

# INDUCTION OF AN ANTIBODY RESPONSE IN CULTURES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

BY ALMA L. LUZZATI, MICHAEL J. TAUSSIG, TOMMASO MEO, AND  
BENVENUTO PERNIS

(From the Basel Institute for Immunology, Basel, Switzerland)

The possibility to induce an antibody response in vitro with human lymphocytes is an important prerequisite for the study of the mechanisms and of the genetic control of the immune response in man. A better insight into these phenomena would be of obvious clinical importance since the nature of allergic and autoimmune disorders and of immunodeficiencies could be better understood. Antibody responses of low magnitude to sheep red blood cells (SRBC) have been obtained with spleen cells or with syngeneic combinations of lymph node and peripheral blood lymphocytes (PBL)<sup>1</sup> (1). Strong plaque-forming cell (PFC) responses were obtained when cells from tonsils, either alone or in allogeneic combinations, were stimulated with a number of different antigens (2-5 and Baumöhl, S., and I. Lefkovits, unpublished results).

For many obvious reasons the blood represents a more advantageous source of lymphocytes than any surgical material. However, few reports exist of an antibody response in vitro to SRBC with PBL (6, 7), in some instances (8) dependent on a prior stimulation with allogeneic blood lymphocytes. In an attempt to stimulate these cells in vitro to respond to SRBC we met with a number of failures.

Having previously observed in rabbits some peculiar features when inducing antibody responses in vitro with blood lymphocytes (9-11), we decided to investigate extensively and systematically the conditions necessary to elicit a good and reproducible antibody response in vitro with human PBL.

In this paper we present evidence that, under appropriate conditions, human PBL depleted of an adherent inhibitor cell can be triggered to give a small antibody response. The magnitude of this response is highly increased by an antigen-specific mouse T-cell factor.

## Materials and Methods

*Preparation of Peripheral Blood Leukocytes.* Heparinized blood obtained from healthy donors was treated with nylon wool according to the method of Hammond et al. (12). Briefly 20 ml of whole blood were pipetted into a 50-ml syringe packed with 3 g of nylon wool (Coop, Basel, Switzerland, no. 609.002). With the aid of the syringe plunger the blood was forced to enter the wool. After 45 min of incubation at 37°C the cell suspension was squeezed out of the syringe. The wool was washed two times with 10 ml of warm Hanks' balanced salt solution (BSS). The resulting

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<sup>1</sup> *Abbreviations used in this paper:* BSS, balanced salt solution; FCS, fetal calf serum; HRBC, horse red blood cells; MEM, Eagle's minimal essential medium; PBL, peripheral blood lymphocytes; PFC, plaque-forming cells; PWM, pokeweed mitogen.

cell suspension was layered on a Ficoll (Pharmacia Fine Chemicals Inc., Uppsala, Sweden)-Urovison (Shering, Berlin, West Germany) gradient (density 1,077) and centrifuged for 40 min at 20°C with an interface force of 400 *g* (13). The cells at the interface were harvested and washed in Hanks' BSS.

In some experiments the nylon-treated blood was mixed with pig skin gelatin (Eastman Kodak Co., Rochester, N. Y., no. 5247) at a final concentration of 0.6%, and the red cells were allowed to settle at 37°C for 30 min. The leukocyte-containing supernate was centrifuged at room temperature, and the pellet was washed in Hanks' BSS.

*Tissue Cultures.* The cells were suspended at the required density in a modified Mishell-Dutton medium (14), containing  $5 \times 10^{-5}$  M 2-mercaptoethanol, 0.01 mg/ml of adenosine, guanosine, uridine, and cytosine (15) and 8% fetal calf serum (FCS) (Table 1A).

50  $\mu$ l per ml of a 1% SRBC or HRBC suspension were added as antigen. The cells were distributed in aliquots of 0.1 ml per well in plastic trays (Falcon Plastics, Oxnard, Calif., no. 3040) and incubated in a CO<sub>2</sub> incubator without rocking.

After the first 24 h of culture the cells were fed with 50  $\mu$ l per well of a nutritional cocktail slightly modified from the one described by Mishell and Dutton (14) and containing 0.02 mg/ml of the four nucleosides and 26% FCS (Table 1B). In some experiments the cultures were rocked at 7-10 cycles/min on a rocking platform (Bellco Glass, Inc., Vineland, N. J.) and were kept in an atmosphere of 7% O<sub>2</sub>, 10% CO<sub>2</sub>, and 83% N. Some of the cultures were performed in a vol of 1 ml in plastic Petri dishes (Falcon Plastics, no. 3001).

A number of experiments were also performed with the culture conditions found to be optimal for rabbit (9) and for monkey (unpublished results) PBL, that is the cells were suspended in RPMI 1640 medium containing 10% FCS and incubated in 1-ml aliquots in plastic tubes (Falcon Plastics, no. 2003) kept in an upright position in a CO<sub>2</sub> incubator without any further treatment.

*Evaluation of the Antibody Response In Vitro.* At the time of harvesting four wells were pooled, the cells washed once in Hanks' BSS, and resuspended in 0.2 ml of Eagle's minimal essential medium (MEM). When 1-ml cultures (in tubes or dishes) were made, duplicates were pooled, and the cells, after washing, were resuspended in 0.5 ml of MEM. Cells were counted and the viability tested with the eosin exclusion method.

The number of direct (IgM) and indirect (IgG) PFC in the cultures was assayed using a modification of the method originally described by Jerne et al. (16) and adapted for microscope slides by Mishell and Dutton (14) as previously described in detail (11). Briefly, 20  $\mu$ l of a 10 mg/ml solution of DEAE-dextran (Pharmacia Fine Chemicals, Inc.), 20  $\mu$ l of 20% SRBC or HRBC, and 10-50  $\mu$ l of cell suspension were added to tubes containing 0.5 ml of 0.7% agar at 46°C. The mixture was poured onto microscope slides precoated with agar and allowed to solidify. The slides were then incubated at 37°C for 1 h followed by 2½ additional h of incubation with a 1:30 dilution of SRBC-absorbed guinea pig complement (Behringwerke AG, Marburg-Lahn, West Germany) or with rabbit anti-human IgG diluted 1:800 in 1:30 guinea pig complement.

The culture fluids were assayed for presence of anti-SRC antibody by hemagglutination performed in microtiter V-shaped plates. Mercaptoethanol sensitivity of the synthesized antibody was checked by preincubating the culture fluids with 0.1 M 2-mercaptoethanol at 37°C for 30 min.

*Preparation of the Mouse T-Cell Factor.* T-cell factor specific for SRBC or HRBC was prepared in C57BL/10ScSn mice using the method described in detail by Taussig (17). Briefly, 10<sup>6</sup> thymocytes were injected into irradiated (750 R) recipients together with 0.1 ml of 10% SRBC or HRBC. 7 days later the spleens of the recipient animals were removed and made into single cell suspension in MEM (Microbiological Associates, Bethesda, Md.) with 10-20 spleen equivalents of cells per 5 ml of medium. The corresponding antigen was added in the amount of 0.1 ml of 0.1% RBC suspension per ml of culture, and the cells were cultured for 6 h at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. At the end of the culture period the cells were spun down, the cell-free supernate represented our preparation of the T-cell factor.

The freshly prepared factor was added to the human PBL at a final concentration corresponding to the equivalent of one mouse spleen per ml of human cell suspension. Antigen was added (0.05 ml of 1% suspension per ml), and the mixture was incubated in ice for 30 min with frequent mixing. At the end of the incubation time the cells were centrifuged, resuspended in the same volume of fresh medium, and distributed in the culture vessels.

TABLE I  
*Ingredients for Cultures*

(A) Culture medium			
Triple distilled water			ml 81
Hanks' BSS (10 ×)	GIBCO*	No. 406	ml 9
Essential amino acids (50 ×)	GIBCO*	No. 113	ml 2
Nonessential amino acids (100 ×)	GIBCO*	No. 114	ml 1
Sodium pyruvate (100 ×)	GIBCO*	No. 136	ml 1
Sodium bicarbonate (7.5%)	GIBCO*	No. 508	ml 3.75
Penicillin-streptomycin	GIBCO*	No. 507	ml 1
HEPES (1 M)	Microbiol‡	No. 17-737	ml 1
Glutamine (200 mM)	GIBCO	No. 503	ml 1
Vitamins (100 ×)	GIBCO	No. 112	ml 1
Guanosine (1 mg/ml)	Koch-Light§		ml 1
Adenosine, cytosine, uridine (1 mg/ml)	Koch-Light§		ml 1
2-mercaptoethanol (0.01 M)	Fluka		ml 0.5
Fetal calf serum (rehatuin)	Reheis¶	Batch K22502	ml 9
(B) Nutritional cocktail			
Hanks' BSS (1 ×)	GIBCO	No. 406	ml 9
Essential amino acids (50 ×)	GIBCO	No. 113	ml 0.4
Nonessential amino acids (100 ×)	GIBCO	No. 114	ml 0.2
Sodium bicarbonate (7.5%)	GIBCO	No. 508	ml 0.75
Glucose (50%)	Difco	No. 0973-60	ml 1
Glutamine (200 mM)	GIBCO	No. 503	ml 0.3
Guanosine (1 mg/ml)	Koch-Light		ml 0.4
Adenosine, cytosine, uridine (1 mg/ml)	Koch-Light		ml 0.4
Fetal calf serum (rehatuin)	Reheis	Batch K22502	ml 4.5

\* GIBCO, Grand Island Biological Co., Grand Island, N. Y.

‡ Microbiological Associates, Bethesda, Md.

§ Koch-Light Lab. Ltd., Coinbrook Bucks, England.

|| Fluka, A. G., Buchs, S. G., Switzerland.

¶ Reheis Chemical Co., Chicago, Ill.

## Results

*Establishment of Culture Conditions.* The experiments performed before the nylon filtration step was introduced in the cell separation procedure gave negative results. After the finding of this crucial requirement, responses of low magnitude to SRBC could be obtained, and it became therefore possible to try to optimize the culture conditions.

Antibody responses could not be induced when the cells were cultured at different densities in RPMI 1640 medium containing 10% FCS, under conditions (A in Table II) which were shown to be optimal for a secondary in vitro response to SRBC or to streptococcal carbohydrates with rabbit PBL (9, 10) or to SRBC with monkey PBL (unpublished results). Negative results were also obtained when FCS was substituted with human AB serum or when different culture vessels were used. With a modified Mishell-Dutton medium (B in Table II) no responses were obtained when the cultures were performed in plastic dishes and kept in an atmosphere of 7% O<sub>2</sub>, 10% CO<sub>2</sub>, and 83% N with constant rocking.

TABLE II  
*Effect of Different Culture Variables on the In Vitro Response of Human PBL to SRBC*

Culture conditions*	Cell density $\times 10^{-6}$ /ml	Vessels	PFC/ $10^6$ initial cells		
			day 5	day 9	day 14
A	9.0	Trays	1	0	0
	4.5	Tubes	0	0	0
	4.5	Trays	0	0	0
B	9.0	Dishes	0	0	0
	9.0	Trays	7	46	4
	4.5	Dishes	0	0	0
	4.5	Trays	0	0	0

\* See text for details.

Positive results were, on the other hand, observed with the same medium when the cultures were performed in a vol of 0.1 ml in the wells of plastic trays. Under these conditions the gas mixture and the rocking did not seem to play an important role, therefore, in the later experiments the cultures were kept in a CO<sub>2</sub> incubator without rocking.

A relatively high cell density was crucial for the induction of the response (Table II). Feeding with a nutritional cocktail 24 h after initiation of the cultures was another important requirement (Table III). With these culture conditions a PFC response could be obtained which peaked at about day 8, with 30-300 PFC per  $10^6$  initial cells. Fig. 1 illustrates the results obtained with five different donors.

Plaques were all of the direct type. The response was dependent on the presence of antigen, controls showed very few if any PFC. However, in more than 50% of the experiments no response or a very low response was obtained, even with donors that have previously given good results. This was true also with gelatin separated PBL.

In the attempt to find out whether this variability is due to the presence of a very small number of circulating precursors able to be triggered by SRBC, we performed an experiment where several independent pools were assayed at each harvesting day. If the assumption was true there should be a big variability among different pools due to sampling error. The results reported in Table IV show that this is not the case, in fact, all the pools responded and with approximately the same number of plaques.

*Search for Possibilities to Amplify the Response.* Experiments were performed to investigate whether it is possible to amplify the B-cell response to the antigen and hence to obtain better and more consistent results. In a series of experiments the effect of various mitogens added to the cultures together with the antigen was investigated. No effect on the antibody response was observed when 5-20  $\mu$ g/ml of lipopolysaccharide (LPS) (*Escherichia coli* LPS, Difco Laboratories, Detroit, Mich., no. 3122-25) or a 1:100-1:500 dilution of pokeweed mitogen (PWM) (Grand Island Biological Co., Grand Island, N. Y., no. 536) were added.

TABLE III  
*Effect of Feeding with the Nutritional Cocktail on the In Vitro  
 Response of Human PBL to SRBC*

Cocktail	PFC/10 <sup>6</sup> initial cells			
	day 6	day 7	day 8	day 9
Yes	2	21	23	9
No	3	0	0	0

The cells were cultured in the modified Mishell-Dutton medium at a density of  $9 \times 10^6$ /ml in the wells of plastic trays and incubated in a CO<sub>2</sub> incubator without rocking.

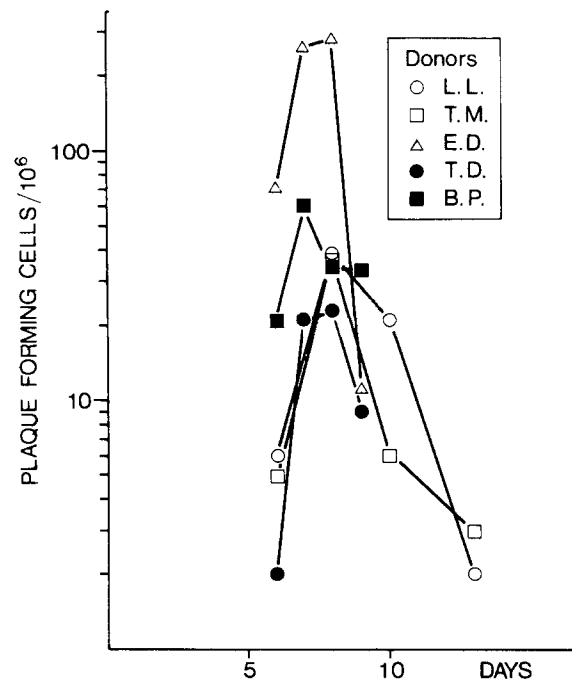


FIG. 1. Antibody response in vitro to SRBC with PBL of five different donors.

In other experiments a modification of the cell surface with enzymes or with Na periodate was attempted. The cells were treated with pronase (Protease VI, Sigma Chemical Co., St. Louis, Mo.) at concentrations of 100, 200, or 400  $\mu\text{g}/\text{ml}$  or with neuraminidase (*Cl. perfringens*, Worthington Biochemical Co., Freehold, N. J.) at concentrations of 20 or 1.5  $\mu\text{g}/\text{ml}$ , or with 1 mM NaIO<sub>4</sub> for 20 min at 37°C. The cells were then washed and put in culture with antigen or with antigen and PWM (final dilution 1:500). None of these treatments improved the response to SRBC.

When, on the other hand, human lymphocytes were incubated with an antigen-specific mouse T-cell factor a striking increase in the response to the antigen was observed. Fig. 2 shows the plaques obtained when equivalent

TABLE IV  
*Comparison of the Anti-SRBC Response in Different Cultures*

Donor	Ag	Day in culture			
		6	7	8	9
B. P.	-	0,0	5,0,0	0,0,0	0,0,0
	+	21,17	85,30,47	75,12,7	6,15,66,36
I. L.	-	3,1	0	-	-
	+	53,23	11,34	-	-

Figures represent PFC per pool of two wells.

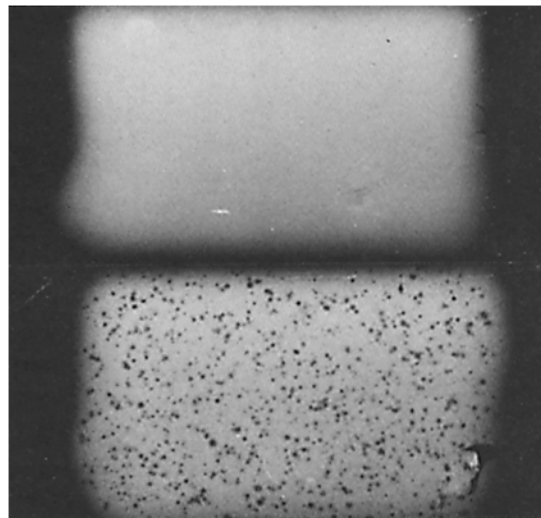


FIG. 2. PFC to SRBC observed at day 5 from cultures nontreated (top) and treated (bottom) with mouse T-cell factor.

volumes of nontreated and treated cultures were assayed for their response to SRBC. The best results were obtained when  $6 \times 10^6$ /ml PBL were treated with the factor and cultured at the same density (Table V). With  $9 \times 10^6$ /ml cells the response was slightly lower and with  $3 \times 10^6$ /ml no response whatsoever could be observed.

When human PBL at a cell concentration of  $6 \times 10^6$ /ml were treated with a mouse T-cell factor specific for SRBC, PFC specific for the antigen could already be observed at day 4, then their number increased and a peak was reached around day 8 (Fig. 3).

Parallel to the increase in PFC there was an increase in cell number. The cells recovered from the treated cultures were at all the times more numerous than in the nontreated cultures (Fig. 3). Similar results were obtained in a number of different experiments (Table VI) although the height of the response, both proliferative and antibody-producing, varied from experiment to experiment, probably due to variations from one batch of factor to another. It is clear from

TABLE V  
*Anti-SRBC Response In Vitro of Human PBL Treated with T-Cell Factor: Effect of Cell Concentration*

Cell input per well $\times 10^{-5}$	Recovered cells per well $\times 10^{-5}$	PFC per well
3	0.4	0
6	12.4	910
9	20.2	710

Cells at different concentrations ( $3 \times 10^6$ ,  $6 \times 10^6$ ,  $9 \times 10^6$ /ml) were treated with the factor, centrifuged, brought to the initial volume with fresh medium, and cultured in 0.1-ml aliquots per well. The response was measured at day 6.

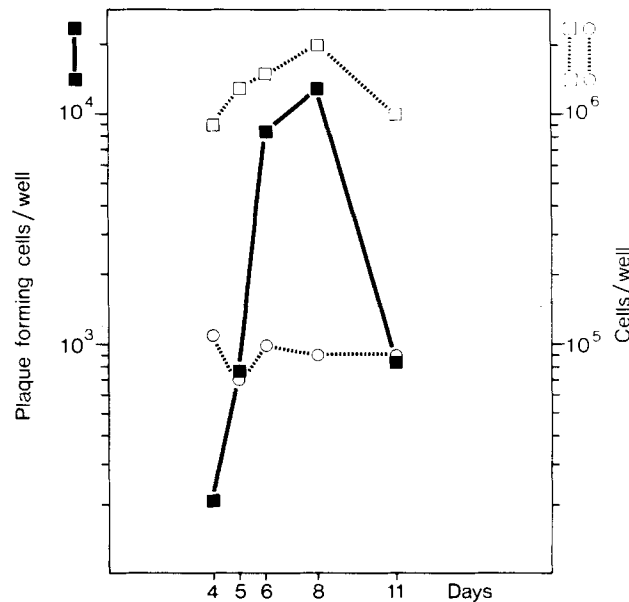


FIG. 3. Response in vitro to SRBC of human PBL treated with mouse T-cell factor. (■—■) PFC/well; (□--□) recovered cells/well; (○--○) recovered cells/well (control without factor treatment); Cell input/well  $6 \times 10^5$ . The cultures that were not treated with the factor did not give PFC.

the data shown in Table VI that a higher proliferative response accompanies a higher PFC response. In experiment no. 3 the controls without factor showed a good cell recovery and a late appearing small PFC response.

The results presented in Table VII show that the effect of the mouse T factor is antigen dependent and antigen specific. In fact a factor induced with SRBC, when used alone or in presence of HRBC, did not cause any proliferative and antibody response. Both responses were obtained when the factor and its corresponding antigen were added to the cells. The activity of the factor could be removed by absorption with packed SRBC or by passage over a Sepharose column coated with a B10A anti-B10 serum (anti-*H-2<sup>b</sup>*).

TABLE VI  
*Anti-SRBC Response In Vitro With Human PBL Treated with T-Cell Factor: Three Independent Experiments with the Same Donor*

Exp.	Day	Cells ( $\times 10^{-5}$ )	PFC
1	6	12.4	910
	8	14.3	1,423
2	6	14.7 (1.0)	8,360 (0)
	8	20.5 (0.9)	13,000 (0)
3	6	5.5 (1.5)	61 (0)
	8	9.9 (3.4)	124 (16)

The results are given as cell yield and PFC per well. The initial input was  $6 \times 10^5$  cells per well. In parenthesis, controls without factor.

TABLE VII  
*Some Properties of the Mouse T-Cell Factor Helping the In Vitro Response of Human PBL*

Present in culture		Day 5		Day 8	
Factor	Antigen	Cells $\times 10^{-5}$	PFC	Cells $\times 10^{-5}$	PFC
No	SRBC	0.7	0	0.9	0
No	HRBC	0.7	0	0.9	0
Yes	No	0.8	0	0.8	0
Yes	SRBC	13.0	750	20.5	13,000
Yes	HRBC	0.2	0	1.1	0
Anti-H-2 Abs	SRBC	1.0	0	0.6	0
SRC Abs	SRBC	0.8	0	1.2	0

The results are given as cell yield and PFC per well. The initial cell input was  $6 \times 10^5$  cells per well. The factor was induced in presence of SRBC.

The specificity of the effect was further investigated by using a mouse factor specific for HRBC (Table VIII). The response obtained to HRBC was rather low, and it was dependent on the presence of the specific antigen only at day 6, while at day 8 a small response to HRBC was also induced in the presence of SRBC. A strong stimulatory effect was seen when both antigens were used in the presence of HRBC factor.

Cultured cells were stained for their content of immunoglobulins with a fluorescent rabbit anti-human Ig serum. The proportion of cultured cells which contained Ig was in no instance higher than 2-3% and usually around 1%. The results of one experiment are shown in Table IX. In the treated cultures there



TABLE VIII  
*Antigen Specificity in the Activation of Human Lymphocytes by Mouse T-Cell Factors*

Type of immunogen for		Day 6			Day 8		
Factor production	Addition to cultures	Cells ( $\times 10^{-5}$ )	PFC to		Cells ( $\times 10^{-5}$ )	PFC to	
			SRBC	HRBC		SRBC	HRBC
—	SRBC	1.5	0	0	3.4	16	0
SRBC	SRBC	5.5	61	1	9.9	124	0
SRBC	HRBC	1.0	0	0	2.0	0	0
HRBC	HRBC	3.0	3	24	2.8	3	25
HRBC	SRBC	2.2	0	0	2.5	16	22
HRBC	HRBC and SRBC	2.5	66	14	3.7	1,011	465

Results are given as cell yield and PFC per well. The initial cell input was  $6 \times 10^5$  cells per well.

TABLE IX  
*Effect of Specific Mouse T-Cell Factor on Human PBL Cultured with SRBC*

Day	Factor	Cells per well	Ig <sup>+</sup> per $10^6$ cells	PFC to SRBC per $10^6$ cells
5	—	$0.7 \times 10^5$	600 (300)	0
	+	$13.0 \times 10^5$	1,600 (1,000)	577
8	—	$0.9 \times 10^5$	<100 (<100)	0
	+	$20.5 \times 10^5$	27,000 (6,000)	6,500
11	+	$10.2 \times 10^5$	1,500 (1,000)	834

Ig<sup>+</sup>, IgM and IgG (in parenthesis) containing cells.

was an increase in the number of Ig-containing cells which paralleled the increase in PFC, being two to four times higher.

The class of the antibody produced was examined; all the plaques detected in the present experiments were direct. We could not detect any additional plaque upon treatment with a rabbit anti-human Ig serum. Accordingly all the antibody secreted in the culture fluid and which in some experiment reached a titer of 1/64 in hemagglutination was mercaptoethanol sensitive.

### Discussion

The data presented in this paper show that in human PBL there are cells which, under appropriate conditions, can be triggered in vitro with SRBC to

give a specific antibody response. We report evidence that the number of such cells must be relatively high, in fact no fluctuation of the response was observed among different cultures when culturing  $10^6$  cells/well, thus indicating that each well must have received at least one precursor cell. Triggering can occur only after removal of adhering inhibitor cells, which are likely to be analogous to the adhering inhibitor cells previously described in rabbit PBL (11).

Unfortunately, under the conditions described, positive results were obtained in not more than 50% of the experiments, even when the same blood donors were used and even though the same procedure was always followed, the ingredients were from the same batch and the same operators were involved in any given step. Addition of mitogens or modification of the cell surface by enzymatic treatment or with sodium periodate did not change the magnitude of the response or the proportion of positive experiments. On the basis of the results obtained with the T-cell factor this variability can perhaps be ascribed to insufficient number of T-helper cells in human peripheral blood or to a blocking of their function. In fact when human PBL were treated with a mouse T-cell factor specific for SRBC, in the presence of the corresponding antigen, a striking effect was observed. The magnitude of the antibody response was increased up to 100-fold, without any change of the kinetics, a peak being still obtained after about 8 days of culture. This increase in the antibody response was paralleled by an increase in cell proliferation and/or cell survival, in comparison with cultures performed with cells not treated with the factor or treated with the factor in the absence of the specific antigen.

The observation that the proportion of Ig-containing cells is not much larger than the proportion of PFC indicates that the effect of the factor treatment is uniquely directed toward the cells able to respond to the specific antigen. However, as the proportion of Ig-containing cells was not higher than 1-2% of the cells recovered from the cultures, the question arises as to what all the other cells are. It may be that they represent human helper T cells which may also come into play as will be discussed later.

The mouse factor used in the present investigation was obtained under the conditions previously shown to yield a product of educated murine thymocytes able to cooperate with bone marrow cells *in vivo* (17) even in spite of *I*-region incompatibility (18). It was postulated that the factor has an antigen-specific variable section joined to an *H-2*-linked gene product (19).

The molecule active in helping the human PBL to give an anti-SRBC response displays basic similarities in that it can be removed by absorption with SRBC or by treatment with anti-*H-2* serum. There are many reports indicating that various factors produced by T cells, either after contact with a specific antigen or after stimulation by allogenic cells or mitogens, can replace the requirements for T cells in antibody formation (20-23). These factors may or may not have antigen specificity and in many instances have been shown to act across an allogenic or even xenogenic histocompatibility barrier (24, 25, and Estroff, T. W. and P. Galanaud, personal communication).

It has been shown that the mouse T-cell factor used in the present work binds to human cells and that when factors specific for synthetic antigens, such as (T,G)-A--L and (Phe,G)-A--L, are used, donors can be found whose cells do not

bind either one or the other or both factors (Taussig, M. J. and D. Bernocco, unpublished results). On the other hand, it is not known if the binding is exclusively to B cells, through a specific acceptor molecule, as postulated for the mouse system (19). In fact it could also be that human T cells are involved in the binding and that this in turn causes them to produce a human factor which may or may not be antigen specific. If this were the case many of the non-Ig-containing cells recovered from the cultures could be human T cells actively collaborating to the anti-SRC response.

Participation of human T cells to the *in vitro* response in the presence of the mouse T-cell factor could perhaps explain the mutually potentiating response obtained when two possibly cross-reacting antigens are present in culture at the same time, with a mouse factor specific for one of them (Table VIII). Consistent with this view is also the observation that when (T,G)-A--L is added with SRBC to cultures of human cells treated with SRBC-specific factor a response to (T,G)-A--L is also obtained (unpublished results).

The question now arises why human circulating lymphocytes, more than 60% of which are T cells (26), are difficult to induce to an *in vitro* antibody response, unless supported by a mouse factor and its specific antigen or by a mixed lymphocyte culture reaction (8). In this respect it is noteworthy that the requirement for an additional help is not absolute and that under certain conditions, which we have failed to standardize, a sizable antibody response can be induced in cultures of human PBL solely in presence of the antigen. Additional factors might counteract unfavorable conditions due to functional heterogeneity of peripheral T cells in terms of ratios of helper and inhibitory effects.

It is obvious that further experiments are required to elucidate the mechanisms that are at the basis of the antibody response *in vitro* described in this paper. However, we believe that this system, perhaps after some improvement and extension to other antigens, may allow a study of the immune response in man.

### Summary

A culture system is described which provides adequate conditions for *in vitro* immunization of human peripheral blood lymphocytes to heterologous erythrocytes. Making use of this method we could obtain, with a number of different donors, an antibody response which peaked at about day 8 of culture with 30-300 plaque-forming cells (PFC) per  $10^6$  input lymphocytes. However, in a number of experiments poor or negative results were obtained, even with donors that had previously given good response. This variability in the results was shown not to be due to a too low number of precursor cells present in the blood and could be overcome by treating the cells, before initiation of the culture, with a factor produced by mouse T cells educated to sheep erythrocytes (SRBC). Under these conditions a PFC response was obtained which peaked at about day 8 and which in some experiments could be as high as 20,000 PFC per  $10^6$  input lymphocytes. Paralleling the increase in PFC was an increase in cell number. The cells recovered from the treated cultures were at all times more numerous than in the non-treated cultures. The height of both the proliferative and antibody-producing re-

sponses varied from experiment to experiment, a higher proliferative response accompanying a higher PFC response.

Although the mechanisms that are at the basis of the antibody response in vitro described in this paper still need to be clarified, this system may become a useful tool in studying the immune response in man.

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