Effect of Immunoglobulin G on the Responsiveness of Human Neutrophils to Soluble Staphylococcal Protein A-Induced Neutrophil Migration Inhibition Factor from T-Lymphocytes

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Staphylococcal protein A is a bacterial cell wall product that binds human immunoglobulin G and thereby interferes with opsonization and phagocytosis of *Staphylococcus aureus* by neutrophils. Phagocytic cells are also responsive to various non-immunoglobulin lymphocyte mediators. We utilized the detection of a newly recognized mediator, a neutrophil migration inhibition factor from T-lymphocytes (NIF-T), to show that aggregates of staphylococcal protein A and immunoglobulin G could inhibit the responsiveness of neutrophils to NIF-T. That such aggregates may alter the responsiveness of neutrophils to lymphocyte mediators that amplify or modulate phagocytic functions may have important pathogenetic implications in staphylococcal infection.

Human neutrophils have Fc receptors that bind immunoglobulin G (IgG) and promote opsonization (6, 7, 9). Staphylococcal protein A (SPA) is a bacterial cell wall product that competes with neutrophil Fc receptors by binding the Fc portion of IgG subclasses 1, 2, and 4 (2, 3, 8, 11). Accordingly, SPA on the surface of *Staphylococcus aureus* prevents opsonization and thereby interferes with phagocytosis of these organisms by neutrophils (2, 3, 11). Neutrophils are also effector cells for various lymphocyte mediators, and the possibility that SPA-IgG complexes might interact with neutrophils to block their responsiveness to non-immunoglobulin mediators as well has not been tested.

Our laboratory recently described a newly recognized mediator, neutrophil migration inhibition factor from T lymphocytes (NIF-T) (13, 14). Although SPA is a polyclonal B-cell activator, recent work demonstrates that it also activates T lymphocytes (4, 12). We previously showed that NIF-T is produced in response to SPA (14). The following studies were designed to determine whether the interaction between IgG and SPA would interfere with the responsiveness of neutrophils to NIF-T.

MATERIALS AND METHODS

Mitogens. SPA and concanavalin A (Con A) (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) were diluted with Hanks balanced salt solution, filtered through 0.45- μ m-pore size membrane filters (Millipore Corp., Bedford, Mass.), and used at a final concentration of 20 μ g/ml and 5 μ g/ml, respectively, in cell

cultures. SPA-Sepharose was washed four times with Hanks balanced salt solution and used in lymphocyte cultures at a final concentration of 20 μ g/ml.

Preparation of IgG. IgG from healthy subjects and myeloma IgG subclasses 1 to 4 were isolated by diethylaminoethyl cellulose chromatography (5). Each isolated preparation of IgG (5 mg/ml) was shown to give a single arc on immunoelectrophoresis against rabbit anti-whole human serum. IgG subclasses were determined with subclass-specific antisera. Deaggregation was performed by centrifuging the IgG at $106,000 \times g$ for 2 h with a Beckman type 40 fixed-angle rotor in a Beckman L5-65 ultracentrifuge. The upper half of each supernatant was carefully removed, filtered, and used the same day in lymphocyte cultures.

Preparation of IgM. Myeloma IgM was prepared from the serum of a patient with IgM myeloma by euglobulin precipitation, agar gel chromatography, and deaggregation by ultracentrifugation as described for IgG (10).

Depletion of IgG from human serum and culture supernatants. IgG was removed from serum by incubating and intermittently mixing serum for 30 min at 37°C with SPA-Sepharose (Pharmacia) prewashed six times with Hanks balanced salt solution. The serum was filtered (0.45- μ m pore size) to remove the Sepharose beads. Supernatants (30 μ l) containing human serum were depleted of IgG by incubating them with similarly prepared SPA-Sepharose beads. After the beads settled, 10 μ l of supernatant was carefully removed from the top and assayed. Absorbed serum contained less than 10% of the original IgG as determined by radial immunodiffusion against anti-human IgG (G-Que Plate; Helena Labs, Beaumont, Tex.).

Lymphocyte cultures. Human peripheral blood lymphocytes from healthy donors were isolated by Ficoll-Hypaque gradient centrifugation (1). The pe

ipheral blood lymphocytes were washed with Hanks balanced salt solution and cultured in Falcon Test II plates (Falcon Plastics, Oxnard, Calif.) in 100 μ l of medium 199 with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer, penicillin (100 U/ml), and streptomycin (100 μ g/ml). The effect of human serum (2, 5, and 10 μ l) on SPA- and Con A-induced NIF-T production by peripheral blood lymphocytes (7.5×10^5 /ml) was assessed in microcultures that contained 10% fetal calf serum (FCS). Similarly, we examined the effect of IgG-depleted human serum (10 μ l) on SPA-induced NIF-T production. The effect of isolated, pooled, deaggregated human IgG, IgG subclasses, and IgM (10 μ l at 2.5 to 5.0 mg/ml) on SPA and SPA-Sepharose-induced NIF-T production was assessed in serum-free cultures with 3×10^6 lymphocytes per ml.

Lymphocytes were cultured at 37°C in the presence of 5% CO₂. After 24 h, 30 μ l of supernatant was harvested from each culture, and the nonstimulated supernatants were reconstituted with the same concentration of SPA or Con A that was used in the stimulated cultures. All cultures were viable as determined by trypan blue exclusion. The supernatants were stored at -20°C until assayed.

Neutrophil migration assay. The agarose microassay to detect NIF-T activity was performed as previously described (15). Each test consisted of quadruplicate control (supernatant from nonstimulated cultures) and test (supernatant from stimulated cultures) migrations. The data are expressed as percent inhibition of the area of neutrophil migration of the test as compared to the control migrations. *P* values were determined by Student's *t* test.

RESULTS

Effect of human serum on SPA- and Con A-induced NIF-T activity. NIF-T production is manifested as a reduced area of neutrophil migration (15). NIF-T activity was produced in response to both SPA and Con A. Table 1 demonstrates the effect of human serum from seven healthy subjects on SPA-induced NIF-T production by lymphocytes from four healthy donors. SPA stimulated lymphocytes to produce NIF-T in FCS in the absence of human serum (mean response, $49 \pm 4\%$ inhibition in the area of neutrophil migration). The addition of 5 and 10 μ l of human serum produced 45 and 71% blocking, respectively, of the NIF-T response to SPA. In contrast, human serum had no effect on Con A stimulation of NIF-T production at a concentration (5 μ g/ml) of Con A which was shown in preliminary studies to be the lowest effective dose.

Effect of IgG-depleted human serum on SPA-induced NIF-T activity. Lymphocytes from four subjects produced NIF-T in response to SPA in the presence of FCS ($30 \pm 4\%$ inhibition of the area of neutrophil migration) (Table 2). The addition of fresh human serum from

three subjects to the FCS-containing cultures, however, blocked SPA-induced NIF-T production by 80% ($P < 0.001$). IgG-depleted serum from two subjects, however, failed to inhibit SPA stimulation of the NIF-T response (10% blocking). It appears, therefore, that the IgG present in human serum was primarily responsible for blocking the SPA-induced NIF-T response.

Effect of isolated IgG and IgM on SPA- and SPA-Sepharose-induced NIF-T activity. The specificity of IgG inhibition of SPA-induced NIF-T production was assessed by comparing it with IgM (Table 3). Two pools of isolated IgG (2.5 mg/ml) (each pool contained equal volumes of serum from five subjects) blocked NIF-T production by 69% ($P < 0.01$) in four subjects. In contrast, monoclonal IgM (3.7 mg/ml) produced only 22% inhibition of the NIF-T response which was not significantly different from the controls. SPA-Sepharose-induced NIF-T activity was not blocked by IgG, however. This indicates that soluble SPA is nec-

TABLE 1. Effect of human serum (HUS) on SPA- and Con A-induced NIF-T activity^a

Reaction mixture	Re- sponse ^b	% Block- ing	<i>P</i>
SPA	49 ± 4^c		
SPA plus 2 μ l of HUS	35 ± 4^d	29	
SPA plus 5 μ l of HUS	27 ± 3^d	45	<0.005
SPA plus 10 μ l of HUS	14 ± 3^d	71	<0.001
Con A	33 ± 3^e		
Con A plus 10 μ l of HUS	30 ± 4^e	9	

^a All cultures contained 10% FCS.

^b Percent inhibition in the area of neutrophil migration $\pm$ standard error of the mean.

^c Mean of duplicate determinations in four subjects.

^d Mean of 28 responses of lymphocytes from four subjects with serum from seven subjects.

^e Mean of six responses of lymphocytes from one subject with serum from six subjects.

TABLE 2. Effect of human serum (HUS) and IgG-depleted HUS on SPA-induced NIF-T activity^a

Reaction mixture	No. of tests	Re- sponse ^b	% Block- ing	<i>P</i>
SPA	4	30 ± 4		
SPA plus 10 μ l of HUS	12	6 ± 1	80	<0.001
SPA plus 10 μ l of IgG-depleted HUS	8	27 ± 6	10	

^a All cultures contained 10% FCS.

^b Percent inhibition in the area of neutrophil migration $\pm$ standard error of the mean.

essary for blocking of NIF-T activity.

Effect of human myeloma IgG subclasses on SPA-induced NIF-T activity. Table 4 shows the mean response of the lymphocytes of four subjects to SPA-induced NIF-T production in the presence and absence of myeloma IgG subclasses 1 to 4. IgG subclasses 1, 2, and 4 produced 79, 89, and 63% inhibition of SPA-induced NIF-T activity, respectively, whereas IgG3 at the same concentration gave only 29% inhibition. The difference between the blocking of IgG1, 2, 4, and IgG3 was significant at $P < 0.005$. Therefore, those subclasses of IgG which bind SPA blocked SPA-induced NIF-T activity.

Effect of IgG on the response of neutrophils to SPA-induced NIF-T activity. Experiments were designed to assess whether the inhibition of SPA-induced NIF-T activity by IgG was due to inhibition of NIF-T production or to inhibition of responsiveness of neutrophils to NIF-T. To do this, IgG was added to supernatants that contained SPA-induced NIF-T, and the supernatants were assayed before and after addition of IgG. Table 5 shows that the mean response of lymphocytes (duplicate determinations) from three subjects to SPA was $34 \pm 5\%$ inhibition in the area of neutrophil migration. The addition of $10 \mu\text{l}$ of serum from each subject to the activated supernatants from each subject abolished these responses ($-13 \pm 10\%$ inhibi-

tion). This result was further confirmed by removing IgG from SPA-stimulated culture supernatants that failed to show NIF-T activity in the presence of IgG. The mean response of lymphocytes from three subjects to SPA in the presence of $10 \mu\text{l}$ of serum from each of the three subjects was $4 \pm 8\%$ inhibition in the area of neutrophil migration. After these supernatants were adsorbed with SPA-Sepharose to remove IgG, the mean response was $27 \pm 4\%$ inhibition in the area of migration. These results indicate that IgG inhibited NIF-T activity by inhibiting the responsiveness of neutrophils to NIF-T. IgG did not interfere with NIF-T production by T lymphocytes.

DISCUSSION

Phagocytic dysfunction predisposes to opportunistic infections. The interaction between IgG and SPA interferes with opsonization and phagocytosis of *Staphylococcus aureus* (2, 3). Phagocytic cells are responsive to a variety of non-immunoglobulin lymphocyte mediators that amplify or modulate their activity. We wondered whether the interaction between IgG and SPA could also inhibit the responsiveness of phagocytic cells to other lymphocyte mediators.

TABLE 5. Effect of IgG on the response of neutrophils to SPA-induced NIF-T activity^a

Reaction mixture	No. of tests	Response ^b	P
SPA-induced NIF-T, no HUS	6	34 ± 5	
SPA-induced NIF-T, $10 \mu\text{l}$ of HUS	9	-13 ± 10	<0.005
SPA-induced NIF-T, with HUS	9	4 ± 8	
SPA-induced NIF-T, with HUS after adsorption with SPA-Sepharose	8	27 ± 4	<0.025

^a All cultures contained 10% FCS. HUS, Human serum.

^b Percent inhibition in the area of neutrophil migration $\pm$ standard error of the mean.

TABLE 3. Effect of isolated IgG and IgM on SPA- and SPA-Sepharose-induced NIF-T activity^a

Reaction mixture	No. of tests	Response ^b	% Blocking	P
SPA	4	36 ± 4		
SPA plus IgG	8	11 ± 5	69	<0.01
SPA plus IgM	4	28 ± 2	22	
SPA-Sepharose	12	32 ± 2		
SPA-Sepharose plus IgG	12	40 ± 2	0	

^a Serum-free cultures.

^b Percent inhibition in the area of neutrophil migration $\pm$ standard error of the mean.

TABLE 4. Effect of human myeloma IgG subclasses on SPA-induced NIF-T activity^a

Reaction mixture	No. of IgG's tested	No. of tests ^b	Response ^c	% Blocking
SPA		16	38 ± 4	
SPA plus IgG1	4	16	8 ± 8	79
SPA plus IgG2	3	12	4 ± 6	89
SPA plus IgG4	2	8	14 ± 2	63
SPA plus IgG3	3	12	27 ± 6	29

^a Serum-free cultures.

^b Mean response of peripheral blood lymphocytes from four healthy subjects.

^c Percent inhibition in the area of neutrophil migration $\pm$ standard error of the mean.

Our laboratory recently described a newly recognized neutrophil migration inhibition factor from T cells, NIF-T (14). NIF-T was distinguished from other migration inhibition factors (14). In addition, NIF-T is produced as a result of the interaction between T and B cells or T cells and monocytes (16). NIF-T has been shown to be produced spontaneously by a T-lymphoblast cell line derived from a patient with a T-cell variant of hairy cell leukemia. In contrast, numerous other lymphoblast cell lines including B cells, T cells, null cells, and myeloblast cells have been shown not to produce this factor (13). Moreover, the mediator spontaneously produced by the T-lymphoblast cell line and that produced by peripheral blood leukocytes in response to mitogens appeared to be the same (14). These data strongly suggest that NIF-T is produced selectively by T lymphocytes. We decided, therefore, to utilize the detection of this T-cell mediator to assess the effect of IgG on SPA-induced NIF-T activity.

The studies reported here show that SPA-stimulated T cells produce NIF-T in serum-free medium as well as in FCS. These results indicate that SPA induces T-cell mediator production in addition to its previously described effects on T-lymphocyte transformation and polyclonal B-cell activation. However, SPA-induced NIF-T activity was not apparent in the presence of human serum. IgG in human serum was shown to be responsible for inhibiting SPA-induced NIF-T activity. Removal of IgG from serum by SPA-Sepharose prevented inhibition of NIF-T activity by serum, and the addition of isolated IgG to serum-free and FCS-containing cultures produced inhibition of NIF-T activity. Soluble SPA (present in the NIF-T-containing supernatants) was necessary for the inhibition of NIF-T activity, since Con A- or SPA-Sepharose-induced NIF-T activity was not blocked by IgG. Furthermore, inhibition of NIF-T activity by IgG was specific for those IgG subclasses (1, 2, and 4) that bind SPA. Inhibition of SPA-induced NIF-T activity by IgG was shown to occur at the level of the effector cell response, since removal of IgG from inactive supernatants restored NIF-T activity, and IgG prevented neutrophils from responding to previously generated NIF-T in the presence of SPA. Thus it appears most likely that the inhibition of NIF-T activity is mediated by complexes of IgG and soluble SPA.

The inhibition of SPA-induced NIF-T activity on neutrophils in the presence of IgG may have pathogenetic implications in staphylococcal infections. If NIF-T plays an important role in the accumulation of neutrophils at sites where infection is spreading, high-rate protein A-producing strains might be able to protect themselves from

neutrophil attack. Moreover, future investigation may show that other circulating immune aggregates in patients with infections, neoplasia, and connective tissue diseases may act in a way similar to SPA-IgG complexes and modify the response of phagocytic cells to lymphocyte mediators.

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