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Polyclonal Activation of Human Lymphocytes by Bacteria

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The proliferative response of human unseparated lymphocytes, T cells, and B cells to various bacterial strains was investigated. All the bacteria tested induced a proliferative response in unseparated lymphocytes and B cells. T cells were stimulated only after reconstitution with monocytes. Stimulation of umbilical cord blood lymphocytes suggests that the response is polyclonal. We interpret these and previous data as an indication of a common mechanism of resistance against infectious diseases.

In the course of our studies on lymphokine synthesis by human lymphocytes, we tested various staphylococci as potential B-cell activators. Quite unexpectedly, all the staphylococci, regardless of their protein A content, triggered the cells to elaborate lymphokines. Protein A and protein A-containing staphylococci had previously been reported to be human B-cell mitogens (6).

Our observation that the lymphocyte-activating capacity of staphylococci does not depend on protein A led to a more systematic study with various bacteria. All the bacterial strains tested stimulated B cells polyclonally to synthesize lymphokines (14). In the continuation of these experiments on bacterium-induced lymphokine production, we investigated bacterium-induced transformation, another manifestation of lymphocyte activation. The present report deals with the proliferative responses of unseparated lymphocytes, T cells, and B cells to various bacteria.

MATERIALS AND METHODS

Separation and identification of cells. Mononuclear cells were obtained by Ficoll-Isopaque centrifugation of heparinized or citrated peripheral or umbilical cord blood (4). The cord blood was obtained immediately after birth. These cells contained on the average 89% lymphocytes and 11% monocytes. In order to obtain T and B cells, the cell suspension was rosetted with sheep erythrocytes and centrifuged on Ficoll-Isopaque as described earlier (14). B lymphocytes were then incubated with 0.2 g of carbonyl iron in Hanks balanced salt solution supplemented with 10% fetal bovine serum (Flow Laboratories, Irvine, Scotland) at 37°C for 45 min, after which the phagocytic cells were removed with a magnet. T cells were not treated with carbonyl iron since their monocyte contamination was very small.

Monocytes were purified as follows. Mononuclear cells (40×10^6) were incubated for 30 min in a tissue culture flask (growth area, 25 cm^2; Falcon Plastics, Oxnard, Calif.) in 10 ml of Hanks balanced salt solution supplemented with 10% fetal bovine serum. In order to remove the nonadherent cells, the flasks were shaken vigorously with Hanks balanced salt solution, after which phosphate-buffered saline containing 0.2% ethylenediaminetetraacetate was added to the flasks. After a 2-h incubation at room temperature, the adherent cells were removed by pipetting vigorously with a Pasteur pipette. The viability of the recovered cells exceeded 85%.

The purity of the cell populations was studied as described earlier (14), using sheep erythrocyte rosette formation to demonstrate T cells, staining of surface membrane immunoglobulin to demonstrate B cells, and staining of nonspecific esterase to demonstrate monocytes. The average contaminations of T and B cells by monocytes were <0.1% and 0.8%, respectively. The purity of monocytes was 95.1%. T lymphocytes were contaminated with an average of 2.4% B cells, and B cells were contaminated with 2.5% T cells.

Cell cultures. The cells were suspended at a concentration of 10^6 cells per ml in minimal essential medium for suspension cultures (Flow Laboratories) supplemented with 10% autologous plasma. Triplicate cultures containing 10^5 cells per well were set up in U-bottomed microplates (Sterilin Ltd., Middlesex, England). The cells were cultured in the absence (control cultures) or presence of various concentrations of the following bacteria: *Staphylococcus aureus* strain Wood 46 (with a very low content of protein A, a gift from A. Forsgren, Malmö, Sweden), *S. aureus* strain Cowan I NCTC 8530 (with a high content of protein A), *Mycobacterium bovis* BCG (Statens Seruminstitut, Copenhagen, Denmark), *Escherichia coli* B6:O26 (originally obtained from the Department of Medical Microbiology, University of Turku, Finland), *Staphylococcus albus*, *Neisseria catarrhalis*, group A beta-hemolytic *Streptococcus*, alpha-hemolytic *Streptococcus*, and a diphtheroid (these bacteria were isolated from clinical samples in our laboratory). The bacteria had been killed by incubation in 0.5% formaldehyde for 3 h at room temperature and heating for 3 min at 80°C.

The cell cultures were incubated at 37°C in a 5% CO_2 atmosphere for 4 days unless otherwise stated. Sixteen hours before harvesting, 0.125 μCi of 5-[^{125}I]iodo-2'-deoxyuridine (Amersham Radiochemical Centre, Amersham, England) containing 1 μM fluoro-

deoxyuridine was added per well. The uptake of the isotope was measured with a gamma counter. The stimulation index was defined as follows: transformation (as counts per minute) in the presence of bacteria/transformation in the absence of bacteria.

Mitomycin treatment of cells. In order to ascertain that the proliferating cells in mixtures of T lymphocytes and monocytes were T cells and not monocytes, T cells or monocytes were treated with mitomycin and cultured together with untreated cells. The cells were incubated with 50 μg of mitomycin (Sigma Chemical Co., St. Louis, Mo.) per ml, after which they were washed five times and combined with untreated cells. Treatment of the cells with 50 μg of mitomycin per ml caused >90% inhibition of proliferation as assessed by the incorporation of iododeoxyuridine.

RESULTS

Bacterium-induced proliferation of adult cells. In the preliminary experiments, various culture periods were tested. As can be seen in Fig. 1, with *S. aureus* Cowan I and *E. coli*, peak proliferation occurred on day 4 of incubation. The optimal response was obtained on day 4 also with all other bacteria (data not shown), and hence a 4-day culture period was used in subsequent experiments.

All the bacterial strains tested induced a proliferative response in mononuclear cells (Table 1) and B lymphocytes (Table 2) but not in purified T lymphocytes (data not shown). The optimal bacterium/lymphocyte ratio varied between 0.2 and 20, being 2 in most cases. The lack of stimulation at high doses of bacteria may be due to a toxic effect. A more likely explanation is the mechanical effect of abundant bacteria hindering cell-cell contacts which are important in immunological activation. Except for BCG, there were no great differences in the proliferative responses between mononuclear and B cells (the smaller stimulation indices of B cells are due to higher control values; the controls of T cells and mononuclear cells were about the same).

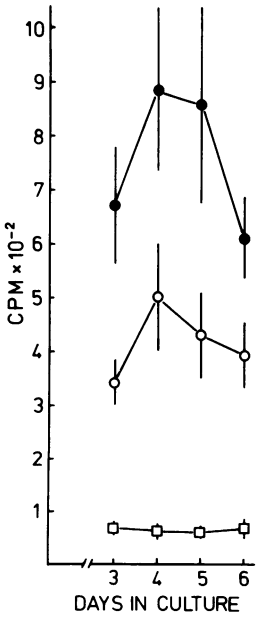

FIG. 1. *Kinetics of the proliferative response of mononuclear cells to Escherichia coli* (○) *and S. aureus Cowan I* (●). (□) *Control cultures.*

TABLE 1. *Bacterium-induced transformation in mononuclear cells*[a]

Bacterial strain	Bacterium/lymphocyte ratio				
	0.02	0.2	2	20	200
Staphylococcus aureus Cowan I		11.6[b]	12.0	11.0	4.2
S. aureus Wood 46		6.3	6.3	5.3	0.7
S. albus		10.2	10.5	8.7	1.2
Escherichia coli B6:O26	4.5	4.9	4.9	5.1	
Neisseria catarrhalis	2.8	3.9	3.9	0.3	
Diphtheroid	3.1	5.7	7.9	0.5	
Alpha-hemolytic *Streptococcus*	4.1	5.3	5.6	0.5	
Beta-hemolytic *Streptococcus*, group A	5.6	7.8	7.5	9.4	
Mycobacterium bovis BCG	15.4	28.1	19.6	10.2	

[a] Mononuclear cells containing lymphocytes and monocytes were stimulated with bacteria, and cellular proliferation was assessed by the incorporation of iododeoxyuridine.
[b] Mean stimulation index of four to nine experiments.

TABLE 2. *Bacterium-induced transformation in B lymphocytes*[a]

Bacterial strain	Bacterium/lymphocyte ratio				
	0.02	0.2	2	20	200
Staphylococcus aureus Cowan I		3.5[b]	4.5	6.9	0.5
S. aureus Wood 46		2.3	4.0	4.1	0.2
S. albus		2.8	4.1	5.8	0.4
Escherichia coli B6:O26	1.9	2.3	2.5	4.2	
Neisseria catarrhalis	2.3	4.1	2.5	0.5	
Diphtheroid	1.8	4.2	3.7	0.4	
Beta-hemolytic *Streptococcus*, group A	1.8	2.8	3.9	3.7	
Mycobacterium bovis BCG	2.0	3.1	5.0	4.6	

[a] B lymphocytes, contaminated with an average of 0.8% monocytes and 2.5% T cells, were obtained by sheep erythrocyte rosette formation and centrifugation on Ficoll-Isopaque.
[b] Mean stimulation index of four to eight experiments.

Reconstitution of T-cell proliferation by monocytes. Our T-cell populations contained less than 0.1% monocytes, which might have caused the lack of their response. The effect of additional monocytes on T-cell transformation was therefore investigated. Table 3 shows the results of these experiments. As anticipated,

TABLE 3. *Proliferation of bacterium-stimulated T cells in the presence of monocytes*[a]

Bacterial strain	Bacterium/ lymphocyte ratio	T cells	Monocytes	T cells + indicated % monocytes			T cells + 10% mitomycin-treated monocytes	Mitomycin-treated T cells + 10% monocytes
				2.5%	5%	10%		
Staphylococcus aureus Cowan I	2	0.9[b]	1.0	9.8	13.4	13.7	12.0	1.3
Escherichia coli B6:O26	20	1.0	1.0	3.5	3.2	4.2		
Beta-hemolytic *Streptococcus*	2	1.4	1.1	12.6	13.6	15.8	14.3	1.2
Mycobacterium bovis BCG	0.2	1.2	1.2	8.0	12.4	15.7		

[a] Purified T cells and monocytes were cultured either alone or in various combinations in the presence of optimal doses of bacteria. The proliferation of T cells or monocytes in mixtures of T lymphocytes + monocyte cells was blocked by treating the other cell population with 50 μg of mitomycin per ml before co-culture.
[b] Mean stimulation index of four experiments.

streptococci and BCG stimulated T lymphocytes to proliferate in the presence of additional monocytes. Quite unexpectedly, the cells stimulated with staphylococci and *E. coli* transformed as well. Monocytes alone did not proliferate. The possibility that monocytes in the presence of T cells could have incorporated radioactive label was ruled out in a control experiment with cells pulse treated with mitomycin. Treated T cells cultured with intact monocytes did not proliferate, whereas T cells with treated monocytes responded like the untreated combination (Table 3).

A possibility exists that staphylococcus- and *E. coli*-induced proliferation in T-cell populations in the presence of monocytes was due to contaminating B cells. However, the finding that B cells do not seem to require the presence of additional monocytes in order to proliferate speaks against this possibility (Table 2). Further confirmation was acquired by studying the effect of B cells on the proliferative response of T-cell populations. It was thought that if the observed proliferation in T-cell fractions was due to B cells, higher amounts of B cells in T-cell populations would cause a higher response. When T cells contained 2.5 to 10% B cells and 1.25% monocytes, no correlation between the amount of B cells and the magnitude of the response was observed (Table 4).

Bacterium-induced proliferation in umbilical cord blood cells. In order to investigate whether bacteria are able to act polyclonally, cord blood cells were stimulated with bacteria. The results of these experiments are shown in Table 5. Again, all the strains tested activated mononuclear, T, and B cells to transform, which supports the concept that bacteria contain mitogenic components. Most bacteria gave lower stimulation indices with cord blood than with adult cells (Table 1). Evidently, the antigenic components of bacteria have partly induced the proliferation of adult cells.

TABLE 4. *B cell independence of T cell proliferation*[a]

Bacterial strain	Bacterium/ lymphocyte ratio	T cells + 1.25% monocytes + indicated % B cells		
		2.5%	5%	10%
Staphylococcus aureus Cowan I	20	4.6[b]	4.8	4.7
Escherichia coli B6: O26	20	4.1	4.0	3.8

[a] T and B cells were isolated by sheep erythrocyte rosette formation and centrifugation on Ficoll-Isopaque. The B cell contamination of T cell populations was checked by staining of surface membrane immunoglobulin. The B-cell content of T cells supplemented with 1.25% monocytes was then adjusted to 2.5, 5, or 10% before culturing with optimal doses of bacteria.
[b] Mean stimulation index of four experiments.

DISCUSSION

It is demonstrated here that all the bacterial strains tested induced a proliferative response in human T and B lymphocytes. T cells responded only in the presence of additional monocytes, whereas no monocyte reconstitution was required for B cells. Experiments with cord blood cells showed that the activation, or part of it, was polyclonal. Bacteria were found to act as both T- and B-cell mitogens. We have shown previously that bacteria are strong stimulators of lymphokine synthesis (14). In contrast, bacterium-induced transformation seems to be rather weak.

Our finding that T lymphocytes cultured together with monocytes proliferated after stimulation with staphylococci and *E. coli* was unexpected. These bacteria or their components have

TABLE 5. *Proliferation of umbilical cord blood cells by bacteria*

Bacterial strain	Bacterium/ lymphocyte ratio	Mononuclear cells	T cells	T cells + 10% monocytes	B cells
Staphylococcus aureus Cowan I	20	7.6[b]	1.2	8.4	7.2
S. aureus Wood 46	20	4.7			
Escherichia coli B6:O26	20	6.2	1.3	6.8	5.5
Diphtheroid	2	5.3			
Beta-hemolytic streptococcus, group A	20	4.3			
Mycobacterium bovis BCG	2	3.5			

[a] Optimal concentrations of bacteria.
[b] Mean stimulation index of 5 to 10 experiments.

been reported to be B-cell stimulants (1, 6), although recently protein A has been found to activate T cells as well (15, 17, 19). The characterization of the responding cells is hindered by difficulties in obtaining pure cell populations. Our T-cell populations contained an average of 2.4% B cells and <0.1% monocytes. Theoretically, the observed response in T-cell populations may reflect T-cell function or alternatively may be due to contaminating B cells. However, when the B-cell populations were stimulated with bacteria, there was no correlation between the magnitude of the response and the amount of contaminating monocytes. Furthermore, in the presence of <0.1% monocytes, B lymphocytes still responded well. These results suggest that B cells do not require the presence of cooperative monocytes in order to proliferate, or the amount of monocytes required is very small. Therefore, it seems improbable that the proliferative response in T-cell populations requiring the presence of additional monocytes would have been due to contaminating B cells.

Data presented here and earlier (14) demonstrate that lymphocytes are activated by the staphylococci, regardless of their protein A content. A similar experience has been reported recently by Ringden et al. (16).

At present, a wide variety of B cell-activating microbes, e.g., *E. coli* (1), *Klebsiella* (12), *Serratia* (11), *Neisseria* (18), *Brucella* (20), staphylococci (6), *Listeria* (13), *Corynebacterium* (21), *Nocardia* (3), *Actinomyces* (5), *Mycoplasma* (2) and *Herpesvirus* (10), are known, and their number is constantly increasing. Bacterium-induced lymphocyte activation manifests itself as lymphokine synthesis, transformation, and antibody formation in vitro. It is, of course, possible that this is an in vitro artifact with no relevance in vivo. However, in animal experiments bacterial products have induced autoantibody production (7, 9) and hyperplasia in the B-cell zones of the spleen (13). Furthermore, there are several infectious diseases such as leishmaniasis, syphilis, toxoplasmosis, and trypanosomiasis in which a polyclonal antibody response has been detected in humans (8). Thus, it is reasonable to think that microbe-induced, polyclonal lymphocyte activation has in vivo relevance.

The significance of a polyclonal lymphocyte response in microbial infections is unclear. It has been suggested that the nonspecific activation represents an early mechanism of protection against microbes (13). As we proposed earlier (14), lymphokines would fit this picture well as mediators of inflammation and as factors of amplification of the host response. Yet, polyclonal proliferation of lymphocytes together with subsequent antibody production would seem a harmful overreaction if autoantibodies are formed. In infectious diseases, depending on the properties of the microbes and the immune reactivity of the host, only specific antibodies or a variety of antibodies including those to self-antigens are detected. A specific antibody response often dominates even if the stimulant is capable of polyclonal activation. The mechanisms by which the cells are able to escape from polyclonal antibody production, if once nonspecifically activated, are unclear.

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