Cellular Reactions With Protein A of *Staphylococcus aureus*[1] (36168)

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The occurrence in most strains of staphylococci of protein A capable of reacting directly with human and various mammalian IgG globulins (1–4), may provide a wider understanding of some of the important intrinsic biologic effects of staphylococcal infections. Recent reports by Coombs and coworkers (5, 6) have indicated the presence of immunoglobulin molecules on the surface of circulating lymphocytes. Previously, the demonstration of lymphocyte stimulation by antisera to various immunoglobulins (7–10) had been interpreted as indicative of the presence of immunoglobulin molecules or their consituents on the surface of test lymphocytes. This phenomenon has now been confirmed using several assay systems including immunofluorescence (11–13). Since protein A combines with IgG globulin, it was considered a reagent with the potential of interacting directly with lymphocytes in a manner analogous to that previously demonstrated for anti-rabbit allotype antisera (7). The studies reported here therefore focused on interaction of protein A of the staphylococcus with various circulating cells, presumably on the basis of its reaction with the Fc portion of IgG globulin. Experiments utilizing lymphocyte cultures to which protein A were added indicated that protein A blocked or impeded lymphocyte transformation induced by anti-lymphocyte antiserum (ALS) or plant mitogens. Furthermore, protein A-IgG interaction did not directly stimulate lymphocyte blast transformation even when cross-linking was made possible using rabbit antisera to protein A. Since protein A exists as the outermost material on the staphylococcal cell wall (14), its reactivity with the cells which it meets in the course of invasion and production of infection is of considerable biologic interest.

Materials and Methods. Protein A. Protein A of *Staphylococcus aureus* was isolated and partially purified as previously described (1, 2). The Cowan I strain of *S. aureus* served as the source of protein A.

Lymphocyte stimulation. Peripheral blood lymphocytes were obtained from human and rabbit whole blood using the technique of Böyum (15) and centrifugation through a gradient of Ficoll. Cells were washed three times with phosphate-buffered-saline pH 7.4, before use in lymphocyte stimulation studies. Lymphocyte transformation was measured after culture for three days followed by incorporation of tritiated thymidine as previously described (16, 17). Cell cultures contained γ-globulin-free fetal calf serum (Hyland Laboratories, Los Angeles, CA). In some experiments protein A was added to cells in the presence of rabbit antiserum to protein A or the isolated pepsin $F(ab)'_2$ fragment of rabbit anti-protein A antibody to achieve cross-linking of cells. Pepsin fragments of anti-protein A antibody were used to exclude any reaction between protein A and the Fc portion of the rabbit anti-protein A antibody.

The technique of mixed leukocyte culture (18, 19) was used in an attempt to document whether previous incubation with staphylococcal protein A affected cells behavior in mixed leukocyte culture (MLC). Leukocytes from two unrelated normal donors were used in the one-way stimulation system where one member of the two admixed donor lympho-

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TABLE I. Summary of Four Human Lymphocyte Cultures Using Varying Amounts of Protein A as Stimulant.

	Expt. 1	Expt. 2
Baseline	239[a]	126[a]
PWM[a]	3010	2717
μg Protein A added to cultures		
0.001	399	—
.01	354	—
0.1	288	—
1.0	314	120
5.0	—	156
10.0	—	132
20.0	—	122
30.0	—	111

[a] Numbers refer to counts/min of ^{3}H-thymidine in pelleted nuclear material after three days of culture.

[b] PWM represents pokeweed mitogen which was utilized as positive control.

cytes is pretreated with mitomycin (18, 19). In addition, cells were incubated with solutions containing 100 μg of protein A/ml for 30 min at 20° and then washed before use in mixed leukocyte cultures. Appropriate baseline controls including a basic MLC without preincubation of either responding or stimulating cells with protein A were included in each experiment.

Results. Lymphocyte stimulation. Initial studies were directed primarily at results of lymphocyte stimulation after addition of protein A. A wide range of concentrations of protein A was used in combination with a fixed number of lymphocytes (2×10^6/2 ml). When human or rabbit peripheral blood lymphocytes were studied, no lymphocyte stimulation occurred over the range of protein A concentrations tested (Table I). Moreover, no lymphocyte transformation was noted when rabbit antisera to protein A, capable of cross-linking, was added (Table II). The same lack of lymphocyte stimulation was recorded when peptic $F(ab)'_2$ fragments of rabbit antibody to protein A were used to cross-link cells and protein A. In some instances increasing amounts of protein A added to lymphocyte cultures resulted in lower counts being recorded at the end of 72 hr of culture. However, no evidence for direct cytotoxicity of protein A on lymphocytes was noted using trypan blue dye exclusion or direct observation under phase microscopy. Absence of lymphocyte stimulation using protein A alone or with protein A plus rabbit antibody to protein A provided no evidence for lymphocyte stimulation or cellular interaction with this staphylococcal cell wall product.

The effect of protein A added to lymphocyte cultures in the presence of stimulating anti-lumphocyte antisera or artificial plant mitogens was next studied. Table III indicates a progressive decrement in labeled thymidine at 72 hr as added protein A was increased from 1.0 to 30.0 μg. Of interest was not the marked stimulation by rabbit anti-lymphocyte antisera (ALS), but inhibition of this stimulation by 10 μg of protein A added to the culture at the same time as ALS. In addition preincubation of cultured lymphocytes with ALS for one hour followed by addition of protein A was also followed by

TABLE II. Human Lymphocyte Culture Using Protein A and Rabbit Antiserum to Protein A for Crosslinking.

Test System	Count Min^{-1} ^{3}H-thymidine 72 hr
Baseline	1603
PWM[a]	37571
PHA[a]	22108
Protein A	
1 μg	969
10 μg	348
100 μg	298
Protein A 1 μg + Rabbit antiserum to Protein A[b]	271
Protein A 10 μg + Rabbit antiserum to Protein A[b]	224
Protein A 100 μg + rabbit Antiserum to Protein A[b]	216
Rabbit antiserum to Protein A	254

[a] PWM represents pokeweed mitogen and PHA, phytohemagglutinin added as positive controls to cultures.

[b] Similar lack of potentiation of cell transformation was recorded when pepsin $F(ab)_2'$ fragments of rabbit anti-protein A antibody were added to protein A + rabbit or human lymphocytes in culture.

TABLE III. Effects of protein A on human lymphocyte cultures stimulated with anti-lymphocyte antisera and with artificial plant mitogens (pokeweed or phytohemagglutinin).

EXPERIMENT #1		EXPERIMENT #2	
Test System	Count Min^{-1} ^{3}H-thymidine 72 hr	Test System	Count Min^{-1} ^{3}H-thymidine 72 hr
Baseline	261	Baseline	629
Pokeweed Mitogen	2,220	Pokeweed Mitogen	12,161
Phytohemagglutinin	2,608	Phytohemagglutinin	4,936
Protein A 1 μg	873	Protein A 10 μg	151
10 μg	350	Anti-lymphocyte antiserum	11,210
30 μg	164	Protein A + Pokeweed Mitogen (10 μg)	588
Anti-lymphocyte antiserum	8,936	Protein A + phytohemagglutinin (10 μg)	299
Anti-lymphocyte antiserum + Protein A 10 μg	236	[Cells preincubated with protein A (10 μg) 1 hr, washed] and pokeweed mitogen added.	6,665
[Anti-lymphocyte antiserum preincubated with lymphocytes 1 hr] + protein A 10 μg	276	[Cells preincubated with protein A (10 μg) 1 hr, washed] and phytohemagglutinin added.	1,717
		[Cells preincubated with protein (10 μg) 1 hr, washed] and anti-lymphocyte antiserum added.	7,332
		[Cells preincubated with protein A (10 μg), washed] and thymidine added.	230
		[Cells preincubated with ALS 1 hr, washed] and thymidine added.	5,420

absence of lymphocyte stimulation. In similar fashion inhibition of stimulation by pokeweed mitogen or phytohemagglutinin was noted when protein A was added to cultures at the same time as the plant mitogens (Table III). However, if cells were preincubated for one hour with protein A, washed, and then pokeweed, phytohemmaglutinin, or ALS added, only partial suppression of lymphocyte stimulation was recorded.

Mixed leukocyte cultures. Attempts were made to see if transient incubation of lymphocyte preparations with protein A altered their behavior in one-way stimulation using paired lymphocytes, one member of which had been pretreated with mitomycin. These experiments were done to attempt to document whether reaction of protein A with γG receptor molecules affixed to lymphocytes altered their ability to respond to strong histocompatibility antigens presented by admized unrelated lymphocytes. The results of several experiments indicated that incubation of protein A with lymphocytes did not appreciably affect mixed leukocyte culture stimulation, either as stimulating or responding members of mixed leukocyte reactions.

Discussion. Of considerable interest was the fact that protein A added to lymphocyte cultures at the same time as potent rabbit anti-human lymphocyte antisera or plant mitogens seemed to interfere with lymphocyte transformation; however, less suppression was noted when protein A was preincubated with test lymphocytes, the cells washed, and then exposed to ALS, pokeweed or phytohemagglutinin. The apparent protein A blockade of lymphocyte transformation induced by rabbit anti-human lymphocyte antisera or plant mitogens added to cultures simultaneously was a highly reproducible, consistent finding. It is possible that interaction of protein A with the rabbit γ-globulins possessing anti-lymphocyte antibody activity prevents their firm binding to lymphocyte surfaces and thereby impedes their ability to deform

cell surfaces enough to induce blast transformation. This explanation cannot, however, be advanced for the apparent blockade of cell transformation when protein A and plant mitogens are both added to cultures. The preincubation and washing experiments recorded here indicate that for maximal blocking effect, continued presence of protein A in cultures is necessary. The results of this study suggest that rather than acting as a stimulant for lymphocyte transformation, protein A of the staphylococcus may possess competitive affinity for lymphocyte receptor sites in cell transformation and could thereby function as an immunosuppressive reagent in staphylococcal infections.

Considerable recent attention has been directed at immunoglobulin receptor molecules on the surfaces of various circulating immunocompetent cells, particularly lymphocytes (20, 21). It seemed of interest to attempt to determine if protein A of the staphylococcus was capable of producing direct lymphocyte stimulation when added to *in vitro* lymphocyte cultures, since a reaction between cell-bound γG and protein A might conceivably set off the mechanisms for cell transformation *in vivo*. The experiments shown here clearly indicate that protein A alone cannot instigate transformation of rabbit or human lymphocytes. Moreover, no lymphocyte transformation was recorded when protein A was added to cultures in the presence of whole rabbit antiserum or $F(ab)'_2$ fragments of such antiserum reacting with protein A. Such a reagent as the latter affords cross-linking between protein A molecules attached to the Fc part of γ-globulin. Cell to cell approximation has recently been shown to be of considerable importance for lymphocyte transformation by Fanger *et al.* (22); however, under the circumstances of the experiments recorded here, no lymphocyte transformation was produced.

Summary. Protein A of *Staphylococcus aureus* reacts with the Fc portion of γG globulin molecules. Rabbit and human lymphocytes were studied in an attempt to document the effects of interactions between protein A and immunoglobulin receptors on such cells. Addition of protein A to lymphocyte cultures simultaneously with stimulating amounts of anti-lymphocyte antiserum, pokeweed mitogen, or phytohemagglutinin resulted in blockade of lymphocyte transformation. If lymphocytes were preincubated with protein A, washed and exposed to the same mitogens, depression of subsequent cell transformation was also noted. No lymphocyte stimulation was noted when protein A was added to cell cultures. Rather than exerting any stimulatory effects on lymphocytes, it is suggested that protein A of *S. aureus* may function as an immunosuppressant.

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