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Polyclonal activation to immunoglobulin secretion in human adenoid lymphocytes induced by bacteria from nasopharynx *in vitro*

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SUMMARY

Adenoid lymphocytes from twenty-six children scheduled consecutively for adenoidectomy because of severe nasal obstruction were stimulated *in vitro* by *D. pneumoniae*, *H. influenzae* and haemolytic streptococci group A. All bacteria induced both increased DNA synthesis and polyclonal antibody secretion, tested in a haemolytic plaque assay. The magnitude of the B-cell response was correlated to the age of the children. Thus, cells from older children showed a significantly higher polyclonal response than those from younger children. No case of unresponsiveness to any stimulant was observed. Susceptibility to infection was more pronounced among the younger children, which might reflect some degree of immaturity in the immune system. However, adenoid lymphocytes are immunocompetent cells, contributing to antibody secretion in the response against infection. The role of the receptor for PBA substances on adenoid cells in immune reactions against infectious micro-organisms is discussed.

INTRODUCTION

The adenoid is an immunocompetent organ containing lymphocytes supposed to be directly stimulated to immune reactivity against natural antigens, such as bacteria and viruses. Several studies have shown that even healthy children sometimes carry pathogenic bacteria, such as *Diplococcus pneumoniae* and *Haemophilus influenzae* in the nasopharynx (Loda *et al.*, 1975). During upper respiratory tract infections, the percentage of positive cultures are higher and those strains of bacteria which are responsible for purulent otitis media are often also found in the nasopharynx (Kamme, Lundgren & Mårdh, 1971).

Immunological studies on immunity against infection have been much concerned with the specific immune response to micro-organisms. However, whole micro-organisms and bacterial products have long been known to induce unspecific antibody secretion *in vivo* (Wollheim & Williams, 1966; Nakashima & Kato, 1974). There is now increasing evidence that this non-specific adjuvant-like effect of bacteria on B cells has a physiological role. Extensive studies on products from micro-organisms, such as lipopolysaccharide from *E. coli* (LPS), dextran, pneumococcal polysaccharides and protein A from *Staphylococcus aureus* have shown that these substances are polyclonal B cell activators (PBA), which are capable of inducing a polyclonal antibody secretion in resting B cells (Andersson, Sjöberg & Möller, 1972; Coutinho & Möller, 1973; Möller & Landwall, 1976; Biberfeld & Gronowicz, 1976; Biberfeld, 1977; Rosén *et al.*, 1977). This unspecific triggering of B cells is not the result of specific receptor binding of antigen, but rather of the existence of receptors for PBA substances on distinct or overlapping subsets of lymphocytes. (Coutinho & Möller, 1975; Gronowicz & Coutinho, 1975). The PBA receptors are distinct from the cell surface immunoglobulin molecules, but may be functionally or structurally linked

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to them (Andersson, Bullock & Melchers, 1974). Thus, lymphocytes have at least two kinds of receptors. The capacity of a B cell to grow and to secrete immunoglobulin depends on the ability of the cell to be mitogen-reactive, due to the presence of a PBA receptor. Low doses of antigen can activate B cell specific clone(s) without the participation of T cells, if the antigen is linked to a carrier substance with PBA properties. Binding of antigen to Ig receptors focusses PBA to the specific clone and triggering is achieved as a result of the existence of PBA receptors on the responding clone of lymphocytes.

Bacteria such as *H. influenzae* and *Neisseria catarrhalis* have been shown to have mitogenic properties which give increased DNA synthesis in adenoid cells (Rynnel-Dagöö, 1976). By the use of a haemolysis-in-gel assay for the detection of antibody production in human lymphocytes (Fauci & Pratt, 1976), it has been possible to show that *Staphylococcus aureus* strain Cowan 1 and Wood act as PBA both for human spleen and blood lymphocytes (Ringdén *et al.*, 1977). In order to elucidate the possible PBA activity of *D. pneumoniae* and *H. influenzae* in an organ stimulated by these bacteria *in vivo*, adenoid lymphocytes from twenty-six children were treated with these substances *in vitro* and parameters such as DNA synthesis and antibody secretion were measured. The relationship of immune response to age and susceptibility to infection is discussed.

MATERIALS AND METHODS

Subjects. Twenty-six children between 2·5 and 13 years of age were investigated. They were scheduled for adenoidectomy in the ENT clinic at Huddinge Hospital because of severe nasal obstruction due to adenoid hypertrophy. A case history for the preceeding year was taken on the basis of a special questionnaire. The frequency of common colds, purulent otitis media, tonsillitis, sinusitis, pneumonia and the occurrence of long-standing rhinitis was noted. Patients with more than seven attacks of the common cold and/or four spells of purulent otitis media per year (Rynnel-Dagöö & Schiratzki, 1978) were recorded as suceptible to infection. Other forms of upper respiratory tract infections were rare.

Cell preparations. The tissue obtained at adenoidectomy was placed in phosphate buffered saline (PBS), cut into small pieces and passed through a steel screen. Clumps were allowed to sediment and the resulting single cell suspension was washed in PBS.

Blood lymphocytes from the same children undergoing adenoidectomy were prepared by centrifugation of defibrinated blood on Ficoll–Isopaque (Lymphoprep, NyCo, Oslo, Norway) as described by Bøyum (1968). Fresh cells were used in all experiments except for tests with surface markers where cells stored in fluid nitrogen were used.

Mitogens. Lipopolysaccharide (LPS) from *E. coli* 055:B5 was prepared by Professor T. Holme, Department of Bacteriology, Karolinska Institute, Stockholm, and was used at a final concentration of 100 μg/ml. Phytohaemagglutinin (PHA) was obtained from Wellcome Reagents Ltd., England and used in a final concentration of 1/100.

Bacteria. Suspensions of whole bacteria of *D. pneumoniae*, *H. influenzae* and haemolytic streptococci group A were provided by H. Alfredsson, Department of Microbiology, Huddinge Hospital. The bacteria were killed by heating to 70°C for 60 min. They were then washed twice and resuspended to a final concentration of 10^9 bacteria/ml. *Staphylococcus aureus* strain Cowan 1 was provided by Dr. P. Landwall, Department of Bacteriology, Karolinska Institute, Stockholm. The bacteria were killed by heating to 80°C for 10 min. They were then centrifuged and resuspended to a final concentration of 10% in balanced salt solution (BSS). Cowan 1 was used in a final dilution of 1/2500.

Culture conditions. The cells were cultured in medium prepared according to the method of Mishell & Dutton (1967). The medium was supplemented with 10% AB serum which had been heat inactivated and absorbed three times with sheep erythrocytes (SRBC). Cultures for antibody-secreting cells were set up in petri dishes (3·5 cm diameter, Nunclon, Denmark) in a final dilution of $2{\cdot}5\times 10^6$ cells/ml. To measure DNA synthesis, triplicates of $0{\cdot}25\times 10^6$ cells in 0·1 ml were cultured in microtitre plates (Falcon 3040). The cultures were incubated at 37°C in a humidified 5% CO_2 in air atmosphere.

Measurement of DNA synthesis. 1·0 μCi of ^{3}H-thymidine (Radiochemical Centre, Amersham, England) in 0·05 ml of PBS was added per well 24 hr prior to harvesting. The cells were harvested using a Skatron harvesting machine (Lierbyen, Norway). Radioactivity was measured in an Intertechnique (Nanotechnique, Sweden) liquid scintillation spectrophotometer counter. Results were computed and plotted using a Hewlett Packard computer. All data are presented as the arithmetic means$\pm$standard error (s.e.) of three individual cell cultures.

Assay for plaque-forming cells. A modified haemolytic plaque assay was used to quantify cellular antibody production in cultures. (Fauci & Pratt, 1976). Cells were harvested using a plastic spatula, washed twice in PBS and adjusted to 10^6 cells/ml. 0·7 ml of 0·5% agar (Bacto Agar, Difco, Laboratories, Detroit, Michigan) in Eagle's MEM solution containing 0·05% DEAE-Dextran (Pharmacia Fine Chemical Inc., Uppsala, Sweden) was added to tubes kept at 45°C in a water bath. 5·0 mg fluorescein isothiocyanate (FITC) in 1·0 ml carbonate bicarbonate buffer with pH 9·23 was coupled to 0·5 ml packed SRBC and diluted 1:5 in PBS. Thereafter, 0·05 ml of FITC coupled SRBC diluted 1:5 in PBS, 0·2 ml of lymphocyte suspension and 0·05 ml guinea-pig complement were added to each tube. Three separate 0·2 ml drops of the mixture were then placed in a plastic petri-dish. A 22 × 32 mm glass coverslip was immediately placed on each drop, leaving an ultra thin layer of gel.

Plates were incubated for 3 hr at 37°C. Plaque-forming cells (PFC) were counted using indirect light or a dissecting microscope. In all experiments representative plaques were checked for the presence of a central lymphoid cell.

Surface markers for T and B cells. Assays for cells with receptors for the Fc portion of IgG and for C′3. The relative proportion of T cells was detected by their ability to bind SRBC spontaneously (SRBC-RFC) according to the method of Jondal, Holm & Wigzell (1972). For the enumeration of B cells, the surface membrane immunoglobulin (SmIg) marker was used (Möller, 1961; Unanue *et al.*, 1971). Lymphocytes were incubated with fluorescein-labelled anti-human Ig serum (Behring Werke, Germany).

Lymphocytes with receptors for the Fc portion of IgG were tested according to the method of Basten *et al.* (1972). Lymphocytes with receptors for C′3 were tested according to the method of Bianco, Patrick & Nussenzweig (1970).

Serum immunoglobulin determinations. IgG, IgM and IgA were measured with a nephelometric technique (Technicon, Geneva, Switzerland).

Bacteriological sampling technique. At the same time as the adenoidectomy was performed a sample was taken from the nasopharynx and from the tonsils. These were placed immediately in transport medium and were inoculated within 4 hr. A small piece of adenoid tissue was put in a sterile tube and was inoculated within the same length of time.

Statistical method. In order to study a possible correlation between age and immune response, Spearman's coefficient of rank correlation (r) was used (Kendall & Stuart, 1973). The number (n) of subjects were ranked from $1-n$ according to age and the magnitude of immune response. The difference (d) between the rank numbers of each pair of observations was calculated and used in the following formula:

$$r = 1 - \frac{6\Sigma^n d^2}{n^3 - n}$$

Significance limits are: $P = 0{\cdot}05$, $r > 0{\cdot}329$; $P = 0{\cdot}01$, $r > 0{\cdot}465$.

RESULTS

Cell surface markers on adenoid lymphocytes

Cells from adenoids from five children of various ages were tested. As can be seen in Table 1, the mean value for SmIg positive cells was $47{\cdot}6 \pm 2{\cdot}0\%$ and for spontaneous rosette-forming cells $42{\cdot}0 \pm 2{\cdot}0\%$. The mean value for cells binding C′3 was $50{\cdot}8 \pm 7{\cdot}8\%$ and for cells binding IgG-coated sheep red blood cells $17{\cdot}6 \pm 1{\cdot}7\%$. These values are in accordance with earlier observations showing that very few adenoid lymphocytes carry Fc receptors (Rynnel-Dagöö, Möller & Waterfield, 1977). The percentage of T cells, however, is slightly higher in this report. A possible explanation is that adenoid lymphocytes in the present study were not separated on Lymphoprep gradients.

TABLE 1. Surface markers on adenoid lymphocytes from five children out of twenty-six

Patient	Age of children	SmIg positive cells	Fc-RFC	C′3-RFC	SRBC-RFC
1 (NC)	13·0	47·7	18·5	53·2	41·5
2 (OS)	5·0	49·5	19·4	55·6	40·2
3 (LT)	3·0	46·0	15·2	59·4	45·5
4 (SS)	3·5	45·1	16·7	40·0	41·2
5 (DR)	3·0	49·7	18·2	46·0	41·7
		47·6±2·0	17·6±1·7	50·8±7·8	42·0±2·0

Optimal dose of PBA inducing PFC response in adenoid cells

Different doses of *D. pneumoniae* and *H. influenzae* ranging from 5×10^7–5×10^3 bacteria/ml were added to cultures of adenoid lymphocytes and the polyclonal PFC response was tested on days 3 and 4. (Fig. 1a, b). The optimal dose for *D. pneumoniae* was found to be a final dilution of 5×10^6 bacteria/ml for both days. This dose was also optimal for *H. influenzae* on day 3 and was used for further tests. The dose of 5×10^6 bacteria/ml was chosen for the haemolytic streptococci group A.

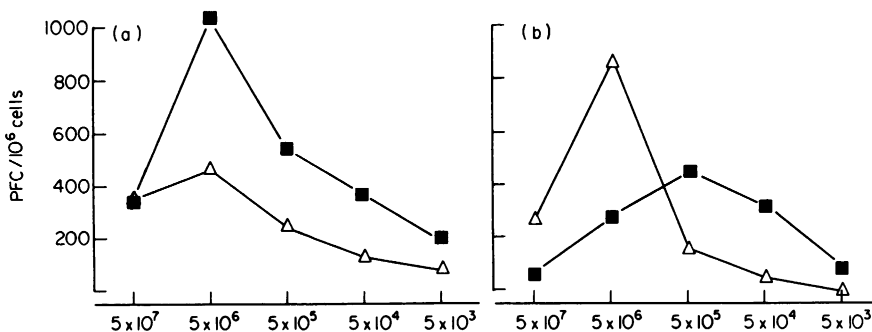

FIG. 1. Plaque formation (PFC/10^6 cells) on day 3 (a) and on day 4 (b) in adenoid lymphocytes induced by various dilutions of *D. pneumoniae* (△——△) and *H. influenzae* (■——■). Backgrounds are subtracted and were 29 and 7 PFC/10^6 on day 3 and 4, respectively.

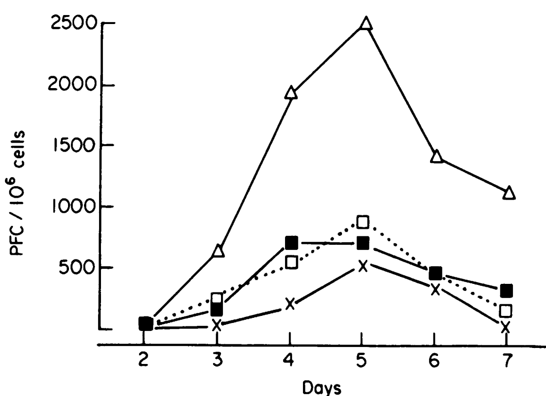

FIG. 2. Kinetic study in adenoid lymphocytes of plaque formation (PFC/10^6 cells) induced by *D. pneumoniae*, 5×10^6 (△——△); *H. influenzae*, 5×10^6 (■——■); Cowan 1, 1/2500 (□ · · · · · □) and LPS, 100 μg (× ——×). Backgrounds are subtracted and were less than 60 PFC/10^6, except for day 7 (108).

Kinetics of response in adenoid lymphocytes

The optimal PFC response induced by the bacteria *D. pneumoniae*, *H. influenzae*, Cowan 1 and LPS occurred on day 3, 4 or 5 in several experiments. One representative experiment is shown in Fig. 2, where optimal response was obtained on day 5. Since stimulation was invariably positive on day 4, this day was chosen for further experiments. The kinetics of DNA synthesis in adenoid lymphocytes from the same child induced by these bacteria and by LPS are shown in Fig. 3. Cells were harvested on days 3, 5 and 7. A peak response occurred on day 5.

DNA synthesis in adenoid and blood lymphocytes

Various dilutions of *H. influenzae* and *D. pneumoniae* have earlier been found to give increased DNA synthesis in adenoid and blood lymphocytes (unpublished data). The same dilutions of bacteria as those used for PFC induction, were used for stimulation to DNA synthesis. Adenoid and blood lymphocytes from fourteen children were harvested on days 3 and 6. LPS induced only a slight increase in DNA synthesis. *D. pneumoniae*, *H. influenzae* and Cowan 1 gave a very variable response in both adenoid cells and blood (Table 2).

The plaque-forming cell (PFC) response in adenoid lymphocytes from twenty-six children

Specimens from twenty-six consecutive experiments were used in this study. The level of polyclonal antibody secretion in adenoid cells induced by *D. pneumoniae*, *H. influenzae*, haemolytic streptococci, Cowan 1 and LPS was investigated by means of the PFC response in cells from twenty-six children.

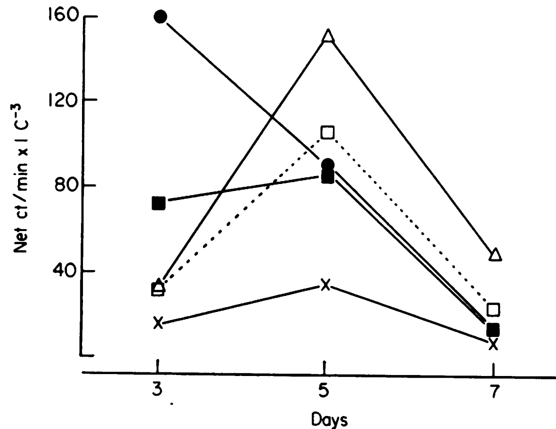

FIG. 3. Kinetic study in adenoid lymphocytes of DNA synthesis harvested on days 3, 5, and 7 induced by *D. pneumoniae*, 5×10^6 (△——△); *H. influenzae*, 5×10^6 (■——■); Cowan 1, 1/2500 (□ · · · · · □); LPS 100 µg (× —— ×) and PHA, 1/100 (●——●). Backgrounds are subtracted and were less than $7 \cdot 0 \times 10^{-3}$ ct min.

TABLE 2. DNA synthesis induced by *D. pneumoniae*, *H. influenzae*, Cowan 1 and LPS in adenoid and blood lymphocytes in fourteen children. (Net ct/min ($\times 10^{-3}$.)

	Adenoid		Blood	
	Day 2–3	Day 5–6	Day 2–3	Day 5–6
D. pneumoniae	21·3±5·9	31·0±7·2	10·7±4·5	32·7±11·5
H. influenzae	58·4±10·8	62·1±15·0	32·9±8·1	37·4±12·8
Cowan 1	28·0±6·0	32·4±4·8	28·4±3·7	30·0±9·0
LPS	9·9±3·5	15·6±5·5	4·5±1·9	3·5±1·4
Background	10·8±1·8	13·9±2·9	5·8±0·9	33·1±9·4

The children were ranked from 1 to 26 according to age (Table 3) and the magnitude of immune response. In order to study a possible correlation between increasing age and increasing immune response, Spearman's rank correlation coefficient was used. The value of this coefficient, due to stimulation to a PFC response by various bacteria and LPS, is shown in Table 4.

The magnitude of the PFC response to stimulation by the bacteria occurring in the nasopharynx showed a significant correlation with increasing age (*H. influenzae* and haemolytic streptococci, $P<0 \cdot 01$; *D. pneumoniae*, $P<0 \cdot 05$). No correlation was seen with Cowan 1 and LPS ($P>0 \cdot 05$).

Occurrence of bacteria in the nasopharynx, tonsils and the adenoid

At the time of operation pathogenic bacteria, such as *D. pneumoniae*, *H. influenzae* and *Staphylococcus aureus* were present in nine out of twenty-six samples from the nasopharynx, in two out of twenty-five from the tonsils and in eleven out of twenty-two from adenoid tissue. In the resting samples either no growth or only an ordinary growth was found.

There was no increased occurrence of pathogenic bacteria in the nasopharynx or the adenoid tissue of the younger children who were more susceptible to infection. Adenoid lymphocytes from children with a growth of bacteria in adenoid tissue did not show unresponsiveness to stimulation by bacteria.

Immunoglobulin determinations

IgG levels in serum were tested in fifteen children. Only two children had levels just below the lower limits and both of these had normal IgM and IgA levels.

TABLE 3. PFC response in adenoid lymphocytes from twenty-six children induced by *D. pneumoniae* (DP), *H. influenzae* (HI), haemolytic streptococci (HS), *Staphylococcus aureus*, strains Cowan 1 (C) and LPS. Backgrounds are subtracted

Patients	Age	Background	DP	HI	HS	C	LPS	Infection prone
1 (MD)	2·0	39	202	59	316	311	244	+
2 (TF)	2·5	125	0	83	105	11	208	+
3 (LT)	3·0	28	614	27	793	643	35	+
4 (ÖH)	3·0	2	0	145	229	102	55	+
5 (DR)	3·0	8	110	180	91	240	292	+
6 (GM)	3·5	10	295	40	515	31	94	+
7 (SS)	3·5	58	137	199	213	588	327	+
8 (ET)	3·5	458	552	475	350	0	264	+
9 (LC)	3·5	35	88	0	72	98	29	−
10 (EL)	4·0	24	547	97	25	184	115	+
11 (RS)	4·0	39	293	0	291	46	75	+
12 (CA)	4·5	8	383	159	135	276	72	+
13 (OS)	5·0	42	177	66	645	180	114	+
14 (SA)	5·5	23	248	310	469	319	227	+
15 (JN)	5·5	31	151	33	35	73	42	+
16 (GM)	6·5	183	1664	157	639	471	0	−
17 (VJ)	7·0	318	1468	1376	1219	1557	3026	−
18 (MN)	7·5	12	758	307	850	105	64	−
19 (LR)	7·5	9	821	264	598	495	306	−
20 (AM)	8·0	170	194	378	970	494	507	−
21 (WN)	9·5	62	1923	701	866	511	216	+
22 (KB)	10·5	71	1717	18	619	250	206	−
23 (HN)	10·5	3	311	652	71	307	76	+
24 (KA)	10·5	0	119	267	392	80	87	−
25 (GA)	12·0	354	695	1098	2786	2291	1960	−
26 (NC)	13·0	0	2583	456	1967	277	357	−

TABLE 4. Values of Spearman's coefficient of rank correlation (r) in twenty-six children. Magnitude of plaque-forming cell (PFC) response induced by various bacteria and by LPS in adenoid lymphocytes from children is correlated to the age of the children

Activator	r	P
D. pneumoniae	0·453	$0{\cdot}05 > P > 0{\cdot}01$
H. influenzae	0·548	$0{\cdot}01 > P > 0{\cdot}001$
Haemolytic streptococci	0·486	$0{\cdot}01 > P > 0{\cdot}001$
Cowan 1	0·318	n.s.
LPS	0·235	n.s.

n.s. (Not significant) = $P > 0{\cdot}05$.

Incidence of upper respiratory tract illness

Fifteen of the twenty-six children were classified as infection prone. In eleven children no increased susceptibility to infection could be shown. The mean age of infection-prone children was $4{\cdot}5 \pm 2{\cdot}4$, and for the healthy children $8{\cdot}6 \pm 2{\cdot}9$, years of age.

DISCUSSION

Three pathogenic bacteria, all of which normally reside in the nasopharynx in children, but which also have the ability to induce upper respiratory tract infections, have been studied. *D. pneumoniae* type 13, *H. influenzae* type b and haemolytic streptococci group A were all able to induce polyclonal antibody secretion in adenoid lymphocytes from twenty-six children, as shown in a haemolytic plaque assay.

The ability of various doses of bacteria to induce antibody secretion was tested and the responses showed the typical bell-shaped curve for PBA activation, super-optimal doses of PBA giving no response. Optimal PFC responses were found after the incubation of cells for 3, 4 or even 5 days, which is in accordance with the kinetics of the optimal response in spleen cells (Ringden *et al.*, 1977).

DNA synthesis was measured in adenoid lymphocytes from fourteen children after stimulation with the same bacteria. A highly variable response was seen. There was generally no correlation between the PFC responses and DNA synthesis, which is in accordance with earlier observations. Antibody secretion and cell proliferation are not necessarily linked (Coutinho *et al.*, 1973; Andersson & Melchers, 1974). Adenoid cells can thus be activated by these three bacteria to antibody secretion and DNA synthesis.

As mentioned above, several bacterial products have PBA properties. It should be said, however, that the cell walls of bacteria often have a complicated structure. Protein A from *Staphylococcus aureus* strain Cowan 1 acts as a PBA for human cells (Ringdén & Rynnel-Dagöö, 1978), but suspensions of whole bacteria Cowan 1 are much stronger stimulants for antibody secretion than protein A in itself. Interestingly, *Staphylococcus aureus* strain Wood 46, which is a mutant lacking protein A, is also a PBA. This would seem to exclude protein A from being the only PBA substance in Cowan 1 bacteria. There are probably several substances in bacteria which interact and behave as PBAs. This was probably the case with all bacteria used in this study.

As with all other Gram-negative bacteria, *H. influenzae* contains LPS, which is also a potent mitogen for human cells (Ringdén, 1976). Nor would it appear to be the only PBA in these bacteria since there was no correlation between LPS and *H. influenzae* responses in our study. The capsule of *H. influenzae* contains repeating units of polyribosephosphate (Zamenhof *et al.*, 1953), acts as an immunogen and has been used as a vaccine (Smith *et al.*, 1974). High doses of this substance might well have mitogenic properties. Capsules from *D. pneumoniae* type 3 contain type-specific polysaccharide (SIII) (How, Brimacombe & Stacey, 1964). SIII has PBA properties in mice (Coutinho & Möller, 1973) and in human adenoid cells (to be published). It is likely that the capsule of *D. pneumoniae* type 13 used in this study has properties similar to those of SIII.

The magnitude of the PFC response in adenoid lymphocytes induced by the bacteria *D. pneumoniae*, *H. influenzae* and haemolytic streptococci correlated significantly with the increasing age of the patients. This was not the case with the response to Cowan 1 or LPS. A similar phenomenon has been seen in mice where the LPS response was age-dependent in murine spleen cells. Newborn spleen contained approximately 10% of the number of reactive cells found at 2 months of age (Andersson, Coutinho & Melchers, 1977). With increasing age, an increased number of adenoid lymphocytes express different PBA receptors and are able to mount unspecific as well as specific immune responses.

A low specific antibody response to bacterial infections has been found in small children. Thus, antibody responses to vaccine from *H. influenzae* would seem to be dependent on age. Only 22% of the children aged 5 to 12 months responded to any dose, whereas 70% of the group aged 13 to 24 months responded (Smith *et al.*, 1973). Among children with acute otitis media due to *D. pneumoniae*, it was found that 12% of infants less than 12 months of age showed a significant response to the infections. In contrast, 48% of children over 24 months responded (Sloyer *et al.*, 1974). As discussed above, this low specific antibody response in small children may be due to the late expression of either the specific Ig receptor or the receptor for PBA.

Partly because of the accidental composition of the patient material in that every child was being operated on because of nasal obstruction, all children but one below 6 years of age could be classified as susceptible to infection. Polyclonal antibody secretion in adenoid cells from children with or without susceptibility to infection could not be compared. It is, however, likely that there is a correlation

between increased susceptibility to infection and an immature immune system in small children.

In the present study we have described how bacterial preparations from different gram-positive as well as gram-negative bacteria contain substances which induce polyclonal B cell activation in human adenoid lymphocytes. If further testing shows that this effect is direct with B lymphocytes, then we must conclude that these bacteria contain PBA substances. A deficient humoral immunity in young children can depend upon the late maturation either of the specific receptor or of the receptor for the PBA substance on B cells.

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REFERENCES

ANDERSSON, J., BULLOCK, W.W. & MELCHERS, F. (1974) Inhibition of mitogenic stimulation of mouse lymphocytes by anti-mouse immunoglobulin antibodies. I. Mode of action. *Eur. J. Immunol.* 4, 715.

ANDERSSON, J., COUTINHO, A. & MELCHERS, F. (1977) Frequencies of mitogen-reactive B cells in the mouse. *J. exp. Med.* 145, 1511.

ANDERSSON, J. & MELCHERS, F. (1974) Maturation of mitogen-activated bone marrow-derived lymphocytes in the absence of proliferation. *Eur. J. Immunol.* 4, 533.

ANDERSSON, J., SJÖBERG, O. & MÖLLER, G. (1972) Mitogens as probes for immunocyte activation and cellular cooperation. *Transplant. Rev.* 11, 131.

BASTEN, A., MILLER, J.F.A.P., SPRENT, J. & PYE, J. (1972) A receptor for antibody on B lymphocytes. I. A method of detection and functional significance. *J. exp. Med.* 135, 610.

BIANCO, C., PATRICK, R. & NUSSENZWEIG, V. (1970) A population of lymphocytes bearing a membrane receptor for antigen antibody complement complexes. *J. exp. Med.* 132, 702.

BIBERFELD, G. (1977) Activation of human lymphocyte subpopulations by Mycoplasma pneumoniae. *Scand. J Immunol.* 6, 1145.

BIBERFELD, G. & GRONOWICZ, E. (1976) Mycoplasma pneumoniae is a polyclonal B cell activator. *Nature (Lond.)*, 261, 238.

BØYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* 21, Suppl. 97.

COUTINHO, A. & MÖLLER, G. (1973) B cell mitogenic properties of thymus-independent antigens. *Nature: New Biology*, 245, 12.

COUTINHO, A. & MÖLLER, G. (1975) Thymus-independent B cell induction and paralysis. *Advanc. Immunol.* 20, 113.

COUTINHO, A., MÖLLER, G., ANDERSSON, J. & BULLOCK, W.W. (1973) *In vitro* activation of mouse lymphocytes in serum-free medium: effect of T and B cell mitogens on proliferation and antibody synthesis. *Eur. J. Immunol.* 3, 299.

FAUCI, A. & PRATT, K. (1976) Activation of human B lymphocytes I. Direct plaque forming cell assay for the measurement of polyclonal activation and antigenic stimulation of human B lymphocytes. *J. exp. Med.* 114, 674.

GRONOWICZ, E. & COUTINHO, A. (1975) Functional analysis of B cell heterogeneity. *Transplant. Rev.* 24, 3.

HOW, M.J., BRIMACOMBE, J.S. & STACEY, M. (1964) The pneumococcal polysaccharide. *Advanc. Carbohyd. Res.* 19, 303.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J. exp. Med.* 136, 207.

KAMME, C., LUNDGREN, K. & MÅRDH, P. (1971) The aetiology of acute otitis media in children. *Scand. J. Infects. Dis.* 3, 217.

KENDALL, M.G. & STUART, A. (1973) *The advanced theory of statistics. Interference and relationship.* Vol. 2, p. 494. Griffin, London.

LODA, F.A., COLLIER, A.M., GLEZEN, W.P., STRANGERT, K., CLYDE, W.A. & DENNY, F.W. (1975) Occurrence of *Diplococcus pneumoniae* in the upper respiratory tract of children. *J. Pediat.* 87, 1087.

MISHELL, R.I. & DUTTON, R.W. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. exp. Med.* 136, 423.

MÖLLER, G. (1961) Demonstration of mouse isoantigens at the cellular level by the fluorescent antibody technique. *J. exp. Med.* 114, 415.

MÖLLER, G. & LANDWALL, P. (1977) The polyclonal B-cell-activating property of Protein A is not due to its interaction with the Fc part of immunoglobulin receptors. *Scand. J. Immunol.* 6, 357.

NAKASHIMA, I. & KATO, N. (1974) Non-specific stimulation of immunoglobulin synthesis in mice by capsular polysaccharide of *Klebsiella pneumoniae*, *Immunology*, 27, 179.

RINGDÉN, O. (1976) Activation of human lymphocyte subpopulations by rabbit anti-human β_2-microglobulin and by lipopolysaccharide. *Scand. J. Immunol.* 5, 891.

RINGDÉN, O. & RYNNEL-DAGÖÖ, B. (1978) Activation of human B and T lymphocytes by protein A of *Staphylococcus aureus*. *Eur. J. Immunol.* 8, 47.

RINGDÉN, O., RYNNEL-DAGÖÖ, B., WATERFIELD, E.M., MÖLLER, E. & MÖLLER, G. (1977) Polyclonal antibody secretion in human lymphocytes induced by killed staphylococcal bacteria and by lipopolysaccharide. *Scand. J. Immunol.* 6, 1159.

ROSÉN, A., GERGELY, P., JONDAL, M., KLEIN, G. & BRITTON, S. (1977) Polyclonal Ig production after Epstein–Barr virus infection of human lymphocytes *in vitro*. *Nature (Lond.)*, 267, 52.

RYNNEL-DAGÖÖ, B. (1976) The immunological function of the adenoid. The proportions of T and B cells. *Acta Oto-Laryngol.* 82, 196.

RYNNEL-DAGÖÖ, B., MÖLLER, E. & WATERFIELD, E. (1977)

Characterization of human adenoid cells using surface and functional markers for lymphocyte subpopulations. *Clin. exp. Immunol.* 27, 425.

RYNNEL-DAGÖÖ, B. & SCHIRATZKI, H. (1978) The effect of adenoidectomy. A controlled two year follow up. *Ann. otol. rhinol. laryngol.* 87, 272.

SLOYER, J.L., HOWIE, V.M., PLOUSSARD, J.H., AMMAN, A.J., AUSTRIAN, R. & JOHNSTON, R.B. (1974) Immune response to acute otitis media in children. *Infec. Immunity*, 9, 1028.

SMITH, D.H., PETER, G., INGRAM, D.L., HARDING, A.L. & ANDERSSON, P. (1973) Responses of children immunized with the capsular polysaccharide of *Haemophilus influenzae type b. Pediatrics*, 52, 637.

UNANUE, E.R., GREY, H.M., RABELLINO, E., CAMPBELL, P. & SCHMIDTKE, J. (1971) Immunoglobulins on the surface of lymphocytes II. The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulin. *J. exp. Med.* 133, 1188.

WOLLHEIM, F.A. & WILLIAMS, R.C. (1966) Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM) increase in infections mononucleosis. *N. Engl. J. Med.* 274, 61.

ZAMENHOF, S., LEIDY, G., FITZGERALD, P.L., ALEXANDER, H.E. & CHARGOFF, E. (1953) Polyribosephosphate the typo-specific substance of *Haemophilus influenzae, type b. J. Biol.* 203, 695.