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Mechanism of Activation of Human Basophils by *Staphylococcus aureus* Cowan 1

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We investigated the capacity of *Staphylococcus aureus* Cowan 1 and *S. aureus* Wood 46 to induce histamine release from human basophils in vitro. *S. aureus* Cowan 1 (10^5 to 10^7/ml), which synthesizes protein A (Staph A), stimulated the release of histamine from basophils, whereas *S. aureus* Wood 46 (10^5 to 2×10^7/ml), which does not synthesize Staph A, did not induce histamine secretion. Soluble Staph A (10^{-3} to 10 μg/ml), but not staphylococcal enterotoxin A, induced histamine secretion from human basophils. Staph A binds through its classical site to the Fc region of human immunoglobulin G (IgG) and through its alternative site to the Fab portion of the different human immunoglobulins. Hyperiodination of Staph A, which destroys over 90% of the original Fc reactivity without altering the Fab-binding site, did not alter the ability of the protein to induce histamine release. The stimulating effect of Staph A was dose dependently inhibited by preincubation with human polyclonal IgG (0.3 to 100 μg/ml) and a human monoclonal IgM (0.3 to 100 μg/ml) which have F(ab')-Staph A reactivity. In contrast, rabbit IgG, which possesses only Fc-Staph A reactivity, and a Staph A-unreactive human monoclonal IgM did not inhibit Staph A activity. Similar results were obtained with intact *S. aureus* Cowan 1. Preincubation with either Staph A or anti-IgE (rabbit anti-Fc$_\varepsilon$) resulted in complete desensitization to a subsequent challenge with the homologous stimulus. Staph A and anti-IgE induced partial cross-desensitization to the heterologous stimulus. Cells preincubated with anti-IgG (rabbit anti-Fc$_\gamma$) lost a small but significant part of their ability to release with Staph A but did not lose their response to anti-IgE. Basophils from which IgE had been dissociated by brief exposure to lactic acid no longer released histamine in response to anti-IgE and Staph A. When basophils from which IgE had been dissociated were incubated with human polyclonal IgE, they regained their ability to induce histamine in response to Staph A and anti-IgE. In contrast, two monoclonal IgEs which do not bind to Staph A did not restore the basophil responsiveness to Staph A. Furthermore, there was complete cross-desensitization between soluble Staph A and *S. aureus* Cowan 1, while cells desensitized to *S. aureus* Wood 46 released normally with Staph A and *S. aureus* Cowan 1. These results indicate that Staph A and *S. aureus* Cowan 1 activate histamine release from human basophils by interacting with the F(ab')$_2$ region of IgE or IgG or both present on the cell surface.

Protein A from *Staphylococcus aureus* Cowan 1 (Staph A) and intact staphylococci induce histamine release from human basophils (23, 25, 26). It has been suggested that the activation of human basophils induced by Staph A is mediated by the interaction with the cell surface-bound immunoglobulin G (IgG) (26). We have found a significant correlation between the maximum percent histamine release induced by anti-IgE and that induced by Staph A, which suggests that these two release mechanisms have a common triggering event (23). However, the mechanism of basophil activation induced by staphylococci and Staph A has yet to be defined.

Staph A is known to bind specifically to the Fc$_\gamma$ region of human IgG subclasses 1, 2, and 4 (3). Binding to other immunoglobulin isotypes was initially reported to be minimal (15, 36). More recently, however, it has been shown that Staph A also reacts with a structure located in the Fab region of immunoglobulin which is shared by human IgM, IgE, IgG, and IgA (7, 9). Unlike rabbit IgG, which reacts with Staph A through the Fc$_\gamma$ region alone, human IgG can react with Staph A via both the Fab and the Fc regions, and the Staph A-binding Fab region is shared by a proportion of human IgE, IgM, and IgA (7, 9).

Human basophils possess specific, distinct membrane receptors for the Fc$_\gamma$ and Fc$_\varepsilon$ portions of IgE and IgG (12, 13). Anti-IgE (rabbit anti-Fc$_\varepsilon$) and anti-IgG (rabbit anti-Fc$_\gamma$) induce histamine secretion by cross-linking membrane-bound IgE and IgG, respectively (4, 10). Therefore, the possibility exists that Staph A and *S. aureus* also induce histamine release from human basophils by cross-linking membrane-bound immunoglobulins.

The experiments described here were designed to investigate the mechanism by which Staph A and Staph A-containing staphylococci activate basophils. The results indicate that Staph A and *S. aureus* Cowan 1 induce the activation of human basophils by cross-linking of sites on the F(ab')$_2$ portion of IgE or IgG or both present on the cell surface.

MATERIALS AND METHODS

Leukocyte donors. Venous blood was obtained from normal subjects, aged 20 to 40 years. The use of human volunteers was approved by the Committee of Clinical Investigations of the University of Naples, II School of Medicine, and informed consent was always obtained.

Buffers. The buffers used in these experiments were P (25 mM PIPES [piperazine-*N*,*N'*-bis(2-ethanesulfonic acid)], 110 mM NaCl, 5 mM KCl, pH 7.4) and PC, which is P buffer with 2.0 mM $CaCl_2$ (18). P-EDTA is P buffer with 4 mM EDTA (24).

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Histamine release assay. After informed consent was obtained, blood was drawn into a final concentration of 0.008 M EDTA and 1.1% dextran 70 and allowed to sediment for 90 min at 22°C (21). The leukocyte-rich upper layer was drawn off, pelleted (200 × *g*, 4°C, 8 min), and washed as previously described (19). A 0.4-ml sample of the cell suspension (10^6 to 2.5×10^6 cells per tube) was placed in Falcon polyethylene tubes (12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.) and warmed to 37°C; 0.2 ml of each stimulus for release was prewarmed and added to the cells, and incubation was continued at 37°C for 45 min (22). After centrifugation (1,000 × *g*, 22°C, 2 min), the cell-free supernatants were assayed for histamine by an automated fluorometric technique developed by Siraganian (20, 38). The net percent release was calculated from the total histamine released from cell aliquots by lysis with 2% perchloric acid minus the histamine released spontaneously from unstimulated samples (17). Spontaneous histamine release in PC was always less than 5% of the total histamine. All experiments were done with cells from at least four separate donors, and each experiment was performed in duplicate or triplicate, with less than 10% variation between replicates.

Purification of human monoclonal and polyclonal IgE proteins. IgE myeloma proteins ADZ (35) and PS (kindly donated by A. Sehon) were purified from the patient sera by repeated gel filtration on Sepharose G-200 followed by elution through a Sepharose CL-4B column. Human polyclonal IgE (isolated by affinity chromatography from the serum of a patient) containing approximately 50,000 IU of IgE was further purified by repeated gel filtration on an Ultrogel AcA 34 column (14). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified human monoclonal and polyclonal IgE proteins demonstrated a single protein with a molecular weight of 180,000 to 200,000. Analysis by radioimmunoassay showed no IgG, IgM, or IgA contamination (30, 32).

Purification of monoclonal IgM proteins. Monoclonal IgM proteins reactive (IgM_R) and nonreactive (IgM_{NR}) with the alternative site of Staph A were isolated from the serum of two patients with Waldenström's macroglobulinemia by repeated euglobulin precipitation with distilled water followed by gel filtration on Sephadex G-200. IgM_R and IgM_{NR} proteins were freed from any contaminating IgG by elution through a column containing rabbit antibodies against human γ-chain coupled to CNBr-activated Sepharose CL-4B (33).

Purification of HIgG and RIgG. Human (HIgG) and rabbit (RIgG) polyclonal IgGs were prepared by precipitation of normal human or rabbit serum with 50% saturated ammonium sulfate followed by chromatography on a DEAE-cellulose column equilibrated with 0.01 M phosphate buffer (pH 7.9) as previously described (33). Nonretained protein was collected.

Anti-immunoglobulin antisera. The preparation and characterization of affinity-purified rabbit antibodies directed against human γ-chain have been described in detail elsewhere (28, 31). Briefly, the antisera were rendered monospecific by repeated solid-phase absorption with appropriate immunoglobulin class determinants. After these absorptions, purified antibodies specific for human γ-chain determinants were obtained by affinity chromatography. The specificity of the anti-human γ-chain antiserum was checked by double diffusion in agarose, by immunofluorescence on bone marrow from patients with IgG, IgD, and IgA myelomas, and by radioimmunoassay performed in polyvinyl plates (Dynatech Laboratories, Inc., Alexandria, Va.). In this assay, antiserum was bound to microtiter plate wells, and IgM, IgE, and IgG molecules were allowed to bind to antibody-coated wells. ^{125}I-radioiodinated, immunosorbent-purified antibodies were then allowed to react with the bound molecules. Anti-γ antiserum reacted only with IgG. Rabbit anti-human IgE, produced by immunization with the Fc fragment of a human IgE myeloma protein and then adsorbed with IgE Fab fragments as previously described (11), was kindly donated by Kimishige and Teruko Ishizaka.

Preparation of radiolabeled reagents. Labeling of RIgG, HIgG, and human IgM_R with ^{125}I-labeled sodium iodide was performed by the chloramine-T method (6).

Iodination of Staph A. Staph A (1 mg/ml) was iodinated with different concentrations of KI (0.001 to 10 mg/ml) in the presence of chloramine-T (1.6 mg/ml), and the reaction was stopped by the addition of sodium metabisulfite (4.8 mg/ml). Iodinated Staph A was then separated on a Sephadex G-25 column (6, 28, 33, 39).

Solid-phase protein-binding assay. The ability of Staph A and hyperiodinated Staph A to react with RIgG, HIgG, and human IgM_R was evaluated by a solid-phase binding assay performed in polyvinyl plates (Dynatech). For this purpose, microtiter plate wells were filled with Staph A at a concentration of 2 μg/ml. After incubation overnight at 22°C, the coating solution was removed, and wells were washed individually three times with phosphate-buffered saline (pH 7.8), and then 10% bovine serum albumin (BSA) in phosphate-buffered saline was added to the wells and left for 6 h to saturate any remaining protein-binding surface. After three washes with phosphate-buffered saline, the labeled reagents to be assayed were added to wells and allowed to incubate overnight at 22°C. Thereafter, the plates were washed three times with 1% BSA in phosphate-buffered saline and eight times with running tap water. The individual wells were separated, and the bound radioactivity was determined.

Staphylococci. *S. aureus* Cowan 1 and Wood 46 were obtained from the National Collection of Type Cultures (London). The bacteria were killed by incubation with 0.5% formaldehyde (3 h, 22°C), heat treated (3 min, 80°C), washed, and finally stored in small aliquots at −80°C. The bacteria were counted in a Neubauer chamber (33). Staph A was obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden) (lot no. GF 19273, HK 28624, and HE 24852). Enterotoxin A was purchased from Serva (Heidelberg, Federal Republic of Germany).

Materials. The following were purchased: PIPES (Sigma Chemical Co., St. Louis, Mo.); 60% perchloric acid (Baker Chemical Co., Deventer, The Netherlands); dextran T 70, Sepharose CL-4B–CNBr, Sephadex G-200, Sephadex G-25 (Pharmacia Fine Chemicals); Ultrogel AcA 34 (LKB Produkter AB, Stockholm, Sweden); DEAE-cellulose (Serva); ^{125}I-labeled sodium iodide (IMS-30; Amersham Corp., Arlington Heights, Ill.).

Statistical analysis. The results are expressed as the mean ± the standard error of the mean.

RESULTS

Effect of *S. aureus* on histamine release from basophils. Increasing numbers of *S. aureus* Cowan 1 produced graded increases in histamine release from human basophils. A typical dose-response curve, selected from 36 donors, is shown in Fig. 1. In the range of 10^5 to 10^7 staphylococci per tube, histamine secretion gradually increased with increasing concentrations of bacteria. *S. aureus* Wood 46 (10^5 to 2×10^7 bacteria per tube), which does not contain Staph A

(34), did not induce histamine release in any of the 12 subjects studied.

Leukocytes were also treated with *S. aureus* Cowan 1 or Staph A in P-EDTA for 30 min at 37°C. At the end of incubation, cells were washed and suspended in PC. Leukocytes pretreated with either *S. aureus* Cowan 1 or Staph A released virtually no histamine when the cells were exposed to optimal concentrations of *S. aureus* Cowan 1 (Fig. 2). In contrast, cells preincubated with *S. aureus* Wood 46 released the same percentage of histamine as cells preincubated in P-EDTA. In the same experiment, cells preincubated with either *S. aureus* Cowan 1 or Staph A released virtually no histamine when challenged in the second incubation with Staph A. Cells preincubated with *S. aureus* Wood 46 released histamine similarly to cells preincubated in P-EDTA. As previously shown, basophils did not release histamine in response to *S. aureus* Wood 46. These results show cross-desensitization between soluble Staph A and intact *S. aureus* Cowan 1.

These findings suggest that Staph A is responsible for the activation of basophils by *S. aureus*. Recently, it has been suggested that contamination of Staph A with enterotoxin A is responsible for both immune interferon induction and T-cell mitogenic activity by commercial Staph A preparations (40). Staphylococcal enterotoxin A (10^{-3} to 10 ng/ml) did not induce a significant release of histamine from human basophils (data not shown).

Effect of hyperiodination. It is now evident that Staph A possesses two binding sites for immunoglobulins. The classical site binds the Fc of IgG1, IgG2, and IgG4 (3), and the alternative site binds the Fab portion of a percentage of IgG, IgE, IgA, and IgM (7, 9). Hyperiodination selectively alters the Fc-binding region of Staph A (33, 39). The histamine-releasing activity of Staph A was only slightly reduced by hyperiodination (10 μg of KI per μg of Staph A) (Fig. 3A), although the same treatment virtually abolished the ability of Staph A to react with RIgG and strongly reduced its reactivity with HIgG (Fig. 3B). As expected, binding of IgM_R with the alternative site of Staph A was not affected by this treatment. These findings suggest that activation of basophils induced by Staph A is not mediated by interaction through the classical site of the protein.

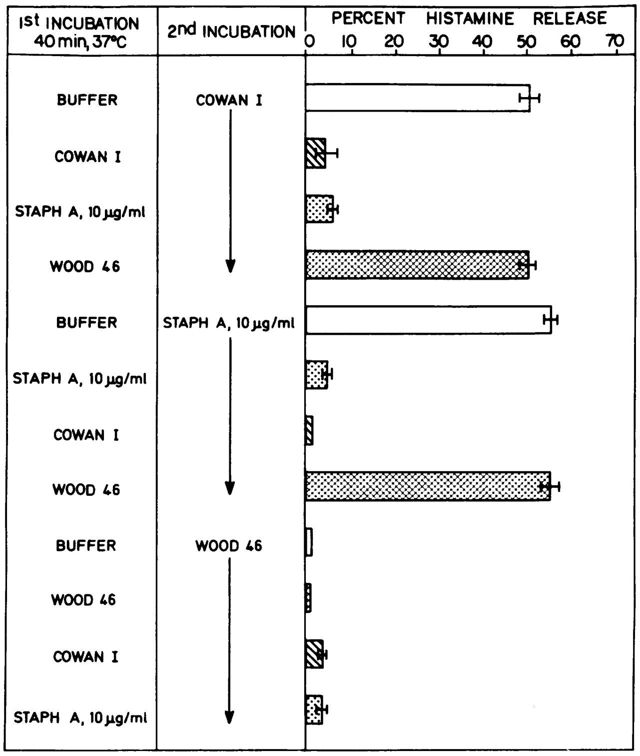

FIG. 2. Effect of desensitization to one stimulus on the response to a second stimulus. Cells were desensitized to *S. aureus* Cowan 1 (10^8 bacteria per ml), *S. aureus* Wood 46 (10^8 bacteria per ml), or Staph A (10 μg/ml) by preincubation with the stimuli in P-EDTA for 40 min at 37°C. Cells were washed (twice at 4°C), suspended in PC, and challenged with *S. aureus* Cowan 1 (3×10^7 bacteria per ml), *S. aureus* Wood 46 (3×10^7 bacteria per ml), or Staph A (10 μg/ml) for 45 min at 37°C. Each bar is the mean ± the standard error of the mean of triplicate determinations. Similar results were obtained in two other experiments.

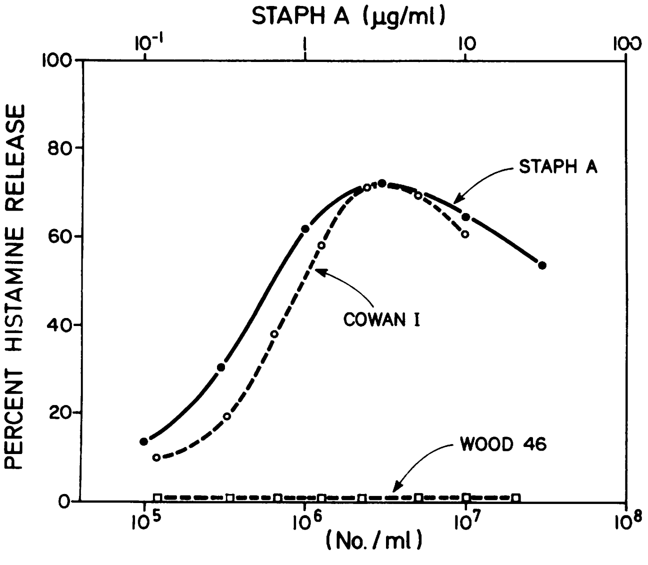

FIG. 1. Effect of *S. aureus* Cowan 1, *S. aureus* Wood 46, and Staph A on histamine secretion from human basophils. Each point represents the mean of duplicate determinations from a typical experiment.

The alternative F(ab')$_2$-binding site on Staph A is responsible for *S. aureus* Cowan 1-induced activation of human basophils. The role of F(ab')$_2$-binding regions of Staph A in the activation of human basophils induced by Staph A-containing *S. aureus* was investigated next. In a first series of experiments, we studied the effect on Staph A-induced basophil activation of molecules that exhibit Fc_γ-Staph A reactivity alone, such as RIgG, those that exhibit F(ab')$_2$-Staph A reactivity alone, such as IgM_R, and those with both Fc_γ and F(ab')$_2$ reactivity, such as HIgG (7, 9, 33). As a control, the effect of IgM_{NR} was also evaluated. Both HIgG and IgM_R dose dependently inhibited Staph A-induced histamine release, whereas RIgG and IgM_{NR} had no such effect (Fig. 4A). In a parallel series of experiments, we tried to inhibit selectively the Fc and F(ab')$_2$ reactivity of intact *S. aureus* by preincubation with the immunoglobulins mentioned above (Fig. 4B). The results were essentially similar to those obtained with purified Staph A. The findings indicate that both Staph A and *S. aureus* induce histamine release, presumably by binding through the alternative site to immunoglobulins present on human basophils.

Cross-desensitization between *S. aureus*, Staph A, anti-IgE, and anti-IgG. We have previously shown an excellent cor-

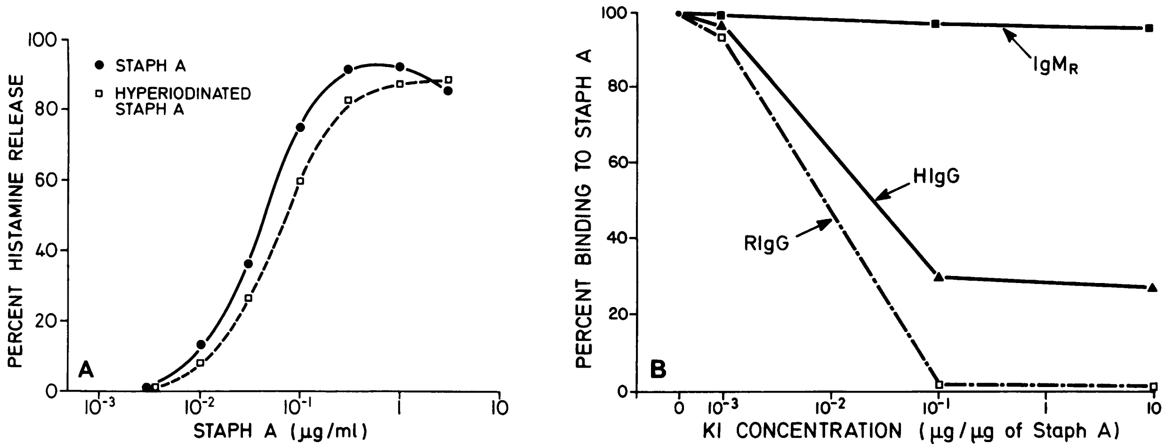

FIG. 3. (A) Effect of inactivation of tyrosyl residues of Staph A on the ability of Staph A to induce histamine secretion from human basophils. Each point represents the mean of duplicate determinations from a typical experiment. Staph A was iodinated with KI (10 μg/10 μg of Staph A) as previously described (28). Similar results were obtained in three other experiments. (B) Effect of inactivation of tyrosyl residues of Staph A on its ability to react with IgM$_R$, HIgG, and RIgG. The binding of ^{125}I-labeled IgM$_R$, ^{125}I-labeled HIgG, and ^{125}I-labeled RIgG to Staph A coupled to polyvinyl plate wells was evaluated before and after inactivation of tyrosyl residues by treatment of Staph A with different concentrations of KI.

relation between maximal release caused by anti-IgE and that caused by Staph A (23). The relationship between anti-IgE and Staph A was further examined to test for cross-desensitization between anti-IgE, anti-IgG, and Staph A. Leukocytes were treated with anti-IgE (1 μg/ml), Staph A (10 μg/ml), or anti-IgG (100 μg/ml) in P-EDTA for 30 min at 37°C. At the end of incubation, cells were washed and suspended in PC. Cells preincubated with P or with anti-IgG released histamine normally with anti-IgE (Fig. 5). In contrast, leukocytes preincubated with anti-IgE released less than 10% histamine. Cells desensitized to Staph A released 60 to 80% less histamine than did control cells. Basophils of this donor released histamine (36% of total content) when challenged with anti-IgG (100 μg/ml) (data not shown).

In reverse experiments, cells were preincubated with anti-IgG, Staph A, or anti-IgE in P-EDTA before challenge with Staph A. As expected, when Staph A-pretreated cells were challenged with Staph A they had lost their ability to release with the homologous stimulus (Fig. 6). Preincubation with anti-IgG and anti-IgE, respectively, partly and almost completely desensitized basophils. Thus, it appears that the releasing activity property of Staph A is mediated mainly by interaction with IgE and partly by interaction with IgG present on the basophil membrane (12, 13).

A second line of evidence that Staph A induces histamine release by binding to IgE is based on its different capacity to induce histamine release from basophils from which IgE had been dissociated versus those to which IgE had been restored (27). IgE is removed from basophil receptors by brief exposure to low pH; it is restored by exposing the unoccupied receptors to IgE. Lactic acid-induced dissociation of IgE from basophils markedly reduced anti-IgE-induced release and completely eliminated Staph A-induced release (Fig. 7). When basophils from which IgE had been dissoci-

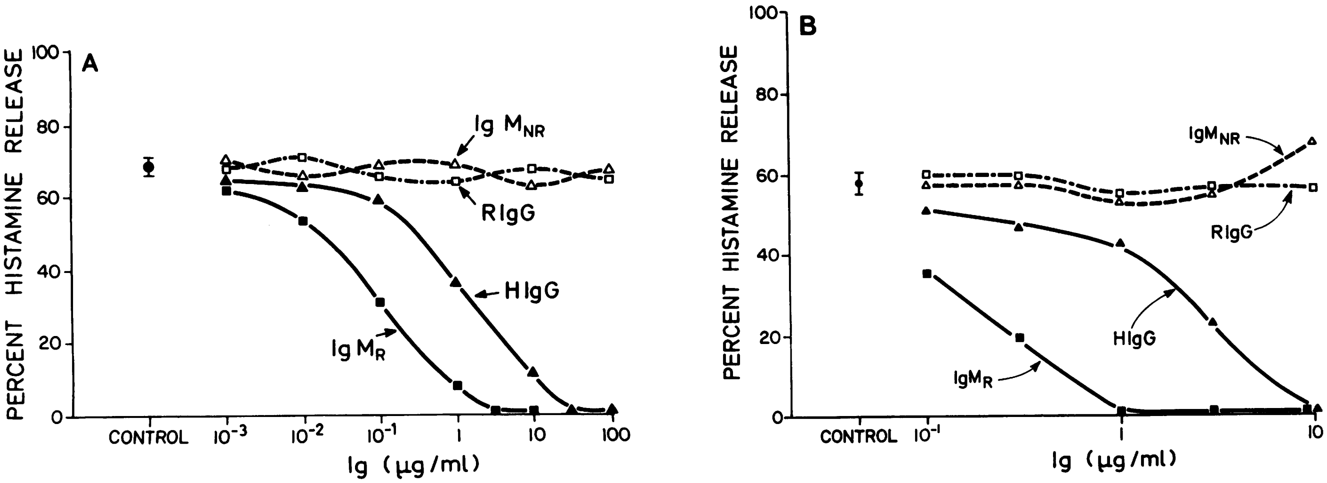

FIG. 4. Effect of preincubation (10 min, 22°C) of Staph A (A) or *S. aureus* (B) with RIgG, IgM$_{NR}$, HIgG, and IgM$_R$ on the activation of human basophils. Each point represents the mean of duplicate determinations from a typical experiment. The mean ± the standard error of the mean of the percent histamine release (CONTROL) in the absence of preincubation with immunoglobulins (Ig) is indicated (●). Similar results were obtained in four other experiments.

TABLE 1. Nonreactivity of two monoclonal IgE proteins with Staph A[a]

Material bound to microwell	^{125}I-labeled Staph A binding (cpm)
BSA	207
Myeloma IgE (PS)	236
Myeloma IgE (ADZ)	245
HIgG	27,653
RIgG	26,541

[a] IgE protein was purified from two different IgE myelomas (PS and ADZ) and linked to wells of polyvinyl microtiter plates by incubation for 12 h at room temperature and pH 7.8. Free binding sites were then saturated by incubation for 4 h with 4% BSA. The wells were then incubated for 2 h at room temperature with ^{125}I-labeled Staph A (40,000 cpm). After washings, individual wells were cut apart, and radioactivity was measured. For a control, wells coated with BSA, HIgG, or RIgG were included in the study. Each value represents the mean of duplicate determinations from a typical experiment.

ated were incubated with human polyclonal IgE, they regained their capacity to release histamine in response to anti-IgE as well as in response to Staph A. In contrast, when basophils were treated with lactic acid and then with two different monoclonal IgEs (ADZ and PS) which do not bind to Staph A (Table 1), they remained unresponsive to Staph A and responsive to anti-IgE. These experiments demonstrate that cell surface polyclonal IgE is a prerequisite for Staph A-induced histamine release from basophils.

DISCUSSION

The results of the present study indicate that soluble Staph A-containing bacteria such as *S. aureus* Cowan 1 induce histamine release from human basophils. *S. aureus* Wood 46, which does not synthesize Staph A, failed to activate these cells. The releasing activity of both Staph A and *S. aureus* Cowan 1 appears to be mediated by interaction of the alternative nonimmune F(ab')$_2$-binding site with IgE or IgG or both present on human basophils. Another *Staphylococcus* component, Staph A-enterotoxin A, which also activates human lymphocytes (29) and which has been found to contaminate some Staph A preparations (40), is not responsible for the releasing property of Staph A.

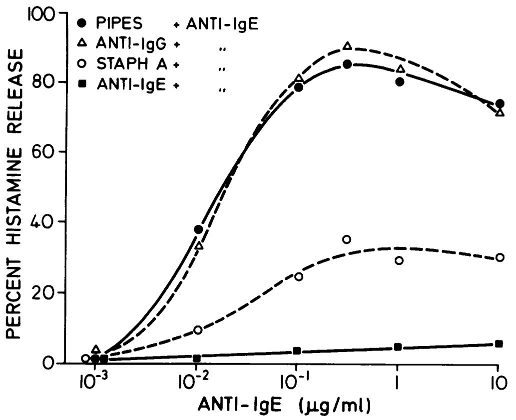

FIG. 5. Effect of desensitization to one stimulus on the response to a second stimulus. Cells were desensitized to anti-IgG (100 μg/ml), Staph A (10 μg/ml), or anti-IgE (1 μg/ml) by preincubation with the stimuli in P-EDTA for 40 min at 37°C. Cells were washed (twice at 4°C), suspended in PC, and challenged with various concentrations of anti-IgE for 45 min at 37°C. Each point represents the mean of duplicate determinations from a typical experiment. Similar results were obtained in four other experiments.

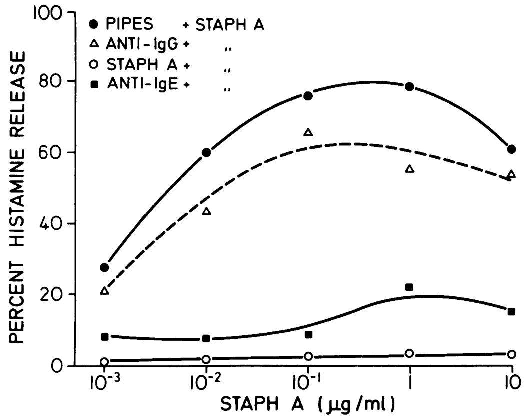

FIG. 6. Effect of desensitization to one stimulus on the response to a second stimulus. Cells were desensitized to anti-IgG (100 μg/ml), Staph A (10 μg/ml), or anti-IgE (1 μg/ml) by preincubation with the stimuli in P-EDTA for 40 min at 37°C. Cells were washed (twice at 4°C), suspended in PC, and challenged with various concentrations of Staph A for 45 min at 37°C. Each point represents the mean of duplicate determinations from a typical experiment. Similar results were obtained in four other experiments.

These findings show that intact *S. aureus* Cowan 1 and soluble Staph A are capable of activating human basophils and releasing chemical mediators of inflammation. Our results demonstrate that there is complete cross-desensitization between soluble Staph A and intact *S. aureus* Cowan 1, whereas *S. aureus* Wood 46 desensitization does not affect the response to these stimuli.

Hyperiodination of Staph A and inhibitory experiments with immunoglobulins indicated that the F(ab')$_2$ region rather than the Fc-binding region is responsible for the activating property of the molecule.

In addition to the classical Fc$_\gamma$-Staph A interaction, several data indicate the existence of a common and variably expressed Staph A reactivity in at least four (IgG, IgM, IgA, and IgE) of five human immunoglobulins (7, 14). The alternative reactivity involves protein fragment B (the one involved in the classical reactivity) (8) and a structure located in the F(ab')$_2$ fragment of Staph A-reactive immunoglobulins (9, 33). It crosses species (9) and does not correlate with the light-chain type, subclass characteristics, or allotypic markers (7). Although there is no direct evidence to support an association between certain invariant or subgroup (framework) regions of the variable domain and the alternative Staph A reactivity, this association cannot be excluded (7). While the Fc fragment of IgG is monovalent in its reaction with Staph A, the alternative protein reactivity is bivalently expressed in F(ab')$_2$ fragments of human immunoglobulins (40). It is therefore possible that *S. aureus* Cowan 1 and Staph A cross-link some of the IgE on human basophils. This is borne out by the previously reported (23) correlation between the maximum percent histamine release induced by anti-IgE and that induced by Staph A. Furthermore, the possibility cannot be excluded that Staph A binds to the F(ab')$_2$ fragments of some of the IgG bound on basophil membranes (12, 13).

The alternative Staph A reactivity is essential for the occurrence of precipitation in vitro between IgG and Staph A as it provides the crucial link between Staph A and IgG for the formation of the three-dimensional lattice required for precipitation (8). Another biological effect of Staph A, complement activation, is at least partially mediated by the

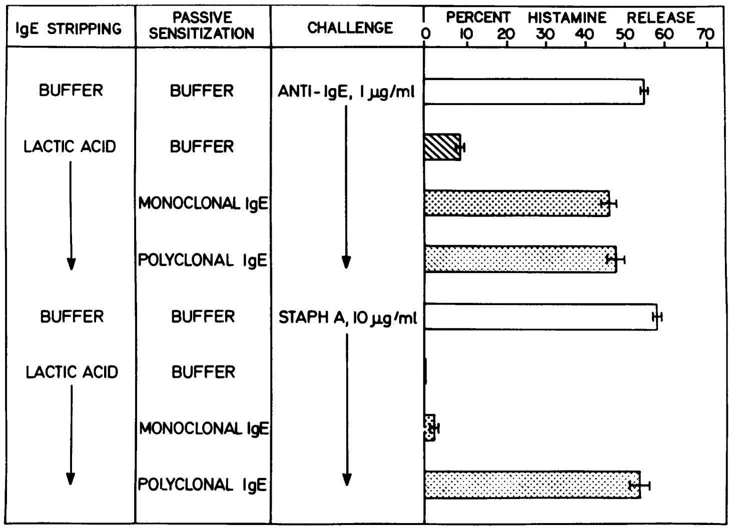

FIG. 7. Effect of treating basophils with lactic acid, followed by passive sensitization with polyclonal or monoclonal IgE on anti-IgE- or Staph A-induced histamine secretion from human basophils. Leukocytes were either untreated (buffer) or treated with lactic acid (0.01 M, pH 3.9, 5 min, 22°C) and then washed and incubated (20 min, 37°C) in the presence of 4×10^{-3} M EDTA and 10 μg of heparin per ml with or without human polyclonal IgE or IgE myeloma (ADZ) containing 50 μg of IgE per ml. These procedures are based on those of Pruzansky et al. (27). The leukocytes were washed extensively and then tested in a 45-min histamine release assay in the presence of anti-IgE or Staph A. Results are presented as mean ± standard error of the mean histamine release. Similar results were obtained in two other experiments with a different IgE myeloma (PS).

Fab-associated reactivity (9). An interaction between the alternative Staph A-binding site and surface IgM has been found to be mainly responsible for the mitogenicity of insolubilized Staph A (Staph A linked to Sepharose beads or present on the cell wall of *S. aureus* Cowan 1 bacteria) on human lymphocytes (29). Our results suggest that the alternative Staph A-binding activity is responsible for the activation of human basophil leukocytes to release inflammatory mediators. Therefore, the alternative Staph A-binding activity may represent an example of biologically significant interaction between bacterial antigens and immunoglobulins. Such an interaction has the potential to trigger defensive effector systems. It has been recently shown that other bacteria, such as *Streptococcus haemolyticus*, synthesize a protein, distinct from Staph A, which also binds to the $F(ab')_2$ fragment of some human polyclonal immunoglobulins (2). Therefore, it is possible that the alternative Staph A reactivity represents some kind of primitive, broad-spectrum, antibacterial antibodylike reaction by which some bacteria activate inflammatory cells. It is of interest that IgE also has this activity, and it suggests a different, perhaps beneficial, role of IgE in the activation of inflammatory cells.

S. aureus is the principal cause of hospital-acquired bacteremias (1) which, in some patients, result in septic and toxic shock syndromes (37) associated with hypotension, rash, and collapse. Furthermore, Staph A alone or immobilized on *S. aureus* Cowan 1 or on Sepharose has been used in serotherapy of cancer in humans (41). During this treatment patients often suffer from symptoms similar to those observed during anaphylaxis (16, 44). Histamine infusion in humans induces hypotension and skin rash (42, 43), and Staph A injection produces systemic anaphylaxis in nonimmunized guinea pigs (5). Therefore, the present results suggest that some of the symptoms associated with *S. aureus* bacteremia or serotherapy of cancer with Staph A are due to the release of histamine from peripheral blood basophils.

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