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Protein A-positive staphylococci serve as a selective B cell mitogen for lymphocytes from primary immunodeficiency patients

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SUMMARY

Staphylococcus aureus protein A-positive bacteria have recently been proposed as selective B lymphocyte mitogens. We have studied the lymphocyte response to such mitogens in bacteria in normal subjects and in patients with primary immunodeficiencies. Patients with primary T cell defects show a normal response to protein A-positive bacteria and impaired responses to PHA and Con A. In contrast, patients with Bruton agammaglobulinaemia respond normally to these T cell mitogens but not to the bacteria. Thus, protein A-positive bacteria fulfil the criteria for being a T cell-independent B cell mitogen for human peripheral blood cells.

INTRODUCTION

Primary immunodeficiency disorders constitute a group of diseases of a complex nature where the underlying defect causing the actual disease is frequently difficult to define exactly. Morphological classification of peripheral blood leucocytes to allow the enumeration of subgroups of lymphocytes has provided an important tool for the analysis and diagnosis of these patients. Likewise, functional parameters such as the ability to stimulate the lymphocytes *in vitro* into increased DNA synthesis using supposed T and/or B lymphocyte selective mitogens have already been shown to be valuable. A particular problem in the human system has been the difficulty of obtaining a mitogen that is able to specifically activate peripheral blood B lymphocytes into division without requiring the simultaneous presence of T cells. A mitogen showing unusual potential in this respect is protein A-positive *Staphylococcus aureus* bacteria (Forsgren, Svedjelund & Wigzell, 1976a; Ringdén *et al.*, 1977), where purified B cells from human blood respond in an enhanced manner to this mitogen whilst purified T lymphocytes fail to become activated. An earlier report, however, suggested that this mitogen may activate T cells (Kronvall & Williams, 1969), but no evidence for this was found in the tests performed subsequently. As certain primary immunodeficiency diseases seem to represent comparatively 'clean' deletions of a given subgroup of lymphocytes, we considered it of importance to analyse the ability of protein A-positive bacteria to activate *in vitro* lymphocytes from patients with various immunodeficiency diseases. Comparisons of morphological markers, as well as activation by T cell mitogens in the same cellular populations were included. The results obtained prove that protein A-positive staphylococci do indeed behave as a completely T-independent, B cell stimulatory agent with the ability to provide otherwise unobtainable information in certain clinical situations.

MATERIALS AND METHODS

Lymphocytes. These were isolated from heparinized peripheral blood on a Ficoll–Sodium Metrizoate gradient (Lymphoprep, Nyegaard, Oslo) and resuspended in TC 199 medium (Difco Laboratories, Detroit), with 100 iu/ml penicillin. Viability of cells was determined using uptake of trypan blue and was always more than 95%.

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Stimulation test. 50 or 100 μl of a Cowan I formaldehyde-fixed *Staphylococcus aureus* suspension at 10^8 bacteria per ml were added to 10^6 lymphocytes in 1·0 ml as described previously (Forsgren *et al.*, 1976). Other cultures were performed without mitogens, with phytohaemagglutinin (PHA, Wellcome Research Laboratories) in a concentration of 1·0 μg/m or with concanavalin A (Con A, Industrie Biologique Francaise) in a concentration of 4·0 μg/ml. It should be noted that stimulation was carried out in the absence of serum.

The cultures were incubated for 3 days at 37°C in a 5% CO_2 milieu. 1·0 μCi of tritiated methylthymidine (0·2 Ci/μg, sp. act.), was added to each culture 22 hr prior to termination. The cells, after precipitation with 5% TCA, were washed and resuspended in scintillation fluid (Unisolve Koch Light Laboratories). The uptake of ^{3}H-thymidine was measured in a Nuclear (Chicago) liquid scintillation counter.

Surface markers. E-rosettes were prepared as described by Jondal, Holm & Wigzell (1972). Surface membrane Ig (SmIg) was determined using a fluorescent antibody technique (Aiuti, Cerottini & Coombs, 1974).

Patients. We studied ten normal donors and six patients with primary immunodeficiencies: two with Bruton type agammaglobulinaemia and four with selective T cell defects.

(1) A.G., male, 20 years old. An uncle died at 5 years of age after several infections. From the first year of life the patient developed recurrent gastrointestinal or bronchopulmonary bacterial infections. In 1967 we made a diagnosis of X-linked agammaglobulinaemia based on the classical clinical and immunological data. Since 1971 we have failed to find B lymphocytes in his peripheral blood (SmIg). Cell-mediated immunity was always intact with normal numbers and normal percentage of sheep erythrocyte rosette-forming cells (SRFC) in the blood, and he had positive delayed hypersensitivity reactions to candida, SK-SD, PPD and DNCB. The patient has been receiving gammaglobulin for 10 years.

(2) M.M., male, 7 years old. Family history of infant death. From 6 months of age he showed an increased susceptibility to infections. Decreased values of serum Ig and absence of secretory Ig, and the absence of circulating B lymphocytes became apparent. Cellular immunity was normal.

(3) P.G., male, 6 years old who developed fungal and viral infections from birth showed a deficiency of non-specific immune reactions (phagocytosis, chemotaxis). In the last 2 years, he has shown highly decreased numbers of T cells (SRFC) (from 5–15%), and negative skin tests, with no blast transformation to PHA and Con A. Last year he developed chronic mucocutaneous candidiasis and herpes infections. Humoral immunity remained close to normal as determined by serum Ig levels and B cells.

(4) C.S., female, 2 years old. Great grandmothers were half-sisters. At 20 months she developed acute bronchitis and anaemia and was referred to us 2 months later. The patient had a positive Coombs autoimmune haemolytic anaemia and cytomegalovirus infection. T cells, studied by anti-T serum and SRFC, were absent. She showed no response to PHA and Con A, and delayed skin tests with candida, SK-SD and PPD were all negative. SmIg lymphocytes and serum Ig levels were normal. The patient's RBC and granulocytes were NP enzyme negative and no uric acid was detectable in the serum. She died after 3 months with interstitial pneumonia. Post-mortem examination revealed thymus aplasia and depletion of cells in T-dependent areas of the lymph nodes (Carapella-De Luca *et al.*, 1978).

(5) B.A., male, aged 2 years, had congenital cytomegalovirus infection. All parameters of cellular immunity were decreased during the observations period of 3 months. Humoral immunity was normal.

(6) S.A., male, 6 years old, had severe mucocutaneous candidiasis and interstitial pneumonia when he came to us. He showed no anti-candida cell-mediated immunity (no blast transformation and no leucocyte migration inhibition to candida antigen). Peripheral T cells were decreased in numbers and displayed no increased DNA synthesis using PHA or Con A. Ig levels and SmIg lymphocytes were normal, but antibody responses towards typhoid and DTP vaccines were impaired. Thymic extract therapy was tried but he died with severe bronchopneumonia after 3 months. Post-mortem revealed thymic aplasia and depletion of paracortical areas of the lymph nodes.

All patients with T cell defects were studied with respect to the present results before any attempted immune therapy.

RESULTS

We established first the ability of protein A-positive bacteria to stimulate normal peripheral blood lymphocytes into increased DNA synthesis in relation to surface markers (SRBC-rosettes and surface Ig-positive cells), and with the T cell mitogens, PHA or Con A. The results, as shown in Table 1, demonstrate that every individual donor showed good stimulation indices using the bacteria-associated mitogen with no individual population having a lower index than 19 and 31, respectively, using the two different doses of bacteria.

We then analysed the ability of peripheral blood lymphocytes from patients with immunodeficiencies to respond *in vitro* against the protein A-positive bacteria (Table 2). Bruton-type agammaglobulinaemia is generally accepted to represent one of the best defined immunodeficiency disorders affecting B lymphocytes in a highly selective manner. We were able to show that lymphocytes from such patients displayed extremely low reactivity when confronted with the bacteria, whereas their reactivity against PHA was normal. The last group of patients consisted of four patients with T cell deficiency, as established by the

TABLE 1. ^{3}H-thymidine uptake (ct/min and stimulatory index) by human peripheral blood lymphocytes cultured with SpA (50λ and 100λ), PHA and Con A

Name	Age (years)	Sex	SRFC (%)	SmIgM	Ct/min*	SpA 50 λ Ct/min†	SpA 50 λ SI	SpA 100 λ Ct/min†	SpA 100 λ SI	PHA/Con A Ct/min†	PHA/Con A SI
SCM	26	F	60	n.d.	585	12520	21	31740	59	43800	79
CT	35	M	39	4	574	17120	29	22140	38	96935	168‡
VW	29	F	64	10	680	41200	60	63000	92	12100	17‡
UM	25	M	64	2	1040	20000	19	49500	47	15200	14‡
CM	40	F	63	n.d.	184	13435	73	34717	188	81330	442
LF	30	F	50	2	185	25690	138	73350	407	99650	588
SA	25	M	70	8	740	21750	29	49040	66	76700	103
FM	28	M	60	5	495	26660	58	38610	78	10850	21
DCM	40	F	70	7	328	20210	61	65640	200	52000	158
MA	50	M	70	4	469	10000	21	14920	31	44900	95
Mean values ± s.e.	—	—	—	—	—	—	51 ± 12		121 ± 37		212 ± 81

SpA = *Staphylococcus aureus* protein A$^+$ (Cowan I strain).
PHA = Phytohaemagglutinin.
Con A = Concanavalin A.
SRFC = Percentage of sheep rosette-forming cells.
SmIg = Percentage cells with surface membrane-bound immunoglobulin M.
* Control samples.
† Stimulated samples.
‡ Concanavalin response.

TABLE 2. ^{3}H-thymidine uptake by human peripheral blood lymphocytes from patients with B or T cell deficiences using SpA bacteria or PHA as mitogens

Bruton-type agammaglobulinaemia Name	Age (years)	Sex	SRFC (%)	SmIgM (%)	SpA 50 λ Ct/min*	SpA 50 λ Ct/min†	SpA 50 λ SI	SpA 100 λ Ct/min†	SpA 100 λ SI	PHA λ Ct/min†	PHA λ SI
AG	20	M	66	0	250	1108	4	1194	4	30992	123
MM	7	M	50	0	1250	6623	5	18958	15	73300	58
Mean values							4·5		9·5		90·5
T cell defects											
PG	6	M	2	22	866	18169	20	64600	74	640	0
CS	3	F	0	8	306	10380	33	45300	148	568	1
BA	2	M	12	12	211	13680	64	21270	100	728	3
SA	6	M	12	13	296	10000	33	10230	34	4148	14
Mean values ± s.e.							38 ± 9		89 ± 24		4·5 ± 3

* Control samples.
† Stimulated samples.

low percentage of SRBC-rosetting lymphocytes and their poor ability to respond to PHA stimulation. The lymphocytes from these patients all displayed normal mitogenic reactivity when confronted with the protein A-positive bacteria.

DISCUSSION

The purpose of the present study was two-fold. We wished to investigate whether protein A-positive bacteria could be used as a truly T cell-independent mitogen for B lymphocytes using peripheral blood lymphocytes from humans as responder cells. Previous investigations have suggested that this mitogen may carry the necessary prerequisites for this, as judged by the failure to activate T cells whilst inducing B lymphocytes into increased DNA synthesis (Forsgren *et al.*, 1976a; Ringdén *et al.*, 1977; Bloom *et al.*, 1976), although conflicting results have been reported using a somewhat different technique (Sakane & Green, 1978). This mitogen would seem to exert a similar B cell activating power in the mouse (Möller & Landwall, 1977). Mitogens used previously for the activation of human B lymphocytes have either failed to activate peripheral blood cells to any significant degree or T lymphocytes would appear to be necessary for optimal stimulation of the B cells (Janossy & Greaves, 1971; Ringdén & Möller, 1975). We felt it to be important in the clinical area to have an accessible T cell-independent B cell mitogen, but were concerned that the above mentioned studies in the human system may still have been under regulation by a minority of T cells contaminating the 'pure' B cells as obtained from healthy donors. To circumvent this problem we studied the activity of the protein A-bacteria mitogen on lymphocytes from immunodeficiency patients selected to contain 'pure' T or B cell deficiencies as judged by a variety of earlier investigations (Good, 1975). By using the peripheral blood lymphocytes from such patients we hoped to discover whether protein A-bacteria mitogen would stimulate T cells at all and/or whether the activation of B lymphocytes could occur in the physical and functional absence of T cells. If so, a second purpose of the investigation could be achieved. Namely, to prove that the protein A-bacteria mitogen could serve as a useful probe in the analysis of immunodeficiency patients when studying the underlying cause. It is already clear that mere morphological markers on peripheral lymphocytes may leave the investigator in doubt as to the functional potential of the cells under investigation, as exemplified by the functional inertia of malignant but morphologically basically intact lymphoid cells. From the present results both purposes would seem to be at least in part fulfilled. Thus, clear-cut B cell deficiencies (Bruton-type agammaglobulinaemias) failed to respond in any significant manner to protein A-bacteria, whilst clear-cut T cell deficiency patients provided lymphocytes with normal reactivity towards the mitogen used.

The present results further support our earlier findings that protein A-positive bacteria can serve as a highly useful and select human B cell mitogen (Forsgren *et al.*, 1976b). Recent reports (Sakane & Green, 1978), using soluble protein A together with serum in *in vitro* stimulation tests, have shown that under such conditions both human T and B cells are stimulated. As protein A are known to interact with IgG molecules leading to complement activation, aggregate formation, etc., we find these later findings difficult to analyse. We would emphasize again that the present test has to be carried out under serum-free conditions where it then behaves as an autonomous human B cell mitogenic assay functioning in the absence of T cells.

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