IGE AND IGG\(\text{A}\) ANTIBODY-MEDIATED RELEASE OF HISTAMINE FROM RAT PERITONEAL CELLS

II. INTERACTION OF IGG\(\text{A}\) AND IGE AT THE TARGET CELL*

BY MICHAEL K. BACH,‡ PH.D., KURT J. BLOCH, M.D., AND K. FRANK AUSTEN, M.D.

(From the Department of Medicine, Robert B. Brigham Hospital, the Clinical Immunology and Arthritis Units, Department of Medicine, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 2120)

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The existence, in several species, of two major types of homocytotropic antibodies has been recognized and reviewed previously (1). In the preceding paper (2) conditions were described for studying in vitro the antigen-induced release of histamine in the rat IGE and IGG\(\text{A}\) antibody-mediated systems. The present paper further characterizes the properties of the IGG\(\text{A}\) antibody-mediated reaction and presents evidence that homologous IGG\(\text{A}\) and IGE antibodies utilize the same cellular receptors in the pathway leading to the release of histamine.

**Materials and Methods**

The media, antigen, and antibody preparations used in this study were the same as those previously described (2). Except where specifically indicated, the conditions of the experiments were those found to yield optimum results in the previous study (2).

**RESULTS**

**Characteristics of the IGG\(\text{A}\) Antibody-Mediated System**

Evidence for the Transient Interaction of IGG\(\text{A}\) Antibody and Target Cell.— It has been previously shown that mouse IGG\(\text{I}\) antibodies prepare mixed mouse peritoneal cells for antigen-induced release of histamine in vitro (3). The interaction of mouse IGG\(\text{I}\) antibodies and peritoneal cells is transient and does not persist after washing of the cells. The characteristics of the rat IGG\(\text{A}\) antibody–target cell interaction were explored in the following experiments.

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‡ Sabbatical awardee of The Upjohn Company; present address: Department of Hypersensitivity Diseases Research, The Upjohn Company, Kalamazoo, Mich. 49001.
Rat peritoneal cell suspensions were exposed to an IgGa anti-dinitrophenyl (DNP) antibody fraction for 1 hr at 30°C, followed by the addition of dinitrophenyl-bovine serum albumin (DNP-BSA) antigen; a net histamine release of 9% was achieved. In contrast, there was no specific release of histamine after the addition of antigen to cells which had been washed once after exposure to the IgGa antibody fraction (Table I). In another experiment, peritoneal cells were incubated in medium I for 1 hr at 30°C, followed by the simultaneous addition of IgGa antibody fraction and antigen; a net histamine release of 13% was achieved. Addition of antigen to cells treated with the IgGa antibody fraction for 1 hr yielded a net histamine release of 16% in this experiment (Table I). The findings in this series of experiments suggest that the IgGa antibody-mediated reaction does not require a latent period for preparing target cells for antigen-induced release of histamine and that this antibody is not firmly bound to the target cell.

Effects of Normal Rat Serum.—Since IgGa antibodies can activate the complement system in the presence of antigen (4), it was of interest to determine whether normal rat serum would potentiate the IgGa antibody-mediated release of histamine. Fig. 1 shows the results obtained by adding normal rat serum to the IgGa antibody fraction and antigen.

**TABLE I**

| Prechallenge treatment | Additions made at time of challenge | Net histamine release (%) |
|------------------------|-----------------------------------|--------------------------|
| A. Antibody*           | Antigen                           | 9                        |
| Antibody, wash         | Antigen                           | 0                        |
| B. Antibody‡           | Antigen                           | 16                       |
| None                   | Antigen and antibody              | 13                       |

* Eight tubes, each containing 2.5 × 10⁶ mixed peritoneal cells suspended in medium I (but without added Ca²⁺, Al³⁺, or heparin) were incubated for 1 hr at 30°C. IgGa antibody fraction was added at a dilution of 1:10 as indicated; antigen challenge was initiated by the addition of 0, 100, 500, 1000, 2000, and 5000 ng/ml DNP-BSA to duplicate tubes with mixing. The final volume in each tube was 80 μl and the cell buttons contained a total of 2600 ng histamine. Antigen challenge was continued for 15 min at 30°C. Maximum net release was obtained at 1000 ng/ml DNP-BSA; spontaneous release of histamine was 1.2%.

‡ Conditions as above, except that antibody fraction was used at a dilution of 1:25 and antigen at 0, 500, 1000, 2000, and 4000 ng DNP-BSA/ml. Cells in each tube contained 1280 ng histamine; spontaneous release was 2.5%.

1 Abbreviations used in this paper: DNP, dinitrophenyl; DNP-BSA, dinitrophenyl-bovine serum albumin; SRS-A, slow reacting substance of anaphylaxis.
INTERACTION OF IgGa AND IgE AT THE TARGET CELL

serum \((CH_0 [5] = 120 \text{ units/ml})\) or complement-depleted rat serum \((CH_0 = 5.6 \text{ units/ml})\) to the IgGa antibody-mediated system. Complement-depleted serum was obtained from rats treated 45 hr previously with 3 units of cobra venom factor \((6, 7)\). The presence of either serum in concentrations up to 1\% potentiated the release of histamine; this effect was diminished at higher serum concentrations. The results of these experiments suggest that the effect of serum did not depend upon an intact complement system.

In the range of serum concentration from 3 to 5\%, the effect on histamine release was variable; enhancement was observed in experiments shown in Fig. 1 and inhibition was observed in experiments shown in Fig. 2. However, at higher concentrations of serum, inhibition of histamine release was consistently observed (Fig. 2).

**Effect of Exposure of Peritoneal Cells to an IgGa Antibody Fraction and Antigen at 0°C.**—It has been previously noted in other in vitro systems that exposure of sensitized tissues or cells to antigen at 0°C does not lead to the release of histamine. Furthermore, rewarming of such tissues after a suitable interval also does not lead to the release of histamine, even after the introduction of additional antigen \((8, 9)\). Rat peritoneal cells were exposed to an IgGa anti-DNP antibody fraction and antigen at 0°C for various intervals and were

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**Fig. 1.** Effect of added normal rat serum (\(\bullet, ---\)) and serum from cobra venom factor-treated rats (\(\bigcirc, \ldots\)) on release of histamine in vitro in the IgGa antibody-mediated system. Incubation medium and conditions are cited in Table I. Maximum net release was obtained with 500 ng of DNP-BSA/ml.
then rewarmed and held at 30°C for 30 min. The net release of histamine was 32, 14, and 4% after preincubation for 0, 60, and 150 min at 0°C, respectively. The decrease in the amount of histamine released in this experiment might have been due to the inactivation of the target cells by either cold incubation per se or in the presence of antigen-antibody complexes, or due to the inability of antigen-antibody complexes formed during prolonged incubation to induce the release of histamine after the rewarmed period. To evaluate the first possibilities, peritoneal cells were held at 0°C for 1 hr, washed, and exposed to an IgGα antibody fraction and antigen at 30°C; a net histamine release of 33% was achieved. Control cells challenged without prior incubation at 0°C with the same antibody-antigen mixture showed a net release of histamine of 40%. In contrast, cells exposed to an IgGα antibody fraction and antigen at 0°C for 1 hr, washed, and rechallenged with antibody and antigen at 30°C, showed a reduced net histamine release of 11% (Table II). Thus, while incubation of peritoneal cells alone at 0°C may have impaired slightly their subsequent response to challenge at 30°C, treatment with antigen and antibody at 0°C led to significant inactivation.
The effect of preformed antigen–antibody complexes on antigen-induced release of histamine was also evaluated in this experiment (Table II). An IgGa antibody fraction and antigen were added to medium II at concentrations which ordinarily produce maximum release of histamine from peritoneal cells. The solutions were held for 1 or 10 hr at 0°C before the addition of target cells. Incubation of cells with either of these mixtures for 30 min at 30°C resulted in a net release of histamine of 24%. It should be noted that the release of histamine by complexes formed during 1 hr at 0°C (24%) exceeded the release achieved when the target cells were present together with the complexes during 1 hr at 0°C, and the mixture subsequently rewarmed and re-

**TABLE II**
Specific Inactivation of Target Cells by Treatment with IgGa Antibody and Antigen at 0° and 30°C

| Prechallenge treatment | Challenge treatment | Net histamine release (%) |
|------------------------|---------------------|---------------------------|
| None                   | Normal*             | 40                        |
| IgGa antibody, 0°C for 1 hr, wash | Normal            | 33                        |
| IgGa antibody and antigen, 0°C for 1 hr,† wash | Normal            | 11                        |
| None                   | Preformed complex§  | 24                        |
| IgGa antibody and antigen, 30°C for 1 hr, wash | Normal            | 0                         |

* Conditions as in Fig. 1, paper I(2), except that the concentration of the IgGa anti-DNP antibody fraction was 1:25, and antigen concentration for maximum release was 250 ng DNP-BSA/ml. Duration of challenge was 75 min. The cells in each tube contained 2000 ng of histamine; spontaneous release of histamine was 2.2–6.7%.

† Conditions were the same as for the challenge treatment, using the same concentrations of antigen, antibody, and serum. After the first challenge, cells were washed twice in medium II and resuspended in 80 µl of medium II for challenge treatment. There was no net release of histamine during challenge at 0°C.

§ IgGa anti-DNP antibody fraction and antigen were mixed in medium II and held for 1 hr at 0°C before the addition of peritoneal cells suspended in 80 µl of medium II.

challenged (11%). Based on these observations, it is concluded that the reduction in release of histamine after prolonged incubation of target cells, antigen, and IgGa antibody fraction involves both a partial inactivation of the target cell and a decreased effectiveness of preformed complexes.

Peritoneal cells were also treated with antigen and IgGa antibody fraction for 1 hr at 30°C, washed, and rechallenged with a fresh dose of antigen and antibody. No release of histamine was observed after the second challenge (Table II), although approximately 55% of the initial histamine present remained within the cells.

**Interaction of IgGa and IgE at the Target Cell**

The interaction of IgGa and IgE antibodies at the target cell was evaluated in three different experimental designs: by testing the effect of adding an
IgGa anti-DNP antibody fraction and DNP-BSA antigen at the time of challenge with worm antigen of peritoneal cells prepared with IgE anti-worm antibodies; by testing the effect of challenging cells prepared with IgE anti-worm antibodies with IgGa anti-DNP antibody and DNP-BSA before challenge with worm antigen; and by testing the effect of the presence of an added IgGa anti-DNP antibody fraction during the preparation step of the IgE antibody-mediated system.

The effect of the IgE antibody-mediated system in the fluid phase on the IgGa antibody-mediated reaction was not investigated. Rat anti-worm antisera rich in IgE antibodies could not be used for this purpose because of the presence of IgGa anti-worm antibodies and because of the interference of nonspecific IgGa in the IgGa antibody-mediated system.

**Combined IgGa and IgE Antibody-Mediated Release of Histamine.**—Peritoneal cells prepared with rat anti-worm antibodies showed a net release of histamine of 19% on exposure to worm antigen; similarly prepared cells exposed to an IgGa anti-DNP antibody fraction and DNP-BSA antigen showed a net release of 25%. Cells prepared with IgE antibody and exposed simultaneously to both specific worm antigen and the mixture of IgGa anti-DNP antibody and DNP-BSA showed a net release of histamine of 54% (Table III). The slight synergistic effect observed was not considered to be significant.

**Inactivation of Cells Prepared with IgE Antibody by Subsequent Exposure to an IgGa Antibody-Antigen Mixture.**—Rat peritoneal cells prepared with IgE anti-worm antibodies, washed, and held for 1 hr at 0°C, showed a net histamine release of 31% upon challenge with worm antigen at 30°C (Table IV). Another

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**TABLE III**

**Effect of the Simultaneous Addition of both *Nippostrongylus Brasiliensis* Antigen and IgGa Anti-DNP Antibody Fraction and DNP-BSA Antigen to Cells Previously Prepared with Anti-*N. Brasiliensis* Antiserum Rich in IgE Antibodies**

| Additions during challenge | Cells prepared with IgE antibody | Net histamine release (%) |
|----------------------------|---------------------------------|--------------------------|
| *N. brasiliensis* antigen   |                                 | 19                       |
| IgGa anti-DNP antibody fraction and DNP-BSA | 25                             |
| IgGa anti-DNP antibody fraction, DNP-BSA, and *N. brasiliensis* antigen | 54                             |

* Cells prepared as described in Fig. 1, paper I(2). After washing, cells were suspended in 80 μl of medium II.
† *N. brasiliensis* antigen was added at a dilution of 1:16, IgGa antibody fraction at 1:80 dilution, and DNP-BSA at 250 ng/ml. The cells in each tube initially contained 1000 ng of histamine; spontaneous release was 15%.
sample of the cells prepared with IgE antibodies were held for 1 hr at 0°C in the presence of an IgGa anti-DNP antibody fraction and DNP-BSA antigen and were washed and exposed to worm antigen at 30°C; a net histamine release of only 5% was observed. Exposure of peritoneal cells prepared with IgE antibodies to an IgGa anti-DNP antibody fraction and DNP-BSA at 30°C for 30 min led to a net release of histamine of 26%; after washing and exposure to worm antigen, the net release of histamine achieved was 6%. In contrast, cells prepared with IgE anti-worm antibodies held at 30°C for 30 min and then challenged with worm antigen in the standard procedure showed a net histamine release of 21%. Thus, pretreatment of peritoneal cells prepared with IgE anti-worm antibodies, with IgGa antibody and antigen under conditions which either do (30°C) or do not (0°C) lead to histamine release, markedly inhibited release of histamine provoked by specific worm antigen.

**Competition by IgGa Antibody with IgE Antibody during the Preparation of Peritoneal Cells.**—The addition of IgGa anti-DNP antibody fraction to sera rich in IgE anti-worm antibodies during the preparation of peritoneal cells caused a marked decrease in the amount of histamine released during challenge with worm antigen (Fig. 3). This decrease was more pronounced the more IgGa anti-DNP antibody fraction added during the preparation step, and fur-
thermore, appeared to be reversed when a higher IgE anti-worm antibody concentration was used. It should be stressed that inhibition occurred in these experiments without the addition of DNP-BSA antigen during either phase of the IgE antibody-mediated reaction.

**Fig. 3.** Effect of added IgGa anti-DNP antibody fraction on the preparation of mixed peritoneal cells with anti-*N. brasiliensis* antiserum rich in IgE antibodies. Conditions as in paper I, Fig. 1 (2). The cells in each tube initially contained 1500 ng of histamine; spontaneous release of histamine was 5-7%. Results, obtained with a 1:80 dilution of IgE antibody-rich serum (●, −−−−) and with a 1:16 dilution of IgE antibody-rich serum (○, −−−−), are the averages of quadruplicate determinations and bars represent the standard deviation of the mean.

**DISCUSSION**

Two lines of evidence indicate that firm binding of rat IgGa antibody to receptor sites2 on homologous target cells is not a prerequisite for the subse-

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2 The concept of receptor sites for homocytotropie antibodies on target cells is an attractive hypothesis; little is known of the surface structure which might be involved. This paper suggests that receptor sites may be complex, involving both a binding site and an activation site in the pathway to release of histamine.
quent antigen-induced release of histamine. Release of histamine was not improved by interaction of the target cells with IgGa antibody before challenge with antigen and washing of target cells previously interacted with IgGa antibody completely prevented the subsequent antigen-induced release of histamine (Table I). Furthermore, preformed immune complexes were active at a concentration of antigen and antibody which was the same as that used routinely in the IgGa antibody-mediated system. In contrast, IgE antibody not only required a latent period for optimal sensitization (2), but also remained bound to the target cell through multiple washings (10).

The finding that IgGa antibody does not bind firmly to the target cell may account for the striking difference in the time course of histamine release observed in the IgGa antibody-mediated system (2) as compared with that observed in the IgE antibody-mediated system (10). As noted in the companion paper (2), release of histamine from cells prepared with rat anti-\(N.\) brasiliensis antiserum and challenged with worm antigen was complete within 5 min. Release of histamine by IgGa anti-DNP antibody fraction with DNP-BSA antigen did not reach peak values for 90 min. The latter observation suggests that the IgGa antibody–antigen complex may trigger the release of histamine by interacting with multiple activation sites on the target cell because of its transient interaction with any one site. It is of interest that an increase in the ionic strength of the incubation medium (2) not only diminished the release of histamine in the IgGa antibody-mediated system but also restricted release to the first 5 min of interaction. Alteration in ionic strength of the medium may limit the capacity of IgGa antibody–antigen complexes to interact with multiple sites.

The effect of normal serum in the IgGa antibody-mediated system was twofold. The inhibitory action of normal rat serum in relatively high concentration (Fig. 2) was presumably due to competition by nonspecific IgGa and IgE antibodies in the fluid phase. Normal mouse serum had been previously reported to inhibit the release of histamine from mouse peritoneal cells treated with IgG1 antibody and antigen (11). The basis of the enhancing effect of serum (Fig. 1) is not clear. The enhancement did not require the presence of an intact complement system, since C3-deficient serum obtained from cobra venom factor-treated rats was fully active.

The inactivation of target cells by exposure to IgGa antibody and antigen in the cold (Table II) most likely reflects the sterile activation of the pathway to histamine release. Presumably, the slowing of energy-generating metabolic steps at 0°C prevents release of histamine in the cold, while the concomitant decay of some essential intermediate prevents the subsequent release of histamine on rewarming either in the presence or absence of added immune reactants. Two lines of evidence suggest that inactivation results from a specific effect of treatment in the cold on the target cells. Cells incubated alone or in
the presence of IgGa antibody fraction at 0°C and subsequently rewarmed and challenged with IgGa antibody fraction and antigen at 30°C demonstrated nearly normal release of histamine, and immune complexes preformed at 0°C were capable of initiating the release of histamine from target cells under standard challenge conditions. The release of histamine provoked by preformed immune complexes was about two-thirds as great as that achieved with complexes formed at 30°C in the presence of the target cell. While it is possible that a tightly formed complex is less able to interact with the activation site on the target cell than is a complex in the earlier stages of its formation, the effect noted here is less than that observed in a mouse IgG1 system (3).

In four experiments not presented, rat peritoneal mast cells prepared with anti-N. brasiliensis antiserum rich in IgE antibodies failed to be inactivated by exposure to worm antigen at 0°C for up to 120 min. On rewarming the reaction mixture, histamine release achieved was the same as that of a duplicate cell preparation first challenged at the usual release temperature of 30°C. The failure of the IgE antibody-mediated system studied to inactivate cells upon interaction with antigen at low temperature is in contrast to the observations with the IgGa antibody-mediated system investigated (Table II). Several explanations may be considered. If inactivation of the target cell requires sterile activation of a large proportion of the receptor sites on the cell, then the firm fixation of IgE antibodies to relatively few sites would limit such a process. Secondly, the biochemical requirements of the IgE antibody-mediated pathway may be so limited that the pathway does not decay appreciably after sterile activation. Finally, and perhaps most likely, the interaction of IgE antibody and the worm antigen may not activate the release mechanism at reduced temperature.

The interaction between the IgGa and IgE antibodies at the target cell appears to involve a common receptor site and, at least in part, a common pathway to the release of histamine. The evidence for a common receptor was demonstrated by the ability of IgGa anti-DNP antibody to diminish the capacity of IgE anti-N. brasiliensis antibodies to prepare the target cell during a 2 hr incubation period at 30°C for subsequent worm antigen-induced release of histamine (Fig. 3). Since IgGa antibodies did not bind to the target cell, the effective competition between IgGa and IgE antibodies observed may be due to the continual, but transient, occupation of receptor sites by IgGa antibodies present in the fluid phase. Such competition between two immunologically distinct homologous immunoglobulins for sites on the target cell has not been previously observed in model systems. It is not based upon competition for antigen since the specificity of the two immunoglobulins was different.

The interrelationship between IgGa and IgE antibodies at the target cell is not limited to the receptor site, but also involves some common point in the
pathway to mediator release. The latter was indicated by the finding that incubation of target cells with IgGa anti-DNP antibody and antigen at low temperature for an appropriate time period not only inactivated the cell for histamine release by the IgGa antibody-mediated system (Table II), but also inactivated the cell for histamine release by worm antigen in the IgE antworm antibody-mediated system (Table IV). Additional evidence for a common point in the release pathway initiated by interaction of either immunoglobulin with specific antigen comes from the finding that activation of an IgE antibody-prepared cell by treatment with the IgGa antibody-mediated system at 30°C so as to achieve histamine release prevents further histamine release upon challenge at the same temperature with the worm antigen.

The observation that the effects of the IgGa and IgE antibody-mediated systems were additive when combined challenge was carried out (Table III), but that they were competitive when employed in sequence to challenge the target cell (Table IV) or when both immunoglobulins were present during the IgE antibody preparation step (Fig. 3), requires further comment. It seems most likely that the additive effect observed was related to the ability of IgGa antibody-antigen complexes to find unoccupied sites on the IgE antibody-prepared cell during the prolonged challenge period. Under these circumstances, activation of the pathway to histamine release by the IgE antibody-mediated system appears not to interfere with the utilization of the same pathway by the IgGa antibody-mediated system.

Heat-labile homocytotropic antibodies have been recognized functionally in the mouse (12) and guinea pig (13) and by both functional and immunochemical characteristics in the rabbit (14). These same species have also been shown to possess heat-stable 7S immunoglobulins which prepare a skin site for cutaneous anaphylaxis (3, 15, 16), or certain target tissues or cells in vitro for histamine release (3, 16, 17). In the rat, IgE (18) and IgGa (5) antibodies have been previously shown to mediate both cutaneous anaphylaxis and the intraperitoneal release of histamine. Monospecific antisera directed against either of these immunoglobulins removes the activities attributed to one without affecting those of the other (18, 19). The finding that despite their immunochemical differences these immunoglobulins share common points of interaction at the target cell may have broad implications. It has been suggested that, in the rat, mast cell-bound IgE antibodies mediate the antigen-induced release of histamine, while complexes of IgE antibodies and specific antigen formed in the fluid phase induce the release of slow reacting substance of anaphylaxis (SRS-A) from the same target cell (18). Under in vivo conditions in which there is no time limit for the fixation of IgE antibodies to the target cell, nonspecific IgGa would not be expected to compete effectively for receptor sites. On the other hand, under circumstances in which the biologic effects of IgE antibodies depended upon a complex with antigen present transiently in the fluid phase, nonspecific IgGa would be expected to com-
pete successfully for receptor sites on target cells. If under these circumstances the IgGa and IgE antibodies were of the same specificity, then additive effects would be expected. Terr and Betz (20) have reported finding in man a short latency period heat-stable antibody to horse serum which mediates a wheal-and-flare reaction in human skin. This antibody may represent an equivalent of the rat IgGa. The possibility that such antibodies develop during the course of immunotherapy of human allergic disease must be considered, and if found, their influence on the course of human disease investigated.

SUMMARY

IgGa, in contrast to IgE, antibodies mediated the antigen-induced release of histamine from rat peritoneal mast cells without a requirement for a latent period and without the capacity to bind firmly to the target cell. Nonetheless, IgGa anti-DNP antibody interfered with the capacity of rat anti-*N. brasiliensis* antiserum rich in IgE antibodies to prepare the target cells for histamine release by worm antigen. Further, interaction of IgE antibody-prepared cells with IgGa anti-DNP antibody and DNP-BSA at 0°C so as to achieve sterile activation, or at 30°C to permit histamine release, inactivated such cells as determined by the subsequent failure to release histamine upon challenge with worm antigen. Thus, although IgE and IgGa antibodies are immunologically distinct homologous immunoglobulins and exhibit different functional characteristics, their interaction at the target cell involves a common receptor and at least one common point in the pathway to the release of pharmacologic agents from the cell.

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