HELPER FUNCTION OF T CELLS DEPLETED OF ALLOANTIGEN-REACTIVE LYMPHOCYTES BY FILTRATION THROUGH IRRADIATED F_1 HYBRID RECIPIENTS

I. Failure to Collaborate with Allogeneic B Cells in a Secondary Response to Sheep Erythrocytes Measured In Vivo

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The question of whether *H-2*-incompatible T and B lymphocytes can collaborate in a humoral immune response is controversial. The main difficulty encountered in designing experimental systems to answer this question has been to prevent the helper cells concerned from stimulating the B cells nonspecifically, i.e., through an "allogeneic" effect. Various approaches have been employed to overcome this problem.

By irradiating the helper cells to prevent an allogeneic effect, Katz et al. (1–3) demonstrated that carrier-primed T cells collaborated effectively with syngeneic and semiallogeneic (F_1) hapten-primed B cells but did not stimulate allogeneic B cells; this applied irrespective of whether responses were measured in vivo (1, 2) or in vitro (3). Studies with congeneic mouse strains led to the conclusion that T and B cells collaborate only when they share *I*-region determinants of the *H-2* complex.

Recently studies with chimeric mice have challenged this hypothesis. Bechthol et al. (4, 5) reported that allophenic mice derived from fused embryos of strains giving a high and low response respectively to the synthetic polypeptide, poly-L-(Tyr,Glu)-poly-D, L-Ala – poly-L-Lys (TGAL),[1] produced TGAL-specific antibody of low responder allotype. These findings implied, though they did not prove, that the antibody response reflected collaboration between the (*H-2*-incompatible) high responder T cells and low responder B cells. Evidence directly supporting this notion has come from studies with tetraparental bone marrow chimeras (TBMC), i.e. irradiated F_1 hybrid mice containing mature T cells derived from both parental strains, each population being mutually unresponsive to the alloantigens of the other (6). T cells from such chimeras collaborated as effectively with allogeneic B cells (cells of the opposite parental strain)

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[1] *Abbreviations used in this paper*: [^{3}H]TdR, tritiated thymidine; LN, lymph node; MLC, mixed-lymphocyte culture; PFC, plaque-forming cells; TBMC, tetraparental bone marrow chimeras; TDL, thoracic duct lymphocytes; TGAL, poly-L-(Tyr,Glu)-poly-D, L-Ala--poly-L-Lys; SRC, sheep erythrocytes.

as with syngeneic B cells in a secondary response to sheep erythrocytes (SRC) measured in vivo (7). In vitro studies with a hapten-carrier system have since confirmed these findings (8).

In rats, Heber-Katz and Wilson (9) reported essentially similar results for T cells depleted of specific alloantigen-reactive lymphocytes by filtration from blood to lymph through irradiated semiallogeneic hosts. Such T cells collaborated effectively with both syngeneic and allogeneic B cells in a primary IgM response to SRC measured in vitro.

The present paper documents evidence that, in mice, filtered T cells obtained by the above technique collaborate well with syngeneic and semiallogeneic B cells in a secondary response to SRC measured in vivo. Such cells fail, however, to stimulate allogeneic B cells.

Materials and Methods

Mice. CBA/ca (CBA) ($H\text{-}2^k$), C57BL/6 (C57BL) ($H\text{-}2^b$) and (CBA × C57BL)F_1 mice were used and kept under conventional conditions.

Cells. Thoracic duct lymphocytes (TDL) and cells from spleen and lymph nodes (LN) were obtained as described elsewhere (10, 11).

Alloantisera. Anti-C57BL serum was obtained from CBA mice given six intraperitoneal injections of 10^7 C57BL spleen cells at weekly intervals. The mice were bled at 10 days after the last injection and the serum stored frozen. Similarly, anti-CBA serum was obtained from C57BL mice hyperimmunized against CBA spleen cells. Anti-θ-C3H (thy 1.2) antiserum was made by injecting AKR mice with 2×10^7 C3H thymus cells intraperitoneally at weekly intervals for 10 wk. The mice were bled 10 days after the last injection.

Cell Identification with Alloantisera. Cells (5×10^6/ml) were incubated with antiserum (1:4 dilution) at 4°C for 30 min and then, after washing, with guinea pig complement (1:6 dilution) at 37°C for another 30 min. Cytotoxic indices were measured by dye exclusion as described by Boyse et al. (12). Under the conditions used the alloantisera invariably lysed >98% of the appropriate target lymphoid cells; the anti-θ-serum lysed 70–80% of TDL from normal CBA mice. In the case of TDL, lysis with cells treated with antiserum alone, complement alone or normal mouse serum plus complement was usually <10%.

Irradiation. Mice were exposed to whole body γ-irradiation from a ^{60}Co source at a rate of 18 rads/min. The mice used as filtration hosts received a dose of 1,000 R. Mice used as recipients for measuring adoptive collaborative responses received 750 R; cells and antigen were injected within 4–8 h of irradiation.

Injections. All cell suspensions and antigen were injected intravenously via the tail vein unless stated otherwise.

Plaque-Forming Cell (PFC) Assays. Direct (19S, IgM) PFC were detected by the method of Cunningham and Szenberg (13). 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 a specific goat anti-mouse μ-chain serum to suppress IgM PFC. The anti-μ-serum (kindly provided by Dr. M. Feldmann) suppressed 95% of total splenic PFC on day 3 of a primary response to SRC but <20% of PFC on day 8 of a secondary response; the antiserum thus appeared to suppress IgM but not IgG PFC.

Preparation of B Cells. Spleen cells were resuspended in neat anti-θ-serum at a concentration of 1.5-2 × 10^8/ml of serum 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 serum (1:6 dilution, 3×10^7 cells/ml) as a source of complement. The cells were then washed once and resuspended to a volume suitable for injection. This treatment lysed 35–45% of the spleen cells.

Preparation of T Cells. To obtain purified populations of T cells, suspensions of mesenteric LN cells were passed through nylon-wool columns as described by Julius et al. (14).

Priming with SRC. SRC were stored in Alsever's solution and washed three times in saline before use. For priming, mice were injected with 0.1 ml of 25% SRC intraperitoneally and used 6–12 wk later.

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Mixed-Lymphocyte Cultures (MLC). Responses in MLC were measured by a modification (15) of the microculture method described by Nabholz et al. (16). Briefly, 2×10^5 lymphocytes (responders) were cultured for 4 days in Linbro Microtrays (Linbro Medlabs Ltd., Dublin, Ireland) with 10^6 mitomycin-C-treated spleen cells as stimulators. Cultures were harvested after addition of tritiated thymidine ([^{3}H]TdR) at 90–96 h.

Results

Characteristics of Lymph-Borne Cells Recovered from Irradiated (CBA × C57BL)F$_1$ Mice Given CBA LN Cells. Filtered T cells were obtained by injecting 10^8 viable CBA LN cells (pooled from the mesenteric, axillary, cervical, and inguinal nodes) intravenously into 8-wk-old (CBA × C57BL)F$_1$ mice exposed to 1,000 R irradiation 4–6 h before. Thoracic duct fistulas were inserted in the recipients 16 h later and TDL collected between 18 and 40 h after injection.

The data shown in Table I are from a single experiment involving 10 recipients; 11 other experiments gave comparable findings. The lymph-borne cells collected at 18–40 h represented only a small proportion (~8%) of the injected LN cells. Of the cells collected, however, a high proportion (90–98%) were of donor origin, i.e. were resistant to lysis by CBA anti-C57BL alloantiserum (see footnote to Table I). Furthermore, nearly all of the cells (98–100%) were θ-positive; this confirmed previous findings that the B cells contaminating the injected LN cells were unable to recirculate in the irradiated hosts (15). Table I also shows the response of the filtered cells in MLC. The cells gave no detectable response against the "selecting" determinants (C57BL) but reacted well against third-party determinants (DBA/2).

In all of the experiments to be considered below, the proportion of donor lymphocytes in the lymph-borne cell suspensions was >90%; the proportion of θ-positive lymphocytes invariably exceeded 97%. The reactivity of the cells in MLC was measured in four experiments and gave results comparable to those shown in Table I.

Helper Function of CBA T Cells Filtered Through Irradiated (CBA × C57BL)F$_1$ Mice. The helper function of the filtered CBA T cells was investigated by transferring the cells intravenously in varying doses to irradiated (750 R) (CBA × C57BL)F$_1$ mice together with viable syngeneic, allogeneic, or semiallogeneic B cells (anti-θ-serum-treated spleen cells). The cells were transferred together with 0.1 ml of 5% SRC (also given intravenously) and direct (IgM) and indirect (IgG) PFC measured in the spleens of the recipients at various times later, usually at 7 days. Unless stated otherwise, the T- and B-cell populations were from mice primed with SRC 6–12 wk previously; the B cells were transferred in a dose of 5–6 $\times 10^6$ viable cells. In any one experiment the cells used and the irradiated recipients were all of the same sex.

When filtered CBA T cells (2×10^6) were adoptively transferred with CBA B cells, a good collaborative IgM and IgG response to SRC was obtained when PFC were measured at 7 days (Table II). Transfer of the cells with C57BL B cells, by contrast, failed to produce a collaborative response, i.e. the numbers of both IgM and IgG PFC were no higher than the background values in mice given C57BL B cells alone. An augmented response was obtained, however, when the C57BL B cells were transferred with (CBA × C57BL)F$_1$ T cells, viz. either (*a*) 10^8 thymus cells from normal unprimed mice (Tables II, III) or (*b*) 3×10^6 nylon-wool-purified T cells from LN of SRC-primed mice (Table IV).

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TABLE I

Properties of Lymphocytes from Thoracic Duct Lymph of Irradiated (1,000 R) (CBA × C57BL)F_1 Mice Injected with CBA LN Cells

No. of lymph-borne cells (× 10^{-6}) from irradiated (CBA × C57BL)F_1 mice collected 18–40 h after injection of 10^8 CBA LN cells*	Percentage		MLR ([^{3}H]TdR incorporation: cpm ± SE) against‖		
	Donor origin‡	θ-Positive§	CBA	(CBA × C57BL)F_1	(CBA × DBA/2)F_1
8.2 ± 0.5¶	94	99	316 ± 40	290 ± 63	17,842 ± 1534
	Normal CBA LN cells		530 ± 115	14,154 ± 1908	29,536 ± 1213

* LN cells injected intravenously 6 h after irradiation.

¶ Mean number (± SE) of lymph-borne cells from eight mice.

‡ Calculated from cytotoxic index of 6% when TDL pooled from the eight filter hosts were incubated with CBA anti-C57BL alloantiserum in the presence of complement (see Materials and Methods); this antiserum lysed 100% of TDL from (*a*) normal (CBA × C57BL)F_1 mice and (*b*) (CBA × C57BL)F_4 mice given 1,000 R 2 days before.

§ Cytotoxic index with anti-C3H-θ-antiserum in the presence of complement; this antiserum lysed 70–75% of normal CBA LN cells.

‖ Mitomycin-C-treated spleen cells as stimulators. Cultures were harvested after addition of [^{3}H]TdR at 90–96 h. Each value represents mean of three cultures.

TABLE II

Numbers of IgM and IgG PFC in Spleens of Irradiated (CBA × C57BL)F_1 Mice Given Filtered CBA T Cells Together with Either Syngeneic or Allogeneic B Cells

Cells transferred to irradiated (CBA × C57BL)F_1 mice plus SRC*		PFC/spleen at 7 days	
B cells‡	T cells§	IgM	IgG
5 × 10^6 CBA anti-θ-spleen	–	540 (700–400)‖	1,170 (1,510–910)
5 × 10^6 CBA anti-θ-spleen	3 × 10^6 CBA $T_{filt.}$	9,860 (11,380–8,540)	40,180 (46,400–34,800)
5 × 10^6 CBA anti-θ-spleen	10^8 (CBA × C57BL)F_1 thymus	3,910 (4,200–3,640)	10,410 (10,960–9,880)
5 × 10^6 C57BL anti-θ-spleen	–	490 (840–290)	350 (820–150)
5 × 10^6 C57BL anti-θ-spleen	3 × 10^6 CBA $T_{filt.}$	280 (630–120)	200 (280–140)
5 × 10^6 C57BL anti-θ-spleen	10^8 (CBA × C57BL)F_1 thymus	6,830 (8,530–5,470)	10,060 (12,000–8,840)
–	3 × 10^6 CBA $T_{filt.}$	<100	<100
–	10^8 (CBA × C57BL)F_1 thymus	<100	<100

* Viable lymphocytes transferred intravenously with 0.1 of 5% SRC into irradiated (750 R) mice.

‡ Anti-θ-serum-treated spleen cells from mice primed with SRC 6–10 wk before.

§ Filtered cells were from thoracic duct lymph of irradiated (1,000 R) (CBA × C57BL)F_1 mice injected intravenously with 10^8 LN cells from CBA mice primed with SRC 2 mo before; thoracic duct cells (99% θ-positive) were collected at 18–40 h after injection of LN cells. Thymus cells were from unprimed mice.

‖ Geometric mean of data from four to six mice per group; figures in parenthesis represent upper and lower limits of SE.

The effect of varying the dose of filtered CBA T helper cells is shown in Fig. 1. In the case of CBA B cells, appreciable responses were observed with as few as 0.33 × 10^6 helper cells. At no cell dose, however, was collaboration observed with C57BL B cells.

Despite the inability of the filtered CBA T cells to stimulate C57BL B cells, excellent collaboration was found with (CBA × C57BL)F_1 B cells (Table III, IV and Fig. 2.). The capacity to stimulate semiallogeneic not allogeneic B cells thus

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TABLE III

Numbers of IgM and IgG PFC in Spleens of Irradiated (CBA × C57BL)F₁ Mice Given Filtered CBA T Cells plus Syngeneic, Semiallogeneic, or Allogeneic B Cells

Cells transferred to irradiated (CBA × C57BL)F_1 mice plus SRC*		PFC/spleen at 7 days	
B cells‡	T cells§	IgM	IgG
6 × 10^6 CBA anti-θ-spleen	–	620 (780–490)‖	5,610 (6,080–5,190)
6 × 10^6 CBA anti-θ-spleen	2 × 10^6 CBA T_{FILT}	7,840 (8,840–6,950)	89,060 (90,420–87,720)
6 × 10^6 (CBA × C57BL)F_1 anti-θ-spleen	–	3,130 (3,770–3,350)	6,790 (7,800–5,910)
6 × 10^6 (CBA × C57BL)F_1 anti-θ-spleen	2 × 10^6 CBA T_{FILT}	18,690 (23,190–15,070)	60,700 (72,320–50,940)
6 × 10^6 C57BL anti-θ-spleen	–	690 (800–590)	2,900 (4,190–2,010)
6 × 10^6 C57BL anti-θ-spleen	2 × 10^6 CBA T_{FILT}	970 (1,170–810)	3,170 (4,140–2,420)
6 × 10^6 C57BL anti-θ-spleen	10^8 (CBA × C57BL)F_1 thymus	3,850 (4,910–3,030)	16,520 (20,090–13,590)
6 × 10^6 C57BL anti-θ-spleen	10^8 (CBA × C57BL)F_1 thymus + 2 × 10^6 CBA T_{FILT}	3,900 (4,530–3,430)	17,080 (19,380–15,050)
–	2 × 10^6 CBA T_{FILT}	<100	<100
–	10^8 (CBA × C57BL)F_1 thymus	<100	<100

*, ‡, §, ‖, as for footnote to Table II.

TABLE IV

Number of IgM and IgG PFC in Spleens of Irradiated (CBA × C57BL)F₁ Mice Given Filtered CBA T Cells plus Semiallogeneic or Allogeneic B Cells: Inability of Filtered Cells to Inhibit Helper Activity of Nylon Wool-Purified F₁ T Cells

Cells transferred to irradiated (CBA × C57BL)F_1 mice plus SRC*		PFC/spleen at 7 days	
B cells‡	T cells§	IgM	IgG
5 × 10^6 (CBA × C57BL)F_1 anti-θ-spleen	–	950 (1,100–820)‖	5,790 (6,090–5,510)
5 × 10^6 (CBA × C57BL)F_1 anti-θ-spleen	3 × 10^6 CBA T_{FILT}	8,630 (9,900–7,520)	85,350 (95,440–76,340)
5 × 10^6 (CBA × C57BL)F_1 anti-θ-spleen	3 × 10^6 (CBA × C57BL)F_1 T_{LN}¶	9,740 (11,150–8,510)	97,550 (117,270–81,150)
5 × 10^6 C57BL anti-θ-spleen	–	430 (610–300)	1,070 (2,080–550)
5 × 10^6 C57BL anti-θ-spleen	3 × 10^6 CBA T_{FILT}	690 (940–500)	1,890 (2,870–1,240)
5 × 10^6 C57BL anti-θ-spleen	3 × 10^6 (CBA × C57BL)F_1 T_{LN}¶	6,260 (7,210–5,430)	63,070 (76,480–52,010)
5 × 10^6 C57BL anti-θ-spleen	3 × 10^6 CBA T_{FILT} + 3 × 10^6 (CBA × C57BL)F_1 T_{LN}¶	4,560 (5,350–3,890)	59,310 (66,490–52,910)
–	3 × 10^6 CBA T_{FILT}	190 (310–120)	250 (440–140)
–	3 × 10^6 (CBA × C57BL)F_1 T_{LN}¶	110 (180–80)	150 (300–70)

*, ‡, §, ‖, as for footnote to Table II.

¶ Nylon wool-purified T cells from LN of mice primed with SRC 2 mo before.

appeared to exclude the possibility that, despite their filtration, the helper cells exerted an inhibitory effect against B cells carrying C57BL alloantigens. In this respect it was also found that the stimulation of C57BL B cells by F_1 T cells was not impaired when these two cell populations were transferred together with the filtered CBA T cells (Tables III, IV).

In the preceding experiments, numbers of PFC were measured arbitrarily at 7 days after transfer. Fig. 3 shows that similar results were obtained when PFC numbers were measured at 5, 7, and 9 days. At no stage of assay was the response of the C57BL B cells significantly augmented ($P < 0.05$) by the addition of the filtered CBA T cells.

The basic failure to obtain collaboration between CBA T cells and C57BL B cells was observed in a total of seven out of eight experiments. In the one experiment in which significant cooperation was seen the response was in-

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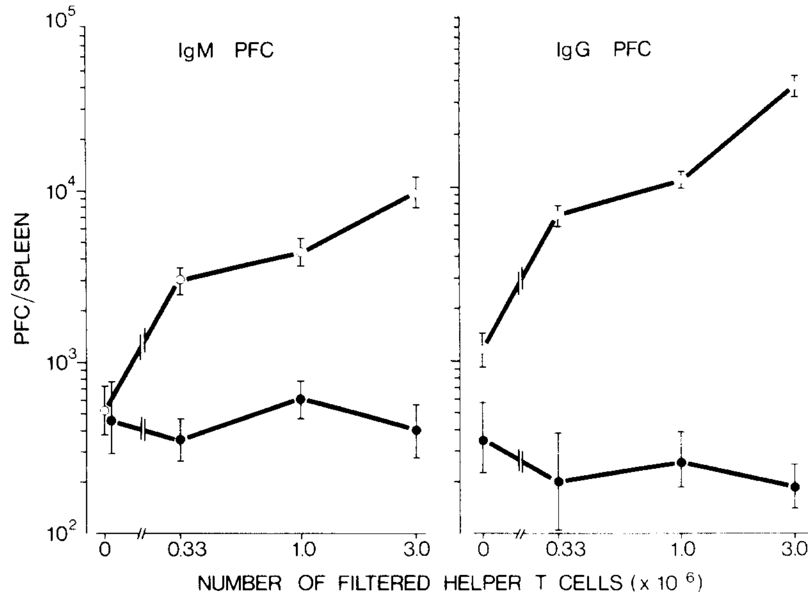

FIG. 1. Numbers of PFC in spleens of irradiated (CBA × C57BL)F_1 mice injected intravenously 7 days previously with varying numbers of filtered CBA T cells together with SRC and 5 × 10^6 anti-θ-serum-treated spleen cells from CBA (○) or C57BL (●) mice. The T- and B-cell populations were from mice primed with SRC 8 wk previously. Each point shows geometric mean of results from four to five mice; vertical bars represent upper and lower limits of SE.

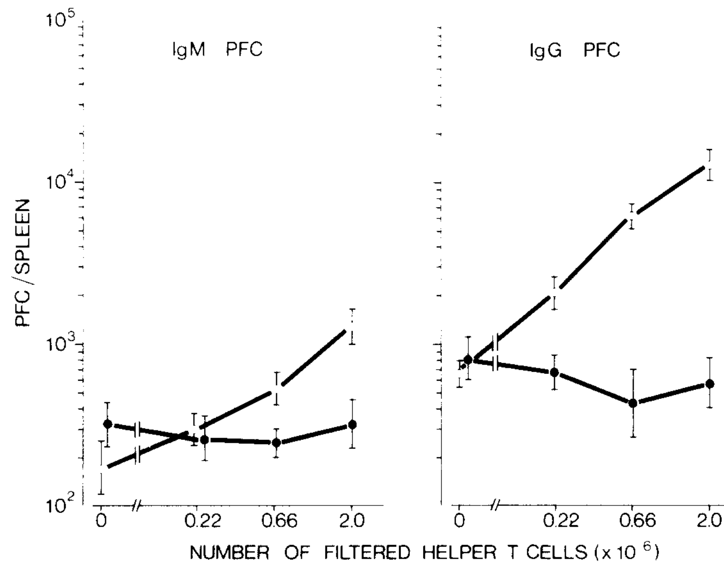

FIG. 2. Numbers of PFC in spleens of irradiated (CBA × C57BL)F_1 mice injected intravenously 7 days previously with varying numbers of filtered CBA T cells together with SRC and 5 × 10^6 anti-θ-serum-treated spleen cells from (CBA × C57BL)F_1 (○) or C57BL (●) mice. The T- and B-cell populations were from mice primed with SRC 8 wk previously. Each point shows geometric mean of results from four to six mice; vertical bars represent upper and lower limits of SE.

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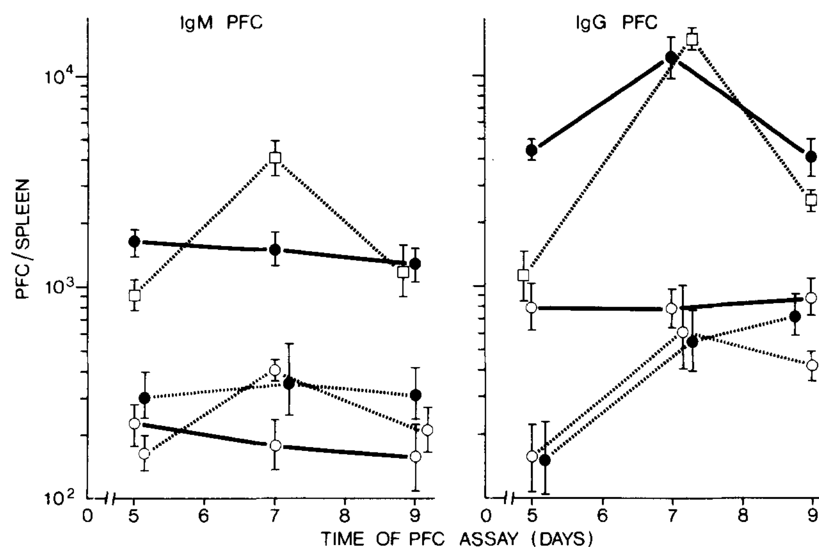

FIG. 3. Numbers of PFC in spleens of (CBA × C57BL)F_1 mice measured at various times after injection of the following cell suspensions: ○——○, 5 × 10^6 (CBA × C57BL)F_1 B-(anti-θ-serum-treated) spleen cells alone; ●——●, 2 × 10^6 filtered CBA T cells plus 5 × 10^6 (CBA × C57BL)F_1 B cells; ○- - -○, 5 × 10^6 C57BL B cells alone; ●- - -●, 2 × 10^6 filtered CBA T cells plus 5 × 10^6 C57BL B cells; □- - -□, 10^8 (CBA × C57BL)F_1 thymus cells plus 5 × 10^6 C57BL B cells. All of the cell suspensions were from mice primed with SRC 10 wks previously. The transferred cells were injected intravenously together with SRC. Each point represents geometric mean of data from four to five mice; vertical bars represent upper and lower limits of SE.

creased by only 3-fold above background (compared to 15-fold for the syngeneic [CBA] B cells).

Helper Function of C57BL T Cells Filtered Through Irradiated (CBA × C57BL)F_1 Mice. Studies with filtered C57BL T cells proved difficult because of poor recoveries from the filter hosts. In fact the average recoveries were in the order of only 2–3% of the injected cell dose. Furthermore, the lymph-borne cells tended to contain a high proportion (25–50%) of host-derived lymphocytes. To use the lymph-borne cells as helpers, it was therefore first necessary to eliminate the host-derived cells with alloantiserum and complement. Such manipulation inevitably impaired the function of the surviving cells.

The helper function of filtered C57BL T cells was investigated in two experiments. In both experiments, one of which is illustrated in Table V, the cells cooperated with semiallogeneic B cells but not with allogeneic B cells. Only the IgG responses are shown; numbers of IgM PFC were very low (<500 PFC/spleen) and have therefore not been included. The filtered cells used in Table V were tested in MLC and found to have no detectable reactivity towards CBA determinants but responded well to third party (DBA/2) determinants (data not shown).

Discussion

The technique of filtering parental strain lymphocytes through irradiated F_1 recipients—first described by Ford and Atkins (17) using rats—has proved a highly effective method for removing a specific population of alloantigen reac-

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TABLE V

Numbers of IgG PFC in Spleens of Irradiated (CBA × C57BL)F_1 Mice Given Filtered C57BL T Cells Plus Semiallogeneic or Allogeneic B Cells

Cells transferred to irradiated (CBA × C57BL)F_1 mice plus SRC*		IgG PFC/Spleen at 7 days
B cells‡	T cells§	
6×10^6 (CBA × C57BL)F_1 anti-θ-spleen	–	820 (880–760)‖
6×10^6 (CBA × C57BL)F_1 anti-θ-spleen	1.5×10^6 C57BL $T_{filt.}$	5,890 (6,240–5,560)
5×10^6 CBA anti-θ-spleen	–	1,170 (1,260–1,080)
5×10^6 CBA anti-θ-spleen	1.5×10^6 C57BL $T_{filt.}$	1,230 (1,360–1,120)
5×10^6 CBA anti-θ-spleen	10^8 (CBA × C57BL)F_1 thymus	7,840 (8,300–7,410)
5×10^6 CBA anti-θ-spleen	1.5×10^6 C57BL $T_{filt.}$ + 10^8 (CBA × C57BL)F_1 thymus	8,000 (8,280–7,730)
–	1.5×10^6 C57BL $T_{filt.}$	<100
–	10^8 (CBA × C57BL)F_1 thymus	<100

* Viable cells transferred intravenously with 0.1 ml of 5% SRC into irradiated (750 R) mice.

‡ Anti-θ-serum-treated spleen cells from mice primed with SRC 6 wk before.

§ Filtered cells were from thoracic duct lymph of irradiated (1,000 R) (CBA × C57BL)F_1 mice injected intravenously with 10^8 LN cells from C57BL mice primed with SRC 6 wk before. Of the thoracic duct cells collected at 18–40 h after injection, 98% were θ-positive and 25% were of host origin, i.e., were susceptible to lysis by C57BL anti-CBA antiserum. To eliminate the host cells, the TDL were treated with anti-CBA serum before use as helper cells.

‖ Geometric mean of data from four to six mice per group; figures in parenthesis represent upper and lower limits of SE.

tive lymphocytes. With the rat model the filtered cells show virtually no reactivity towards the selecting determinants as measured by a graft vs. host assay (17) or rejection of skin allografts (18). In mice, the specific unresponsiveness of the filtered cells applies also to the mixed-lymphocyte reaction, measured either in vitro (this paper, Table I) or in vivo (15). With respect to the present studies it would therefore seem unlikely that the failure of the filtered cells to collaborate with allogeneic B cells reflected a destructive reaction against specific alloantigens on the B cells. Indeed such a possibility would seem to be ruled out by the fact that the filtered cells (*a*) collaborated well with F_1 hybrid B cells, and (*b*) did not interfere with the capacity of F_1 T cells to stimulate the allogeneic B cells. An alternative explanation is that the allogeneic B cells reacted against the filtered helper cells, e.g. by production of (T-cell-independent) alloantibody. The fact that F_1 T cells provided effective help would seem to exclude this possibility. Moreover, if such alloantibody has been produced it would presumably have been absorbed rapidly by the transfer (F_1) hosts themselves.

The present findings contrast with the report of Heber-Katz and Wilson (9) that filtered rat lymphocytes collaborate well with allogeneic B cells. It is important to emphasize, however, that the latter findings were based on a study of a primary IgM response to SRC measured in vitro. For this reason it is difficult to make a comparison with the present data in which secondary IgM and IgG responses were measured with an adoptive transfer system. Experiments are currently in progress to determine whether or not the failure of allogeneic primed T and B cells to collaborate in vivo in the present system also applies to unprimed cells and can be observed in vitro.

As they stand, the present data are clearly consistent with the evidence of Katz et al. that T and B lymphocytes fail to collaborate unless they share certain determinants (*I*-region products) coded for by the major histocompatibility complex. How then can one explain the contrasting evidence that T cells

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tolerized to alloantigens of the opposite parental strain in TBMC collaborate well with B cells bearing these alloantigens (7)? Although there are a number of possible explanations (reviewed in reference 19), only further experimentation will resolve this paradox. In this respect it may be mentioned that the next paper in this series will add to the complexity of the problem by showing that when filtered helper T cells are transferred with a mixture of syngeneic and allogeneic B cells, both B cell populations are stimulated *pari passu,* i.e., there is no syngeneic preference for cooperation in this situation.

Summary

Helper T cells were obtained by injecting heavily irradiated semiallogeneic mice with lymph node cells from *H-2*-incompatible parental strain mice primed with sheep erythrocytes (SRC) 2 mo before. Thoracic duct lymphocytes collected from the recipients 18–40 h later (nearly all of which were θ-positive and of donor origin) were totally and specifically unresponsive against host-type determinants in mixed-lymphocyte culture. The filtered cells were transferred to irradiated semiallogeneic mice together with SRC and anti-θ-serum-treated (B) cells from SRC-primed syngeneic, semiallogeneic, or allogeneic mice. When antibody-forming cells were measured in the spleen 5–9 days later, effective IgM and IgG collaborative responses were observed with both syngeneic and semiallogeneic B cells but not with allogeneic B cells. No evidence was found that the failure to obtain collaboration with the allogeneic B cells was due to inhibition of the B cells by the T cells or vice versa.

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