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Pokeweed mitogen induced differentiation of human B cells: evaluation by a protein A haemolytic plaque assay

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Summary. Using the protein A plaque assay, the number of human cells secreting immunoglobulin of various classes after pokeweed mitogen stimulation was determined. At optimal response (on day 5–7) a mean of 58,354 IgM PFC/10^6, 34,207 IgG PFC/10^6 and 10,525 IgA PFC/10^6 cells was found when using peripheral blood lymphocytes. In spleen cells, peak values which were slightly higher than in blood, were obtained at day 4–6. The proportions of cells secreting light chains of either type were found to be comparable to those of unstimulated cells thus supporting the notion of the polyclonality of the response. Pokeweed mitogen stimulation of peripheral blood lymphocytes was found to be totally T-cell dependent whereas the response of spleen cells was not. When assayed for antigen-specific precursor cells in cultures stimulated by mitogen, the frequency of SRBC-specific IgM producing cells was found to be 1·3/1000 cells. This frequency was regularly found to be independent of medium supplement.

INTRODUCTION

In experimental immunology the functional capacity of B lymphocytes can be measured by certain procedures, such as enhanced uptake of radioactively labelled DNA precursors in response to stimulation or by the increased synthesis of antibodies with differing antigenic specificities. Similar functional studies in man have long been impeded by the lack of a suitable method for detection of *de novo* antibody production by B cells.

The adaption of the original haemolytic plaque assay (Jerne & Nordin, 1963) to human cells (Fauci & Pratt, 1976a, b), which made it possible to detect individual antibody secreting cells *in vitro*, was therefore considered to be a valuable tool when investigating the mechanism involved in human B-lymphocyte activation. The application of this technique has, however, met with certain technical difficulties and furthermore, has not been readily reproducible. One essential feature of the Fauci & Pratt plaque system (1976a, b) has been the use of heavily SRBC absorbed human serum for supplementation of the culture media. This procedure may remove sheep cell antigen-specific suppressive factors (Smith & Hammarström, 1979) normally contained in serum, or alternatively, add sheep erythrocyte antigens to the serum (for discussion, see Möller, 1979) and therefore, evaluation of mitogenic stimulation in this model may constitute a measure of adjuvant effects on antigen-specific humoral immunity and not a 'true' polyclonal activation (Ringdén, Rynnel-Dagöö, Kunori, Freijd & Möller, 1978). This assumption is also in agreement with the

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rather limited number of polyclonal PFC detected utilizing heavily haptenated red cells as targets in the plaque assay (Fauci & Pratt, 1976a; Ringdén, Rynnel-Dagöö, Waterfield, Möller & Möller 1977; Ringdén *et al.*, 1978).

In 1976 a system was developed for the enumeration of the total number of mouse lymphocytes secreting immunoglobulin of any given class by using different developing sera in association with protein A coupled red cells as targets in the PFC assay (Gronowicz, Coutinho & Melchers, 1976). Recently, this method has been successfully adapted for the study of human cells (Bird & Britton, 1979). In the present paper the differentiation of B cells secreting different Ig classes after stimulation with pokeweed mitogen is reported. Kinetics, media requirements, and T-cell dependence and independence of the response of blood and spleen lymphocytes, respectively, are described.

MATERIALS AND METHODS

Cells

Peripheral blood lymphocytes (PBL) were purified by Ficoll–Isopaque (Nyegaard A/S, Oslo, Norway) separation of a leucocyte concentrate (buffy coat) from adult healthy donors (Böyum, 1968). Functionally, lymphocytes obtained from buffy coat do not differ from cells separated from whole blood (Smith, Hammarström, Bird, Holme, Gustavsson & Kunori, 1979). Spleen cell suspensions were prepared from cadaveric kidney donors which were removed within 30 min of cardiac arrest. PBL were always used fresh, whereas spleen cells were either fresh or stored frozen in liquid nitrogen until use.

Culture conditions

All human cells were cultivated at a concentration of 10^6 cells/ml in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) supplemented with human AB serum, either unabsorbed or absorbed with SRBC as described by Fauci & Pratt (1976a, b), FCS (Batch 41405, Rehatuin, Armour Pharmaceutical Co., Kankakee, Illinois, U.S.A.) or rabbit serum (obtained from our own colony of vit lantras rabbits). All sera were heat-inactivated at 56° and stored frozen at −20°. Human albumin was purchased as a lyophilized powder (Batch 53859, KABI AB, Stockholm, Sweden). Cultures of 5 ml were set up in 50 ml plastic flasks Falcon 3013 (Falcon, Oxnard, California, U.S.A.) and kept in a mixture of 10% CO_2, 83% N_2 and 7% O_2. Cell suspensions were prepared by vigorously shaking the flasks and thereafter rinsing the flasks with balanced salt solution (BSS). Cells were washed in the salt solution three times prior to plating. Cell viability was determined by trypan blue exclusion. In pokeweed mitogen (Techtum Instruments, Umeå, Sweden) stimulated cultures, 20 μg/ml was added at onset of culture.

Coupling of protein A to erythrocytes

Protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) was coupled to sheep red blood cells (SRBC) using $CrCl_3$ as described earlier (Gronowicz *et al.*, 1976). SRBC stored in Alsever's solution were washed three times in 0·9% NaCl. Thereafter one part of protein A (0·5 mg/ml) was mixed with 10 parts of $CrCl_3$ ($2{\cdot}5 \times 10^{-4}$ M) and one part of packed SRBC. All reagents were diluted in 0·9% NaCl. The mixture was incubated at 30° for 1 h and thereafter washed once in 0·9% NaCl and twice in BSS and kept at 4°. Cells were usable up to 4 days after coupling.

Plaque assay

One hundred microlitres of cells suspended in balanced salt solution (BSS) were directly added together with 25 μl of protein A coupled erythrocytes (diluted 1:3), 25 μl of the IgG fraction of the antiserum diluted 1:30 (anti-human μ, γ, α, κ and λ chain immunoglobulin produced in rabbit, DAKO-Immunoglobulins Ltd, Copenhagen, Denmark) and 25 μl of SRBC-absorbed guinea-pig complement (diluted 1:4) (Flow Laboratories, Irvine, Scotland) into 700 μl 0·5% agar (Bacto Difco agar, Difco Laboratories, Detroit, Michigan, USA) in BSS containing 0·45 mg/ml DEAE-dextran (Pharmacia Fine Chemicals, Uppsala, Sweden). Two 0·2 ml drops of the mixture placed on a plastic 9 cm petri dish (Heger Plastics A/S, Oslo, Norway) and a 24 × 32 mm glass cover slip (Chance Proper Ltd, Warley, England) was immediately placed on each drop. Dishes were incubated for 4 h at 37° and plaque forming cells (PFC) were counted at 4× magnification using indirect light. For detection of SRBC plaques the previously published Fauci system was used (Fauci & Pratt, 1976a, b).

Assay for T cells

The capacity of human lymphocytes to bind SRBC (E-RFC) was used to determine the relative numbers of T cells since this has been described as a stable marker for resting as well as activated human T cells (Greaves, Janossy & Doenhoff, 1974). Enrichment of

T cells was performed by passage of cells through a nylon wool column using a slight modification (Persson, Bick, Hammarström, Möller & Smith, 1978) of the method described by Julius, Simpson & Herzenberg (1973). B-cell enriched cell populations were obtained by teasing the nylon wool columns thereby recovering the attached cells (Persson *et al.*, 1978).

Protein synthesis inhibitors

Cycloheximide {3[2(3,5-dimethyl-2-oxo-cyclohexyl)2-hydroxyethyl]glutarimide}, LOT 77c-0390 was purchased from Sigma Chemical company, St Louis, Missouri, U.S.A. Ouabain (G-strophanthin) 99·99% pure, was obtained from the WHO Centre for chemical reference substances, Solna, Sweden.

RESULTS

Selection of serum supplement

Emphasis has previously been placed on the necessity of adding SRBC absorbed human sera to supplement the cultures when examining the appearance of antibody-secreting cells using the Fauci plaque assay. In our system, the use of absorbed human AB serum did not markedly increase the total amount of Ig-secreting cells generated after mitogen stimulation and occasionally SRBC absorbed serum was even less supportive than unabsorbed serum (Table 1).

There was apparently no specific need for human serum in the system since foetal calf serum and rabbit serum also supported PFC induction in PBL (Table 2). Normally, however, the use of FCS in the culture increased the number of background PFC generated. Human albumin may also be used for supplementation although addition of this protein alone only occasionally allows differentiation of lymphocytes to antibody-secreting cells and when sufficient, these cultures generally gave rise to fewer PFC than normal AB serum supplemented cultures (data not shown).

The appearance of PFC was dependent on *de novo* protein synthesis since parallel cultures of PWM-stimulated cells grown in the presence of cycloheximide for 4 h prior to harvest gave rise to only 1·5% of the response in unblocked cultures (Table 3). Ouabain (G-strophanthin), added 12 h before harvesting the cells, completely blocked the expression of PFC without affecting viability as did also omission of complement or antiserum (Table 3).

Table 1. Effect of SRBC absorbtion on the supportive capacity of human AB serum for immunoglobulin production

Expt No.	Serum supplement	PFC/10^6 cells IgM	IgG	IgA	SRBC PFC/10^3 IgM-producing cells
1	Unabsorbed AB, batch I	160,202	88,786	21,786	0·1
	SRBC absorbed AB batch I	161,458	84,895	17,708	3·3
	SRBC absorbed AB batch V	70,772	27,022	6,863	1·1
2	Unabsorbed AB, batch III	35,760	76,277	12,364	1·2
	SRBC absorbed AB batch I	20,074	31,011	7,335	0 6
	SRBC absorbed AB batch III	34,228	22,390	5,790	0·5
	SRBC absorbed AB batch IV	16,013	17,325	4,125	1·8
3	Unabsorbed AB, batch IV	24,791	32,813	NT*	0
	SRBC absorbed AB batch IV	18,867	14,492	NT	0
4	Unabsorbed AB batch VI	72,625	50,750	1,750	2·9
	SRBC absorbed AB batch VI	39,246	46,324	0	1·6
	SRBC absorbed AB batch VII	98,170	91,768	4,268	1·5

* NT, not tested.

5×10^6 human peripheral blood lymphocytes were cultured in normal or SRBC absorbed human AB serum for 5–7 days. Results are expressed as net PFC/10^6 surviving cells.

Table 2. Supportive effect of sera from different species on the differentiation of human B cells

Expt No.	PWM	IgM PFC/10^6 cells: Human AB	Foetal calf serum	Rabbit serum
1	–	88	274	36
	+	19,250	20,416	9,479
2	–	1,416	1,926	968
	+	54,062	50,712	42,314

Peripheral blood lymphocytes were cultered at 5×10^6 cells/5 ml for 6 days in medium supplemented with 10% serum of different origin. Results represent IgM PFC/10^6 surviving cells.

Table 3. Impairment of immunoglobulin production by protein synthesis inhibitors

Pre-treatment	Antiserum + complement	Omitting complement	Omitting antiserum
None*	94,060	0	0
Cycloheximide (10 μg/ml)†	4,073	0	0
Cycloheximide (100 μg/ml)	1,418	0	0
Ouabain (1 μg/ml)‡ (G-strophanthin)	0	0	0

Survival in the different cultures were $1{\cdot}2 \times 10^6$/culture, $1{\cdot}0 \times 10^6$/culture, $0{\cdot}8 \times 10^6$/culture and $1{\cdot}3 \times 10^6$/culture respectively.

* IgM PFC/10^6 response of 5×10^6 peripheral blood lymphocytes stimulated by 20 μg/ml PWM as measured on day 6.

† Pre-incubation for 4 h.

‡ Pre-incubation for 12 h.

Kinetics of PWM-induced immunogloblin secretion

At optimal concentration (20 μg/ml), PWM regularly induced the appearance of a large number of IgM-secreting cells with a peak around day 5–7 in peripheral blood lymphocytes (Fig. 1) and slightly earlier in spleen cells (Fig. 1). Stimulation of IgG- and IgA-producing cells may also be observed and the kinetics of the IgG response (Fig. 2) and the IgA response (data not shown) roughly appear to follow that of IgM both in PBL and spleen cells. Although the number of immunoglobulin-secreting cells was generally high when assayed on the optimal day, there was still a great variability between individual donors with

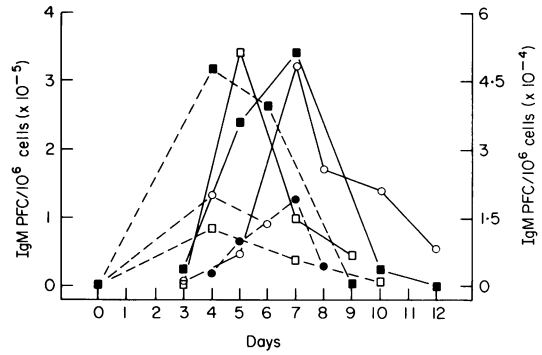

Figure 1. Kinetics of the PWM-induced differentiation of 5×10^6 human peripheral blood lymphocytes (——) (right axis) or spleen lymphocytes (– – –) (left axis) from different donors cultivated in RPMI 1640 medium supplemented with 10% unabsorbed human AB serum. Results are expressed as IgM PFC/10^6 viable cells recovered.

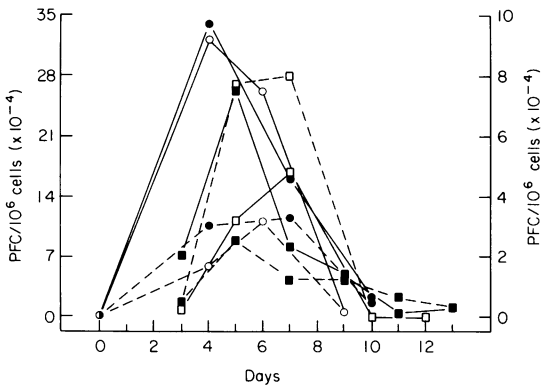

Figure 2. Kinetics of the PWM-induced differentiation of IgM- (——), and IgG- (– – –) producing cells in cultures of 5×10^6 human peripheral blood lymphocytes (square) (right axis) or spleen lymphocytes from different donors (circles) (left axis) cultivated in RPMI 1640 medium supplemented with 10% unabsorbed human AB serum. Results are expressed as PFC/10^6 viable cells recovered.

regard to both total numbers of IgM-, IgG- and IgA-synthesizing cells (Table 4) and their individual proportions (Table 4). In most cases, a majority of the cells secreting Ig are of the IgM class. Occasionally, however, there is a dominance of IgG-synthesizing cells in the stimulated cultures (10–20% of normal blood donors) although these normally comprise only 59% of the responses observed when assaying for IgM. IgA-producing cells rarely account for more than 18% of the IgM response and on the average only 10% of the total response measured. The relative proportions

Table 4. Individual variability of cells synthesizing immunoglobulin of various classes in cells stimulated by PWM

Expt No.	Day of assay	Cell source	PWM (20 μg/ml)	PFC/10^6 cells IgM	IgG	IgA	% E-RFC
1	7	Blood	−	766	1,313	219	72
			+	51,076	80,174	610	39
2	7	Blood	−	538	2,827	135	62
			+	23,438	14,063	4,689	45
3	6	Blood	−	350	350	NT*	
			+	100,000	75,000	NT	
4	7	Blood	−	292	0	0	
			+	29,667	11,757	1,367	
5	7	Blood	−	8,333	1,958	NT	
			+	62,000	5,500	NT	
6	7	Blood	−	833	500	NT	
			+	22,125	5,500	NT	
7	7	Blood	−	1,458	4,375	2,916	
			+	181,034	90,517	54,310	
8	7	Blood	−	11,703	984	109	76
			+	22,969	11,758	3,555	44
9	7	Blood	−	0	146	0	61
			+	37,223	26,814	7,586	38
10	6	Blood	−	1,416	1,166	417	
			+	54,062	23,125	1,560	
11	7	Spleen	−	4,375	27,125	7,000	
			+	128,333	11,667	67,812	
12	5	Spleen	−	3,588	525	NT	
			+	97,222	12,638	NT	
13	6	Spleen	−	7,802	2,698	146	29
			+	262,500	109,375	17,986	13
14	6	Spleen	−	5,250	NT	NT	
			+	273,334	NT	NT	
15	4	Spleen	−	7,291	972	0	28
			+	83,611	26,250	1,458	20

* NT, not tested.

B-cell responses and E-RFC percentages of 5×10^6 PWM-stimulated human lymphocytes. Measurements which were usually performed at peak response, represent Ig-secreting cells/10^6 viable cells.

of cells belonging to different Ig classes did not change markedly during the kinetics of the response (Fig. 2).

In a limited number of experiments, the proportions of cells secreting κ or λ chains were determined. The data from mitogen-activated cells, which are summarized in Table 5, are similar to the proportions normally found in unstimulated cells (Seligmann, Preud'-Homme & Brouet, 1973), thereby indicating no preferential stimulation of cells synthesizing either type of light chain. When assayed separately, the total number of Ig-secreting cells exceeded the number of plaques detected using anti-light chain antiserum by a mean of 49% (Table 5).

T-cell dependence of B-cell differentiation

In our hands, PWM stimulation of B cells in peripheral blood is T-cell dependent (Table 6), since purified B cells do not develop into plaque-forming cells when cultured together with PWM in spite of the relative enrichment of non-T cells found in optimally PWM stimulated cultures (Table 4), nor are any PFC

Table 5. Proportions of light chain secreting cells in PWM-stimulated cultures

Expt No.	Cell source	% of total PFC detected using anti-light chain Ig*		
		κ	λ	$\frac{(\kappa+\lambda)\text{ PFC}}{(\mu+\gamma+\alpha)\text{ PFC}} \times 100$†
1	Blood	66	34	33
2	Blood	49	51	35
3	Blood	57	43	51
4	Blood	58	42	72
5	Blood	63	37	40
6	Blood	60	40	61
7	Spleen	59	41	43
8	Spleen	61	39	70

5×10^6 peripheral blood lymphocytes or spleen cells were stimulated by PWM. At the day of optimal response the frequency of light chain producing cells was determined.

* IgG fraction of antiserum directed against κ and λ chain.

† Percentage of PFC detected using anti-light chain Ig fraction of antiserum (anti-κ + anti-λ-Ig) of PFC detected using anti-heavy chain Ig (anti-μ + anti-γ + anti-α Ig).

detected in the purified T-cell population probably due to lack of B cells, or possibly due to a suppressive effect exerted by T cells. In contrast, the response in spleen appears to be clearly T-cell independent (Table 6 thereby supporting similar data reported by Janossy and Greaves (Greaves *et al.*, 1974, Janossy & Greaves, 1975, Janossy, Gomez de la Concha, Luquetti, Snajdr, Waxdal & Platts-Mills, 1977).

Frequency of antigen-reactive cells

In an attempt to assess the number of antigen-specific cells, the frequency of anti-SRBC cells generated after PWM stimulation was measured in single cultures supplemented with unabsorbed or SRBC absorbed human AB serum. Since the anti-red cell plaques produced are all of IgM type (Dosch & Gelfand, 1976;

Table 6. T-cell dependence of PWM responses in different organs

Expt No.	Day tested	Cell source	PWM	Unseparated	T-cell enriched	B-cell enriched
1	6	Blood	−	350	0	0
			+	100,000	0	0
2	7	Blood	−	0	0	0
			+	3,200	0	0
3	6	Spleen	−	7,802	NT*	7,656
			+	262,500	NT	149,722
4	7	Spleen	−	1,313	NT	16,450
			+	38,181	NT	40,625

* NT, not tested.

PWM induced responses in peripheral blood lymphocytes and spleen cell preparations enriched for T or B cells. Results are expressed as IgM PFC/10^6 viable cells.

Fauci & Pratt, 1976b; Ringdén *et al.*, 1977), the frequencies have all been computed for the number of IgM-secreting cells actually plated out in the assay. A great variability between different donors was found ranging from 0 to 6·4/1000 IgM-producing cells, but on the average, 1·3/1000 IgM-producing cells was found to be SRBC specific (data not shown). This frequency was not readily enhanced in cultures supplemented with SRBC absorbed serum (Table 1).

DISCUSSION

Utilizing the protein A assay for detection of cells secreting different Ig classes (Gronowicz *et al.*, 1976) as adopted for human cells (Bird & Britton, 1979), the experiments reported in this paper show the generation of large numbers of IgM-, IgG- and IgA-secreting cells in cultures of peripheral blood lymphocytes (PBL) or spleen lymphocytes stimulated with PWM. Peak response for all Ig classes tested is generally found on days 5–7 (Fig. 2) when testing PBL and slightly earlier with spleen cells (Fig. 2). These findings agree with studies of similar cultures assessing intracellular Ig (Janossy & Greaves, 1975) or estimating PFC by either a reversed plaque method (Ginsburg, Finkelman & Lipsky, 1978), or by a direct assay as modified by Fauci (Fauci & Pratt, 1976b). Since cells secreting different Ig classes appeared simultaneously and increased and decreased in a parallel fashion, these data suggest that under the culture conditions used, lymphocytes do not appear to mature from IgM to IgG or IgA producers. These observations are similar to those made analysing the supernatants in PWM-stimulated cultures (Saxon, Stevens & Ashman, 1977) or by using a different reversed haemolytic plaque assay (Ginsburg *et al.*, 1978). In the present system, five- to ten-fold more PFC were observed as compared to the number of Ig-producing cells reported when using their reversed plaque assay (Ginsburg *et al.*, 1978). This discrepancy could either be due to more optimal culture conditions, or more likely, due to a greater sensitivity of the protein A method. The latter possibility was supported by the finding of many cells stained for intracellular Ig in PWM-stimulated cultures (Ginsburg *et al.*, 1978).

The proportion of cells from peripheral blood secreting IgM and IgG, as analysed by the reversed haemolytic plaque assay, has been reported to be dependent upon the sex and race of the donor (Ginsburg *et al.*, 1978). Exceptions to this rule were encountered in the present study. Although the donors were all of Caucasian origin, there was a preponderance of IgG-producing cells in cultures from occasional donors (Table 4) (10–20% of all blood donors tested).

In spleen cells, a vast majority of the cells staining for intracellular Ig are reported to be of IgM class (Janossy & Greaves, 1975). When assayed for antibody secretion by the protein A method, a significant proportion of the cells was found to produce IgG and IgA (Table 4). The apparent difference between these studies may have resulted from the chance selection of donors, because results from different individuals can vary greatly (Finkelman & Lipsky, 1978) (Table 4). Alternatively, higher rate of antibody secretion by IgG- and IgA-producing cells, thereby enhancing their ability to form easily detectable plaques may be reflected in the present results.

The proportion of cells expressing light chains of either type appears to be largely unaltered in cultures stimulated by PWM as compared to normal unstimulated cells (Seligmann *et al.*, 1973) supporting the notion of true 'polyclonality' of the response. The total number of cells secreting immunoglobulin was greater than the number secreting light chains (Table 5), suggesting the existence of cells simultaneously secreting more than one Ig class. Similar observations have previously been reported by Keightly, Cooper & Lawton) (1976), who found that in PWM-stimulated human lymphocyte cultures, cells staining for both intracellular IgM and IgG constituted up to 10% of the total staining for either class. Furthermore, the data reported by Ginsburg *et al.* (1978) using an indirect plaque method, suggested that the frequency of double producers actually secreting immunoglobulin may exceed 30%. Our own data as to the proportion of double producers must, however, be interpreted with certain caution because no rigorous testing of the optimal anti-light chain antiserum dilution in each individual experiment was performed, although titration curves made in some experiments indicate that the concentrations used were indeed optimal. The commercial antisera used may also differ in their mean affinity of antibodies, thereby making any analysis of this kind futile. Cells secreting incomplete antibodies, i.e. only heavy chains, could also account for the number of double producers observed. Although we do not favour this possibility, it does gain support from the kinetics of the individual Ig classes. Simultaneous production is preferentially found in cells switching from synthesis of one Ig class to another but, in our experiments, no evidence for maturation from

IgM to IgG secretion was found (Fig. 2), thus possibly reflecting suboptimal culture conditions for the appearance of double producers.

Generation of PFC as detected by the protein A assay is clearly dependent upon the *de novo* production of protein since addition of cycloheximide or G-strophanthin (Ouabain) 4–12 h before assay, virtually abolished the plaque response (Table 3). Induction of antibody secreting cells was, however, not dependent on the use of SRBC-absorbed human sera as media supplement since unabsorbed human AB serum, foetal calf serum and rabbit serum could all support B-cell differentiation although not to an entirely equal extent (Table 2).

When cultures were assayed by the original plaque assay using unmodified SRBC as targets (Jerne & Nordin, 1963) a mean of 1·3 anti-SRBC PFC/10^3 IgM-secreting cells was noted. This estimation also agrees reasonably well with the frequencies observed in cultures of mouse cells (Andersson, Coutinho & Melchers, 1977) but appear to be slightly higher than those calculated from previous reports in man (Dosch, Percy & Gelfand, 1977; Fauci & Pratt, 1976b). These data, however, must again be interpreted with caution since only a single culture was routinely tested for direct SRBC PFC and therefore would represent the chance survival of one to two precursors in certain cultures. The appearance of SRBC PFC was, at least in our hands, not influenced by replacing unabsorbed AB serum with SRBC absorbed AB serum (Table 1). Because this procedure is claimed to enhance greatly the number of anti-SRBC antibody producing cells in the cultures (Fauci & Pratt, 1976a, b), the lack of SRBC PFC in cultures supplemented with absorbed as compared to the PFC response of cells cultured in parallel in unabsorbed serum indicate that appearance of anti-SRBC PFC when assaying single cultures truly represents a random event. These data therefore only add to the present confusion on the requirements of antigen-specific human B cells and emphasizes the need for an analysis of precursor frequencies of multiple pooled cultures grown in the presence of various supplements.

The total T-cell dependence of the PWM response in PBL as compared to the relative independence in spleen cells suports the previous observations by Janossy and Greaves (Greaves *et al.*, 1974; Janossy & Greaves, 1975; Janossy *et al.*, 1977). The lack of PFC in the purified T-cell population may then be due either to lack of B cells or alternatively a suppressive effect of T cells on the residual B cells as suggested previously (Janossy *et al.*, 1977). Since PWM has been suggested to consist of two distinct mitogenic moieties (Waxdal & Basham, 1974), each stimulating either T or B cells, our findings may indicate that the B cells responding to the PBA fraction of PWM are absent from peripheral blood but present in the spleen. Such compartmentalization of human lymphocytes has been claimed for cells responding to LPS (Ivanyi & Lehner, 1974; Oppenheim & Perry, 1965), although this point has been disputed (Ringdén *et al.*, 1977; Smith *et al.*, 1979). This would then imply that PBL B cells may actually respond to a T-cell factor induced by PWM similar to what is observed in Con A- and PHA-stimulated cultures (A. G. Bird, L. Hammarström & C. I. E. Smith, in preparation), indicating a fundamental difference with regard to triggering receptors on PWM responsive B cells of different origin.

The analysis of B-cell functions in PWM-stimulated cultures using the protein A assay is technically simple, highly sensitive and readily reproducible. This may therefore constitute the method of choice to investigate patients with suspected immunodeficiency states and with disease conditions.

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