PURIFICATION AND PROPERTIES OF AN EXTRACELLULAR BLASTOGEN PRODUCED BY GROUP A STREPTOCOCCI*

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The growth of beta hemolytic streptococci is accompanied by the elaboration of extracellular products which constitute a complex mixture of proteins, many with specific biologic, enzymatic, and antigenic properties. The ability to induce lymphocyte blastogenic transformation has been reported in several fractions and present evidence indicates that there are multiple blastogenic activities in these preparations (1, 2). Blastogenic activity is produced by a variety of strains of group A streptococci (3) in amounts generally larger than those produced by group C, D, and G strains.

Streptococcal blastogenic substances from group A strains which have been partially purified include a glycopeptide which is extractable from cells (4, 5), at least two fractions described by Abe and Alouf (6), and a heterogeneous group of substances from a column separation by Seravalli and Taranta (1). Streptococcal pyrogenic exotoxins A, B, and C are the only entities with lymphocyte stimulating activity that have thus far been obtained in a homogeneous state (7–9).

The blastogenic response to the group C products present in the commercial preparation streptokinase/streptodornase varies among individuals and has been shown to be associated in responders with histocompatibility antigen specificity HLA B_5 (10). As an initial step toward determining whether the response to a blastogen of group A streptococci is also genetically linked, the present study was undertaken to obtain a blastogen in pure form. This report describes the purification of a blastogen of group A streptococci (blastogen A) and some of its properties.

Materials and Methods

Bacterial Strains. Strain C203S of group A streptococci was used as the principal source of extracellular products. This strain was originally obtained from Dr. Alan Bernheimer, New York University, New York. S43 is a prototype M6 strain originally obtained from Dr. Rebecca Lancefield, The Rockefeller University, New York. The other strains used in this study were from the collection of this laboratory.

Streptococcal Pyrogenic Exotoxins. Purified streptococcal pyrogenic exotoxins A, B, and C and rabbit antisera to those proteins were generously provided by Dr. D. Watson and Dr. P. Schlievert, Department of Microbiology, University of Minnesota, Minneapolis, Minn.

Growth of Organisms and Preparation of Extracellular Products. Streptococcal strains were grown overnight to stationary phase in 5- to 10-liter batches of Todd-Hewitt dialysate medium. The cells were harvested by centrifugation and the supernate adjusted to 85% saturation in $(NH_4)_2SO_4$, and allowed to precipitate overnight at 4°C. The precipitate was collected by

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centrifugation, dissolved in 0.05 vol of 0.001 M Tris HCl, 0.001 M $CaCl_2$, 0.001 M $MgCl_2$ (pH 7.0) and dialyzed overnight against the same buffer.

Ion Exchange Chromatography. DEAE-cellulose (Whatman DE-23; H. Reeve Angel & Co., Inc., Clifton, N. J.) was equilibrated with the starting buffer (0.001 M Tris HCl [pH 7.0], 0.001 M $CaCl_2$, 0.001 M $MgCl_2$) and packed in a 45 × 2.5-cm column. The sample of crude extracellular products was applied and the column was washed with 5 vol of starting buffer and eluted with a linear gradient of NaCl to 0.5 M.

CM-cellulose (Whatman CM-52; H. Reeve Angel & Co., Inc.) was equilibrated with 0.01 M sodium acetate (pH 5.0) and packed in a 45 × 2.5-cm column. The pooled blastogenic activity from the DEAE column fractions, previously dialyzed against the starting buffer, was applied and the column eluted with a gradient of NaCl from 0.1 to 0.35 M.

Nuclease Assay. Nuclease activity was assayed by the acid-soluble technique as previously described (11).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was carried out essentially as described by Davis (12) with protein bands visualized with Coomassie Blue G250 (13) sodium dodecyl sulfate (SDS)[1] polyacrylamide gel electrophoresis was done by the method of Weber and Osborne (14).

Lymphocyte Blastogenic Transformation Assay. Blastogenic activity was measured by [^{3}H]thymidine incorporation by a gradient-separated monocyte population from human blood.

Heparinized whole blood, diluted with an equal volume of Eagles' minimal essential medium containing 100 U of penicillin and streptomycin and 2 mmol of L-glutamine/ml (MEM) (Grand Island Biological Co., Grand Island, N. Y.) was layered on Ficoll-Hypaque (Pharmacia Fine Chemicals Inc., Piscataway, N. J.). After centrifugation, the lymphocyte fraction was withdrawn and washed three times with MEM. The washed lymphocytes were then resuspended at a concentration of 1 × 10^6/ml in MEM containing 10% pooled human serum. To assay column fractions or eluates of polyacrylamide gel slices, 10–50 μl of the fraction was diluted with 1 ml of MEM–10% pooled human serum. Triplicate 100-μl samples of each dilution were transferred to wells of standard tissue culture microtiter plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) containing 100 μl of the lymphocyte preparation (100,000 cells). The plates were incubated for 5 d at 37°C in a humidified atmosphere containing 5% CO_2. After this period, 0.5 μCi of [*methyl*-^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear, Boston, Mass.) was added and after a further incubation of 18 h, the cells were harvested and washed on glass fiber disks using a semi-automatic cell harvester. The disks were dried and radioactivity measured in a toluene-based cocktail by liquid scintillation counting.

Enzyme Immunoassay of Streptococcal Blastogen A Antibodies. Levels of antibodies against streptococcal blastogen A were measured using an enzyme immunoassay which was similar to that described by Engvall and Perlmann. (15) Polystyrene tubes (12 × 75 mm) (Falcon Labware) were coated with homogeneous blastogen A by incubating at 37°C for 60 min with 1 ml of the protein (10 ng/ml) dissolved in 0.1 M Na_2CO_3 (pH 9.6). The tubes were stored overnight at 4°C then washed three times with 0.9% NaCl, 0.05% Tween-20. Serum to be assayed was diluted in phosphate-buffered saline (PBS) 0.05% Tween-20, 0.02% NaN_3, and 1 ml of a series of dilutions (1/100, 1/300, 1/900, 1/2,700) were incubated in the coated tubes at 37°C for 30 min. The tubes were washed as before and then incubated with 1 ml of goat anti-human gamma chain globulin–horse radish peroxidase conjugate. Conjugate was prepared (16) from the IgG fraction of goat anti-human gamma chain sera (N. L. Cappel Laboratories Inc., Cochranville, Pa.) and horse radish peroxidase, type VI (Sigma Chemical Co., St. Louis, Mo.). After washing as before, 1 ml of peroxidase substrate (0.04% orthophenylenediamine, 0.006% H_2O_2 in citrate-phosphate buffer, pH 5.0) (16) was incubated in the tubes at room temperature for 30 min when 0.5 ml of 2 M H_2SO_4 was added to stop the reaction. The absorbance at 493 nm of the colored product was measured by spectrophotometry. Control tubes not coated with antigen were treated identically with each serum dilution and the A_{493} values were subtracted. In sera with low antibody levels these control values represented a large fraction of the absorbance observed with antigen-coated tubes and therefore were critical for the determination

[1] *Abbreviations used in this paper:* MEM, Eagles' minimal essential medium; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SPE, streptococcal pyrogenic exotoxin.

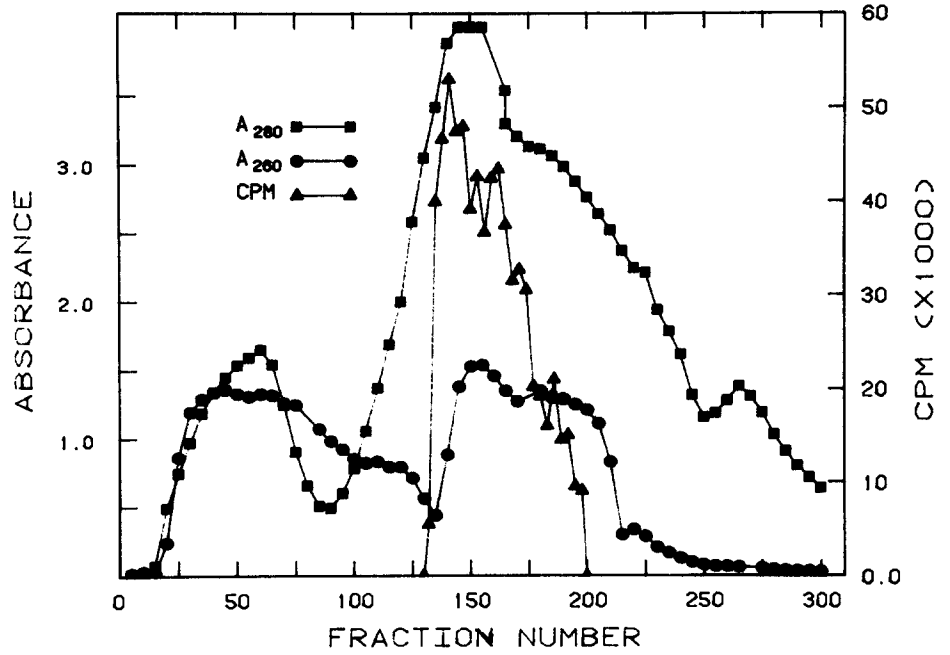

FIG. 1. DEAE chromatography of extracellular products from streptococcal strain C203S. The extracellular products were concentrated by ammonium sulfate precipitation and chromatographed on a DEAE-cellulose column as described in Materials and Methods. ■, absorbance at 280 nm; ▲, blastogenic activity (cpm/100,000 cells); ●, nuclease activity (A_{260} nm/10 min).

of meaningful titers. The corrected A_{493} values were plotted against the serum dilutions on a log/log scale. The antibody titer was arbitrarily set at that extrapolated serum dilution which would produce an A_{493} of 0.100.

Anti-DNase B levels were determined by the microtiter technique of Nelson et al. (17).

Enzyme Treatment of Streptococcal Blastogen A. A sample of a homogeneous preparation of blastogen A (1 μg/ml) was incubated separately with 100 μg pancreatic RNase, 100 μg pronase, and 100 μg trypsin (all from Sigma Chemical Co.) for 60 min at 37°C. The samples, including a control incubated without enzyme, were heated at 100°C for 10 min to inactivate enzymes and then the residual activity was assayed for blastogenic activity.

Results

Purification of Streptococcal Blastogen A. The extracellular products of strain C203S contained a single major peak of blastogenic activity when fractionated on DEAE-cellulose (Fig. 1). The blastogenic activity overlapped streptococcal nuclease A and was completely separated from the earlier eluting nucleases B and D, which were assayed as reference markers. The blastogen peak was pooled and chromatographed on CM-cellulose at pH 5 (Fig. 2). This procedure separated the blastogen from the bulk of other protein as well as from nuclease A. In most preparations, the blastogenic activity, hereafter referred to as streptococcal blastogen A, was homogeneous after CM chromatography (Fig. 3). An occasional contamination with nuclease A could be removed by rechromatography on CM-cellulose. The single band of protein on SDS polyacrylamide gel electrophoresis had a mobility characteristic of a mol wt of 17,500 (Fig. 3).

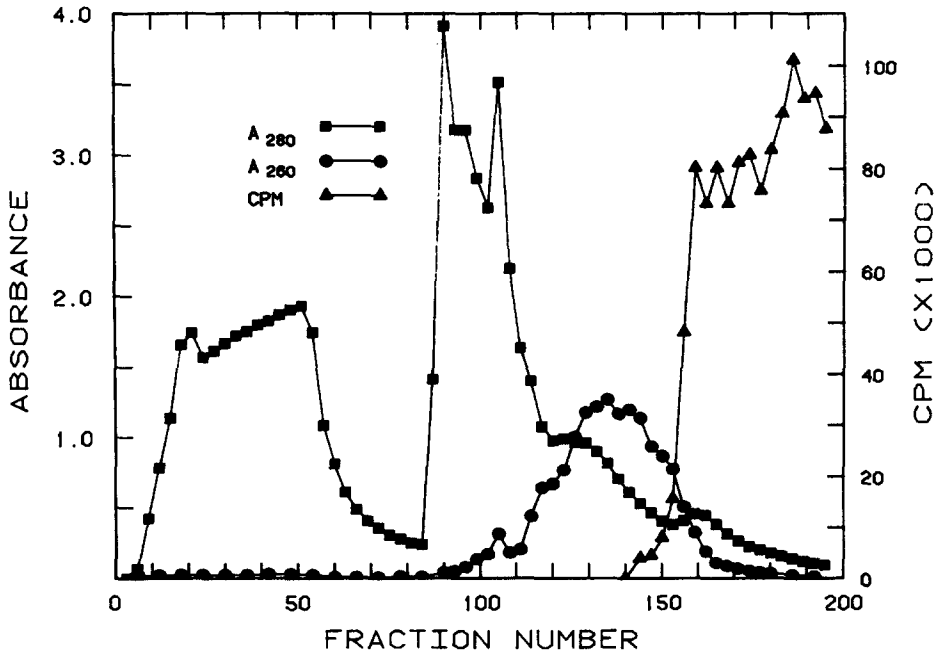

FIG. 2. CM-cellulose chromatography of blastogenic activity from DEAE-cellulose column eluate. ■, A_{280} nm; ▲, blastogenic activity (cpm/100,000 cells); ●, nuclease activity (A_{260} nm/10 min).

Effect of Enzymes on Streptococcal Blastogen A. The nature of blastogen A was examined by digestion with various enzymes. The activity is unaffected by pancreatic RNase but is destroyed by pronase and trypsin. The active portion of the blastogen is therefore likely to be a protein.

Heat Stability of Streptococcal Blastogen A. The heat stability of blastogen A was examined by measuring the activity remaining after various times of heating at 100°C. Blastogen A was rapidly and apparently irreversibly denatured by this treatment with <10% of the activity remaining after 5 min of heating. The purified material and the blastogenic activity in crude extracts from strain C203S were both affected. The blastogenic activity in crude extracellular products from all of the streptococcal strains listed in Table I showed kinetics of heat denaturation similar to the purified material from strain C203S.

Production of Blastogenic Activity by Various Streptococcal Strains. The production of extracellular blastogenic activity by several strains of group A streptococci was examined in crude extracts prepared from stationary phase cultures (Table I). The levels of blastogenic activity varied from 0.1 to nearly 30 stimulation U/μg of protein but all of the strains examined produced measurable lymphocyte transforming activity. It is possible that the blastogenic activity in some of the cultures was due to another streptococcal product but evidence from the enzyme immunoassay of anti-blastogen A-IgG suggests that the production of blastogen A is a common property of group A streptococci.

Polyacrylamide Gel Electrophoresis of Streptococcal Blastogen A. The electrophoresis of homogeneous blastogen A preparations in nondenaturing polyacrylamide gels resulted

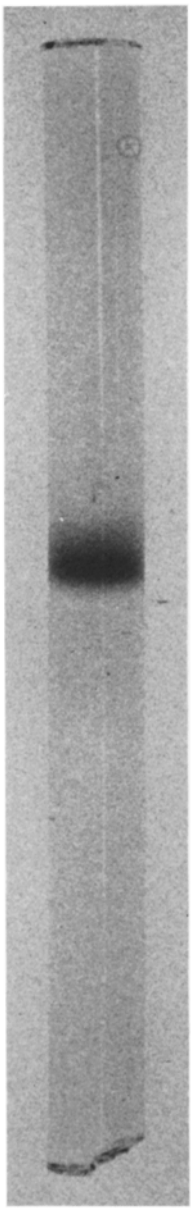

FIG. 3. SDS polyacrylamide gel of blastogen A.

in the appearance of multiple bands of protein with a broad spectrum of electrophoretic mobility (Fig. 4a). A companion gel was sliced and eluted and blastogenic activity was measured in the eluates. The peak of activity was not correspondent to the major protein bands that had barely detectable blastogenic activity. Instead, the biological activity was associated with minor protein bands of much higher mobility. In another experiment (data not shown) in which a similar gel was fractionated into thinner slices, two separate peaks of blastogenic activity was apparent, corresponding to the two protein bands. One explanation of these observations could be the

TABLE I

Extracellular Blastogen Activity from Culture Supernates of Various Strains of Group A Streptococci

Strain	Source	M type	T type	Stimulation units*
				per μg protein
GT-71-675	Univ. of Minn.	M1	T1	29.6
GT-71-676	Univ. of Minn.	M2	T2	3.4
GT-71-677	Univ. of Minn.	M3	T3	0.8
GT-75-194	Univ. of Minn.	M4	T4	0.2
GT-71-681	Univ. of Minn.	M6	T6	0.1
C203S	A. Bernheimer	M3	T1	27.1

* Stimulation unit was calculated as follows: (cpm of dilution of blastogen/cpm of maximum stimulation) × dilution factor = stimulation units (in undiluted blastogen preparation). The value reported is from a dilution on the linearly decreasing portion of the titration curve (see Fig. 6).

formation of oligomeric forms of blastogen A through disulfide cross linkage. In fact, the treatment of the blastogen A preparation with dithiothreitol before electrophoresis (Fig. 4 b) resulted in a marked reduction in the major protein bands and the generation of a previously absent protein species coincident with the blastogenic activity. This latter protein is of greater mobility than either of the two active species in the non-reduced blastogen A gel. If dithiothreitol treatment is prolonged, all of the slower migrating proteins are absent with the single band of protein remaining coincident with the blastogenic activity.

Relationship of Streptococcal Pyrogenic Exotoxins to Blastogen A. The streptococcal pyrogenic exotoxins are mitogens produced by many strains of group A streptococci. To compare blastogen A with these entities, the electrophoretic mobilities of streptococcal pyrogenic exotoxins A, B, and C and that of blastogen A in polyacrylamide gels were determined (Table II). Exotoxins A and C are obviously different in mobility from blastogen A, which has a mobility in the range of that of exotoxin B; this latter protein was present as two-charge isomers.

To further examine the possibility that blastogen A was identical with one of the streptococcal pyrogenic exotoxins, specific rabbit antisera were used in the lymphocyte stimulation assay. The blastogenic response to various concentrations of blastogen A was measured in the presence of antisera to the pyrogenic exotoxins. The results of such an experiment (Fig. 5) indicate that none of the antisera, with the possible exception of anti-exotoxin A, significantly affected the stimulation of lymphocytes by blastogen A, suggesting that the pyrogenic exotoxins are dissimilar antigenically from blastogen A.

A possibility existed that these antisera, even if directed toward blastogen A, could not inhibit the lymphocyte response. In a separate experiment, human serum shown to contain anti-blastogen A-IgG by enzyme immunoassay was incubated with lymphocytes and varying concentrations of blastogen A (Fig. 6). As the results indicate, the lymphocyte transformation response to blastogen A was markedly inhibited in the presence of the anti-blastogen A serum. It would appear that inability of the anti-pyrogenic exotoxin sera to inhibit the lymphocyte response to blastogen A (Fig. 5) reflects antigenic differences between blastogen A and the exotoxins.

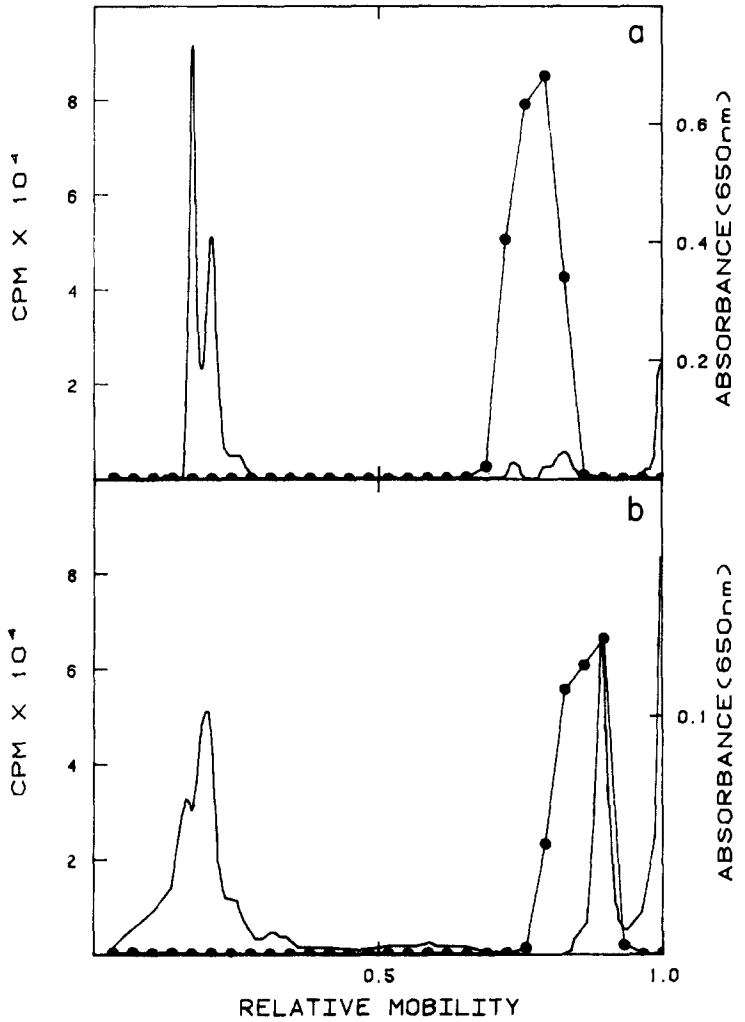

FIG. 4. Electrophoresis of streptococcal blastogen A in nondenaturing polyacrylamide gels. (a) A homogeneous preparation of blastogen A was electrophoresed in polyacrylamide gels as described in Materials and Methods. One gel was stained for protein and a companion gel was sliced with a template into equal slices ≅3 mm thick. The slices were eluted with 1.0 ml of 0.001 M Tris(pH 7), 0.001 M $MgCl_2$, 0.001 M $CaCl_2$ overnight at 4°C and the blastogenic activity was assayed in the eluates. (b) The same blastogen A preparation was made 0.01 M with respect to dithiothreitol and incubated at 37°C for 1 h before electrophoresis as in (a). ●, cpm/100,000 cells; —, A_{595} nm (protein).

Antibodies to Streptococcal Blastogen A in Human Sera. The humoral immunogenicity of blastogen A was examined by means of an enzyme immunoassay for antibodies directed against this antigen in human sera. The results of a survey of sera with a range of antibody titers against streptococcal DNase B are shown in Table III. Antibodies to blastogen A appear to be present in sera in which anti-DNase B levels are elevated. The relative levels of anti-blastogen A, with some exceptions, correspond to those of anti-DNase B. In certain sera, the anti-blastogen A level was undetectable although low levels of anti-DNase B were present. In this small group of sera, the

TABLE II

Comparison of Electrophoretic Mobility of Blastogen A with Streptococcal Pyrogenic Exotoxins

		Rm
Streptococcal blastogen A		0.75, 0.84
Streptococcal pyrogenic exotoxin	A	0.27
	B	0.64, 0.68
	C	0.39

The purified materials were electrophoresed on 7.5% polyacrylamide gels as in Materials and Methods. The mobilities of the proteins are relative to that of bromophenol blue.

infecting strains of streptococci were not available; however, it seems likely that blastogen A is a common antigen produced by a variety of streptococci.

Discussion

The present study has demonstrated the isolation in homogeneous form of a group A streptococcal extracellular product capable of stimulating lymphocyte blastogenesis. The protein, termed streptococcal blastogen A, has a mol wt of 17,500 and is susceptible to heat denaturation and readily forms disulfide cross-linked species, most of which are relatively inactive on lymphocytes.

A variety of streptococcal cellular, cell-bound, and extracellular proteins have the ability to induce lymphocyte blastogenesis. Abe and Alouf (6) have partially purified an extracellular blastogenic substance which appeared to have a mol wt of 50,000 as estimated by gel filtration and an isoelectric point near 4.7. This substance corresponds in size to a glycopeptide mitogen extracted from resting streptococci by Plate and Amos (4, 5). Seravalli and Taranta (1) have described an extracellular mitogen from the same group A streptococcal strain (C203S) that was the principal source of material used in the present study. In this case, the mitogenic substance appeared to be heterogeneous, with a major species of mol wt 26,000 and an isoelectric point of 6.9. However, in the present study the DEAE chromatograms of crude extracellular products from two distinct group A strains (C203S and S43) show the same pattern of blastogenic activity, a single major peak with little evidence for other blastogens. If indeed the mitogens described by Seravalli and Taranta (1) are separate species, they are not detectable by the fractionation techniques used to purify blastogen A. A possible explanation for the heterogeneity reported by these authors could be related to the observations of multiple disulfide linked forms on polyacrylamide gel electrophoresis (Fig. 4).

Another group of mitogenic proteins that are produced by group A streptococci are the streptococcal pyrogenic exotoxins (SPE), also known as the streptococcal erythrogenic toxins (7–9). Three distinct species of SPE exist with mol wt of 8,000, 17,500, and 13,000, respectively, for SPE A, B, and C. The molecular weight and electrophoretic mobility of SPE A and C are clearly different from blastogen A. Exotoxin B, however, is similar in mobility to blastogen A and has an identical molecular weight. Furthermore, exotoxin B exists as two charge isomers and two active forms of blastogen A are separable by gel electrophoresis. Nauciel et al. (18) have reported that erythrogenic toxin (SPE-A) can be isolated in two distinct forms which are convertible to a

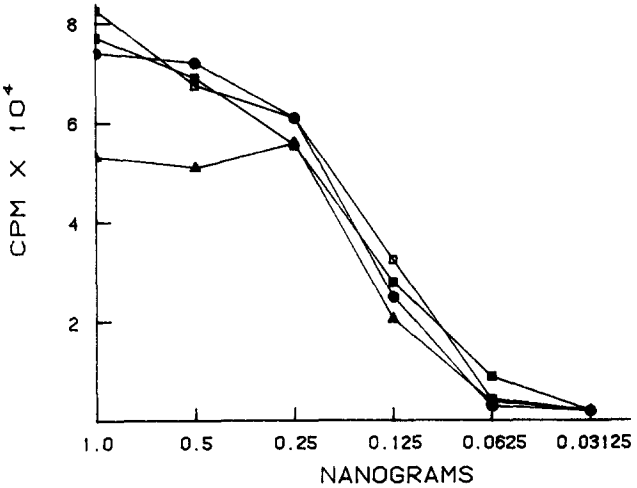

FIG. 5. Effect of antisera to streptococcal pyrogenic exotoxins A, B, and C on the stimulation of lymphocyte blastogenesis by streptococcal blastogen A. Lymphocyte stimulation by varying concentrations of blastogen A was measured as described in Methods in the presence of specific rabbit antisera to either SPE A, B, or C diluted 1:100. The control curve was an assay performed without rabbit sera. ●, control; ▲, anti-SPE A; ■, anti-SPE B; □, anti-SPE C.

single species by reduction. Similar behavior was observed with blastogen A in the present investigation, although multiple inactive and two active forms of blastogen A were converted to a single active species. This property appears to be shared by streptococcal nuclease B, which exists in multiple forms (19), some of which are absent after reduction with dithiothreitol (Gray, E. D. Unpublished results). It may, therefore, be a more general property of streptococcal extracellular products.

The lack of significant inhibition of lymphocyte stimulation by blastogen A in the presence of antisera to the streptococcal pyrogenic exotoxins appears to indicate that blastogen A is antigenically dissimilar to these entities. An alternative interpretation of these results is that the antigenic sites which specify the humoral antibodies are different from those involved in the lymphocyte blastogenic response. This appears to be the case with some systems (20) and under these circumstances, even if blastogen A was identical to one of the pyrogenic exotoxins, the antibodies against the toxins might not be directed at the antigenic site which interacts with lymphocytes. However, a recent study indicates that the mitogenic activity of erythrogenic toxin can be inhibited by specific rabbit antisera to the toxin (21). Furthermore, the blastogenic activity of blastogen A was inhibited by serum containing antibodies against this protein (Fig. 6). It appears that blastogen A is not closely antigenically related to the pyrogenic exotoxins. Further studies will be required to exclude the possibility that blastogen A may be identical with or related to one of the other known extracellular products of group A streptococci.

The lymphocyte stimulating substances isolated from streptococcal extracellular products have been termed mitogens, because they induce blastogenesis in lymphocytes from animals presumably unsensitized by streptococcal antigens (1, 2). In the absence thus far of definitive evidence, the stimulating substance purified in the present investigation has been termed a blastogen. The detection of antibodies to

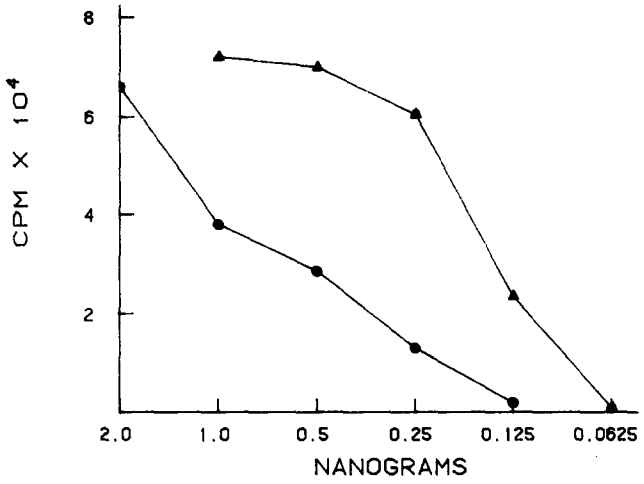

FIG. 6. Effect of human antiserum to streptococcal blastogen A on lymphocyte blastogenesis. Lymphocyte stimulation by varying concentrations of blastogen A was measured in the presence of 10% human serum containing anti-blastogen A-IgG (titer of 2,500). The control was a similar assay performed with the usual 10% pooled human sera. ▲, control; ●, anti-blastogen A.

blastogen A in human sera and their inhibition of lymphocyte stimulation suggest that the human response to blastogen A is by lymphocytes sensitized to the antigen. The possibility exists that blastogen A is also a nonspecific mitogen, stimulating unsensitized lymphocytes, and this will be a subject for future studies on lymphocytes from human cord blood.

Streptococcal blastogen A is evidently produced by a wide variety of streptococci as evidence by the presence of antibodies to this protein in a small, randomly selected group of human sera. Furthermore, blastogenic activity has been directly detected in several group A strains (Table I). Although this activity could be due to a variety of streptococcal products, it has similar chromatographic properties in two unrelated strains. If, indeed, blastogen A is a common streptococcal extracellular product, most adults will have been exposed to this antigen. The blastogenic response to blastogen A will, therefore, represent an index of the reactivity of an individual's immune system to a streptococcal antigen. A survey of such responses may allow the demonstration of altered reactivity in individuals with clinical evidence of post-streptococcal disease (acute nephritis, acute rheumatic fever, and rheumatic heart disease) and perhaps allow the identification of those at risk for these diseases. Such a study employing the purified blastogen A is presently underway.

Summary

An extracellular product of group A streptococci which induces lymphocyte blastogenesis has been purified to homogeneity by DEAE-cellulose and CM-cellulose chromatography. The protein, termed streptococcal blastogen A, has a mol wt of ≅ 17,500 and is inactivated by protease treatment and by heating at 100°C. The purified blastogen gave rise to multiple protein bands on nondenaturing polyacrylamide gel electrophoresis, only two of which possessed blastogenic activity. Treatment of the protein with dithiothreitol before electrophoresis resulted in the apparent

TABLE III

Comparison of Levels of Antibody to DNase B and Blastogen A

Serum No.	Antibody titer	
	Anti-DNase B	Anti-blastogen A
9793	<50	0
9788	240	0
9761	320	0
9747	480	265
9810	200	465
9743	480	1130
9765	800	1400
9746	800	2125

A group of human sera selected for differing levels of anti-DNase B was assayed for anti-blastogen A levels by an enzyme immunoassay as described in Materials and Methods.

conversion of the multiple forms to a single active species. Blastogen A differs in electrophoretic mobility from the streptococcal pyrogenic exotoxins and its lymphocyte stimulating activity is not inhibited by rabbit antisera to the exotoxins. An enzyme immunoassay has been developed to measure human antibodies against blastogen A. A selection of sera with varying levels of anti-DNase B contained anti-blastogen A-IgG.

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References

1. Seravalli, E., and A. Taranta. 1974. Lymphocyte transformation and macrophage migration inhibition by electrofocused and gel-filtered fractions of group A streptococcal filtrate. *Cell. Immunol.* **14**:366.
2. Alouf, J. E., and C. Damais. 1977. Purification et proprietes d'un materiel mitogene exocellulaire streptococcvque non specifique. *Ann. Immunol. (Paris).* **128C**:37.
3. Taylor, A. G. 1972. Lymphocyte transforming activity of streptococci belonging to various Lancefield groups. *J. Med. Microbiol.* **5**:61.
4. Plate, J., and B. Amos. 1971. Lymphocyte stimulation by a glycopeptide isolated from *Streptococcus pyogenes* C203S. *Cell. Immunol.* **1**:476.
5. Plate, J., and B. Amos. 1971. Lymphocyte stimulation by a glycopeptide isolated from *Streptococcus pyogenes* C203S. II. Kinetics of the response. *Cell. Immunol.* **1**:488.
6. Abe, Y., and J. E. Alouf. 1976. Partial purification of exocellular lymphocyte mitogen from *Streptococcus pyogenes. Jpn. J. Exp. Med.* **46**:363.
7. Barsumian, E. L., C. M. Cunningham, P. M. Schlievert, and D. W. Watson. 1978. Heterogeneity of group A streptococcal pyrogenic exotoxin type B. *Infect. Immun.* **20**:512.
8. Schlievert, P. M., K. M. Bettin, and D. W. Watson. 1977. Purification and characterization of group A streptococcal pyrogenic exotoxin type C. *Infect. Immun.* **16**:673.

9. Cunningham, C. M., E. L. Barsumian, and D. W. Watson. 1976. Further purification of group A pyrogenic exotoxin and characterization of the purified toxin. *Infect. Immun.* **14:** 767.
10. Greenberg, L. J., E. D. Gray, and E. Yunis. 1975. Association of HL-A5 and immune responsiveness *in vitro* to streptococcal antigens. *J. Exp. Med.* **141:**935.
11. Marker, S., and E. D. Gray. 1972. A simple method for the preparation of streptococcal nucleases. *Appl. Microbiol.* **23:**368.
12. Davis, B. J. 1964. Disc gel electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121:**404.
13. Holbrook, I. B., and A. G. Leaver. 1976. A procedure to increase the sensitivity of staining by Coomassie Brilliant Blue G-250-perchloric acid solution. *Anal. Biochem.* **75:**634.
14. Weber, K., and M. Osborne. 1969. The reliability of molecular weight determinations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:**4406.
15. Engvall, E., and P. Perlmann. 1972. Enzyme linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme labeled anti-immunoglobulin in antigen coated tubes. *J. Immunol.* **109:**129.
16. Mathiesen, L. R., S. M. Feinstone, D. Wong, P. Skinhoej, and R. H. Purcell. 1978. Enzyme-linked immunosorbent assay for detection of hepatitis A antigen in stool and antibody to hepatitis A antigen in sera: Comparison with solid phase radioimmunoassay, immune electron microscopy and immune adherence hemagglutination assay. *J. Clin. Microbiol.* **7:** 184.
17. Nelson, J., E. M. Ayoub, and L. W. Wannamaker. 1968. Streptococcal anti-desoxyribonuclease B microtechnique determination. *J. Lab. Clin. Med.* **71:**867.
18. Nauciel, G., T. Blass, R. Mangalo, and M. Raynaud. 1969. Evidence of two molecular forms of streptococcal erythrogenic toxin, conversion to a single form by 2-mercaptoethanol. *Eur. J. Biochem.* **11:**160.
19. Gray, E. D. 1972. Nucleases of group A streptococci. *In* Streptococci and Streptococcal Diseases: Recognition, Understanding and Management. L. W. Wannamaker and J. M. Matsen, editors. Academic Press, Inc., New York. 143.
20. Gell, P. G. H., and B. Benacerraf. 1959. Studies on hypersensitivity. II. Delayed hypersensitivity to denatured proteins in guinea pigs. *Immunology.* **2:**64.
21. Petermann, F., H. Knöll, and W. Köhler. 1978. Mitogenic activity of erythrogenic toxins. 1. Type specific inhibition of the mitogenic activity of erythrogenic toxins by antitoxic antisera from the rabbit. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Erste Abt. Orig. Reihe A Med. Mikrobiol. Parasitol.* **240:**366.