negative cells by passage over nylon-wool columns as described by Julius et al (17). The majority of the effluent cells recovered from the columns were T cells, since 86–95% (mean - 91%, 15 experiments) were susceptible to lysis by anti-thy 1.2 antiserum in the presence of complement. The recovery of T cells after nylon-wool passage was usually on the order of 60–80% (established with respect to the number of thy 1.2-positive cells in the LN cell preparation before passage [50–70%]). The viability of the cells recovered from the column was usually >85%.

Positive Selection to Heterologous Erythrocytes in Irradiated Mice. In most experiments, 5×10^7 viable, unprimed, nylon-wool-passed (CBA × B6)F_1 T cells in a 0.5-ml volume were mixed with 0.5 ml of 25% SRC (or HRC) and injected intravenously into CBA, B6, or (CBA × B6)F_1 mice (2–5 mice per group) given 800 rads 1 day previously. (DBA/2 × B6)F_1 T cells were used in one experiment, and these cells were transferred with SRC into irradiated B6 or DBA/2 mice. Thoracic duct fistulas were inserted in the mice 5 days later. TDL were collected overnight and used as helper T cells after establishing their identity with appropriate alloantisera.

Preparation of B Cells. Spleen cells from mice primed with SRC or with both SRC and HRC were resuspended in undiluted anti-thy 1.2 antiserum (0.1 ml of antisera/2×10^7 spleen cells) and kept at 4°C for 30 min. After washing twice by centrifugation, the cells were incubated at 37°C for 30 min with guinea pig complement (1:6 dilution, 2×10^7 cells/ml). The cells were then washed once and resuspended to a volume suitable for injection. This treatment lysed 20–45% of the spleen cells.

Measurement of T-B Collaboration. In most experiments, helper T cells (usually 0.8×10^6 TDL from irradiated mice given F_1 T cells plus SRC 5 days before) were transferred with 0.1 ml of 5% SRC into irradiated (750 rads 1 day before) F_1 hybrid mice with B cells (anti-thy 1.2-treated spleen cells) prepared from SRC-primed F_1 hybrid or parental strain mice. With one exception, B cells were transferred in a dose of 5×10^6 viable cells. The exception occurred when B6 B cells were transferred to irradiated (CBA × B6)F_1 recipients; here, presumably because of the strong *Hh* barrier existing in this situation (18), a higher dose of cells ($8–10 \times 10^6$) had to be transferred to get responses equivalent to those given by the other B-cell populations. In experiments in which responses to HRC were measured, both SRC and HRC (0.1 ml of 5% of each) were added to the injected mixture of T and B cells; in this situation, the B cells were prepared from mice primed to both SRC and HRC.

Plaque-Forming Cell (PFC) Assays. Direct (19s, IgM) PFC were detected by the method of Cunningham and Szenberg (19). Numbers of indirect (7s, IgG) PFC were measured by adding a polyvalent rabbit anti-mouse immunoglobulin reagent to the reactive mixture in the presence of specific goat anti-mouse μ-chain serum. As described elsewhere (16), the anti-μ serum (kindly provided by Dr. M. Feldmann, University College, London, England) suppressed IgM but not IgG PFC.

Serum Hemagglutinin Assays. These were performed by the method of Dietrich (20).

Priming with Heterologous Erythrocytes. SRC and HRC (obtained from Gibco Diagnostics, The Mogul Corp., Chagrin Falls, Ohio) were stored in Alsever's solution and washing three times before use. Mice were primed intraperitoneally with 0.1 ml of 25% SRC (or also with a similar dose of HRC) and used as B-cell donors 2–4 mo later.

Peritoneal Exudate (PE) Cells. PE cells were obtained from mice given a 1-ml intraperitoneal injection of 4% thyoglycollate solution (Difco Laboratories, Detroit, Mich.) 4 days earlier. The cells were treated with anti-thy 1.2 serum and complement according to the procedure used for preparing B cells from spleen.

Statistical Analysis. Geometric means and values used to derive the upper and lower limits of the SE of the mean (these values represent the anti-log of SE of $\log_{10}$ geometric mean) were calculated from the $\log_{10}$ of the PFC counts. Arithmetic means and standard errors of the mean were calculated from the reciprocal of the $\log_2$ values for the hemagglutinin titers. *P* values were determined by Student's *t* test. In the comparison of the means 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 transfer LN T cells from unprimed (CBA × B6)F_1 mice into irradiated CBA, B6, and (CBA × B6)F_1 mice together with SRC, and then to study the helper function of the SRC-activated T cells recovered from the thoracic duct lymph of the recipients 5 days later. Before transfer, the LN 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 5×10^7 cells with SRC (0.5 ml of 25% solution), also given intravenously. To limit the

TABLE I

Identity of TDL Recovered from Thoracic Duct Lymph of Irradiated CBA, B6, and (CBA × B6)F_1 Mice Injected with Unprimed Nylon-Wool-Purified (CBA × B6)F_1 LN Cells Plus SRC 5 Days Previously

Recipients of (CBA × B6)F_1 T cells plus SRC*	Average no. of TDL (× 10^{-6}) collected from each recipient over 18 h‡	Cytotoxic index with alloantisera + complement§			Host origin
		B6 anti-CBA	CBA anti-B6	Anti-thy 1.2	
					%
Irrad. CBA	14.5	99	95	97	5
Irrad. B6	12.1	95	100	98	4
Irrad. (CBA × B6)F_1	14.3	99	99	97	–

* Nylon-wool-purified LN T cells (93% thy 1.2-positive) were transferred intravenously in a dose of 5 × 10^7 viable cells with 0.5 ml of 25% SRC; the cell recipients (three mice per group) were exposed to 800 rads 1 day before.

‡ Thoracic duct fistulas were inserted at 5 days after cell transfer. TDL were collected overnight, the collections commencing at about 4 h after establishing the fistulas. Lymph samples were pooled from the three mice in each group before counting.

§ See Materials and Methods. Background lysis with cells treated with normal mouse serum plus complement was <3%.

possibility of a host-versus-graft reaction, the transfer hosts received irradiation at a high dose (800 rads) 1 day before cell transfer. Thoracic duct fistulas were inserted in the cell recipients at 5 days post-transfer, i.e. during the stage of positive selection when it was presumed that the progeny of the cells responding to the injected antigen in the lymphoid tissues entered the circulation in large numbers (21, 22). The features of the lymph-borne cells collected over a 16-h period are shown in Table I; the data are from one experiment which is representative of many others. Testing with anti-thy 1.2 serum and appropriate alloantisera in the presence of complement showed that the lymph-borne cells consisted almost entirely (≥97%) of T cells, nearly all of which were of donor origin (>94%). Cell viability was 99–100%. The yield of cells (compared with the numbers initially injected) was high (≈30%).

To study their helper function, the cells were transferred intravenously in small numbers (0.8 × 10^6) into irradiated (750 rads) (CBA × B6)F_1 mice, together with SRC (0.1 ml of 5% solution), and B cells (5–8 × 10^6 anti-thy 1.2-serum-treated spleen cells from SRC-primed mice). Numbers of direct (IgM) and indirect (IgG) PFC to SRC were measured in the spleen 7 days later.

As shown in Table II, (CBA × B6)F_1 T cells activated to SRC in irradiated (CBA × B6)F_1 mice (F_1 $T_{+(SRC-F_1)}$), provided effective help for B cells derived from either of the two parental strains, as well as for F_1 B cells. By contrast, F_1 T cells activated to SRC in irradiated B6 mice (F_1 $T_{+(SRC-B6)}$) or in CBA mice (F_1 $T_{+(SRC-CBA)}$) cooperated well with B cells derived from only one of the two parental strains, namely the strain in which the T cells were initially activated. The failure to stimulate more than minimal responses by B cells of the opposite parental strain did not seem to be the result of active suppression, since a supplemental injection of F_1 $T_{+(SRC-F_1)}$ cells led to high responses. Moreover,

TABLE II

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated CBA, B6, and (CBA × B6)F_1 Mice Injected 5 Days Previously with Unprimed (CBA × B6)F_1 T Cells Plus SRC**

T-cell group	T cells (0.8×10^6)‡	B cells§	Anti-SRC PFC/spleen at 7 days in irradiated F_1 mice	
			IgM	IgG
A	F_1 $T_{+(SRC-B6)}$	CBA	1,150 (1.08)‖	8,610 (1.01)
		B6	40, 590 (1.13)	118,870 (1.15)
		(CBA × B6)F_1	7,370 (1.28)	84,660 (1.16)
B	F_1 $T_{+(SRC-CBA)}$	CBA	30,490 (1.02)	275,400 (1.17)
		B6	3,940 (1.05)	9,360 (1.15)
		(CBA × B6)F_1	13,060 (1.13)	74,500 (1.11)
C	F_1 $T_{+(SRC-F_1)}$	CBA	21,190 (1.25)	125,690 (1.08)
		B6	13,890 (1.18)	56,180 (1.14)
		(CBA × B6)F_1	14,680 (1.16)	89,360 (1.06)
Group A + group C		CBA	19,170 (1.30)	131,390 (1.25)
Group B + group C		B6	20,380 (1.23)	64,960 (1.27)
–		CBA	200 (1.40)	510 (1.07)
–		B6	990 (1.39)	1,740 (1.26)
–		(CBA × B6)F_1	<100	610 (1.22)
Group A		–	<100	<100
Group B		–	<100	<100
Group C		–	<100	<100

* Cell recipients were exposed to 800 rads 1 day before being injected intravenously with 5×10^7 unprimed nylon-wool-purified (CBA × B6)F_1 T cells plus 0.5 ml of 25% SRC.

‡ Recipients of F_1 T cells and SRC were cannulated at 5 days post-transfer and the lymph-borne cells were collected overnight. The helper activity of the cells was measured by transferring 0.8×10^6 TDL (nearly all of which were T cells of F_1 origin; Table I) intravenously into irradiated (750 rads) (CBA × B6)F_1 mice with B cells and 0.1 ml of 5% SRC. When transferring mixtures of T cells to check for suppression, a dose of 0.8×10^6 of each T-cell population was used.

§ Anti-thy 1.2-treated spleen cells from mice primed with SRC 2–4 mo previously; 5×10^6 (CBA and (CBA × B6)F_1) or 8×10^6 (B6) viable cells transferred.

‖ Geometric mean of data from four mice per group (two mice only for T cells transferred without B cells); number in parenthesis refers to value by which mean is multiplied by or divided to give upper and lower limits, respectively, of SE. All values shown are significantly above ($P < 0.05$) the background values of B cells transferred without T cells.

effective cooperation was observed with F_1 B cells. Five other experiments gave similar results.

Unless stated otherwise, the design of the experiments to be considered below was identical to the protocol used in Table II. In all experiments, the helper cells were recovered from thoracic duct lymph at day 5–6 post-transfer, and they were pooled from at least 2 mice per group. In each experiment the identity of the lymph-borne cells was checked with anti-thy 1.2 serum and appropriate *H-2* alloantisera. By these parameters, the lymph-borne cells were invariably >90% (and usually >95%) T cells of donor F_1 origin. For simplicity, the background values obtained when B cells were transferred without T cells have been subtracted from the data shown in Tables III–VIII. These values were generally

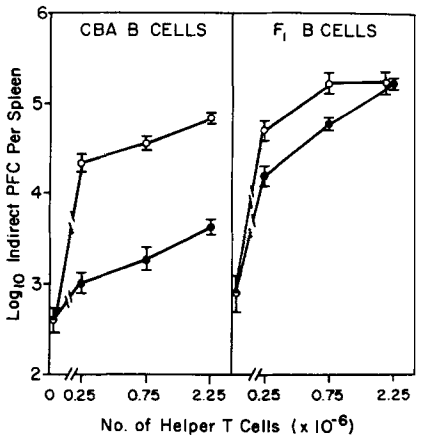

FIG. 1. Dose response of helper activity of (CBA × B6)F_1 T cells activated to SRC in irradiated B6 (●) or irradiated (CBA × B6)F_1 (○) mice and harvested from thoracic duct lymph of the recipients 5 days later. The fig. shows numbers of anti-SRC IgG PFC/spleen in irradiated (CBA × B6)F_1 mice injected intravenously 7 days previously with varying doses of one of the two T-cell populations plus 0.1 ml of 5% SRC and either CBA B cells or (CBA × B6)F_1 B cells. ◓, B cells transferred without T cells; PFC numbers with T cells transferred without B cells were < 100 PFC/spleen. The B cells (5×10^6 viable anti-thy 1.2-serum-treated spleen cells) were from mice primed with SRC 2 mo previously. Each point represents geometric mean of data from four mice; vertical bars show upper and lower limits of SE.

quite low (200–2,000 PFC/spleen) and are shown in the footnotes to the tables. T cells transferred without B cells gave negligible responses (<200 PFC/spleen). It should be stressed that the irradiated recipients used for measuring T-B collaboration were invariably F_1 hybrids, i.e. mice syngeneic or semi-syngeneic with the T and B cells.

Effect of Varying the Dose of Helper T Cells. Although the helper function of F_1 T cells activated to SRC in mice of one parental strain was very low for B cells of the opposite parental strain, it was significant. This is illustrated in Fig. 1 where it can be seen that F_1 $T_{+(SRC-B6)}$ cells gave a linear response when transferred in varying doses with a constant number of CBA B cells. With the highest dose of T cells (2.25×10^6), the response was 20-fold above background. It is apparent that the slope of the dose-response curve obtained in this situation paralleled the slope of the (far greater) response observed when CBA B cells were transferred with varying doses of F_1 $T_{+(SRC-F_1)}$ cells.

In the case of responses with F_1 B cells, it is of interest that the helper activity of F_1 $T_{+(SRC-B6)}$ cells was appreciably less than that of F_1 $T_{+(SRC-F1)}$ cells; this was apparent where low doses of T cells were transferred, though not with high doses. The significance of this curious finding with low T-cell doses (which is also evident in Table IV, V, and Fig. 2) will be considered in the following paper.

Effect of Transferring F_1 T Cells to Irradiated Parental Strain Mice with or without Antigen. When (CBA × B6)F_1 T cells were transferred to irradiated B6 mice without SRC, the lymph-borne cells collected at day 5 (F_1 T_{+B6}) gave significant though small anti-SRC and anti-HRC responses with CBA, B6, and

TABLE III

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated B6 Mice Injected 5 Days Previously With Unprimed (CBA × B6)F_1 T Cells Given with or without SRC**

T-cell group	T cells	Dose of T cells ($\times 10^{-6}$)	B cells‡	PFC/spleen at 7 days in irradiated F_1 mice		
				Anti-SRC		Anti-HRC
				IgM	IgG	IgM
A	F_1 T_{+B6}	0.4	CBA	1,180 (1.29)§	1,390 (1.29)‖	20 (1.65)‖
		2.0	CBA	4,210 (1.12)	10,760 (1.20)	830 (1.30)
		0.4	B6	1,300 (1.55)‖	1,930 (1.34)‖	1,250 (1.58)‖
		2.0	B6	3,560 (1.25)	5,680 (1.19)	3,690 (1.20)
		0.4	(CBA × B6)F_1	150 (1.09)‖	3,350 (1.15)	140 (1.46)‖
		2.0	(CBA × B6)F_1	4,680 (1.15)	21,870 (1.18)	4,510 (1.23)
B	F_1 $T_{+(SRC-B6)}$	0.4	CBA	1,620 (1.21)	1,550 (1.06)‖	0
		2.0	CBA	4,750 (1.25)	12,420 (1.25)	1,180 (1.20)
		0.4	B6	26,340 (1.20)	66,050 (1.12)	1,750 (1.23)
		2.0	B6	55,060 (1.23)	100,440 (1.13)	6,950 (1.19)
		0.4	(CBA × B6)F_1	8,140 (1.25)	35,900 (1.24)	640 (1.43)‖
		2.0	(CBA × B6)F_1	58,130 (1.36)	188,680 (1.28)	6,540 (1.53)

* As for footnote to Table II; group A. T cells were TDL from irradiated B6 mice given 5×10^7 unprimed (CBA × B6)F_1 T cells, but not SRC, 5 days previously; group B. T cells were from irradiated B6 mice given F_1 T cells with SRC (0.5 ml of 25%) 5 days before.

‡ As for footnote to Table II except that the B cells were taken from mice primed with both SRC and HRC.

§ As for footnote to Table II except that background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA 950(1.18) (IgM SRC), 2,050(1.36) (IgG SRC), 440(1.41) (IgM HRC); B6 850(1.50) (IgM SRC), 1,890(1.70) (IgG SRC), 430(1.45) (IgM HRC); (CBA × B6)F_1 1,000(1.17) (IgM SRC), 2,840(1.19) (IgG SRC), 870(1.36) (IgM HRC). Values for T cells transferred without B cells were all <100 PFC/spleen. In the table values in parentheses used to derive limits of SE (see footnote to Table II) were calculated before subtraction of the background PFC numbers.

‖ Not significantly above background values of B cells transferred alone ($P > 0.05$).

F_1 B cells (Table III). These responses were dose-dependent, i.e. 2×10^6 T cells produced moderate numbers of PFC, whereas 0.4×10^6 T cells gave responses which were generally not significantly higher than the background values observed when B cells alone were transferred.

Markedly different results occurred when SRC were added to the F_1 T cells transferred to the irradiated B6 mice. Three points can be made concerning the helper activity of these cells. First, even small doses of these cells (F_1 $T_{+(SRC-B6)}$) gave high anti-SRC responses with B6 and F_1 B cells, though not with CBA B cells. Second, the low response observed with CBA B cells was similar in magnitude to that given by T cells from recipients not given SRC (F_1 T_{+B6} cells). Third, the response to a different antigen, HRC, was low with all three populations of B cells.

Specificity of Positive Selection. To obtain further information on the specificity of the F_1 T cells positively selected to SRC in the above experiments, F_1 T cells were activated in (*a*) irradiated B6 mice given SRC (F_1 $T_{+(SRC-B6)}$), (*b*) irradiated B6 mice given HRC (F_1 $T_{+(HRC-B6)}$), and (*c*) irradiated F_1 mice given

TABLE IV

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated B6 Mice Injected 5 Days Previously with (CBA × B6)F_1 T Cells Plus Either SRC or HRC**

T-cell group	T cells (0.8×10^6)‡	B cells§	IgG PFC/spleen at 7 days in irradiated F_1 mice	
			Anti-SRC	Anti-HRC
A	F_1 $T_{+(SRC-B6)}$	CBA	960 (1.27)‖	1,160 (1.28)
		B6	40,770 (1.28)	5,830 (1.08)
		(CBA × B6)F_1	16,360 (1.15)	3,180 (1.16)
B	F_1 $T_{+(HRC-B6)}$	CBA	460 (1.23)¶	940 (1.53)¶
		B6	1,940 (1.37)	61,420 (1.13)
		(CBA × B6)F_1	2,500 (1.13)	32,110 (1.09)
C	F_1 $T_{+(SRC+HRC-F_1)}$	CBA	35,310 (1.14)	26,550 (1.17)
		B6	12,390 (1.24)	29,400 (1.26)
		(CBA × B6)F_1	42,360 (1.13)	44,990 (1.18)

* As for footnote to Table II; T cells were taken from lymph of irradiated B6 mice given, 5 days previously, 5×10^7 unprimed (CBA × B6)F_1 T cells with 0.5 ml of 25% SRC (group A) or HRC (group B); group C. T cells were from irradiated (CBA × B6)F_1 mice given F_1 T cells together with both SRC and HRC.

‡ As for footnote to Table II except that the T and B cells were transferred with both SRC and HRC (0.1 ml of 5% of each).

§ As for footnote to Table II except that B cells were prepared from mice primed with both SRC and HRC.

‖ As for footnote to Table II except that background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA 350(1.45) (SRC), 470(1.33) (HRC); B6 1,000(1.26) (SRC), 800(1.19) (HRC); (CBA × B6)F_1 950(1.36) (SRC), 290(1.07) (HRC). In the table the values in parentheses used to derive limits of SE (see footnote to Table II) were calculated before subtraction of the background PFC numbers. Numbers of PFC for T cells transferred without B cells were all <100/spleen.

¶ Not significantly above background values of B cells transferred alone ($P > 0.05$).

both SRC and HRC (F_1 $T_{+(SRC+HRC-B6)}$). The helper function of these three groups of cells is shown in Table IV. In the case of F_1 $T_{+(SRC-B6)}$ cells, anti-SRC responses were high with B6 and F_1 B cells but not with CBA B cells; anti-HRC responses were low with all three B-cell populations. Reciprocal results were found with F_1 $T_{+(HRC-B6)}$ cells, i.e. anti-SRC responses were all low, whereas anti-HRC responses were high with B6 and F_1 B cells, but not with CBA B cells. F_1 $T_{+(SRC+HRC-B6)}$ cells stimulated all three B-cell populations to produce high responses to both SRC and HRC.

Time of PFC Assay. In the preceding experiments, numbers of PFC were measured arbitrarily on day 7 post-transfer. Fig. 2 shows that essentially similar results occurred when PFC were assayed on days 5, 7, and 9. Thus, at no stage of assay did F_1 $T_{+(SRC-B6)}$ cells give > 1,800 anti-SRC PFC/spleen with CBA B cells. By contrast, F_1 $T_{+(SRC-F1)}$ cells elicited high responses with CBA B cells, PFC numbers reaching a peak on day 7 (46,730 IgG PFC), and then declining.

Serum Hemagglutinin Titers. It might be argued that despite the failure of F_1 $T_{+(SRC-B6)}$ cells to stimulate CBA B cells to differentiate into PFC in the spleen, effective collaboration could have occurred in other regions. If so, this

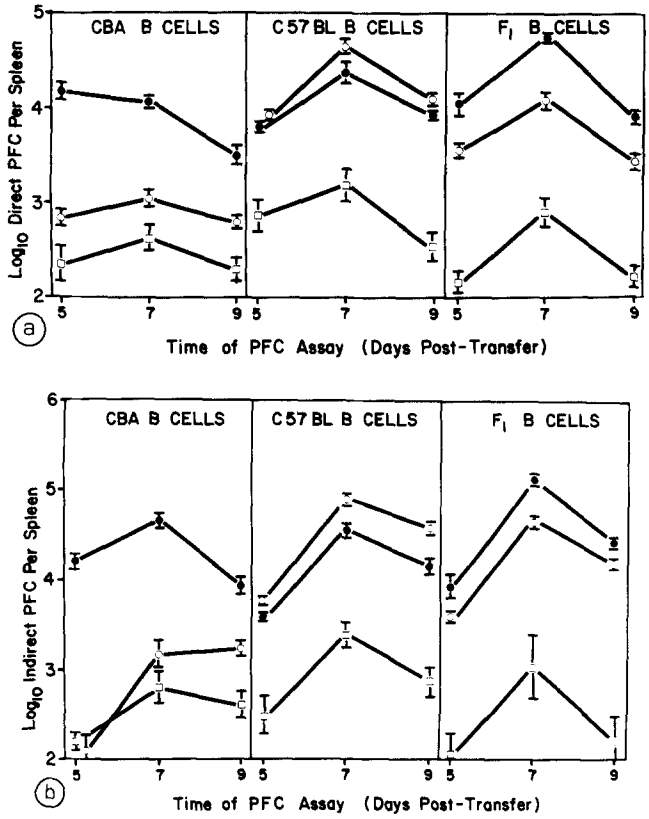

FIG. 2. Time response of helper activity of (CBA × B6)F_1 T cells activated to SRC in irradiated B6 (○) or (CBA × B6)F_1 (●) mice and harvested from thoracic duct lymph of the recipients 5 days later. The fig. shows numbers of anti-SRC IgM (direct) (a) and IgG (indirect) (b) PFC/spleen in irradiated (CBA × B6)F_1 mice at various times after intravenous injection of one of the two T-cell populations (0.8×10^6 cells) plus 0.1 ml of 5% SRC and B cells from CBA, B6 (C57BL), or (CBA × B6)F_1 mice. □, B cells transferred without T cells. The B cells (anti-thy 1.2-serum-treated spleen cells) were from SRC-primed mice and were transferred in a dose of 5×10^6 viable cells (8×10^6 for B6 B cells). Number of PFC with T cells transferred without B cells was < 100 PFC/spleen at all time points. Each point represents geometric mean of data from four mice; vertical bars show upper and lower limits of SE.

would presumably be reflected in the levels of serum hemagglutinins. To investigate this possibility, the mice used for PFC assay at day 9 in the above experiment (Fig. 2) were exsanguinated at the time of sacrifice, and these sera were measured for anti-SRC hemagglutinin activity. As shown in Table V, the serum hemagglutinin titers closely reflected the results obtained by measuring numbers of PFC in the spleen (compare with Fig. 2).

Effect of Adding PE Cells during Positive Selection. Macrophages present in the irradiated parental strain mice used for positive selection might be responsible for the restricted helper function observed after selection. If so, the restriction would be expected not to occur if the mice used for selection were supplemented with macrophages of the opposite parental strain. To investigate

TABLE V

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated B6 and (CBA × B6)F_1 Mice Injected 5 Days Previously with (CBA × B6)F_1 T Cells and SRC: Serum Hemagglutinin Titers after Transfer of T and B Cells to Irradiated (CBA × B6)F_1 Mice**

T cells (0.8×10^6)	B cells	Anti-SRC hemagglutinin titers ($1/\log_2$) at 9 days after transfer to irradiated F_1 mice
F_1 $T_{+(SRC-B6)}$	CBA	1.6 (±0.8)‡
	B6	5.7 (±0.5)
	(CBA × B6)F_1	6.7 (±0.5)
F_1 $T_{+(SRC-F_1)}$	CBA	6.1 (±0.9)
	B6	4.8 (±0.8)
	(CBA × B6)F_1	9.4 (±1.1)

* For details see legend to Fig. 2. Sera tested were from mice assayed for splenic PFC at day 9 after transfer of T and B cells to irradiated (CBA × B6)F_1 mice; the numbers of PFC for these mice are shown in Fig. 2.

‡ Arithmetic mean (±SE) after subtraction of background values given by B cells transferred without T cells. These values were: CBA 1.9 (±0.5); B6 3.6 (±0.9); (CBA × B6)F_1 2.9 (±0.7). SE were calculated before background subtraction. Titers from mice given T cells but not B cells were <1.4 mice per group. The value asterisked is not significantly above background value of B cells transferred alone; all other values are significantly above background values ($P < 0.05$).

this possibility, (CBA × B6)F_1 T cells were positively selected to SRC in irradiated B6 mice injected with 3×10^7 anti-thy 1.2-serum-treated PE cells derived from either CBA or B6 mice (groups B, C; Table VI); of these cells, >80% had features of macrophages, i.e. they were large cells and contained numerous granules. The F_1 T cells and SRC were mixed with the PE cells and injected intraperitoneally; attempts to transfer this mixture intravenously caused respiratory distress and rapid death, presumably because of clumping of the PE cells. For controls, F_1 T cells and SRC were transferred intravenously into irradiated B6 and CBA mice (groups A, D; Table VI).

The helper activity of TDL recovered from these four groups of mice at day 5 post-transfer is shown in Table VI. It is apparent that addition of CBA PE cells during positive selection in irradiated B6 mice did not lead to restriction in helper function, i.e. the TDL recovered from these mice collaborated well with either CBA or B6 B cells. By contrast, TDL recovered after positive selection in the presence of B6 PE cells led to high responses with B6 B cells, but not with CBA B cells. With respect to the helper cells recovered from Group B, it should be mentioned that >98% of the cells were susceptible to lysis with either anti-thy 1.2 serum or CBA anti-B6 alloantiserum. Thus, there was no evidence that the CBA PE cells given to these mice reached the central lymph.

Positive Selection with (DBA/2 × B6)F_1 T Cells. To determine whether or not the above findings with (CBA × B6)F_1 T cells also applied to other strain combinations, (DBA/2 × B6)F_1 T cells were activated to SRC in irradiated DBA/

TABLE VI

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated B6 Mice Injected 5 Days Previously with Unprimed (CBA × B6)F_1 T Cells Plus CBA PE Cells and SRC**

T cell group	T cells (0.8×10^6)‡	PE cells added during positive selection	B cells§	Anti-SRC PFC/spleen at 7 days in irradiated F_1	
				IgM	IgG
A	F_1 $T_{+(SRC-B6)}$	–	CBA	1,770 (1.12)‖	1,580 (1.52)¶
			B6	17,280 (1.14)	37,050 (1.13)
B	F_1 $T_{+(SRC-B6)}$	3×10^7 CBA PE	CBA	26,950 (1.16)	67,740 (1.13)
			B6	21,160 (1.19)	47,880 (1.16)
C	F_1 $T_{+(SRC-B6)}$	3×10^7 B6 PE	CBA	2,150 (1.34)	8,140 (1.37)
			B6	57,330 (1.24)	114,910 (1.02)
D	F_1 $T_{+(SRC-CBA)}$	–	CBA	19,740 (1.12)	59,400 (1.06)
			B6	1,300 (1.30)	2,160 (1.14)

* As for footnote to Table II except that in the case of groups B and C, the F_1 T cells (5×10^7 nylon-wool-passed), PE cells and SRC were all injected intraperitoneally in one injection; in groups A and D, the F_1 T cells and SRC were transferred intravenously. The PE cells were from mice given thyoglycolate 4 days before and were treated with anti-thy 1.2 antiserum and complement before injection.

§ As for footnote to Table II.

‖ As for footnote to Table II except that background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA 190(1.57) (IgM), 700(1.76) (IgG); B6 130(1.44) (IgM), 370(1.46) (IgG). Values for T cells transferred without B cells were all <400 PFC/spleen. In the table values in parentheses used to derive limits of SE (see footnote to Table II) were calculated before subtraction of background PFC numbers.

¶ Not significantly above background value of B cells transferred without T cells ($P > 0.05$).

2 or B6 mice. Their helper activity was then assayed in irradiated (DBA/2 × B6)F_1 mice. As shown in Table VII, F_1 $T_{+(SRC-DBA/2)}$ cells collaborated well with DBA/2 and F_1 B cells, but not with B6 B cells. By contrast, F_1 $T_{+(SRC-B6)}$ cells stimulated B6 and F_1 B cells, but not DBA/2 B cells. Injection of both populations of T cells with either of the two parental strain B-cell populations led to high responses, i.e. suppression was not observed.

Positive Selection Using Activated T Cells Recovered from the Spleen. All of the preceding experiments involved the use of activated helper cells recovered from thoracic duct lymph of the intermediate irradiated hosts. Two experiments were also performed with helper cells recovered from the spleen. Cells from this region were less satisfactory to work with because of their low viability (20–40%) and greater contamination with radioresistant host cells (15–30%). Nevertheless, the qualitative helper activity of these cells was similar to that of the cells obtained from the central lymph. Thus, it can be seen in Table VIII that (CBA × B6)F_1 T cells positively selected to SRC in irradiated B6 mice and recovered from the spleen of the recipients 5 days later collaborated well with (CBA × B6)F_1 B cells, but poorly with CBA B cells. By contrast, F_1 T cells activated in irradiated F_1 mice stimulated both CBA and F_1 B cells. Mixtures of these two T-cell populations gave high responses with CBA B cells.

TABLE VII

*Helper Activity of T Cells Recovered from Thoracic Duct Lymph of Irradiated B6 and DBA/2 Mice Injected 5 Days Previously with Unprimed (DBA/2 × B6)F_1 T Cells Plus SRC**

T-cell group	T cells (0.8×10^6)‡	B cells§	Anti-SRC PFC/spleen at 7 days in irradiated (DBA/2 × B6)F_1 mice	
			IgM	IgG
A	F_1 $T_{+(SRC-B6)}$	B6	17,790 (1.13)‖	34,400 (1.26)
		DBA/2	310 (1.18)	390 (1.60)
		(DBA/2 × B6)F_1	7,840 (1.23)	35,860 (1.09)
B	F_1 $T_{+(SRC-DBA/2)}$	B6	390 (1.38)	250 (1.28)
		DBA/2	4,340 (1.05)	17,990 (1.10)
		(DBA/2 × B6)F_1	9,360 (1.14)	29,010 (1.02)
	Group A + group B	B6	17,980 (1.48)	32,690 (1.35)
	Group A + group B	DBA/2	3,730 (1.27)	18,180 (1.14)

* Cell recipients were exposed to 800 rads 1 day before being injected intravenously with 5×10^7 unprimed nylon-wool-purified (DBA/2 × B6)F_1 T cells plus 0.5 ml of 25% SRC.

‡ As for footnote to Table II except that irradiated (DBA/2 × B6)F_1 mice were used as the recipients of the T and B cells. When transferring a mixture of group A and group B cells to check for suppression, a dose of 0.8×10^6 of each T-cell population was used.

§ Anti-thy 1.2-treated spleen cells from mice primed with SRC 3 mo previously; 5×10^6 (DBA/2 and (DBA/2 × B6)F_1) or 8×10^6 (B6) viable cells transferred.

‖ As for footnote to Table II except that background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: B6 100(1.37) (IgM), <100 (IgG); DBA/2 < 100 (IgM), 140(1.24) (IgG); F_1 160(1.30) (IgM), 340(2.27) (IgG). PFC numbers for T cells transferred without B cells were <100/spleen. In the table values in parentheses used to derive limits of SE were calculated before subtraction of background PFC numbers. All values shown in the Table are significantly above the values of B cells transferred without T cells ($P < 0.05$).

Discussion

A priori, the restriction in helper function observed in the present studies might have been due to the induction of suppressor cells during T-cell activation. In this respect it is of particular relevance that Skidmore and Katz (14), using a hapten-carrier system, recently reported that F_1 T cells activated to the carrier in irradiated mice of one parental strain stimulated anti-hapten responses by B cells of this strain, but not those of the opposite strain. In marked contrast to the present studies, however, cooperation was not observed with F_1 B cells. The authors concluded that this failure to trigger F_1 B cells reflected the presence of suppressor cells generated during T-cell activation. It was not determined whether the suppressors were of host or of donor origin and whether or not they were antigen-specific. On the basis of these findings the authors argued that F_1 T cells contain two subpopulations of helper cells, each able to collaborate with only one of the two populations of parental strain B cells. However, it is difficult to justify this reasoning since the presence of suppressor cells in their system made it impossible to determine whether the failure to observe collaboration with B cells of the opposite parental strain reflected an innate restriction in the specificity of the helper cells rather than simple destruction of either the T cells, B cells, or both by the suppressor cells.

TABLE VIII

*Helper Activity of T Cells Recovered from Spleen of Irradiated B6 and (CBA × B6)F_1 Mice Injected 5 Days Previously with Unprimed (CBA × B6)F_1 T Cells Plus SRC**

T-cell group	T cells‡ (10^6)	B cells§	Anti-SRC PFC/spleen at 7 days in irradiated F_1 mice	
			IgM	IgG
A	F_1 $T_{+(SRC-B6)}$	CBA	980 (1.22)‖	6,930 (1.17)
		(CBA × B6)F_1	11,890 (1.24)	60,760 (1.21)
B	F_1 $T_{+(SRC-F_1)}$	CBA	10,010 (1.13)	60,900 (1.18)
		(CBA × B6)F_1	38,580 (1.25)	108,380 (1.07)
	Group A + group B	CBA	19,280 (1.47)	85,140 (1.22)

* As for footnote to Table II.

‡ T cells were harvested from the spleens of four mice per group at 5 days after transfer of F_1 T cells plus SRC. Most of the cells recovered from the spleens were (*a*) thy 1.2-positive, i.e. 93% for group A and 90% for group B, and (*b*) of donor F_1 origin, i.e. 85% of group A cells were killed by B6 anti-CBA alloantiserum. When transferring a mixture of group A and group B cells to check for suppression, a dose of 10^6 viable cells of each T-cell population was used.

§ As for footnote to Table II.

‖ As for footnote to Table II except that background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA 160(1.07) (IgM), 170(1.22) (IgG); (CBA × B6)F_1 630(1.27) (IgM), 2,490(1.27) (IgG). In the table values in parentheses used to derive limits of SE (see footnote to Table II) were calculated before subtraction of background PFC numbers. Numbers of PFC for T cells transferred without B cells were all <200/spleen. All values shown in the table are significantly above the background values of B cells transferred without T cells ($P < 0.05$).

In the present studies, three lines of evidence suggest that the restriction in helper function was not due to the presence of suppressor cells. First, the failure of F_1 T cells activated to antigen in one parental strain to collaborate with B cells of the opposite strain was not infectious, i.e., high responses were invariably observed when these populations of T and B cells were transferred in the presence of F_1 T cells activated either in mice of the other parental strain or in F_1 mice (Tables II, VII, VIII). Second, F_1 T cells activated in parental strain mice cooperated well with F_1 B cells. Third, the poor responses found with B cells of the opposite parental strain, although very low in magnitude, were not insignificant. With small numbers of helper cells these responses were close to background levels but rose in a linear fashion to appreciable levels as the dose of T cells was increased (see Fig. 1). Moreover, the magnitude of these responses was comparable to the responses observed with similar doses of unprimed F_1 T cells (unpublished data) or with unprimed F_1 T cells passaged through irradiated parental strain mice without antigen (Table III).

In view of the evidence mentioned earlier that F_1 T cells contain two discrete populations of antigen-reactive cells, it would seem reasonable that such a dichotomy accounted for the present findings. If so, can the data be taken to indicate that F_1 T cells do consist of two subpopulations of helper cells, each able to collaborate with B cells derived from only one of the two parental strains? Before approaching this question it is first necessary to consider the nature of positive selection to antigen in the irradiated parental strain

environment. Since the function of positively selected T cells was measured in terms of B-cell stimulation, one might argue that antigen presentation in the irradiated intermediate hosts was controlled by host B cells. This seems unlikely since B lymphocytes are exquisitely radiosensitive and die within a few hours of irradiation (23). Moreover, there is no evidence that B lymphocytes can present antigen to T cells. Indeed there is evidence against this notion. Thus, if antigen bound to B cells could trigger helper cell induction, removal of B cells from the inoculum of F_1 T cells used for activation should be a critical step for demonstrating restriction in helper function. Preliminary experiments suggest that this is not the case since restriction in helper function after activation in parental strain mice was no less evident when normal LN (which contain 30–40% B cells but few macrophages) rather than nylon-wool-passed T cells were used for activation (unpublished data). Macrophages (or related cells) are more likely candidates for antigen presentation in the present system, not only because of their well-accepted role in T-cell triggering, but also because they are highly radioresistant (23). The fact that the restriction in helper function could be overcome by the addition of PE cells (>80% macrophages) of the opposite parental strain (Table VI) supports this view. However, the precise identity of the cell(s) presenting antigen has yet to be established.

Whatever the nature of the cell presenting antigen to the T cell, it is clearly necessary to assume that despite the particulate nature of the antigen used (heterologous erythrocytes), the T cells were unable to recognize the antigen in terms of helper cell induction unless the antigen was first processed in a specific way in the irradiated environment. Thus, of the two putative T-cell subgroups in the F_1 T cell inoculum, only one apparently underwent clonal expansion when exposed to antigen in irradiated mice of one parental strain; the other subgroup failed to recognize the antigen and therefore remained in an unprimed (nonexpanded) state. Although this interpretation is in line with the generally accepted view that T-macrophage interactions are under MHC control, it is less easy to understand why, in the present system, such interactions were associated with apparent restrictions at the level of T-B collaboration.

One explanation for this paradox is that the data reflect restrictions acting at both the level of T-macrophage interactions and during T-B collaboration. Accordingly, one could argue that each subgroup of F_1 T cells is reactive to MHC-associated antigen presented first on host macrophages during helper cell induction in one of the two parental strain environments, and second, on the corresponding population of parental strain B cells during T-B collaboration. T-cell activation in one parental strain would thus induce clonal expansion of the subgroup of F_1 T cells able to collaborate only with B cells of that parental strain (or with F_1 B cells). The other T-cell subgroup would not be stimulated and would consequently give only a low primary response with its corresponding B-cell population, i.e. B cells of the opposite parental strain. The magnitude of this response would be equivalent to that of unprimed F_1 T cells.

The data, in toto, are consistent with this viewpoint. Nevertheless, it is clearly difficult to assess this interpretation without information on the genetic control of the restriction(s) observed. It is also necessary to consider another

possibility; that the failure of F_1 T cells positively selected in one parent to collaborate with B cells of the opposite parent simply reflected a lack of appropriate macrophages during the stage of T-B collaboration. In other words, the cells failed to manifest their helper function because on subsequent transfer with B cells of the strain opposite to that used for activation, the macrophages presenting antigen to the helper cells were now different from those encountered during the initial activation. Although this possibility might seem unlikely since F_1 mice were always used for measurement of T-B collaboration, it needs to be excluded.

The following paper is addressed to this question and shows that the restriction(s) observed in the present paper cannot be explained in terms of a lack of appropriate macrophages during the stage of T-B collaboration. The data are interpreted as indicating that restrictions do exist at the level of both T helper cell induction and T-B collaboration, each restriction mapping to the *K*-end of the *H-2* complex.

Summary

Unprimed (CBA × C57BL/6)F_1 lymph node T cells were transferred with sheep erythrocytes (SRC) into heavily irradiated F_1 or parental strain mice and recovered from thoracic duct lymph or spleens of the recipients 5 days later. To study their helper function, the harvested F_1 T cells were transferred with antigen into irradiated F_1 mice plus B cells from either the two parental strains or from F_1 mice. F_1 T cells activated in F_1 mice gave high IgM and IgG anti-SRC responses with all three populations of B cells. By contrast, F_1 T cells activated in mice of one parental strain collaborated well with B cells of this strain, but poorly with B cells of the opposite strain. Active suppression was considered an unlikely explanation for this result since (*a*) good responses were found with F_1 B cells, and (*b*) addition experiments showed that the poor response with B cells of the opposite parental strain (which was equivalent to that produced by unprimed F_1 T cells) could be converted to a high response by a supplemental injection of F_1 T cells activated in F_1 mice. The phenomenon (*a*) was specific for the antigen used for activation (criss-cross experiments were performed with horse erythrocytes), (*b*) was reflected in levels of serum hemagglutinins as well as in numbers of splenic plaque-forming cells, (*c*) applied also to comparable activation of (DBA/2 × C57BL/6)F_1 T cells, and (*d*) could be prevented by activating F_1 T cells in mice of one parental strain in the presence of peritoneal exudate cells of the opposite parental strain.

The hypothesis was advanced that F_1 T cells contain two discrete subpopulations of antigen-reactive cells, each subject to restrictions acting at two different levels: (*a*) during T-macrophage interactions and (*b*) during T-B collaboration. It was suggested that when F_1 T cells are activated to antigen in a parental strain environment, radioresistant macrophages activate only one of the two subgroups of T cells, and this subgroup is able to collaborate with B cells of the strain used for activation (and with F_1 B cells) but not with B cells of the opposite parental strain. The other subgroup of T cells remains in an unprimed (nonactivated) state.

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