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IN VITRO IMMUNE RESPONSE OF HUMAN PERIPHERAL LYMPHOCYTES

I. The Mechanism(s) Involved in T Cell Helper Functions in the Pokeweed Mitogen-Induced Differentiation and Proliferation of B Cells[1]

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Human peripheral lymphocytes (PBL) upon stimulation with PWM proliferate and differentiate to IgM- and IgG-producing cells. The PWM-induced Ig production in B cells was dependent on T cells, and cell-free supernatant (CFS) obtained from PWM-stimulated PBL or T cell-rich fraction replaced T cell helper functions. The active substance(s) in CFS were most likely derived from T cells. The kinetic studies showed that the proliferation of B cells took place in advance of the final differentiation to Ig-producing cells and that T cells or T cell product(s) had to exist at the initiation of cultures in order to give the maximum helper effect. However, the final differentiation of B cells to Ig-producing cells was not dependent on T cells. The helper effect of T cells or T cell product(s) on PWM-induced proliferation and differentiation of B cells was exerted across the MHC barrier. This may make it possible to apply this experimental system to the assessment of quantitative and/or qualitative changes in human helper T cells in several immunologic diseases.

In the past decade, subsets of lymphocytes and the functions of these subsets in immune responses have been detected in experimental animals, especially mice (see References 1, 2). The knowledge obtained from experimental animals suggests that quantitative or qualitative changes in T and/or B cells may induce several immunologic disorders in human diseases. In view of this assumption it is desirable to establish an experimental system in which the immunologic functions of human T or B cells can be assessed.

Pokeweed mitogen (PWM)[2]-induced Ig production in human lymphocytes is highly reproducible and the response has been shown to be dependent on T cells (3–6). In this sense, polyclonal differentiation of B cells induced by PWM could be considered to mimic specific antibody formation and would provide an ideal system to assess the regulatory function of T cells. Actually, Waldmann and his colleagues (6, 7) showed the disorders of the helper or suppressor functions of T cells in several immunologic diseases by applying this experimental system.

In the present experiment, we attempted to study the mechanism by which helper T cells exerted their function in PWM-induced differentiation and/or proliferation of B cells in order to give the fundamental basis for this system. The results will show that the proliferation of B cells proceeds in advance of the differentiation to Ig-producing cells and that the helper function of T cells is exerted mainly on the proliferation of B cells that takes place at an early stage of the triggering events. Furthermore, it will be shown that the helper function of T cells is mediated by soluble products released from PWM-stimulated T cells and that the helper T cells or their products exert their function across the major histocompatibility complex (MHC) barriers.

MATERIALS AND METHODS

Reagents. Pokeweed mitogen (lot R657412) was obtained from Grand Island Biological Co. (Grand Island, N. Y.). Cytosine arabinoside (Ara-C), hydroxyurea (HU), and neuraminidase were obtained from Sigma Chemical Co. (St. Louis, Mo.). Mitomycin-C (MMC) was obtained from Kyowa Hakko Co. (Tokyo, Japan).

Immunoglobulins and antisera. Human IgG was purified from pooled normal human sera by 33% ammonium sulfate precipitation and passage through a DEAE cellulose column equilibrated with 0.005 M phosphate buffer, pH 8.0. Human IgM was purified from serum obtained from a patient with macroglobulinemia by Sephadex G-200 gel filtration and zone electrophoresis (8). Normal rabbit IgG was obtained from pooled rabbit sera, extensively absorbed with human IgG and IgM coupled to Sepharose 6B (9) and used for radioimmunoassay as carrier protein. Antiserum against human IgG or IgM was obtained by immunizing rabbits with 200 μg of IgG or IgM included in complete Freund's adjuvant and made mono-specific for heavy chain determinants by sequential absorptions with Sepharose-coupled Fab fragment and IgM or Sepharose-coupled Fab fragment and IgG, respectively. Goat anti-rabbit IgG serum was the same preparation as that used in our previous experiment (10). The antiserum was extensively absorbed with human IgG and IgM coupled to Sepharose 6B to remove the antibodies cross-reactive to human Ig.

Preparation of peripheral lymphocytes. Human peripheral

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[1] This work was supported by research grants from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare, Japan.
[2] Abbreviations used in this paper: PWM, pokeweed mitogen; MHC, major histocompatibility complex; Ara-C, cystosine arabinoside; HU, hydroxyurea; MMC, mitomycin-C; PBL, peripheral blood lymphocytes; CFS, cell-free supernatant; ^{3}H-Tdr (^{3}H-thymidine); TRF, T cell replacing factor.

blood lymphocytes (PBL) were separated from heparinized peripheral blood obtained from healthy adult volunteers by centrifugation on a Ficoll-Isopaque gradient (specific gravity 1.078) at 1000 × G for 20 min (11). Cells collected from the interface were washed three times with MEM (Flow Laboratories, Rockville, Md.) containing 5% FCS (Microbiological Associates, Bethesda, Md.) and resuspended in RPMI 1640 (Flow Laboratories) supplemented with 10% FCS, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 5×10^{-5} M of 2-mercaptoethanol.

Separation of B and T cells. Sheep erythrocytes (SRBC) were treated with neuraminidase at a concentration of 0.5 unit/ml for 30 min at 37°C (12). After incubation, SRBC were washed with MEM three times and adjusted to a concentration of 1% (v/v). Two volumes of neuraminidase-treated SRBC suspension, 1 volume of PBL (10 to 20 × 10^6/ml) suspension and 1 volume of heat-inactivated FCS absorbed with SRBC were mixed, centrifuged at 2000 rpm for 2 min, and allowed to stand for 15 min at room temperature. Then the pellet was gently resuspended, centrifuged again at 2000 rpm for 2 min, and allowed to stand for 60 min at room temperature. After that the pellet was gently resuspended, placed on a Ficoll-Isopaque gradient, and centrifuged at 400 × G for 30 min (13). Cells collected from the interface were washed three times with MEM containing 5% FCS, resuspended in RPMI 1640 culture medium, and used as a B cell fraction (non-rosette forming cells). Rosette forming cells in the bottom of the gradient were collected and SRBC were lysed with Tris-ammonium chloride buffer. Cells were washed three times with MEM containing 5% FCS and used as a T cell fraction. B cell fraction contained 30 to 60% of Ig-positive cells, whereas T cell fraction contained less than 1% of Ig-positive cells as judged by a direct immunofluorescence technique using fluoresoein-conjugated polyvalent goat anti-human immunoglobulin (Meloy Laboratories, Springfield, Va.).

IN VITRO *culture system.* Unless otherwise stated, 2 × 10^5 cells suspended in 200 μl of culture medium were placed in a Micro Test II culture plate (Falcon Plastics Co., Oxnard, Calif.) and cultured in 5% CO_2 in air at 37°C for 7 days. Preliminary experiments employing different doses of PWM indicated that optimal Ig-production occurred in the dose range of 1 to 10 μl/ml. Therefore the concentration of 2.5 μl/ml of PWM was used for all the experiments. On the 4th day of culture, the culture plate was centrifuged at 1500 rpm for 5 min and the culture medium was replaced with a fresh one without PWM. At the termination of the culture, the culture plate was centrifuged and culture supernatant was harvested for radioimmunoassay.

Preparation of cell-free supernatant (CFS) from PWM-stimulated lymphocytes. Two million lymphocytes in 1 ml of culture medium were incubated with 2.5 μl/ml of PWM in 12 x 75 mm culture tubes (Falcon Plastics Co.). After 48 hr the culture tubes were centrifuged and supernatant was used as CFS. Control supernatant was obtained from lymphocytes incubated for 48 hr in the absence of PWM. The same concentration of PWM used for CFS was added to control supernatant. CFS and control supernatant were dialyzed against culture medium and passed through the millipore filter.

Measurements of the amounts of IgG and IgM in culture supernatant. The amounts of IgG and IgM were determined by an inhibition radioimmunoassay described by Platts-Mills and Ishizaka (14). Briefly, purified IgG from normal human serum and IgM from the serum of a patient with macroglobulinemia were labeled with ^{125}I (New England Nuclear, Boston, Mass.). Fifty μl of appropriately diluted culture supernatants or 2-fold dilutions of control IgG (or IgM) (2000 ng to 7 ng) were mixed with 0.1 ml of rabbit anti-human IgG or rabbit anti-human IgM serum appropriately diluted in 100 μg/ml of normal rabbit IgG. After 90 min incubation at room temperature, 0.1 ml of ^{125}I-labeled IgG or IgM (100 to 200 ng/ml) was added and allowed to stand at room temperature for another 90 min. Then 0.1 ml of goat anti-rabbit IgG serum was added and allowed to stand overnight at 4°C. The precipitate was washed three times with cold borate-buffered saline and radioactivity in the precipitate was counted by a well type gamma counter (Aloka, Tokyo, Japan).

Measurement of ^{3}H-thymidine uptake. Cells were cultured under the same conditions as those used for Ig production. The incorporation of ^{3}H-thymidine (^{3}H-Tdr) was determined by adding 0.4 μCi of ^{3}H-Tdr (5 to 15 Ci/m mole specific activity, New England Nuclear, Boston, Mass.) to each culture well. After 4 hr pulse, cells were harvested on a glass filter by Dynatech Automash cell harvester (Dynatech, England). The radioactivity was measured by a liquid scintillation counter with Bray's solution as a scintillator.

Mitomycin-C treatment. Cells were treated with MMC at a concentration of 40 μg/ml for 45 min at 37°C. After incubation, MMC-treated cells were washed three times with MEM containing 5% FCS and resuspended in culture medium.

RESULTS

T cell dependency of PWM-induced Ig production by B cells. As shown in Table I, both IgM and IgG were produced by 2 × 10^5 PBL stimulated with PWM at the final concentration of 2.5 μl/ml for 7 days. In order to study whether PWM-induced Ig production was dependent on T cells, we cultured partially purified B cells, T cells, and a mixture of B and T cells in the presence of PWM. As shown in Table I, neither B cells nor T cells alone produced any significant amount of Ig. In marked contrast, 1 × 10^5 B cells when co-cultured with 1 × 10^5 T cells produced much more Ig than did 2 × 10^5 PBL. In addition, as shown in Figure 1, when 1 × 10^5 B cells were cultured with various numbers of T cells, the amounts of IgM and IgG produced in the culture fluids were proportional to the numbers of T cells added. The maximum Ig production by 1 × 10^5 B cells was observed in the presence of 1 × 10^5 through 4 × 10^5 T cells. These results showed that the PWM-induced Ig production by human PBL was dependent on T cells.

In order to see whether proliferation of T cells was required for their helper function, T cells were treated with MMC and co-cultured with B cells. As shown in Table II, 1 × 10^5 MMC-

TABLE I

Requirement for T cells for PWM-induced Ig-production by human B cells

Expt. No.	Lymphocytes[a] Cultured	IgG[b]	IgM[b]
		ng/ml	
1	2 × 10^5 PBL	413 ± 28	560 ± 168
	2 × 10^5 B	41 ± 4	38 ± 1
	2 × 10^5 T	<10	<10
	1 × 10^5 B + 1 × 10^5 T	6400 ± 680	4900 ± 636
2	2 × 10^5 PBL	1300 ± 176	1650 ± 200
	2 × 10^5 B	45 ± 4	35 ± 3
	2 × 10^5 T	18 ± 5	<10
	1 × 10^5 B + 1 × 10^5 T	7000 ± 800	7400 ± 500

[a] Both T and B cells were obtained from the same donor.
[b] Mean of triplicate cultures ± S.E.

treated T cells showed a helper effect on Ig production by 1 × 10^5 B cells comparable to that of nontreated T cells, indicating that cell proliferation was not essential for the helper function of T cells. In contrast, the Ig production was completely abolished by the treatment of B cells with MMC.

Collaboration of allogeneic T cells with B cells. In order to study whether T cells could collaborate with B cells across the MHC barrier, 1 × 10^5 T cells derived from several donors were co-cultured with 1 × 10^5 B cells. As shown in Table III, the helper effect of allogeneic T cells was almost comparable with that of autologous T cells, indicating that allogeneic T cells were able to collaborate with B cells across the MHC barrier.

Involvement of factor(s) in T-B collaboration. From the accumulated data obtained from experimental animals (15–19), it is highly conceivable that factor(s) released from T cells upon stimulation with PWM may replace the helper function of T cells in PWM-induced Ig production. In order to show this, 100 μl of CFS obtained from PBL, T cells, or B cells were added to 1 × 10^5 B cells suspended in 100 μl of fresh culture medium containing PWM and the Ig production was examined. As shown in Table IV, CFS obtained from either PBL or T cells was able to reconstitute partially the helper function of T cells. On the other hand, CFS obtained from B cells failed to function. However, we did not expect a B cell supernatant to be active because although, as shown in Table IV, we tested the B cell supernatant only once, we had tested purified B cells for PWM stimulation many times, always with negative results. CFS obtained from allogeneic PBL was able to cooperate well with B cells. The helper effect of added CFS on Ig production was proportional to the concentration of CFS. These results indicated that CFS released from T cells upon stimulation with PWM mediated the helper function of T cells in PWM-induced Ig production.

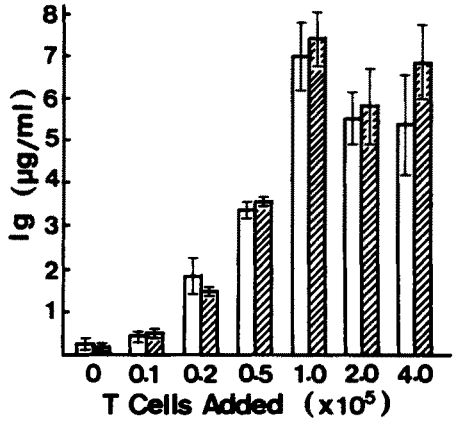

Figure 1. Effect of various numbers of T cells added to B cells on Ig-production. One × 10^5 B cells were co-cultured with various numbers of T cells and stimulated with PWM. The amounts of both IgG (□) and IgM (▨) in the culture fluids were assessed on day 7. Data represent mean of triplicate cultures ± S.E.

TABLE II

No requirement for T cell proliferation for its helper function in PWM-induced Ig-production

Lymphocytes[a] Cultured	IgG[b]
	ng/ml
2 × 10^5 B	15 ± 0.3
2 × 10^5 T	<10
1 × 10^5 B + 1 × 10^5 T	3100 ± 100
1 × 10^5 B + 1 × 10^5 MMC-treated T	3180 ± 560
1 × 10^5 MMC-treated B + 1 × 10^5 T	29 ± 5

[a] Both T and B cells were obtained from the same donor.
[b] Mean of triplicate cultures ± S.E.

TABLE III

No requirement for MHC-identity between B and T cells for T cell helper function in PWM-induced Ig-production

Expt. No.	Donors of B cells[a]	Donors of T cells[a]	IgG[b]
			ng/ml
1	H. K.	H. K.	6400 ± 680
		T. H.	6870 ± 290
2	M. O.	M. O.	2183 ± 109
		Y. N.	2383 ± 73
		T. H.	2017 ± 357
3	Y. N.	Y. N.	3800 ± 306
		M. K.	2220 ± 740
		T. K.	3867 ± 67
		T. H.	2800 ± 612
		M. O.	2167 ± 385

[a] 1 × 10^5 B cells were co-cultured with 1 × 10^5 T cells.
[b] Mean of triplicate cultures ± S.E. The amounts of IgG produced by 1 × 10^5 B cells in the absence of T cells were 41 ng/ml in experiment 1, 64 ng/ml in experiment 2, and 15 ng/ml in experiment 3. The amounts of IgG produced by 1 × 10^5 T cells were less than 20 ng/ml in all experiments.

TABLE IV

Replacement of T cell helper function with CFS obtained from PWM-stimulated lymphocytes

Expt. No.	Donors of B cells[a]	CFS Obtained from	IgG[b]
			ng/ml
1	Y. N.	PBL (Y. N.)[c]	361 ± 6 (68 ± 6)
		PBL (T. K.)	355 ± 20 (80 ± 13)
		T (Y. N.)	265 ± 44 (81 ± 40)
2	H. K.	T (H. K.)	265 ± 15 (71 ± 9)
3	M. O.	PBL (T. K.)	100 ± 30 (29 ± 1)
		T (H. K.)	98 ± 11 (29 ± 9)
4	Y. N.	PBL (Y. N.)	175 ± 25 (17 ± 0.5)
		PBL (M. K.)	267 ± 69 (32 ± 4)
		PBL (T. K.)	188 ± 62 (24 ± 3)
		T (M. K.)	153 ± 40 (N.D.[d])
		B (M. K.)	30 ± 4 (N.D.)

[a] 1 × 10^5 B cells were cultured with PWM in the presence of CFS at the concentration of 50%.
[b] Mean of triplicate cultures ± S.E. IgG produced in the presence of control supernatants is shown in parentheses.
[c] Initials in parentheses represent the donor of lymphocytes.
[d] N.D., not done.

Effect of T cells on the proliferation of B cells induced by PWM. The helper effect of T cells on Ig production might be due to their enhancing effect on B cell proliferation. In order to study this possibility, B cells and T cells were cultured separately and both of them were co-cultured in the presence of PWM. As shown in Table V, when 2 × 10^5 B cells were stimulated with PWM, a slight increase of thymidine uptake was observed on the 4th day. T cells responded to PWM much more than B cells did. It is striking that when 1 × 10^5 B cells together with 1 × 10^5 T cells were stimulated with PWM much more response was observed than when each population was separately stimulated. In order to see the effect of T cells on

the B cell proliferation, 1 × 10^5 MMC-treated T cells were added to 1 × 10^5 B cells and stimulated with PWM. As shown in Table V, B cell proliferation in the presence of MMC-treated T cells was almost 10-fold more than that observed in the absence of MMC-treated T cells. In addition, CFS obtained from PBL, when added to B cells at the initiation of culture, also augmented the increased uptake of thymidine of B cells. These results suggest that one of the possible mechanism(s) of the T cell helper function in Ig production might be due to the augmentation of B cell proliferation.

Kinetic analysis of PWM-induced Ig productions. In order to study the differentiation process of B cells to Ig-producing cells, the relationship between cell proliferation and Ig production was examined. The amounts of IgG and IgM produced by 2 × 10^5 PBL were assessed daily. As shown in Figure 2, until the 3rd day no significant Ig production was observed and then on the 4th day slight but significant amounts of IgG and IgM were detected. After that a vigorous increase of Ig production was observed. The kinetics of the B cell proliferation induced by PWM was also examined. For this purpose, 1 × 10^5 B cells were stimulated with PWM in either the absence or presence of 1 × 10^5 MMC-treated T cells and the increased uptake of thymidine was measured daily. As shown in Figure 3, B cell proliferation was greatly enhanced in the presence of MMC-treated T cells and the maximum response was observed on day 4. Then the response decreased during the last 3 days.

TABLE V

T cell dependency of PWM-induced B cell proliferation

Lymphocytes[a] Cultured	^{3}H-Tdr Uptake[b] PWM +	PWM −
	cpm	
1 × 10^5 B	494 ± 80	243 ± 16
1 × 10^5 MMC-treated T	<10	N.D.[c]
1 × 10^5 B + 1 × 10^5 MMC-treated T	3,884 ± 308	217 ± 18
1 × 10^5 B + control supernatant	426 ± 11	N.D.
1 × 10^5 B + CFS[d]	1,339 ± 170	N.D.
2 × 10^5 B	708 ± 97	326 ± 8
2× 10^5 T	3,817 ± 108	289 ± 34
1 × 10^5 B + 1 × 10^5 T	17,153 ± 858	472 ± 86

[a] Both T and B cells were obtained from the same donor.

[b] The uptake of ^{3}H-Tdr was determined on day 4. Data represent mean of triplicate cultures ± S.E.

[c] N.D., not done.

[d] CFS was obtained from PWM-stimulated PBL.

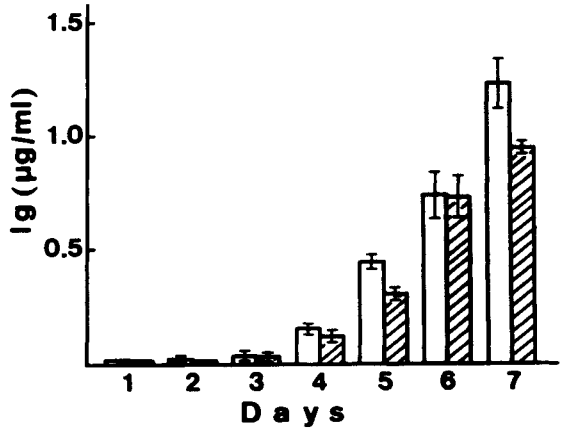

Figure 2. Kinetic study of PWM-induced Ig-production. Two × 10^5 PBL were cultured with PWM and the amounts of both IgG (□) and IgM (▨) in the culture fluids were assessed daily. Data represent mean of triplicate cultures ± S.E.

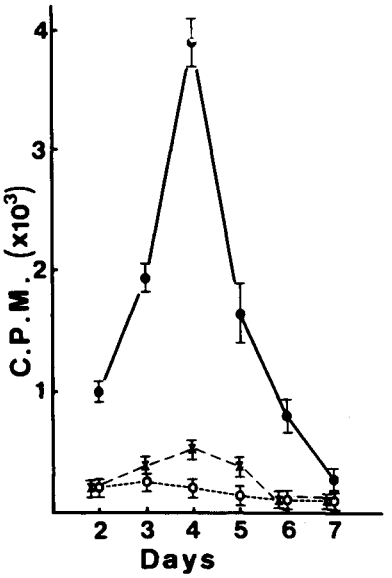

Figure 3. Kinetic study of PWM-induced B cell proliferation. One × 10^5 B cells and 1 × 10^5 MMC-treated T cells were co-cultured in the presence of (●——●) or in the absence (○- - - - -○) of PWM and the increased uptake of thymidine was assessed daily. The increased uptake of thymidine by 1 × 10^5 B cells with PWM in the absence of MMC-treated T cells (x-·-·-·x) was also assessed daily. Data represent mean of triplicate cultures ± S.E.

Further attempts were made to examine the relationship between cell proliferation and Ig production by using mitotic inhibitors such as HU and Ara-C. Either 200 mM of HU or 2 μg/ml of Ara-C were added to the cultures at various times after culture initiation and the IgG production was measured on day 7. As shown in Figure 4, these mitotic inhibitors, if added to the cultures before the 4th day, almost completely inhibited the production of IgG. The inhibitory effect of adding Ara-C or HU rapidly diminished when either was added after the 5th day of culture. Essentially, the same results were also observed in IgM production (data not shown). These observations taken together indicate that during the course of B cell differentiation to Ig-producing cells, cell proliferation occurs in advance of the final differentiation of B cells to Ig-producing cells.

Effect of adding T cells at various times after culture initiation. In order to examine at what level the T cell helper function was exerted, T cells were added to B cells at various times after culture initiation. One tenth of a million B cells were stimulated with PWM and 1 × 10^5 T cells were stimulated independently with PWM. At various times after culture initiation, prestimulated T cells together with the culture medium that was expected to contain the enhancing factor were added to the culture well containing B cells from which culture medium had been removed. On day 7, the amount of IgG in the culture supernatant was assayed. As shown in Figure 5, maximum collaboration occurred when T cells were added to B cells at the initiation of culture. The helper effect of T cells decreased rapidly with the passage of time and no significant effects were observed when T cells were added on day 4. This was true even when CFS obtained from PBL was added to B cells. To get the maximum effect, CFS had to be added at the start of the culture and only a slight enhancing effect was observed when CFS was added on the 4th day (Table

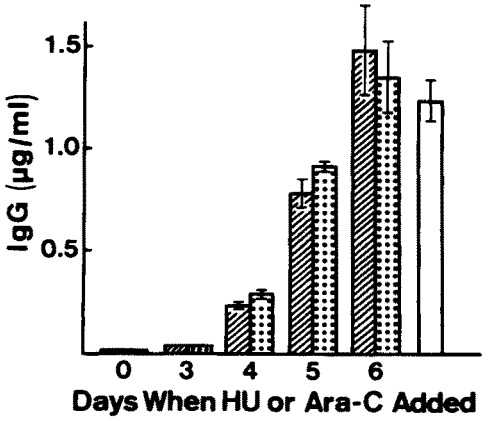

Figure 4. Effect of mitotic inhibitors added at various times after culture initiation on IgG-production. Two × 10^5 PBL were cultured with PWM and at various times after culture initiation, either 200 mM of HU (▨) or 2 μg/ml of Ara-C (▤) were added to the cultures. After 7 days' culture, the amount of IgG in the culture fluids was assessed. As a control, 2 × 10^5 PBL were cultured with PWM without adding HU or Ara-C for 7 days (□). Data represent mean of triplicate cultures ± S.E.

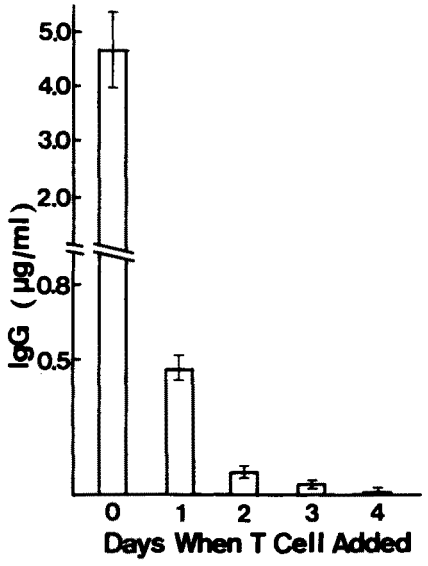

Figure 5. Effect of T cells added at various times after culture initiation on IgG-production by B cells. One × 10^5 B cells were cultured with PWM and at various times after culture initiation, T cells were added to the culture well containing B cells. The amount of IgG in the culture fluids was assessed on day 7. Data represents mean of triplicate cultures ± S.E.

VI). In addition, CFS seemed to have no effect on the final differentiation of B cells taking place during the last 3 days, inasmuch as there was no difference in Ig production between the cultures that contained CFS during the whole 7 days culture period and those containing CFS during only the first 4 days (Expt. 3 in Table VI). These observations taken together indicate that the helper effect of T cells seems to be exerted in the early stages of B cell differentiation.

DISCUSSION

T cell dependency of PWM-induced Ig production in human peripheral B cells was clearly shown in the present experiment. This result is in complete agreement with results reported by several authors (3–6). The helper function of T cells

TABLE VI

Effct of CFS added at various times after culture initiation on PWM-induced IgG production by B cells

Expt. No.	CFS[a] Presented in Cultures				IgG[b]
	day 0	*day 2*	*day 4*	*day 7*	*ng/ml*
1	———	———	———		182 ± 32
	———	- - -	———		70 ± 20
	———	- - -	- - -		22 ± 3
2	———	———	———		175 ± 25
		———	———		51 ± 12
			———	———	59 ± 4
	———	- - -	- - -		17 ± 0.3
3	———	———	———		377 ± 46
	———	———	———	———	379 ± 66
	———	- - -	- - -	- - -	80 ± 13

[a] CFS obtained from PWM-stimulated autologous PBL (——) or control supernatant (- - - -) at the concentration of 50%.

[b] IgG produced by 1 × 10^5 B cells. Data represent mean of triplicate culture ± S.E.

in PWM-induced proliferation or Ig production was MMC resistant, which suggests a similarity with the radiation-resistant helper function of T cells in antigen-specific responses observed in mice (20). Moreover, the fact that the helper function of T cells was replaced by soluble product(s) showed the similarity of PWM-induced Ig production to antigen-specific antibody responses in which antigen-specific (19) or non-specific T cell products (15–18) have been shown to be involved in mediating the helper effect of T cells. Reconstitution of Ig production in human B cells by T cell-derived soluble products has been shown by Janossy and Greaves (3) and Fauci *et al.* (5). In their experiments, purified tonsil B cells produced Ig by stimulation with PWM and PWM-induced T cell products. However, T cell products did not reconstitute Ig production in peripheral B cells (5). On the other hand, in our present experiment a significant enhancement of Ig production was observed in purified peripheral B cells by the addition of CFS from PWM-stimulated PBL. The active factor(s) were most likely released from T cells, since i) the enhancing effect of CFS from the purified T cells was comparable to that from PBL; ii) no enhancing activity was detected in CFS obtained from B cell fraction that was expected to contain more macrophages than T cell fraction; and iii) CFS obtained from PBL depleted of macrophages by the carbonyl iron technique showed the same enhancing effect (unpublished data). These results suggested strongly that the enhancing factor(s) were released from T cells, although the possibility was not completely excluded that T cells or T cell product(s) stimulated macrophages to release the enhancing factor(s) responsible for the differentiation and/or proliferation of B cells (21).

The kinetic studies showed that the proliferation of B cells took place in advance of the final differentiation to Ig-producing cells and that the proliferation was highly dependent on T cells or T cell product(s). However, the T cell dependency of the PWM-induced proliferative response of B cells may be dependent on the maturation stage of B cells. As shown by Janossy and Greaves (3), PWM-induced proliferation of human splenic B cells was T independent, whereas the presence of T cells was essential for the proliferation of peripheral or tonsil B cells. In the present experiment, it was not clear whether the helper function of T cells was involved in only the proliferative response or in both proliferation and differentiation. From the results shown in Table VI and Figure 5, it would be reasonable

to conclude that the final differentiation of B cells to Ig-producing cells is not T dependent, but that the helper function of T cells is exerted in the early stages of the triggering processes of B cells. These results showed a marked contrast to the results reported by Askonas *et al.* (22), in which T cell replacing factor (TRF) was shown to have an effect only on the final differentiation of B cells to Ig-producing cells. The discrepancy between our results and theirs might be explained on the assumption of a difference in the maturation stage of B cells. T cells or T cell factors might not be required for the proliferation of more matured B cells. In our previous series of experiments with rabbit lymphocytes (10, 23, 24), T cell-derived soluble factor showed its effect not only on differentiation but also on proliferation of B cells that had already been triggered via Ig receptors. In these experiments, T cell factor showed the enhancing effect only when it was added to culture 24 hr after stimulation via Ig receptors. In the present experiment with human B cells, however, T cells or CFS maximally exerted their effects when they were added at the initiation of the culture. Since CFS in the present experiment included PWM, it was not clear whether only T cells or T cell product(s) were required for the triggering of human B cells or whether stimulation with both PWM and T cell product(s) was essential. Attempts are now under way to remove PWM from CFS and to analyze the roles of PWM and T cell product(s) in the differentiation and proliferation of B cells.

The question of cell interactions across the MHC barrier continues to be controversial. In the secondary antibody response, Katz *et al.* (25) showed that an identical Ir-region was required for successful interaction between T and B cells. However, this has been challenged by observations in allophenic mice (26) and in tetraparental bone marrow chimera (27). In addition, several T cell replacing helper factors were able to function across the allogeneic (17, 28, 29) or even xenogeneic (30) histocompatibility barrier. In the present experiment, allogeneic T cells showed a helper effect comparable to that of autologous T cells, showing that T cells could cooperate with B cells across the MHC barrier in PWM-induced Ig production. Soluble T cell products also exerted their helper function on allogeneic B cells and the efficiency was comparable to that of the factor released from syngeneic T cells. The results are in complete agreement with those of Keightley *et al.* (4) and Broder *et al.* (6). Since several recent experiments suggest that T cells recognize antigens as a complex of the antigenic determinants and MHC (31–35), it would be supposed that the identity of MHC might be required at the step of antigen recognition in the secondary immune response and that the helper function of T cells or T cell products might be exerted across the MHC barrier in the situation where antigen recognition is not required.

In any case, T cells or T cell products exerted their helper effects across the MHC barrier in PWM-induced Ig production. MHC identity is not a requirement for the application of this experimental system to the assessment of quantitative or qualitative changes in human T cells.

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