INDUCTION OF A HEMOLYSIN RESPONSE IN VITRO

Interaction of Cells of Bone Marrow Origin and Thymic Origin*

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An immune response, hemolysin synthesis and proliferation of the antibody-producing cells (PFC),[1] can be triggered in mouse spleen cell cultures by the addition of immunogenic erythrocytes (1). The events following the immunogenic stimulus and preceding the appearance of the PFC are not yet understood. There is certainly evidence for the existence of lymphoid cells which carry receptor molecules with antigen specificity on their surfaces (2–4); it might be reasonable to assume that these cells are stimulated to synthesize antibody molecules of this specificity after triggering, and that they will be the precursor cells of the antibody-forming clones (5). Their stimulation seems to be dependent on the participation or presence of other cells. Thus it was shown that the removal of glass-adhering cells decreased the number of PFC (6, 7) developing from the precursor cells present in the nonadhering cell population (7); the ability to give rise to PFC could be restored by the addition of irradiated adhering cells (8), by peritoneal exudate cells (7, 9), and even by supernatant of adhering cells (8). A subpopulation of spleen cells rich in precursor cells was obtained by BSA-gradient centrifugation; few PFC developed after cultivation of this fraction. Again, a restoration of the immune response was demonstrated by adding other gradient-cell fractions (10) or adhering cells (8); irradiation of these helping cells did not diminish their activity.

In vivo, in irradiated mice, it had been shown already that the immune response to sheep erythrocytes could be restored only by injection of thymus cells in addition to bone marrow cells (11–13). The PFC are descendants of the injected bone marrow cells (14, 15) and although there is agreement about the specific participation of the cells from the thymus, little is known about their role during the immune response. A review of the many data available about the participation of thymus cells suggests that some immune responses may be more dependent on their presence than others. This influence has also been observed in vitro. Only a few PFC could be detected in cultures of spleen cells of newborn thymectomized (16) and bone marrow chimera

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[1] *Abbreviations used in this paper:* B cells, spleen cells from bone marrow chimeras; BSS, balanced salt solution; HRBC, horse red blood cells; PFC, direct local hemolysin-producing cells; SRBC, sheep red blood cells; T cells, spleen cells from irradiated and thymus cell-grafted animals.

(thymectomized, irradiated, and bone marrow-reconstituted) mice (17, 18); addition of thymus cells or of precursor-depleted spleen subpopulations restored the capability to form PFC. Furthermore, the reduction of the in vitro response by anti-θ serum might point to the involvement of thymus-derived (theta-positive) cells (19).

The work reported here was started in an attempt to learn more about the role of the cells involved and about their interaction (20). Spleen cells of bone marrow chimeras were placed in culture together with spleen cells of irradiated, thymus cell-injected mice. It is shown that only "educated," thymus-derived cells (the irradiated animals had been injected with thymus cells together with immunogenic erythrocytes) were able to assist the development of PFC. These educated cells, in the presence of their antigen, could also assist the formation of PFC to non-cross-reacting erythrocytes from the bone marrow-derived cells.

Materials and Methods

Mice.—Usually BDF_1 mice (C57BL/6 Rij female × DBA/2 Rij male), 8–12 wk old, were used; in those experiments designed to test the origin of the antibody-producing cells, C57BL/6 female or DBA/2 female mice were used. Thymectomy of the adult mice followed the description of Mitchell (21), and the absence of thymus tissue was controlled macroscopically in each mouse at the time of preparing the spleen cell suspension. Irradiation of the mice was done using a small X-ray apparatus (R. Seifert, Hamburg, Germany) at 100 R/min (120 kv, 20 ma) without filters. Mice which had received 850 R did not survive more than 10–12 days without reconstitution with bone marrow cells.

Preparation of the Thymus Cells.—The thymuses of 6-wk old mice were dissected free of the adhering tissue and gently teased apart under sterile conditions. The cells were washed twice in sterile Hanks' balanced salt solution (BSS) before being added to cultures or reinjected intravenously into irradiated animals.

Bone Marrow Cells.—flushed from femurs and tibias and washed twice in BSS.

Spleen Cells.—obtained by gentle teasing in BSS; the cells were washed twice (centrifuged at 100 *g*), and resuspended in complete medium (Eagle's minimal essential medium [MEM] from Microbiological Associates Inc., Bethesda, Md., enriched with glutamine, Na-pyruvate, nonessential amino acid mixture, antibiotics, and 5% fetal calf serum).

Cultures.—Spleen suspensions were cultivated in plastic Petri dishes (Falcon Plastics, Los Angeles, Calif. No. 3001) according to the methods of Mishell and Dutton (1) in the presence of the immunogen (usually 3×10^6 sheep red blood cells [SRBC] or horse red blood cells [HRBC]). In some experiments the cultures were kept according to Marbrook's technique (22): the inner chamber consisted of a glass cylinder 8 cm long with an internal diameter of 1 cm. One end of the cylinder was covered with a dialysis membrane held in place by an elastic silicone rubber band. The cylinder was inserted into a glass vial 2.5 × 5.5 cm; this assembly was autoclaved. 0.3 ml of cell suspension was placed into the inner part, and 10 ml of enriched medium into the outer compartment. An additional 200 μg of asparagine was added to 10 ml of medium. Cultures were incubated in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2.

Erythrocytes.—SRBC or HRBC were stored in Alsever's solution for no longer than 4 wk, and were washed twice in BSS before use.

Cell Harvest and Assay for Hemolysin-Producing Cells (PFC).—The cells were harvested after 4–5 days in culture. The number of PFC was determined by a modification of the technique described by Jerne et al. (23) and Mishell and Dutton (1). The number of PFC was always calculated per 10^6 recovered, nucleated cells. All the experiments were repeated at least

three times; within one experiment the numbers of PFC were counted from two or three cultures which were harvested separately.

The H type of the PFC was determined by treatment with anti-H-2 sera before the hemolytic plaque assay (10).

RESULTS

The Influence of Thymus Cells Added to the Spleen Cell Cultures.—The spleen cells were kept in culture and stimulated with sheep erythrocytes as described by Mishell and Dutton (1); the number of hemolysin-producing cells (direct PFC) after 4 days of incubation was in close agreement with the results published in the original paper. If thymus cells of 8-wk old mice, either carefully suspended in BSS (10^5–10^7 viable cells) or in 1–4 small fragments (approximately 1 mm^3 each), were added to these cultures the number of resulting PFC was decreased compared with the nonsubstituted cultures (Table I); sometimes the formation of PFC was prevented completely. Therefore in the following experiments the thymus cells were first injected intravenously into irradiated hosts and 8 days later the spleens of these animals were made into a dispersed cell suspension (11, 13). It was expected that most of the lymphoid cells of these spleens were descendents of the injected thymus cells. Addition of such cells to the cultures of normal spleen cells did not prevent the development of PFC (Table II). In cultures of spleens from young BDF_1 mice (4–6 wk old) the addition of these thymus-derived cells even had a positive effect and led to an increase in the number of PFC; in spleen cell cultures of older mice (3 months) this helping effect was no longer found.

Cultivation of Bone Marrow-Derived Cells Together with Thymus-Derived Cells.—Bone marrow cells in the desirable cell concentrations could not be maintained under our culture conditions. Again they were injected first into irradiated hosts, and the spleens of the grafted animals were made into suspensions 8 days later, as a source of bone marrow-derived cells. Even then the presence of a large number of hematopoietic cells made culturing difficult; only under the conditions described by Marbrook (22), in a dialysis chamber surrounded by 12 ml of medium, were many cells able to survive during the incubation period (35–50% of the cells survived the 4 day incubation period, the same percentage as in the original Mishell-Dutton cultures [1]).

Cultures of these bone marrow-derived cells together with SRBC did not lead to a detectable development of PFC. Addition of thymus-derived spleen cell suspensions sometimes allowed the development of a few PFC, but the numbers were never significant. Only after educated thymus derived (T) cells were added to the bone marrow-derived spleen cells did a significant number of PFC develop during the 4 day incubation period (Table III). To obtain educated T cells the animals had been irradiated and 10^7 foreign erythrocytes had been injected intravenously together with the 5×10^7 thymus cells. In

TABLE I

In Vitro Responses of Spleen Cells Cultivated Together With Thymus Fragments or Thymus Cells

Spleen cells	Thymus cells	PFC/10^6 cells (day 4)
Exp. No. 60		
1.3×10^7 per culture	—	620
(without SRBC)	—	85
	1×10^5	580
	5×10^5	110
	2×10^6	0
	1×10^7	24
	1 thymus fragment	210
	4 thymus fragments	265

The spleen cells were cultivated for 4 days in the presence of 3×10^6 SRBC. The thymus fragments were removed before harvesting the cells.

TABLE II

In Vitro Responses of Spleen Cells Cultivated Together With Thymus-Derived Cells

Spleen cells	T cells	PFC/10^6 (day 4)
Exp. No. 81		
1.5×10^7 (from 3-month old mice)	—	820
"	10^6	850
"	10^7	540
—	10^7	0
1.1×10^7 (from 4-week old mice)	—	110
"	10^5	180
"	10^7	310

Spleen cells cultivated in the presence of 3×10^6 SRBC. T cells: mice had been irradiated (850 R) and injected with 5×10^7 thymus cells; a spleen cell suspension of these animals was prepared 8 days later and added to the cultures.

TABLE III

Co-Cultivation of Bone Marrow-Derived Cells and Thymus-Derived Cells in Vitro

Cells	PFC/10^6 recovered cells
1.2×10^7 spleen cells	420
1.0×10^7 bone marrow-derived cells	0
1.0×10^7 bone marrow + 8×10^6 T cells	20
1.0×10^7 bone marrow + 7×10^6 educated T cells	120
7.0×10^6 T cells	5

Bone marrow-derived cells: Mice were irradiated (850 R) and reconstituted with 3×10^7 bone marrow cells. Spleen cells were prepared 12 days later. *T cells:* Mice were irradiated (850 R) and injected with 5×10^7 thymus cells; spleen cells were prepared 9 days later. Educated T cells were removed from mice which had received 10^7 SRBC together with the thymus cells. The cells were incubated for 4 days in Marbrook cultures in the presence of 10^7 SRBC.

cultures of these educated T cells none or only a few PFC developed (Table III), but in cultures of both cell suspensions together, the bone marrow-derived cells and the educated T cells, many PFC did develop in the presence of the foreign RBC.

TABLE IV

Cell Cooperation Between Thymus-Derived and Bone Marrow-Derived Spleen Cells in Vitro

Cell suspensions (Exp. 234)		PFC/10^6
B cells	T cells	
8×10^6	—	0
—	11×10^6 (I)	0
4×10^6	5×10^6 (I)	10
—	10×10^6 (II)	32
4×10^6	5×10^6 (II)	265

B cells: Mice were thymectomized, irradiated (850 R), and injected with bone marrow cells 24 days earlier. *T cells:* Mice were irradiated (700 R) and were injected with 5×10^7 thymus cells only (group I), or with thymus cells and 10^7 SRBC (group II). Spleen cell suspensions were cultivated together with 3×10^6 SRBC. PFC were assayed 4 days later.

TABLE V

Cooperation Between Educated Thymus-Derived Cells and Bone Marrow-Derived Spleen Cells In Vitro

Spleen cell suspensions (Exp. 247)		Immunogen (3×10^6 erythrocytes)	PFC/10^6 assayed with	
B cells	T cells		SRBC	HRBC
1.1×10^7	—	SRBC	7	0
1.1×10^7	—	HRBC	0	0
—	3.2×10^6 (I)	SRBC + HRBC	0	0
6.0×10^6	1.6×10^6 (I)	SRBC	420	4
6.0×10^6	1.6×10^6 (I)	HRBC	0	20
—	4.0×10^6 (II)	SRBC + HRBC	0	0
6.0×10^6	2.0×10^6 (II)	SRBC	20	0
6.0×10^6	2.0×10^6 (II)	HRBC	5	475

B cells: Mice were thymectomized, irradiated (850 R), and injected with bone marrow cells 17 days before the experiment. *T cells:* Mice were irradiated (650 R) and injected with 5×10^7 thymus cells and 10^7 SRBC (group I) or 10^7 HRBC (group II) 7 days before the experiment. Cells were cultivated for 4 days in the presence of the erythrocytes; PFC were assayed against SRBC and HRBC.

Cooperation of Bone Marrow-Derived Cells and Educated T Cells.—In the following experiments the preparation of the bone marrow-derived cells was modified in order to exclude most of the hematopoietic tissue from the spleen cell suspension. The mice had been thymectomized (at an age of 6–8 wk) and 1 wk later they were lethally irradiated with 850 R and reconstituted with 3×10^7 isogeneic bone marrow cells; 2–4 wk later spleen cell suspensions of these ani-

mals were prepared. These cells could easily be kept in Mishell-Dutton cultures for 4–5 days. No PFC developed when these cells were incubated in the presence of SRBC without the addition of educated T cells.

If these bone marrow-derived cells were mixed with SRBC-educated T cells and incubated for 4 days in the presence of SRBC, then many PFC could be detected (Table IV). Clearly a synergistic interaction during the development of PFC occurred. This cooperation was dependent on education of the T cells;

TABLE VI

Anti-H-2 Sera Analysis of the Phenotype of PFC Obtained in Cultures of Bone Marrow-Derived and Thymus-Derived Spleen Cells Obtained from Different Strains of Mice

Cell suspensions (Exp 259)			PFC per culture (day 5)* assayed after treatment‡ with		
B cells		T cells	NMS	Anti-H-2b	Anti-H-2d
C57BL (15×10^6)		—	45	0	30
—		C57BL (8×10^6)	10	0	10
C57BL (8×10^6)	+	C57BL (4×10^6)	110	0	145
C57BL (8×10^6)	+	DBA (2×10^6)	515	75	380
DBA (9×10^6)	+	—	40	30	0
—		DBA (4×10^6)	20	30	0
DBA (5×10^6)	+	DBA (2×10^6)	840	700	10
DBA (5×10^6)	+	C57BL (4×10^6)	260	210	20
Normal spleen cells					
C57BL/6 spleen (17×10^6)			800	105 (13%)	900
DBA/2 spleen (15×10^6)			2500	2500	230 (9%)

B cells: Mice were thymectomized, irradiated with 850 R, and injected with 3×10^7 isogenous bone marrow cells. *T cells:* Mice were irradiated with 750 R and injected with 5×10^7 thymus cells and SRBC.

* At the beginning of the culture period 3×10^6 SRBC were added.

‡ The cells were harvested, centrifuged, and resuspended in the original volume. 0.2 ml of cell suspension was incubated with 25 μl NMS or anti-H-2 sera for 30 min at 0°C. After washing in 5 ml of BSS, 25 μl guinea pig serum was added, and the suspension was incubated for 15 min at 35°C. After washing in 5 ml of ice-cold BSS, PFC were assayed,

spleen cells from animals reconstituted with thymus cells and SRBC could interact with bone marrow-derived B cells to form anti-SRBC PFC in the presence of SRBC. HRBC-educated T cells could interact with B cells in the presence of HRBC to form anti-HRBC PFC. In cultures of B cells together with SRBC-educated T cells, and with HRBC as immunogen, no PFC could be detected, neither anti-HRBC PFC nor anti-SRBC PFC (Table V).

Bone Marrow-Derived Cells as Precursors of the Hemolysin-Producing Cells.— The same cooperation during the development of PFC was detectable in cultures of bone marrow-derived cells and educated T cells from different strains

of mice, i.e., from DBA/2 and C57BL/6 mice (Table VI); the number of detectable PFC was partly dependent on the T cell population. Thus, fewer PFC are always seen in spleen cell cultures of our C57BL mice than in comparable cultures of our DBA mice (mice of the same age and sex were always used). In

TABLE VII

Cooperation Between Educated T Cells and Bone Marrow-Derived Spleen Cells In Vitro

Spleen cell suspensions				RBC added to culture	PFC/10^6 assayed with	
B cells		T cells			SRBC	HRBC
Exp. 242						
10×10^5		—	+	SRBC + HRBC	22	8
—		7.0×10^6	+	SRBC + HRBC	49	0
5×10^6	+	3.5×10^6	+	SRBC	450	21
5×10^6	+	3.5×10^6	+	HRBC	14	29
5×10^6	+	3.5×10^6	+	SRBC + HRBC	1010	250
Exp. 249						
8×10^5		—	+	SRBC + HRBC	46	0
—		2.4×10^6	+	SRBC + HRBC	0	0
4×10^6	+	1.2×10^6	+	SRBC	520	22
4×10^6	+	1.2×10^6	+	HRBC	20	85
4×10^6	+	1.2×10^6	+	SRBC + HRBC	525	228
		$3.2 \times 10^{6*}$	+	SRBC + HRBC	0	0
4×10^6	+	$1.6 \times 10^{6*}$	+	SRBC	56	0
4×10^6	+	$1.6 \times 10^{6*}$	+	HRBC	0	440
4×10^6	+	$1.6 \times 10^{6*}$	+	SRBC + HRBC	135	480
Exp. 239						
12×10^6		—	+	SRBC + HRBC	26	22
—		$6 \times 10^{6*}$	+	SRBC + HRBC	10	0
6×10^6	+	$3 \times 10^{6*}$	+	SRBC + HRBC	528	273
Exp. 250						
15×10^6		—	+	SRBC + HRBC	31	0
—		1.1×10^6	+	SRBC + HRBC	0	0
7×10^6	+	0.6×10^6	+	SRBC	1820	0
7×10^6	+	0.6×10^6	+	HRBC	0	10
7×10^6	+	0.6×10^6	+	SRBC + HRBC	980	140

B cells: Mice were thymectomized, irradiated (800 R), and grafted with bone marrow cells 3 wk before the experiment. *T cells:* Mice were irradiated with 600 R (Exp. 242) or 750 R (Exps. 249 and 250), injected with 5×10^7 thymus cells and 10^7 SRBC or 10^7 HRBC* respectively 8–9 days before the experiments. The cells were cultured together with the erythrocytes. PFC were assayed 4 days later.

the cooperation experiments it seems that this quality depends in part on the T-cell population. Addition of DBA–T cells to C57BL–bone marrow-derived cells raised the number of PFC in these cultures, whereas fewer PFC developed in the presence of C57BL–T cells.

Incubation of the harvested cells with anti-H serum prior to the PFC assay suppressed the formation of plaques if its specificity was directed against the histocompatability antigens of the bone marrow-derived cells. Anti-H serum directed against the H antigens of the T cells did not influence the number of PFC. This was taken as evidence that the precursor cells of the PFC had been present in the bone marrow-derived population.

Influence of Educated T Cells on the Response to Heterologous Erythrocytes.— In the preceding experiments, it was shown that SRBC-educated T cells were able to cooperate with bone marrow-derived cells in the presence of SRBC to allow the formation of anti-SRBC PFC, but that in the presence of HRBC none or only a few PFC developed. The results were different if both kinds of erythrocytes, SRBC and HRBC, were present in the cultures of bone marrow-derived cells and SRBC- or HRBC-educated T cells. In these cultures PFC against both SRBC and HRBC could be detected (Table VII). Most, if not all, of these PFC were directed against either SRBC or HRBC. When the cells were assayed in the presence of both kinds of erythrocytes, only hazy plaques developed, and their number was equal to the sum of the two counted separately. Thus, educated T cells, in the presence of the immunogen of their specificity, were also able to assist in the development of PFC against noncross-reacting erythrocytes.

DISCUSSION

The results of these experiments show a synergism of spleen cells from bone marrow-reconstituted mice and of those from thymus cell-injected mice during the in vitro response to foreign erythrocytes. The participating cells are not yet well defined.

Bone marrow chimeras had been employed as a source of bone marrow-derived cells; the mice had been thymectomized and lethally irradiated before reconstitution with bone marrow cells. It is thought that most of the lymphoid cells in these animals have come from the injected bone marrow, and that thymectomy has prevented new contact of the injected cells with thymus epithelium; it does not exclude so-called thymus-derived cells or θ-antigen-carrying cells already present in the original bone marrow (24, 25). Furthermore, it should not be forgotten that hemopoietic cells, reticuloendothelial cells, and irradiation-resistant cells are present in the spleens of the bone marrow-reconstituted animals, and that little is known about their influence during the in vitro response.

Although a positive effect of pure suspended thymus cells in spleen cell cultures was described recently (17, 18), addition of thymus cells to our spleen cell cultures reduced the number of developing PFC; strain differences might be one explanation for this discrepancy. Therefore, the thymus cells were injected into irradiated animals (26) and 8 days later the spleens were used

as the source of thymus-derived cells. Most of the lymphoid cells will have come from the injected thymus cells. Only when foreign erythrocytes were injected together with the thymus cells were these thymus-derivd spleen cells able to cooperate during the in vitro response; these spleen cells were called educated T cells. No PFC could be detected in these spleen suspensions, and few PFC were seen after 4 days in culture. Nothing is known about this "education." Under the influence of the antigen some sensitive lymphoid cells might have selected and settled into the spleen, or they may have been triggered to multiply, or been activated to some "primed" state. Again it should be mentioned that other cells, mainly irradiation-resistant cells, are present in this suspension in addition to the thymus-derived lymphocytes.

The in vitro incubation of bone marrow-derived cells together with SRBC-educated T cells and SRBC allowed the development of many PFC. In experiments in which bone marrow-derived and T cells were taken from different strains of mice, the number of PFC was reduced after treatment with antiserum directed against the histocompatability antigens of the bone marrow-derived cells (Table VI). This was taken as evidence, and it might be the most likely but not the only possible explanation, that the majority of the precursors of the PFC had been present in the bone marrow-derived cell population. (The spleen cells of the bone marrow-reconstituted animals, which are designated as B cells, in some respects appear to be equivalent to avian bursa cells.)

It had been shown by similar criteria that the precursor cells in the in vitro hemolysin response are present in the spleens of neonatally thymectomized mice (16, 17), in the D-band fraction of the BSA gradient (10), and in the nonadhering cell subpopulation (7). It seems justifiable to assume that these precursor cells already have the information to recognize the immunogen and to form clones of antibody-synthesizing cells. They need the help of other cells; adhering spleen cells (6, 7), peritoneal exudate cells (7, 9), attached irradiated cells (8, 16), and even the supernatant of attached cells (8) exert a positive influence on the development of PFC. This influence does not seem to be antigen specific, the supernatant of macrophages is active, even though the cells have not been exposed to antigen (8, 9), and increased recognition and phagocytosis of erythrocytes by the macrophages have destroyed their immunogenicity.[2]

First from the many in vivo experiments, but now also from the in vitro cooperation experiments, it became clear that lymphoid cell subpopulations participate in the antihemolysin response, without being the precursor cells of the antibody-synthesizing clone. The development of PFC from the D

[2] Hartmann, K.-U., and R. W. Dutton. The induction of antibody response *in vitro*; the effect of phagocytosis of the erythrocytes. In preparation.

band of the BSA gradient was dependent on the addition of A band cells (10) in spleen cells of thymectomized animals the response was dependent of addition of thymus cells (16–18). The development of PFC was reduced after treatment with anti-θ serum (19). These helping lymphoid cells could be irradiated in vitro (8, 16, 17) without decreasing their efficiency to assist the response. As was shown in vivo (13) for the hemolysin response and the many carrier–hapten responses (27, 28), these helping lymphocytes are antigen specific. It was suggested that they concentrate the immunogenic molecules and present them to the precursor cells in order to trigger these cells. This antigen dependence is also shown in our experiments. The thymus-derived cells were able to help the response only if they had been educated with SRBC or HRBC; the immunogen of their education, of their specificity, had to be present during the in vitro incubation period.

But (and it is important not to overinterpret the data, since they are obtained in a rather complex in vitro system of mouse cells using erythrocytes as immunogen and measuring only the development of direct PFC) in the presence of immunogen which was used for education, these T cells were able to cooperate in the response to another noncross-reacting erythrocyte. It seems as if the T cells in the presence of their immunogen are producing something which helps the development of PFC. It could be some nutritional substance, but it could be something more specific, such as factors which act on other participating cells (i.e. migration-inhibition factors) or on precursor cells. If these stimulated T cells are producing something which assists the immune response, we do not yet know if these factors act during the triggering of the response or later during the proliferation phase. It is hoped that the system described, the immune response in cultures of bone marrow-derived and of thymus-derived cells, will allow further analysis of the events during an immune response and of the role of the cooperating cells.

SUMMARY

The immune response to foreign erythrocytes was studied in vitro. Two subpopulations of cells were prepared. One was a population of bone marrow-derived spleen cells, taken from thymectomized, irradiated, and bone marrow-reconstituted mice; there was evidence that most of the precursors of the PFC had been present in this cell population, but few PFC developed in cultures of these cells alone in the presence of immunogenic erythrocytes. Another cell suspension was made from spleens of mice which had been irradiated and injected with thymus cells and erythrocytes; these cells were called educated T cells. The two cell suspensions together allow the formation of PFC in the presence of the erythrocytes which were used to educate the T cells, but not in the presence of noncross-reacting erythrocytes. If bone marrow-derived cells and T cells were kept in culture together with two different species of

erythrocytes, and if one of the erythrocytes had been used to educate the T cells, then PFC against each of the erythrocytes could be detected.

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