Activation of Human B Lymphocytes after Immunization with Pneumococcal Polysaccharides

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ABSTRACT The in vivo and in vitro immune response after in vivo immunization with pneumococcal polysaccharides (PPS) has been analyzed in man. Substantial differences were noted in this system when compared with human responses to soluble protein antigens. Within 6 d after immunization, specific PPS antigen-binding cells (ABC), specific plaque-forming cells (PFC), and cells capable of spontaneously synthesizing in vitro large amounts of specific anti-PPS immunoglobulin (Ig) G, IgA, and lesser amounts of specific IgM appeared in the peripheral blood. The ABC, PFC, and the total amount of specific spontaneous antibody production followed nearly identical kinetics after immunization. Low doses of irradiation markedly inhibited spontaneous anti-PPS antibody production by lymphocytes obtained 7 or 8 d after immunization, suggesting a requirement for in vitro proliferation for full expression of antibody-secreting capability of these cells that are activated in vivo and are capable of spontaneous antibody production in vitro. Spontaneous secretion by B lymphocytes in vitro was independent of T cells, unmodified by the addition of T cell factors, and readily suppressible by pokeweed mitogen (PWM).

By 2 wk after immunization, spontaneous anti-PPS antibody production in vitro was no longer detected. Subsequent stimulation of lymphocytes in culture with a wide range of concentrations of specific antigen did not trigger either proliferation or specific antibody synthesis. Despite the unresponsiveness of these cells to antigenic stimulation at this time, they were capable of specific antiPPS antibody production after stimulation with PWM. In vivo booster immunization 4 mo after an initial immunization did not reproduce the increased numbers of ABC, PFC, or in vitro specific antibody production that had been found 4 mo earlier. The dichotomy in capacity for activation of PPS-specific B cells by PWM vs. specific antigen, and the in vivo and in vitro unresponsiveness to in vivo booster immunization with PPS, contrast sharply with previous studies in man with soluble protein antigens such as keyhole limpet hemocyanin and tetanus toxoid. Furthermore, the lack of T cell activation by PPS also contrasts with previous results with tetanus toxoid and other protein antigens. This system should prove useful in delineating certain aspects of human B cell physiology not readily approachable with standard soluble protein antigens.

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INTRODUCTION

Since the humoral immune response to capsular polysaccharides is an important host defense mechanism against many pathogenic bacteria, the serologic response to infection and immunization with polysaccharides in man is an area of considerable interest. Yet little is known concerning the immune response to these antigens at the cellular level in man. In contrast, polysaccharides have been valuable tools in probing the heterogeneity and regulation of murine B cell reactivity. Much of the advantage of the use of polysaccharide antigens in the study of B cell physiology in the mouse resides in their significant differences from protein antigens and the feasibility of their use in approaching certain aspects of B cell activation and immunoregulation that do not readily lend themselves to study with the standard soluble protein and particulate antigens. For example, several polysaccharide antigens are able to induce an antibody response in mice that are deficient in mature T cells, whereas most soluble proteins cannot. Polysaccharides are unable to induce antibody production in mice that carry the CBA/N defect and presumably lack a mature B cell subset (1). They do not produce detectable immune memory, and secondary antibody responses are of low magnitude and affinity compared with responses to soluble proteins (2). Polysaccharides induce limited

isotype diversity with immunoglobulin (Ig) M and IgG3 predominant in the mouse (3) and IgM and IgG2 in man (4). Finally, responses to polysaccharides arise later in ontogeny than do responses to protein antigens, and adult levels are not reached until 8 wk in mice (5) and 2 yr in man (6).

To more precisely delineate the immune response to polysaccharides at the cellular level in man, methodologies recently developed for the study of soluble antigen-induced and antigen-specific triggering of human B cells with protein antigens (7, 8) were adapted to the in vitro study of human lymphoid cells after in vitro immunization with the pneumococcal polysaccharide (PPS)[1] vaccine. As a result, a system was established for examining in vitro B cells spontaneously secreting Ig specific for PPS, and substantial differences from the in vitro immune response to soluble protein antigens were demonstrated.

METHODS

Immunizations. Normal male volunteers, aged 20–35 yr, received a single subcutaneous injection of 0.5 cm^3 of the 14 valent PPS vaccine (Lederle Laboratories, Div., Wayne, NJ). The vaccine consists of a mixture of purified polysaccharide from 14 pneumococcal types (U. S. 1, 2, 3, 4, 6, 51, 8, 9, 12, 14, 56, 19, 23, and 25). 50 μg of each type are present in 0.5 cm^3 of the vaccine.

Cell separation. Peripheral blood mononuclear cells were obtained from Hypaque-Ficoll gradients in standard fashion. T cell-enriched populations were obtained by rosetting with amino s-2 aminoethylisothiouronium bromide-treated sheep erythrocytes (SRBC) and passing the rosetted cells over Hypaque-Ficoll gradients (9). The resulting T cell-enriched suspensions were 92–96% rosette-positive. T cell depletion was accomplished by treating the SRBC-negative population with the anti-T cell hybridoma antibody Leu-1 (Becton, Dickinson & Co., Oxnard, CA) and rabbit serum (Dutchland Laboratories Inc., Denver, PA) as a source of complement as previously described (7). Depletion of T cells was monitored by assessing the blastogenic response of the T cell-depleted (TCD) population to T cell mitogens. Monocyte depletion was performed by plate adherence (10).

Culture conditions. Cultures for measuring blastogenesis were performed as previously described (11) in 96-well round-bottomed microtiter plates (Linbro Chemical Co., New Haven, CT) with 1×10^5 cells/well in 0.2 ml RPMI 1640 with 15% human A sera. Cultures for the measurement of supernatant Ig production were performed in 1 ml RPMI 1640 containing 10% fetal calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) in 24-well flat-bottomed plates (Costar, Data Packaging, Cambridge, MA). These cultures contained various amounts of either PPS types 3, 8, 14; the whole PPS vaccine; pokeweed mitogen (PWM; Gibco Laboratories); or aliquots of mixed lymphocyte culture supernatants as a source of crude T cell factors. Cultures were incubated in a 5% CO_2 atmosphere at 37°C and rocked at 4 cycles/min. Some cultures were washed free of antigen on day 4. Cell densities were 1.5×10^6 unfractionated (UF) mononuclear cells/ml or 0.5×10^6 TCD cells/ml. Cultures for the determination of the spontaneous secretion of Ig were harvested at day 5, while cultures measuring antigen- and PWM-induced supernatant Ig were harvested at day 10. Supernatants were decanted and stored at 4°C until assay, usually within 3 d.

Preparation of polysaccharide-coated SRBC and ox RBC. PPS vaccine was rendered free of preservative and phosphate by dialysis against normal saline. For each milliliter of packed RBC, 1 mg of type III PPS or 1.4 mg of PPS vaccine in 1 ml of normal saline was added, followed by 10 ml of a 0.01% solution of chromic chloride (J. T. Baker Chemical Co., Phillipsburg, NJ) in normal saline. This suspension was incubated in a water bath at 30°C for 40 min, washed, and stored at 4°C in Hanks' balanced salt solution.

Plaque-forming cell (PFC) assay. Cells secreting antibody specific for PPS were detected by using a hemolytic PFC assay originally described by Gronowicz et al. (12) and modified by Fauci et al. (13) for detecting total Ig-secreting cells with staphylococcal protein A-coated SRBC. PPS-coated SRBC were substituted for the staphylococcal protein A-coated SRBC. Developing antiserum was an IgG fraction of rabbit anti-human Ig serum (Cappel Laboratories, Inc., Cochranville, PA). No developing antisera was used when direct PFC were counted.

Antigen-binding cell (ABC) assay. 0.2 ml of UF mononuclear cells at 1×10^7 cells/ml was mixed with 0.2 ml of ox RBC (1×10^8/ml) coated with PPS. Cells were spun for 10 min at 500 rpm at 10°C and then placed on ice for 30 min. The button was gently resuspended and rosettes were counted with a hemocytometer. No ABC were detected with ox RBC coated with chromic chloride, and rosette formation could be inhibited by preincubating mononuclear cells with 100 μg of PPS.

Assays. Specific antibody production was measured by using an enzyme-linked immunosorbent assay as previously described (14) with minor modifications. Flat-bottomed 96-well microtiter plates were first coated with 0.1 ml of a carbonate buffer, pH 9.6, containing 10 μg/ml of PPS types 3, 8, or 14, or 140 μg/ml of whole PPS vaccine and allowed to incubate overnight at 37°C. Plates were then washed three times with phosphate-buffered saline containing 0.05% Tween 20. Samples were appropriately diluted, added to duplicate wells in a volume of 0.1 ml, and incubated at room temperature for 2 h. The plates were then washed as before and 0.1 ml of a 1:1,000 dilution of heavy chain-specific goat anti-human IgG, IgA, or IgM conjugated to alkaline phosphate (Sigma Chemical Co., St. Louis, MO) was added to each well. The plates were incubated overnight at 4°C, washed again, and then allowed to react with 0.1 ml/well of a 1-mg/ml solution of *p*-nitrophenyl phosphate (Sigma Chemical Co.) in a pH 8.6 carbonate buffer containing 0.001 M $MgCl_2$. The subsequent development of color was measured by using a multi-channel spectrophotometer (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, VA) and related to that seen with a standard serum rich in anti-PPS (IgG, IgA, IgM). One PPS_M or PPS_A unit was defined as the amount of PPS-specific IgM or IgA, respectively, present in a 1:10,000 dilution of immune serum and a PPS_G unit was defined as the amount of PPS-specific IgG present in 1:100,000 dilution of immune serum. One vaccine PPS_M unit is approximately equal to 0.6 ng of antibody, one vaccine PPS_A unit is approximately equal to 0.4 ng of antibody, and one vaccine PPS_G unit is approximately equal to 0.3 ng of

[1] *Abbreviations used in this paper*: ABC, antigen-binding cell; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cell; PPS, pneumococcal polysaccharide; PWM, pokeweed mitogen; SRBC, sheep erythrocyte; TCD, T cell-depleted; UF, unfractionated.

antibody. These values were obtained by comparing PPS vaccine-coated plates incubated with immune sera to anti-IgM-, anti-IgA-, or anti-IgG-coated plates incubated with known amounts of IgM, IgA, or IgG.

Lymphocyte blastogenic responses were measured in cultures set up as outlined above. Cultures were pulsed with 2 μCi of [^{3}H]thymidine on day 4 and harvested 4 h later on a Titertek harvestor. Filter disks were placed in scintillation vials with 3 ml Aquasol scintillation fluid and counted in a scintillation counter.

RESULTS

Kinetics of spontaneous antibody production. After immunization with PPS vaccine, lymphoid cells appeared in the peripheral blood that were spontaneously secreting specific antibody directed against the polysaccharides present in the vaccine. This spontaneous production of specific antibody was detected by measuring supernatant antibody production of peripheral blood mononuclear cells after 5 d in culture in the absence of in vitro stimulation with antigen. Fig. 1 demonstrates the kinetics of this response in a representative individual studied at various intervals after in vivo immunization. The amount of total specific IgG, IgM, and IgA anti-PPS antibody against all 14 serotypes is displayed. This spontaneous secretion of antibody by peripheral blood lymphocytes was first detectable on day 6, peaked on day 8, and disappeared by day 13 with no subsequent reappearance. Identical results were found when the response against type 8 alone was measured (data not shown). Substantial

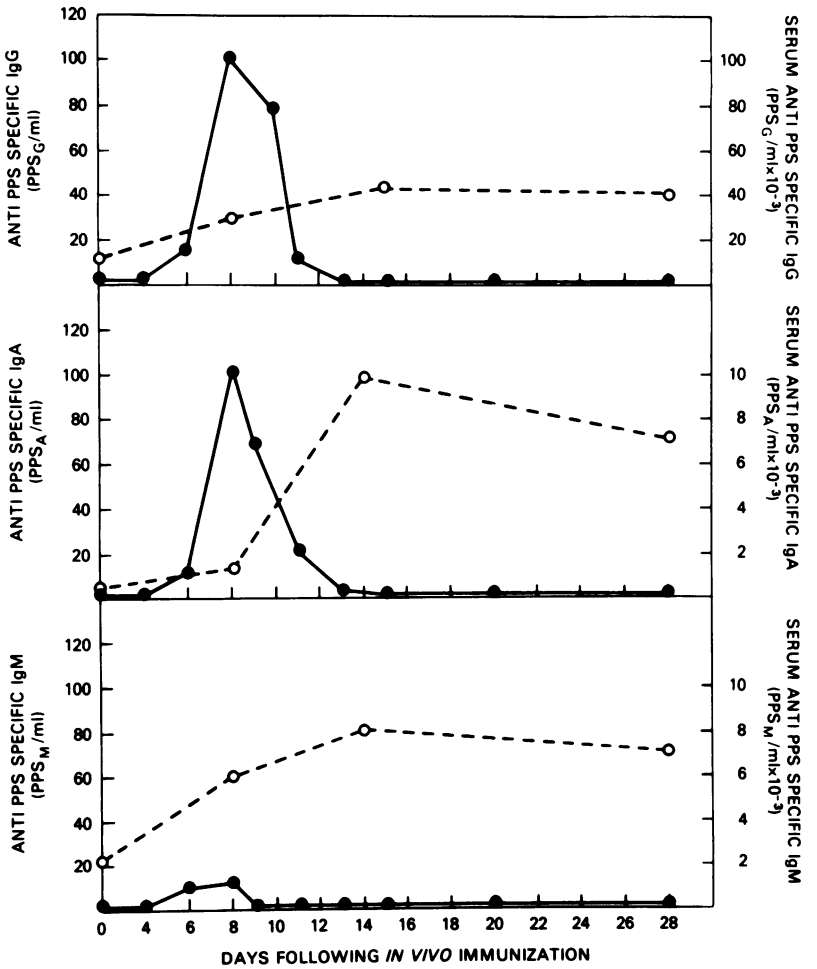

FIGURE 1 Specific amounts of anti-PPS IgG, IgA, and IgM antibody synthesized by mononuclear cells from one individual are expressed. Cultures were established in flat-bottomed vessels with 1.5 × 10^6 UF cells/ml on days shown. Supernatants from quadruplicate cultures were combined before assay. Serum was diluted 1:100 before specific anti-PPS IgA and IgM determination and 1:1,000 for anti-PPS IgG. ● —— ●, spontaneous anti-PPS antibody secretion in vitro; O - - - O, serum anti-PPS antibody levels.

quantities of IgA and IgG were detected, while only small amounts of IgM were found. On the other hand, peak serum responses lagged behind the appearance of spontaneous antibody-secreting cells for both IgG and IgA. Culture supernatants from lymphoid cells from eight normal volunteers immunized and studied 7–8 d after immunization contained 91±30 U of IgG, 13±10 U of IgM, and 75±39 U of IgA. No specific anti-PPS antibody was found in culture supernatants established before immunization; and after immunization no spontaneous antibody was detected against PPS-7, a serotype not in the vaccine (data not shown).

Comparison of numbers of ABC, PFC, and amount of antibody secretion in culture supernatants. To further examine the nature of the cells responsible for the spontaneous secretion of anti-PPS antibody in culture, ABC and PFC assays specific for PPS were developed. As demonstrated in Table I, indirect PFC responses, numbers of ABC, and the amount of specific antibody secreted into culture supernatants follow identical kinetics. Negligible numbers of direct PFC were detected, confirming the small amount of supernatant IgM present. The PFC were antigen-specific as they could be totally inhibited by free polysaccharides. No PFC could be detected against a non–cross-reacting antigen, tetanus toxoid. The large number of ABC and PFC found over the test period indicates that the peripheral blood B cell pool had been markedly, but transiently, enriched with cells that have specificity for PPS. On day 9, 0.14% of the total peripheral blood mononuclear cells were spontaneously secreting specific anti-PPS antibody as detected in the PFC assay.

Modulation of spontaneous Ig production. Initial studies revealed that most of the spontaneous antibody production in vitro could be detected within 3 d of culture. Despite this brief period, these B cells were markedly sensitive to low doses of irradiation (Fig. 2) suggesting that they were actively dividing in vitro and that this cell division over a restricted period of time was critical for the expression of their spontaneous antibody-secreting capability. 500 rad of irradiation reduced the amount of spontaneous IgG PPS-specific antibody production by 75%. In addition, the protein synthesis inhibitor cycloheximide (15 μg/ml) completely abrogated any anti-PPS antibody production (data not shown).

A series of additional studies were performed attempting to enhance or to suppress specific in vitro antibody production. Fig. 3 demonstrates the effect of adding PWM to cell cultures. The amount of specific anti-PPS antibody was reduced one-third to one-half after a 5-d culture in the presence of PWM. The results are for six donors whose cultures were initiated on day 7 after immunization. Similar results were found when cultures were assayed after only 3 d in culture (data not shown).

Fig. 4 demonstrates the effect of T cells or T cell factors on the spontaneous in vitro secretion of anti-PPS antibody by B cells from PPS-immunized individuals. Spontaneous anti-PPS antibody was not modified by the presence or absence of T cells or exogenous T cell factors. In preliminary studies of the PPS antibody-specific, enzyme-linked immunosorbent assay, it was observed that doses of antigen > 100 ng began

TABLE I

Comparison of the Number of ABC, PFC, and the Amount of Supernatant Ig Specific for PPS at Various Times after Immunization

Days after immunization	Anti-PPS antibody*			Anti-PPS PFC		
	IgG	IgA	IgM	Indirect	Direct	ABC
	PPS U/ml			*PFC/10^6 cells*		*ABC/10,000 cells*
0	0	0	0	ND	ND	ND
4	1.0	0.75	0	0.5	0	<1
6	40.5	27.4	5.0	96±4.2‡	7	8.3
7	100.6	128.6	9.9	ND	ND	45
9	111.1	224	5.0	1,382±47	1	343
11	43.6	112.7	0.1	233±182	3	70
13	2.5	13.7	0	42±2	3	13
15	1.1	2.8	1.2	11±2	2	8.5
21	0	0	0	ND	ND	ND
28	0	0	0	6±1	1	4.7

* Supernatants are derived from 5-d lymphocyte cultures in the absence of antigen. Data are from one representative series of experiments.

‡ Mean±SEM.

ND, not done.

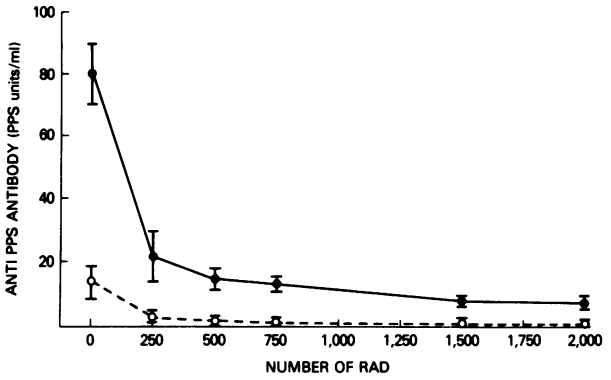

FIGURE 2 The effect of various amounts of irradiation on specific anti-PPS IgG (● —— ●) and IgM (O - - - O) antibody by UF mononuclear cells. Lymphocytes were obtained 7 d postimmunization and cultured for 5 d. Data are from one donor and expressed as an arithmetic mean±SEM of triplicate cultures and are representative of two other experiments.

to inhibit the assay. To study the effect of addition of antigen to cultures on the secretion of anti-PPS antibody, antigen > 0.1 ng–1 μg was added to culture either on day 0 or on day 4, 16 h before the assay. No discernible difference in the amount of anti-PPS supernatant IgG or IgM could be detected (data not shown).

Lymphocyte blastogenic responses to PPS antigen. Proteins such as keyhole limpet hemocyanin (KLH) and tetanus toxoid trigger considerable levels of in vitro T cell proliferation when added to mononuclear cell cultures of immune donors (15, 16). However, no significant proliferative response to PPS in either nonimmune or immune donors was found over a wide range of in vitro antigen doses (Table II). Concentrations as high as 100 μg/ml of PPS did not interfere with cell viability as measured by trypan blue dye exclusion and did not elicit a blastogenic response. This lack of a significant proliferative response to PPS is consistent with findings in the mouse (17). To assess the effect of PPS on B cell proliferation, TCD populations from immune donors were stimulated with PPS. Again, no significant proliferative response was found.

In vitro induction of anti-PPS antibody responses. Previous studies with KLH and tetanus toxoid have demonstrated that after in vivo immunization, B cells appear in the circulation after a defined period of time that are capable of being triggered to produce specific antibody by either the antigen in question or the polyclonal B cell activator PWM (7). In contrast, no antigen-inducible antibody production was noted after immunization with PPS vaccine in 10 donors over a broad range of antigen concentrations and times after immunization. Manipulation of the system by depletion of T cells, depletion of monocytes, addition of T cell factors, or varying culture conditions did not alter this unresponsiveness. However, although antigen could not trigger specific Ig production, PWM-inducible B cells specific for PPS were detected 2–3 wk after immunization (Fig. 5). The predominant antibody isotype induced by PWM was IgM, although lesser amounts of IgG and IgA were detected. This is the reverse of the spontaneous secretion of anti-PPS antibody noted 8 d after immunization. Mononuclear cells from one subject studied 8–12 mo after immunization produced > 100 U of IgG and IgM after PWM stimulation and yet were unresponsive to stimulation with PPS antigen. Thus, PWM-responsive anti-PPS antibody-producing B cells may persist for a prolonged period of time after immunization.

Effect of repeat in vivo immunization. Studies with PPS vaccine in man have demonstrated little change in existing serum anti-PPS antibody titers after a repeat immunization 1 yr after the primary immunization (18). To assess the effect of booster immunization with PPS on spontaneous anti-PPS antibody secretion, a previously immunized donor whose lymphoid cells had synthesized large quantities of spontaneous anti-PPS antibody was boosted with 0.5 ml of the PPS-vaccine 4 mo after the initial immunization. 4–6 h after the booster immunization, a marked local reaction occurred at the site of injection that abated over the next 48 h. Cultures were established 3, 5, 7, 9, and 13 d after booster immunization.

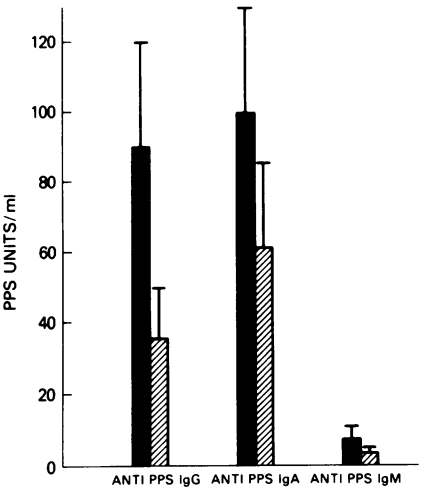

FIGURE 3 The effect of adding PWM to UF mononuclear cells obtained 7 d after immunization. Lymphocytes were cultured for 5 d in the presence (striped bars) or absence (black bars) of PWM. Data are from culture supernatants from six donors and are expressed as arithmetic mean±SEM. The differences are statistically significant at $P < 0.05$ by using a paired comparison test with a Student's t distribution for both anti-PPS IgG and IgA.

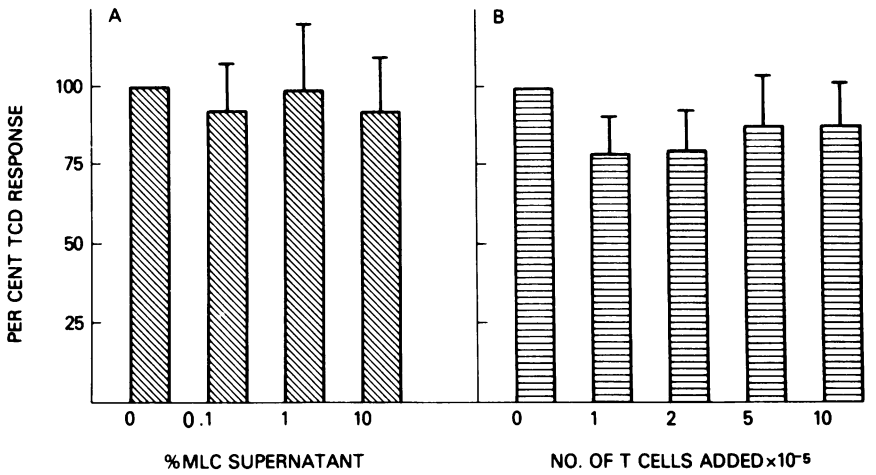

FIGURE 4 The lack of modulation of PPS B lymphoblasts by T cells and T cell factors. Part A illustrates the effect of mixed lymphocyte culture supernatants and part B the effect of adding back T cells. The data are normalized to the TCD PPS-specific IgG response and are expressed as the arithmetic mean±SEM for four subjects in part A and two subjects in part B. UF mononuclear cells synthesized 77±16% of TCD cells, whereas T cells alone only produced 1%. TCD cells were cultured at a cell density of 0.5×10^6 cells/ml, whereas UF mononuclear cells were cultured at 1.5×10^6 cells/ml.

No detectable spontaneous anti-PPS IgG, IgA, or IgM was found. PFC and ABC assays done on days 7 and 9 did not detect either specific PFC or ABC (data not shown). In concordance with the lack of spontaneous production of anti-PPS antibody in vitro, no changes in serum anti-PPS IgG or IgM were observed, although a small increase in IgA was noted.

DISCUSSION

In the present study we demonstrate the rapid appearance and disappearance of lymphoid cells in the circulation that spontaneously secrete antibody specific for PPS after in vivo immunization with this antigen. These spontaneously secreting cells were not modified in vitro

TABLE II
*Proliferative Responses to PPS at Various Time Intervals after Immunization with PPS Vaccine**

	PPS vaccine‡				PPS type 8		PPS type 3	
PPS/ml	Day 7	Day 14	1 Yr	1 Yr§	Day 7	Day 42§	Day 7	Day 43
0	219	245	508	107	219	401	319	408
10 ng	119	357	480	334	520	380	780	259
100 ng	648	373	827	279	584	745	674	581
1 μg	1,287	445	664	219	372	346	637	335
10 μg	961	409	335	251	810	415	551	257
100 μg	ND	ND	ND	ND	718	379	862	453
PWM	13,370	12,509	21,928	242	13,370	ND	9,911	21,011

* Mononuclear cells were stimulated for 4 d in culture with varying amounts of different types of PPS and tritiated thymidine incorporation measured as described in Methods.
‡ Indicated concentration of PPS is for each capsular polysaccharide in the vaccine. Hence the total PPS in culture is 14 times the amount indicated.
§ TCD cultures.
ND, not done.

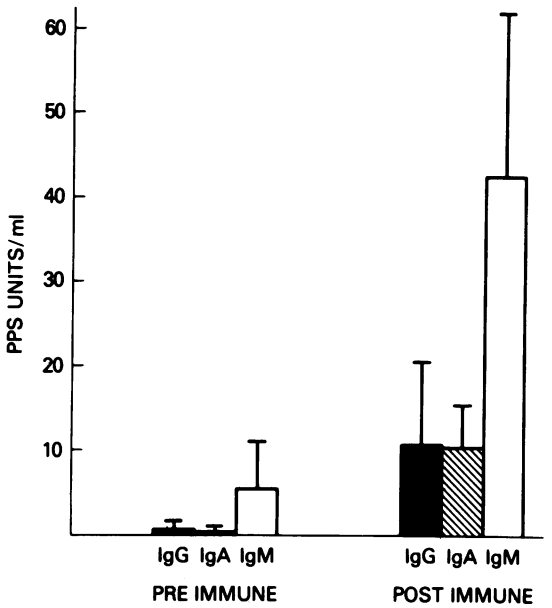

FIGURE 5 PWM induction of specific anti-PPS IgG, IgA, and IgM in culture supernatants. 10-d cultures were established either before immunization or 2–4 wk after immunization. Data are from duplicate cultures from six different donors. The differences between preimmune and postimmune anti-PPS G, A, and M are statistically significant at $P < 0.05$.

by unactivated T cells, T cell factors, or antigen; however, they were readily inhibited by low doses of irradiation and a protein synthesis inhibitor, as well as by PWM. By 2 wk after immunization, spontaneous secretion of anti-PPS antibody by peripheral blood lymphocytes could no longer be detected. However, specific antibody production could be induced with PWM subsequent to the disappearance of spontaneously secreting cells from the circulation. Despite extensive manipulations, peripheral blood mononuclear cells could not be induced by in vitro antigen to secrete specific antibody up to 1 yr postimmunization.

Since most individuals are exposed to pneumococcal antigens during childhood, the human immune response to the PPS vaccine may represent a secondary response. Nevertheless, in many respects the human in vitro and in vivo immune response to PPS parallels the response to this antigen in mice. There is a requirement for in vivo priming with antigen, a lack of secondary in vitro response with antigen stimulation, a period of prolonged unresponsiveness after immunization (19), lack of T cell activation as measured by T cell proliferation (17), and a possible regulatory role for nonspecific T cell suppression (20). The kinetics of the in vitro response differ slightly as the murine splenic PFC appear and peak earlier than the PFC in human peripheral blood. The predominant murine PFC isotype detected is IgM (19); however, the inability of many developing antisera to detect IgG3 may have been responsible for not detecting IgG PFC (21). This latter point may also explain the inability to find PFC in mouse peripheral blood.

Human peripheral blood lymphocytes spontaneously synthesized predominantly IgG and IgA anti-PPS antibody. Despite a substantial IgM serologic response, only low levels of spontaneous anti-PPS IgM antibody secretion by peripheral blood lymphocytes were detected. There was also a difference in the appearance of ABC in the peripheral blood of humans compared with mouse spleen. In the mouse spleen, ABC appear several hours after immunization and reach peak levels 2 d later (22), whereas in human peripheral blood, ABC followed the same kinetics as the PFC. These differences in the kinetics of in vitro antibody production, time course of detection of ABC, and the type of isotype produced may be accounted for by differences in the B lymphocyte populations present in the spleen vs. the peripheral blood.

As in the mouse, substantial differences exist in the human immune system between the response to polysaccharide and the response to protein antigens. The major differences found in this study compared with previous work with soluble protein antigens are the lack of T cell activation as measured by T cell proliferation, the lack of in vitro induction of antibody production by antigen, and the lack of immune memory to in vivo booster immunization. The inability to trigger B cells with antigen in vitro, despite the success of in vivo immunization, is perplexing although, as stated above, consistent with results in the mouse. The increased number of ABC and the response to PWM stimulation noted 2–4 wk after immunization constitute strong evidence that B cells are present in the circulation that are specific for PPS.

In contrast to PPS, the soluble protein KLH can induce specific antibody production in vitro by the addition of nanogram amounts of antigen to UF mononuclear cells obtained 2 wk after booster immunization (8). The addition of antigen to cultures is necessary for triggering KLH-specific T helper cells that induce Ig secretion by the recently in vivo activated B cells. The KLH-primed B cells do not need to be reexposed in vitro to antigen for the induction of specific antibody production (unpublished observation). When the nonspecific T cell help is provided by PWM, both KLH (7) and PPS-specific B cells synthesized specific Ig. Potent T cell supernatants, in the absence of antigen and when added to T cell-depleted cultures, are also able to trigger B lymphocytes from either PPS- (unpublished observation) or KLH-immunized donors (23) to synthesize the appropriate specific antibody. Thus, the differences in the requirement for conventional T cell help in response to immunization with

PPS may explain the differences between KLH or other protein antigens and PPS in their ability to trigger an in vitro antibody response after immunization.

Of particular note is the resemblance of certain of the phenomena observed here to that of the tetanus toxoid system reported previously (24–28), particularly with regard to the appearance of spontaneously secreting cells and the subsequent PWM-inducible specific antibody production. One of the major differences between the two systems is the lack of a detectable T cell response in the PPS system. We interpret this later finding to reflect either a true thymus independence of the response or an absence of PPS-specific T cells within the circulating T cell repertoire.

The absence of B cell memory in murine studies has also been attributed to the T cell independence of the system (29, 30). It has been shown that type III PPS can induce an IgG memory response only if it is coupled to a thymus-dependent carrier such as horse RBC (31). As in the mouse, booster immunization of humans with the PPS vaccine is not accompanied by a memory response (18).

The appearance of lymphoblastoid B cells in the peripheral blood after in vivo perturbation of the human immune system appears to be a general phenomenon and has been demonstrated after immunization with protein antigens such as tetanus toxoid (26), after intranasal inoculation with a cold-adapted influenza virus (32), after immunization with *Salmonella typhi* (33), and in the present study after immunization with PPS. During the peak of the response after immunization with PPS, 1–3% of the peripheral blood lymphocytes formed rosettes with ox RBC coated with PPS and 0.1% formed spontaneous PFC in a hemolysis-in-gel PFC assay. Thus, lymphoblastoid B cells specific for PPS represent a relatively large proportion of the peripheral blood B cell pool. A possible explanation for the 10-fold difference in ABC and PFC frequencies is the likelihood that ABC assays detect cells in various states of differentiation, whereas the PFC assay identifies only B cells that are actively secreting anti-PPS antibody.

The origin, destination, and regulation of these lymphoblastoid B cells are presently not understood. Since the spleen plays a major role in the immune response to PPS (34), these cells may originate there. The bone marrow has been reported to be a major source of Ig production and may be the final destination of these cells (35). Of note is the fact that the lymphoblastoid B cells are sensitive to low levels of irradiation in that spontaneous secretion of antibody can be markedly inhibited by exposure to 500 rad. Thus, at least a proportion of them appear to be proliferating and this level of proliferation may be essential for the full expression of antibody-secreting capability. Results in the present study show that these lymphoblastoid cells are not modified by unactivated T cells or by T cell factors present in a mixed lymphocyte reaction supernatant, but are suppressed by PWM in culture. The role of antiidiotype, T cell suppression, and immune complexes on the proliferation and differentiation of these cells is currently under investigation.

In conclusion, a PFC assay, an ABC assay, and an in vitro culture system for examining B cells actively synthesizing and secreting anti-PPS antibody after in vivo immunization have been described for man, and a close analogy between the human and murine immune response to PPS was found. The dichotomy between PWM and antigen in triggering anti-PPS antibody after immunization with PPS contrasts with previous results in man with KLH and tetanus toxoid antigens, and may be related to the absence of PPS-specific T cell help. Thus, this system may prove useful in delineating the mechanisms of activation and immunoregulation of subsets of human B cells not amenable to study with the commonly used soluble protein antigens.

REFERENCES

1. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, D. R. Barthold, and P. J. Baker. 1972. Genetic control of the antibody response to type III pneumococcal polysaccharide in mice. I. Evidence that an X-linked gene plays a decisive role in determining responsiveness. *J. Exp. Med.* **136:** 931–949.
2. Sharon, R., P. R. B. McMaster, A. M. Kosk, J. D. Owen, and W. E. Paul. 1975. DNP-Lys-Ficoll: a T-independent antigen which elicits both IgM and IgG anti-DNP antibody secreting cells. *J. Immunol.* **114:** 1585–1589.
3. Perlmutter, R. M., D. Hansburg, D. E. Briles, R. A. Nicolotti, and J. M. Davie. 1978. Subclass restriction of murine anti-carbohydrate antibodies. *J. Immunol.* **121:** 566–572.
4. Yount, W. J., M. M. Dorner, H. G. Kunkel, and E. A. Kabat. 1968. Studies on human antibodies. VI. Selective variations in subgroup composition and genetic markers. *J. Exp. Med.* **127:** 633–646.
5. Mosier, D. E., I. M. Zitron, J. J. Mond, A. Ahmed, I. Scher, and W. E. Paul. 1977. Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. *Immunol Rev.* **37:** 89–104.
6. Peltolta, H., H. Kayhty, A. Sivonen, and P. H. Mäkelä. 1977. The haemophilis influenza type B vaccine in children. A double blind field study of 100,000 vaccines 3 months to 5 years of age in Finland. *Pediatrics.* **60:** 730–737.
7. Lane, H. C., D. J. Volkman, G. Whalen, and A. S. Fauci. 1981. In vitro antigen-induced, antigen-specific antibody production in man. Specific and polyclonal components, kinetics, and cellular requirements. *J. Exp. Med.* **154:** 1043–1057.
8. Volkman, D. J., H. C. Lane, and A. A. Fauci. 1981. Antigen-induced in vitro antibody production in humans: a model for B cell activation and immunoregulation. *Proc. Natl. Acad. Sci. USA.* **78:** 2528–2531.

9. Falkoff, R. J. M., L. P. Zhu, and A. S. Fauci. 1982. Separate signals for human B cell proliferation and differentiation in response to *Staphylococcus aureus*. Evidence for a two signal model of B cell activation. *J. Immunol.* **129:** 97–102.
10. Musson, R. A., and P. M. Henson. 1979. Humoral and formed elements of blood modulate the response of peripheral blood monocytes. I. Plasma and serum inhibit and platelets enhance monocyte adherence. *J. Immunol.* **122:** 2026–2031.
11. Fauci, A. S., and D. C. Dale. 1975. Alternate-day prednisone therapy and human lymphocyte subpopulations. *J. Clin. Invest.* **55:** 22–32.
12. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6:** 588–590.
13. Fauci, A. S., G. Whalen, and C. Burch. 1980. Activation of human B lymphocytes. XVI. Cellular requirements, interactions, and immunoregulation of pokeweed mitogen-induced total-immunoglobulin producing plaque-forming cells in peripheral blood. *Cell. Immunol.* **54:** 230–240.
14. Hosea, S. W., C. Burch, E. J. Brown, R. A. Berg, and M. M. Frank. 1981. Impaired immune response of splenectomized patients to polyvalent pneumococcal vaccine. *Lancet.* **I:** 804–807.
15. Geha, R. S., and E. Merler. 1974. Response of human thymus-derived (T) and non-thymus (B) lymphocytes to mitogenic stimulation in vitro. *Eur. J. Immunol.* **4:** 193–198.
16. Curtis, J. E., E. M. Hersh, J. E. Harris, C. McBride, and E. J. Freireich. 1970. The human primary immune response to keyhole limpet haemocyanin: interrelationships of delayed hypersensitivity, antibody response and in vitro blast transformation. *Clin. Exp. Immunol.* **6:** 473–491.
17. Paul, W. E., G. W. Siskind, B. Benacerraf, and Z. Ovary. 1967. Secondary antibody responses in haptenic systems: cell population selection by antigen. *J. Immunol.* **99:** 760–770.
18. Carlson, A. J., W. L. Davidson, A. A. McLean, P. P. Vella, R. E. Weibel, A. F. Woodhour, and M. R. Hilleman. 1979. Pneumococcal vaccine: dose, revaccination, and coadministration with influenza vaccine. *Proc. Soc. Exp. Biol. Med.* **161:** 558–563.
19. Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott. 1971. Characterization of the antibody response to type III pneumococcal polysaccharides at the cellular level. I. Dose-response studies and the effect of prior immunization on the magnitude of the antibody response. *Immunology.* **20:** 469–480.
20. Baker, P. J., N. D. Reed, P. W. Stashak, D. F. Amsbaugh, and B. J. Prescott. 1973. Regulation of the antibody response to type III pneumococcal polysaccharide. I. Nature of regulatory cells. *J. Exp. Med.* **137:** 1431–1441.
21. Slack, J., G. P. Der-Balian, M. Nahm, and J. M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response to thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. *J. Exp. Med.* **151:** 853–862.
22. Baker, P. J., and M. J. Landy. 1967. Brevity of the inductive phase in the immune reponse of mice to capsular polysaccharide antigens. *J. Immunol.* **99:** 687–694.
23. Peters, M., and A. S. Fauci. 1983. Selective activation of antigen-specific human B cells in recently immunized individuals by non-specific factors in the absences of antigen. *J. Immunol.* **130:** 678–680.
24. Stevens, R. H., and A. Saxon. 1978. Immunoregulation in humans. Control of anti-tetanus toxoid antibody production after booster immunization. *J. Clin. Invest.* **62:** 1154–1160.
25. Stevens, R. H., and A. Saxon. 1979. Reduced in vitro production of anti-tetanus toxoid antibody after repeated in vivo immunization with tetanus toxoid. *J. Immunol.* **122:** 592–598.
26. Stevens, R. H., E. Macy, C. Morrow, and A. Saxon. 1979. Characterization of a circulating subpopulation of spontaneous anti-tetanus toxoid antibody producing B cells following in vivo booster immunization. *J. Immunol.* **122:** 2498–2504.
27. Stevens, R. H., and A. Saxon. 1980. Antigen-induced suppression of human in vitro pokeweed mitogen-stimulated antibody production. *Cell. Immunol.* **55:** 85–93.
28. Volkman, D. J., S. P. Allyn, and A. S. Fauci. 1982. Antigen-induced in vitro antibody production in humans. Tetanus toxoid specific antibody synthesis. *J. Immunol.* **129:** 107–112.
29. Dresser, D. W., and A. M. Popham. 1979. The influence of T cells on the initiation and expression of immunological memory. *Immunology.* **38:** 265–274.
30. Davie, J. M., and W. E. Paul. 1974. Role of T lymphocytes in the humoral immune response. I. Proliferation of B lymphocytes in thymus-derived mice. *J. Immunol.* **113:** 1438–1445.
31. Braley-Mullen, H. 1980. Antigen requirements for priming of IgG producing B memory cells specific for type III pneumococcal polysaccharide. *Immunology.* **40:** 521–527.
32. Yarchoan, R., B. R. Murphy, W. Strober, M. L. Clements, and D. L. Nelson. 1981. In vitro production of anti-influenza virus antibody after intranasal inoculation with cold-adapted influenza virus. *J. Immunol.* **127:** 1958–1963.
33. Thomson, P. D., and N. S. Harris. 1977. Detection of plaque forming cells in the peripheral blood of actively immunized humans. *J. Immunol.* **118:** 1480–1482.
34. Schiffman, G. 1981. Immune responses to pneumococcal polysaccharide antigens: a comparison of the murine model and the response in humans. *Rev. Infect. Dis.* **3:** 224–232.
35. Brenner, R., W. Hijmans, and J. J. Haaijman. 1981. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. *Clin. Exp. Immunol.* **46:** 1–8.