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Polyclonal antibody formation of human lymphocytes to bacterial components

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SUMMARY

The capacity of various bacterial components to induce antibody formation in human lymphocyte cultures was studied in the present investigation. Antibody levels were determined by an enzyme-linked immunosorbent assay (ELISA). Lipopolysaccharide (LPS), bacterial cell walls (CW, isolated from *Bacillus subtilis* and *Staphylococcus aureus* Wood 46) and peptidoglycans (PG) appeared to stimulate IgM, IgG and IgA secretion, whereas lysozyme-solubilized PG and teichoic acids (TA) were ineffective. Also, umbilical cord blood lymphocytes produced IgM after stimulation with LPS, CW and PG. Coculture experiments with purified lympocytes and monocytes indicated that B-cell differentiation was dependent on both T cells and monocytes, and that T-cell derived factors could partially substitute for T cells.

INTRODUCTION

Polyclonal activation of human lymphocytes by many bacteria and other microbes is an intriguing phenomenon that is evidently also important *in vivo*. It has been suggested that, on the one hand, polyclonal activation may be useful as a common mechanism of resistance in infectious diseases; on the other, polyclonal activation may also have pathogenetic significance in chronic inflammatory diseases (Petit & Unanue, 1974; Clagett & Engel, 1978; Levitt & Bach, 1985).

The mitogenicity of whole bacteria is essentially due to their surface components. We, as others, have shown that LPS, protein A, PG and TA are polyclonal B-cell activators (PBA) in human cell cultures (Forsgren, Svedjelund & Wigzell, 1976; Kunori, Ringdén & Möller, 1978; Miller, Gartner & Kaplan, 1978; Dziarski, Dziarski & Levinson, 1980; Levitt, Duber-Stull & Lawton, 1981; Räsänen & Arvilommi, 1981; Levinson *et al.*, 1983; Ringdén, 1985). The activation of lymphocytes has been determined by measuring DNA, lymphokine or antibody synthesis. However, microbial stimulants do not need to activate the cells in all assay systems, e.g. B lymphocytes may proliferate but do not produce antibodies (McChesney *et al.*, 1985) or vice versa (Chen *et al.*, 1981). It is also less clear whether B-cell activation depends on T lymphocytes and/or adherent cells. Helper cell requirements of proliferative and antibody responses may also be different, even with the same stimulant.

In the present paper, we studied the capacity of CW, PG and TA from *B. subtilis* and *S. aureus* Wood 46 and *E. coli* LPS to induce polyclonal antibody formation in human lymphocytes. In addition, participation of other cells in B-cell activation was investigated.

Abbreviations: CW, bacterial cell walls; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; PBA, polyclonal B-cell activator(s); PG, peptidoglycan; SRBC, sheep red blood cells; TA, teichoic acid.

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MATERIALS AND METHODS

Stimulants

Lymphocytes were stimulated in cultures with LPS (extracted from *E. coli* 026:B6, Difco Laboratories, Detroit, MI) and bacterial cell wall preparations. CW, TA and PG were isolated from *B. subtilis* (NCTC 3610) and *S. aureus* Wood 46 (a gift from Prof. A. Forsgren, Malmö, Sweden) as previously described in detail (Räsänen & Arvilommi, 1981). The purity of the bacterial preparations was checked by thin-layer chromatography and an amino acid analyser. *B. subtilis* CW contained muramic acid, glucosamine, alanine, diaminopimelic acid and glutamic acid. Correspondingly, muramic acid, glucosamine, alanine, glutamic acid, glycine and lysine were detected in *S. aureus* CW hydrolysate. Alanine, ribitol, anhydroribitol and glucose were identified in the acid hydrolysate of *B. subtilis* TA, whereas in *S. aureus* TA there was glucosamine in place of glucose. PG contained the same amino acids and amino sugars as CW, as well as traces of glucose and anhydroribitol (*B. subtilis*) or anhydroribitol (*S. aureus* Wood 46) evidently originating from teichoic acids.

For some experiments PG were solubilized with lysozyme as described elsewhere (Räsänen & Arvilommi, 1981). Before

being used in cell cultures, bacterial preparations were heated at 70° for 30 min and plated for sterility checks.

Separation and identification of cells

Mononuclear cells were obtained by Ficoll–Isopaque centrifugation of heparinized or citrated adult venous or umbilical cord blood. These cells contained over 75% lymphocytes and fewer than 25% monocytes. Mononuclear cells were stimulated in cultures with various bacterial preparations or used for the isolation of lymphocytes and monocytes.

In order to obtain purified T and B lymphocytes and monocytes, mononuclear cells in 10% AB serum–RPMI-1640 were incubated for 90 min at 37° in tissue culture flasks. The flasks were then rocked vigorously to remove the non-adherent lymphocytes, which were used for the isolation of T and B cells. Monocytes were detached from the flasks by incubating with 0·02% ethylenediamine tetraacetate in phosphate-buffered saline for 2 hr and pipetting. Lymphocytes were rosetted twice with 2-aminoethylisothiouronium bromide-treated SRBC and rosette-forming T cells separated from non-rosetted B lymphocytes by Ficoll–Isopaque centrifugation. Contaminating SRBC among T cells were lysed by a hypotonic shock with distilled water. Thereafter, T and B lympocytes were incubated in 10% AB serum–RPMI supplemented with 0·2 g carbonyl iron for 1 hr. Carbonyl iron-fed monocytes were removed with a magnet.

In order to diminish the contamination of B lymphocytes by T cells, the B-cell populations were treated with OKT3 antibodies (Ortho Diagnostic Systems Inc., Raritan, NJ) and rabbit complement (Behringwerke AG, Marburg, W. Germany). Then, 5×10^6 cells/ml in RMPI-1640 containing OKT3 (final dilution 1:20) were incubated for 30 min on ice, washed twice and then incubated at 37° for 45 min with complement (final dilution 1:10). This procedure was repeated and the cells washed three times before cultures were set up.

The numbers of OKT3-reactive, surface membrane immunoglobulin-positive and non-specific esterase-positive cells (Yam, Li & Crosby, 1971) in lymphocyte and monocyte fractions were counted. The separation method yielded T-lymphocyte populations contaminated with $<0{\cdot}2\%$ monocytes and $<3\%$ B lymphocytes, and B lymphocyte populations contaminated with $<1\%$ monocytes and $<0{\cdot}5\%$ T cells. The average purity of monocytes was 94%. B lymphocytes or B cells supplemented with monocytes and/or T cells were stimulated with bacterial preparations.

Cell cultures

Unless otherwise stated, the cells were suspended at a concentration of 2×10^6 cells/ml in RPMI-1640 supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Ayrshire, U.K.). Triplicate cultures containing 2×10^5 cells/well in 0·2 ml of medium were set up in flat-bottomed microplates. In some experiments, when studying the effect of T-cell derived factors on antibody formation, 10^6 T lymphocytes (supplemented with 5×10^4 monocytes) and 10^6 B lymphocytes (also supplemented with 5×10^4 monocytes) were incubated in the separate compartments of double-chambered Marbrook-type culture vessels. The cells were incubated in the absence (controls) or presence of various concentrations of bacterial stimulants. In preliminary experiments culture periods ranging from 5 to 9 days were tested, and it was found that 9-day cultures did not yield essentially higher immunoglobulin levels than 7-day cultures. Subsequently, the cultures were terminated after 7 days of incubation. Culture supernatants were collected and their IgM, IgG and IgA levels determined by ELISA as previously described in detail (Poikonen *et al.*, 1982).

RESULTS

Bacterial PBA-induced formation of immunoglobulins by adult and cord blood mononuclear cells

LPS, CW and PG stimulated adult mononuclear cells to secrete immunoglobulins in the culture supernatants (Fig. 1). Lysozyme-solubilized PG and TA were not able to drive the cells to antibody formation. IgM was the predominant immunoglobulin class produced, even if the cells also synthesized IgG and IgA (Fig. 1). Relatively high stimulant concentrations were required to elicit an antibody response. Even at high stimulant concentrations, bacterial components were considerably weaker inducers of antibody formation than pokeweed mitogen (data not shown).

Table 1 shows the results on bacterial PBA-induced immunoglobulin production by neonate cells. These cells secreted only class IgM antibodies. In addition, the IgM levels of neonate cultures were somewhat lower than those of adult cell cultures.

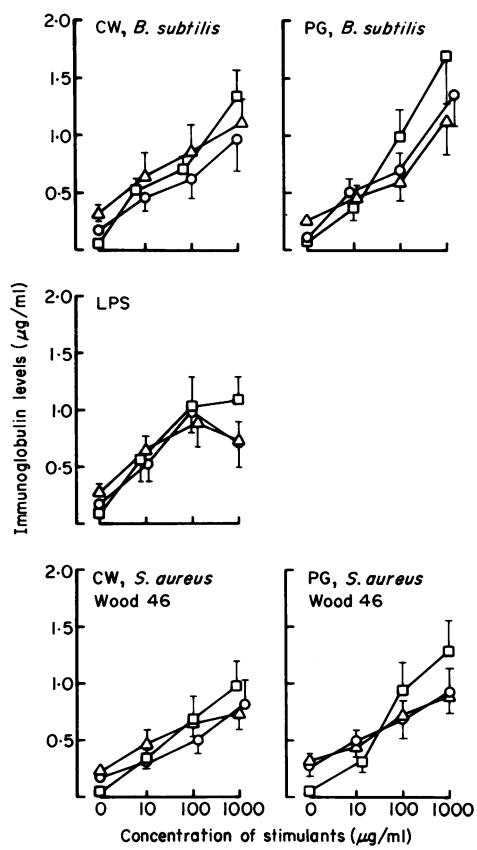

Figure 1. Antibody levels of mononuclear cell culture supernatants. 2×10^5 cells/well were incubated for 7 days and the IgM (□), IgG (○) and IgA (△) concentrations in the culture supernatants determined by ELISA. CW, bacterial cell walls; PG, peptidoglycans; LPS, lipopolysaccharide. The values are means ± SEM of nine individual experiments.

Table 1. Bacterial mitogen-induced production of IgM by umbilical cord blood cells

Stimulant	Concentration of stimulant (μg/ml) 0	10	100	1000
CW	40±20*	30±10	270±80	1260±270
PG	40±20	100±30	410±180	1070±230
LPS	40±20	110±20	180±50	540±160

Neonate mononuclear cells (2×10^5 cells/well) were stimulated with various concentrations of *B. subtilis* CW, PG and *E. coli* LPS and incubated for 7 days.

* Values given as ng/ml, mean±SEM of five experiments.

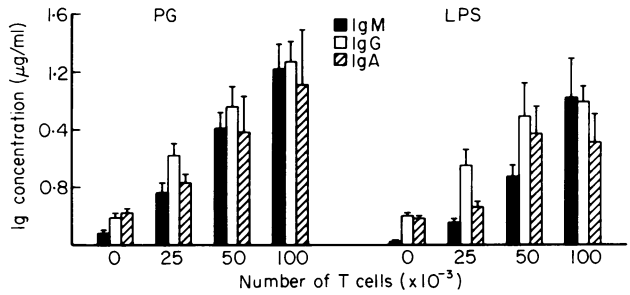

Figure 2. Effect of T cells on immunoglobulin production. 10^5 B lymphocytes + 10^4 monocytes per well were incubated in the absence or presence of various amounts of T lymphocytes. The cells were stimulated with *B. subtilis* PG (500 μg/ml) or *E. coli* LPS (500 μg/ml). The immunoglobulin levels of control cultures containing no mitogens were < 100 ng/ml (IgM) and < 300 ng/ml (IgG, IgA). The data represent the mean±SEM of ten experiments.

Monocytes enhance and suppress immunoglobulin production

Our B-cell preparations contained < 1% monocytes. When B cell–T cell mixtures were stimulated with PG or LPS in the absence of additional monocytes only low levels of immunoglobulins were produced. Table 2 presents the results with IgM; similar results were obtained with IgG and IgA (data not shown). The addition of relatively low amounts of monocytes to B cell–T cell mixtures enhanced immunoglobulin production: (10^4 monocytes among 10^5 B cells and 10^5 T cells appeared to be optimal, and there was a significant difference between the IgM levels of cultures not containing and containing this amount of monocytes ($P < 0.05$, Mann–Whitney *U*-test). When the cultures contained more than 2×10^4 monocytes, immunoglobulin levels started to diminish. However, considerably higher monocyte numbers, about 30% of the total amount of the cells, were required to suppress immunoglobulin production significantly.

B-cell immunoglobulin production is dependent on T cells

Purified B lymphocytes (T-cell contamination < 0·5%) supplemented with 10% monocytes were not driven to antibody synthesis by LPS or *B. subtilis* PG (Fig. 2). The addition of T lymphocytes restored immunoglobulin synthesis. In the presence of $2{\cdot}5 \times 10^4$ T cells, 10^5 B cells (supplemented with 10^4 monocytes) elaborated significant levels of class IgM and IgG antibodies ($P < 0.01$, Mann–Whitney *U*-test, Fig. 2). At higher T-cell additions, significant levels of IgA antibodies were also formed.

In order to investigate whether T-cell help could be mediated by soluble factors, T and B lymphocytes were incubated in the separate compartments of double-chambered culture vessels. In addition, monocytes were added to both compartments. Table 3 shows the results of these experiments. B cells stimulated with PG and supplemented with monocytes did not produce antibodies. Low but statistically significant immunoglobulin levels could be detected in cultures that contained T and B lymphocytes separated by a macromolecule-permeable filter and monocytes. Thus, T-cell derived factors could, at least in part, substitute for T cells.

DISCUSSION

We have here demonstrated that PG and LPS are T-cell and monocyte-dependent polyclonal B-lymphocyte stimulants. TA and soluble PG did not drive the cells to antibody formation. We have earlier shown that TA and lysozyme-solubilized PG were able to stimulate proliferation of lymphocytes (Räsänen & Arvilommi, 1981). Bacterial PBA that induce either antibody formation or proliferation but not both have been described earlier (Chen *et al.*, 1981; McChesney *et al.*, 1985). It thus seems that DNA and antibody synthesis are not correlated, and it may

Table 2. Effect of monocytes of IgM synthesis

Stimulant	Number of monocytes added ($\times 10^{-3}$) 0	5	10	20	40	60
PG	520±80*	1090±330	1430±510	1260±170	1290±280	1020±160
LPS	200±40	620±210	890±240	640±210	550±110	510±150

Mixtures of T cells and B cells ($10^5 + 10^5$ cells/well) were supplemented with various amounts of monocytes and stimulated with *B. subtilis* PG (500 μg/ml) or *E. coli* LPS (500 μg/ml). The IgM levels of control cultures not containing mitogens were < 100 ng/ml.

* Values given as ng/ml, mean±SEM of five experiments.

Table 3. Effect of T-cell derived factors on B-lymphocyte immunoglobulin production

	Concentration of immunoglobulins (ng/ml)					
	IgM		IgG		IgA	
Stimulant	B*	T/B†	B	T/B	B	T/B
None	50±10	70±10	130±30	190±60	180±20	170±50
PG	110±40	720±290	140±50	610±220	160±30	530±150

Values represent the mean ± SEM of six experiments.

* 10^6 B lymphocytes together with 5×10^4 monocytes were incubated in one compartment and 5×10^4 monocytes in the other compartment of a double-chambered culture vessel. *B. subtilis* PG was used at a concentration of 500 μg/ml and the total volume of the cultures was 1 ml.

† 10^6 B lymphocytes + 5×10^4 monocytes in one compartment and 10^6 T lymphocytes + 5×10^4 monocytes in the other.

be possible that different cell populations are responsible for these phenomena (Gronowicz & Coutinho, 1974).

Adherent phagocytic cells co-operate with lymphocytes, taking up, processing and presenting stimulants for them. Lymphocyte-derived factors may, in turn, activate phagocytes to carry out effector functions, e.g. to eliminate microbes. While a minimum amoung of phagocytic cells is required for lymphocyte activation, these cells, when present in excess, may inhibit lymphocyte function (Knapp & Baumgartner, 1978; Gmelig-Meyling & Waldmann, 1981). Inhibition may be mediated directly or by macrophage-derived prostaglandins or other suppressive factors. In the present study, it was found that monocytes, when present up to 10% of the total amount of the cells, enhanced antibody formation, whereas higher monocyte amounts began gradually to lower the response.

Only a few earlier reports exist on the co-operation between human T and B lymphocytes in LPS- or PG-induced antibody synthesis. According to Levitt *et al.* (1981), B cells (T-cell contamination <2%) differentiated to IgM-synthesizing plasma cells in the absence of additional T cells. However, additional T cells enhanced the IgM response and led to the generation of IgG- and IgA-containing plasma cells. Levinson *et al.* (1983) found that PG-stimulated differentiation of B cells (T-cell contamination <3%) to immunoglobulin-secreting cells was relatively T-cell independent. Here again, the responses were augmented by the addition of T cells. In our hands, LPS- and PG-induced antibody synthesis was absolutely T-cell dependent. Our B cells contained <0·5% T cells, which may explain the difference between our results and those of others. Even 2–3% contaminating T cells among B lymphocytes may be sufficient to restore a significant antibody response.

The present results indicate that T-cell derived factors could, at least in part, substitute for T cells in PG-induced antibody formation. We have earlier shown that no additional T cells were required for PG-induced proliferation of B lymphocytes (Räsänen & Arvilommi, 1981). Our results resemble those obtained with *S. aureus* Cowan I, and suggest that cells maturing into antibody-secreting cells and those becoming proliferating cells differ in their helper cell requirement. Proliferation is a T-independent process, whereas antibody production requires participation of helper T cells and factors (Saiki & Ralph, 1981; Falkoff, Zhu & Fauci, 1982; Wrigley & Choi, 1983).

In infectious diseases, polyclonal lymphocyte activation may be both useful and harmful to the host. The advantage of a polyclonal response is its speed; it may provide an early defence mechanism before initiation of specific responses. Recently, it has been suggested that one benefit of polyclonal antibody secretion might be the formation of antibodies protective against infection by a second pathogen (Levitt & Bach, 1985). Excessive formation of autoantibodies and lympokines mediating inflammatory reaction are possible *in vivo* drawbacks of polyclonal lymphocyte activation.

In summary, the present results show that certain bacterial surface components induce a polyclonal antibody response, which is dependent on T cells and monocytes. While there is a great deal of evidence of microbe-induced polyclonal lymphocyte activation both *in vitro* and *in vivo*, the possible protective and harmful effects of this phenomenon in diseases merit further study.

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