Immunology, 1970, **19**, 831.

In vitro Reversed Anaphylaxis: Characteristics of Anti-IgE Mediated Histamine Release

L. M. LICHTENSTEIN, D. A. LEVY AND K. ISHIZAKA

School of Medicine and School of Hygiene, The Johns Hopkins University, Baltimore, Md. and Childrens Asthma Research Institute and Hospital, Denver, Colorado, U.S.A.

(*Received 9th March* 1970)

Summary. Leucocytes from allergic and most normal human donors release histamine when challenged with antibodies against human γ-globulin. This reaction (reversed *in vitro* anaphylaxis) is due primarily to anti-IgE antibodies although there is some response in most donors to antisera against IgG even after it has been absorbed with light and ε chains. The anti-IgE is, however, several 100-fold more potent than the anti-IgG. By passive sensitization of the leucocytes of a normal donor with serum from a ragweed-allergic patient it was shown that the normal cells became sensitive to anti-IgE and ragweed antigen E at the same time; in both cases, there was an inverse relationship between the serum concentration used for passive sensitization and the concentration of antigen or antibody required for histamine release. There is a rough correlation ($r_s = 0{\cdot}42$; $P<0{\cdot}01$) between the serum IgE concentration and the response of leucocytes from allergic donors to anti-IgE and an excellent correlation ($r_s = 0{\cdot}82$; $P<0{\cdot}01$) between the response of the cells to ragweed antigen E and anti-IgE. There is also a strong parallel between the mechanism of direct, antigen mediated histamine release and the reversed reaction induced by anti-IgE. Both appear to be non-serum requiring, non-cytotoxic, secretory-like responses which are inhibited by theophylline, cyclic AMP and colchicine. These data suggest that cell bound IgE is of major importance in the *in vitro* anaphylactic response and that the direct and reversed *in vitro* anaphylactic reactions both operate through cell-bound IgE and share a common reaction mechanism.

INTRODUCTION

Reversed anaphylaxis, the induction of an allergic reaction by the introduction of antibodies against immunoglobulins which are cell 'fixed', has been studied with a number of experimental systems and for a variety of purposes (Ovary, 1960; Keller, 1962; Humphrey Austen and Rapp, 1963). The present study is based on previous work in which we showed that antibodies made against a crude preparation of human γ-globulin (HGG) were able to release histamine from human leucocytes (Osler, Lichtenstein and Levy, 1964). At the time, this reaction was attributed to antibodies against IgG, but with the demonstration that IgE was the major, or perhaps the only human immunoglobulin capable of mediating anaphylactic reactions in man (Ishizaka and Ishizaka, 1967), it seemed appropriate to re-study this reaction. We found that a large part of the histamine releasing ability of the anti-HGG was removed by absorption with human light chains. Specific antisera against

Correspondence: Dr L. M. Lichtenstein, Department of Allergy and Infectious Disease, The Johns Hopkins Hospital, Baltimore, Maryland, 21205, U.S.A.

IgG and IgE were then compared. Although the anti-IgG had some activity, the anti-IgE was far more potent. The main part of this work utilized the latter reagent and was devoted to exploring the characteristics of the reversed *in vitro* anaphylactic reaction as contrasted to the direct antigen-induced response. In these studies we used as a model the technique involving histamine release from isolated human leucocytes (Lichtenstein and Osler, 1964); this report confirms and extends a recent publication (Ishizaka, Ishizaka, Johansson and Bennich, 1969).

MATERIALS AND METHODS

Antiserum

The anti-human γ-globulin was a hyperimmune serum prepared by immunization of rabbits with Cohn Fraction II emulsified in Freund's complete adjuvant. The preparation of guinea-pig antiserum specific for IgE and a rabbit antiserum specific for IgG has been described previously (Ishizaka and Ishizaka, 1968). The immunoglobulins used for immunization were prepared as follows: the fragments of normal IgG were obtained by the method of Porter (1959). The protein was digested with crystalline papain (2× crystallized, Mann Research Laboratory, New York) at an enzyme : substrate ratio of 1 : 100 in the presence of 0·01 M cysteine and 2 m-moles of EDTA. The digest was fractionated by the method of Rowe (1962) to isolate the Fc and Fab fragments. E myeloma protein was isolated from a myeloma patient's serum by DEAE cellulose column chromatography followed by gel filtration. Proteins eluted from a DEAE cellulose column between 0·005 M and 0·025 M phosphate buffer, pH 8·0, were concentrated and applied to a Sephadex G-200 column to remove IgG. A 1 per cent solution of the purified E myeloma protein did not contain any detectable amount of the other serum protein by immunoelectrophoresis.

A rabbit anti-IgE serum, prepared by immunization with an E myeloma protein was also employed. Each rabbit received 1 ml of E myeloma protein included in Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) into footpads and the same amount of antigen every 2 weeks intramuscularly. 2 weeks after the third immunization, the rabbits were bled and anti-light chain antibodies in the antiserum were absorbed with normal IgG. The antiserum gave a single precipitin band of identical specificity with isolated normal IgE and an atopic patient's serum (AR) which contained 16 μg/ml of IgE.

Quantitative measurement of IgE

The concentration of IgE in patients' sera was measured by radial immunodiffusion in antibody-containing agar. Since the concentration of IgE in specimens was low, the sensitivity of anti-IgE immunoplates was increased by employing an additional step with ^{131}I labelled goat anti-RGG (Rowe, 1969). The 7S fraction of a goat anti-RGG serum (Antibody Inc., Davis, California) prepared by gel filtration through a Sephadex G-200 column was labelled with ^{131}I by the method described by McConahey and Dixon (1966). A 3 per cent agarose in 0·3 M phosphate buffer, pH 8·0, was mixed with an equal volume of a 1 : 1600 dilution in 0·1 per cent BSA of the γ-globulin fraction of rabbit anti-IgE serum and immunoplates were prepared. The concentration of anti-ε chain (Fc) antibody in the immunoplate was 0·23 μg N/ml. Antigen wells (4 mm diameter) were filled with serum samples to be tested. Serial two-fold dilutions of an atopic patient's serum (AR) were used as an IgE reference standard. After 2 days incubation at room temperature, the

plates were washed for 2 days and ^{131}I labelled anti-RGG was overlaid on the plates. 24 hours later, the plates were washed again for 2 days, dried and X-ray film (Kodak Medical X-ray film NS 547) applied. The diameters of the radioactive precipitin rings on the X-ray films were measured with magnification. By this method 100 ng/ml or more of IgE could be quantitatively measured and 50 ng/ml of the protein could be detected.

Histamine release techniques

Leucocytes were isolated from the peripheral blood of normal or ragweed pollen sensitive donors by dextran sedimentation, followed by differential centrifugation and washing, as previously described (Lichtenstein and Osler, 1964). Histamine release was induced by the addition of antisera or antigen to the washed cells suspended in a Tris buffer with added Ca^{++}, Mg^{++} and human serum albumin (Lichtenstein and Osler, 1964). Serum was not added unless specified. The reaction was allowed to proceed for 60 minutes at 37°, and the amount of histamine released determined by a fluorometric method (Shore, Burkhalter and Cohen, 1959; Lichtenstein and Osler, 1964). When inhibitors were used, they were dissolved in buffer and added to the cell suspension immediately before the antisera or antigen. Colchicine was purchased from the Sigma Chemical Company and dibutyryl-cyclic-3′, 5′-adenosine monophosphate from Calbiochem. Passive sensitization was carried out by incubating the leucocytes from normal donors in the stated dilutions of reaginic serum (DiGu) for 120 minutes at 37° in the presence of 4×10^{-3} M ethylenediaminetetraacetic acid and 10 μg/ml of heparin (Levy and Osler, 1966; Levy and Osler, 1967a).

K^+ efflux studies

^{42}K or ^{86}Rb was incorporated into leucocytes by incubation of the isolated cells for 30 minutes at 37° in a solution containing 300–500 μCi of the chloride salt of the isotope. The cells then were washed and reacted with appropriate antiserum or antigen. The rate of loss of isotope in experimental tubes was determined in any experiment relative to the rate of efflux in control tubes, as previously described (Lichtenstein and Osler, 1966). Histamine release was determined from the same cell preparations. The interpretational problems that accrue from the differential location of histamine and potassium in the cell population have been discussed previously (Lichtenstein and Osler, 1966).

Patient selection

Donors were judged allergic to ragweed pollen when they gave an appropriate medical history and their leucocytes released histamine on challenge with ragweed antigen E.

RESULTS

When well-washed leucocytes are challenged with an antiserum made against Cohn Fraction II histamine is released without the necessity to add any serum factors (Osler *et al.*, 1964). In order to determine whether this histamine release was actually due to antibodies against IgG molecules, the serum was absorbed with the Fab fragment of normal IgG until there was no residual anti-light chain activity as determined by gel diffusion. This absorption procedure removed over 99 per cent of the histamine releasing activity when the antiserum was tested on several donors: an example is shown in Fig. 1.

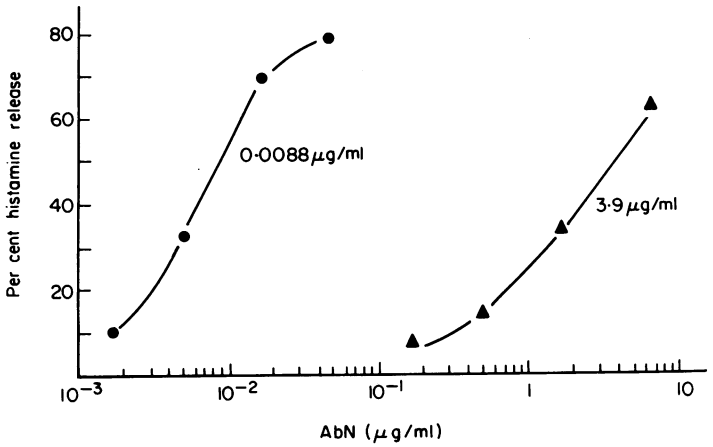

FIG. 1. Histamine release by an antiserum against human Cohn Fraction II before (●) and after (▲) absorption with the Fab fragments of HGG. Loss of activity = 99·8 per cent.

These data suggested that either anti-light chain antibodies were far more effective than anti-γ chain antibodies or that the histamine release was being triggered by antibodies directed against an immunoglobulin other than IgG.

To study the question of specificity we used an antiserum prepared against the Fc fragments of IgG as well as a guinea-pig antiserum made against IgE and absorbed until it was monospecific for the epsilon chain determinants. Dose–response curves were run with these antisera, using leucocytes from ten allergic donors. From 44 to 100 per cent histamine release was obtained with the anti-IgE serum, whereas no more than 36 per cent release was obtained with 0·6 μg of antibody nitrogen per ml of the anti-IgG (Fc) serum, the highest concentration used. It was, however, possible to estimate the minimum concentration of anti-IgG required for 50 per cent histamine release by extrapolation, and the relative effectiveness of the two antisera was then calculated from the ratio of the concentration of each required for 50 per cent histamine release. The anti-IgG : anti-IgE ratio for the cells of these ten donors varied from 100 to 430 (Table 1). Therefore, antibodies specific for IgE appeared much more potent as inducers of the reversed anaphylactic

TABLE 1

HISTAMINE RELEASE FROM HUMAN LEUCOCYTES BY ANTI-IgE AND ANTI-IgG

Patient	Anti-IgE*	Anti-IgG† (per cent)	Minimum ratio $\frac{\text{a-IgE}}{\text{a-IgG}}$
AD	0·013	36	160
DE	0·0038	25	260
CO	0·0093	17	215
MO	0·0037	22	260
FE	0·038	5	210
WA	0·0054	34	185
WE	max. 44%	0	—
DO	0·0023	22	430
LY	0·013	18	230
PA	0·010	25	100

* μg/ml AbN required for 50 per cent histamine release.
† Histamine release at 0·6 μg AbN/ml.

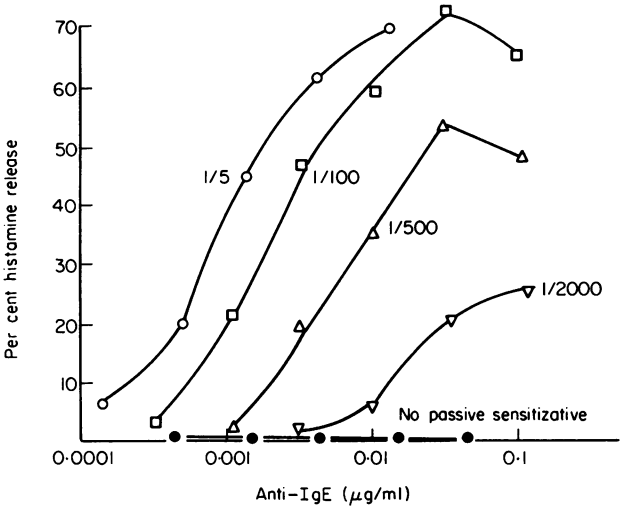

FIG. 2. Histamine release by anti-IgE from cells passively sensitized with various dilutions of serum from a ragweed-sensitive patient.

reaction than did those antibodies specific for IgG. Nevertheless, it should be pointed out that there was a definite response to anti-IgG with cells from most of these donors. Moreover, when the anti-IgG was mixed with myeloma IgE to remove any trace amounts of anti-IgE it did not decrease the activity of the anti-IgG preparation. Whether this release is due to contamination by antibodies other than those against γ chains or is specific for cell bound IgG is under current investigation.

As suggested by the report of Ishizaka *et al.* (1969) and on the basis of the above data, it seemed likely that IgE was the main cell-bound immunoglobulin responsible for histamine release of both the direct and reversed types. This conclusion is strengthened by the following experiments. In the first we used leucocytes from a non-allergic individual which

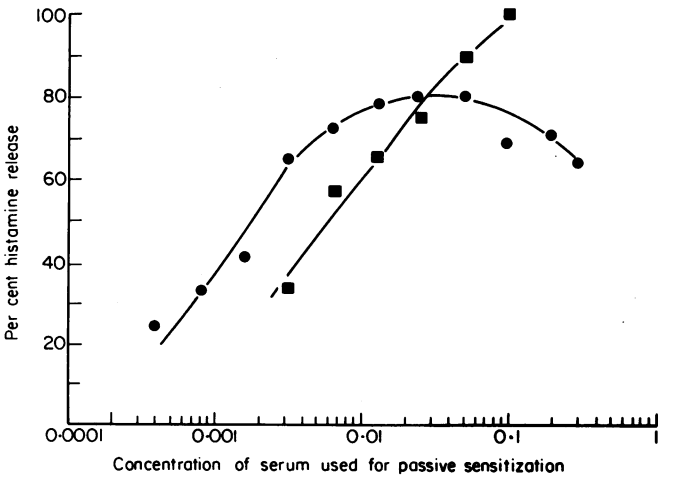

FIG. 3. Histamine release by antigen E (■) and anti-IgE (●) from cells passively sensitized with several concentrations of serum from a ragweed-sensitive patient.

released no histamine when reacted with either anti-IgE or with ragweed antigen E (Fig. 2). The cells were passively sensitized with several dilutions of a potent reaginic serum from a ragweed-sensitive donor (DiGu) and then challenged with graded doses of anti-IgE. The extent of histamine release is a function of the concentrations of both the reaginic serum and the anti-IgE antiserum. Increasing concentrations of reaginic serum in the sensitization stage produced increasing sensitivity to anti-IgE. A similar finding was observed previously with respect to sensitivity to ragweed antigen (Levy and Osler, 1967b). Thus, there is an inverse relationship between the amount of IgE fixed by passive sensitization to a cell membrane and the concentration of either anti-IgE or pollen antigen required for histamine release. Fig. 3 shows another experiment in which we carried out a titration of the same serum on these cells with respect to both the reversed and direct reaction. The antigen or anti-IgE concentration was optimal for each serum dilution. The maximal response to antigen E was 100 per cent and to anti-IgE 80 per cent. The cells appeared relatively more sensitive to anti-IgE than to antigen E, since a 50 per cent response for release with anti-IgE required sensitization in serum diluted 600-fold, whereas release to the same extent with antigen E required sensitization with a 1 : 220 dilution of serum.

The clear-cut increase in sensitivity to anti-IgE after passive sensitization (Fig. 2) was the exception rather than the rule: in testing leucocytes from thirteen other non-allergic donors, an increase in sensitivity to anti-IgE could be demonstrated with leucocytes from only one other individual. As Levy and Osler (1966) have reported, the leucocytes from normal donors are passively sensitized to different degrees. The explanation for these differences between individuals is not known. A reasonable hypothesis, however, was that the ability to be passively sensitized is a function of the amount of IgE already present on the membrane of the leucocytes; that is, cells whose IgE 'binding sites' were already almost saturated would not become sensitized as readily as cells with fewer sites occupied by IgE molecules. If this hypothesis were true, the cells that were the most easily sensitized should

TABLE 2

MAXIMAL HISTAMINE RELEASE AFTER CHALLENGE WITH AgE AND ANTI-IgE*

Donor	AgE† (per cent)	Anti-IgE‡ (per cent)
HN	0	34
ER	0	23
RL	0	51
FC	11	93
JH	24	91
AR	38	26
BH	39	32
RW	43	43
BR	46	24
KL	54	30
BHO	60	54
CC	60	58
GE	65	85
PN	72	85
RS	72	65
DB	73	96
ML	77	86
SL	95	65
SE	100	0

* The antigen E and anti-IgE concentration was optimal for each donor.

† After passive sensitization with a 1/10 dilution of a standard serum from a ragweed sensitive donor.

‡ Prior to passive sensitization.

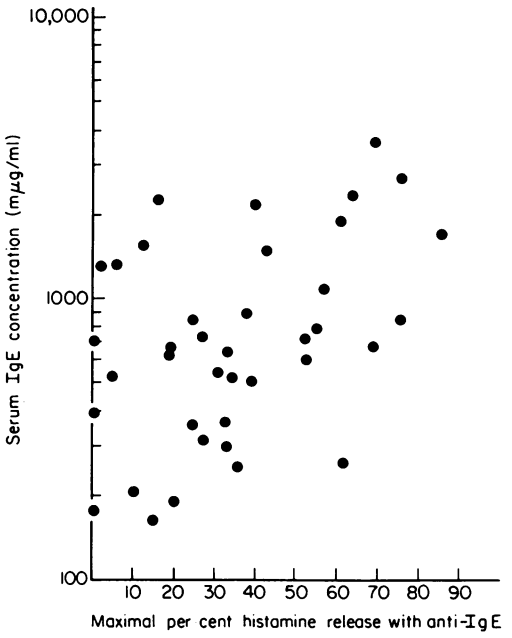

FIG. 4. The correlation between a patient's serum IgE level and the maximal histamine release obtained from his cells by anti-IgE (correlation coefficient (r_S) = 0·42; $P<0·01$). The serum and cells were obtained from twenty ragweed-sensitive children on two occasions.

respond poorly to anti-IgE prior to sensitization whereas the poorly sensitizable cells, having a high level of cell fixed IgE, should be very sensitive to anti-IgE. That this is not the case is shown in Table 2. We have ranked nineteen donors with respect to their ability to be sensitized for antigen-induced histamine release and related this to their ability to respond to anti-IgE before passive sensitization. Some of the best acceptors were most sensitive to anti-IgE, whereas some of the poor acceptors were insensitive. There was no consistent correlation between the two responses. It would appear that the ability of a set of cells to be passively sensitized is not simply a function of the original degree of cell-bound IgE.

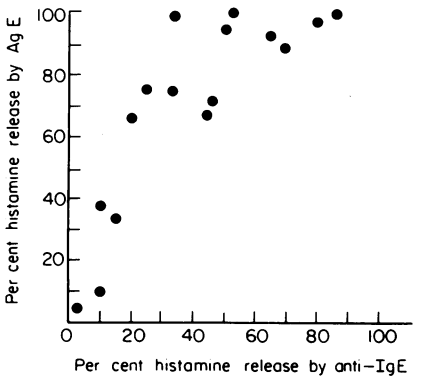

FIG. 5. The correlation between the maximal histamine release obtained from the cells of the patients in Fig. 4 by anti-IgE and antigen E. Correlation coefficient = 0·82; $P<0·001$.

The ability of actively sensitized cells (i.e. those from allergic donors) to release histamine on challenge with anti-IgE does, however, correlate with the serum level of IgE. As shown in Fig. 4 there is a significant correlation ($P<0{\cdot}01$) between the serum IgE level of a cell donor and the maximal response of his cells to anti-IgE. These values were obtained with cells and serum from twenty allergic children undergoing immunotherapy; two bleedings on each were taken and are plotted just before and just after the season of ragweed pollination. The correlation is quite rough and there are interesting exceptions to the general trend, such as the child who had essentially no anti-IgE response in the face of a serum IgE concentration of 1350 ng/ml. With the cells from these same children (Fig. 5), there was an even better correlation between the maximal percentage of histamine releasable by ragweed antigen E and anti-IgE (Lichtenstein, 1969). The cells from all of these children demonstrated the usual range of histamine release to antigen E (70–100 per cent) before therapy but, presumably as a result of this treatment, the cells of some donors became less responsive (Sadan, Rhyne, Mellits, Goldstein, Levy and Lichtenstein, 1969). Interestingly enough, the cells whose responsiveness to antigen E had decreased also showed a decreased responsiveness to anti-IgE (Lichtenstein, 1969).

A parallelism between the reversed *in vitro* anaphylactic reaction induced by anti-IgE and antigen-induced histamine release is also observed when the mechanisms of the reactions are studied. Both proceed with well-washed cells suspended in buffer: the addition of normal rabbit serum to the reaction medium in a final concentration of 1–10 per cent seems to cause little change in the anti-IgE dose–response curve. With no evident need for added serum components the participation of the complement system, as presently understood, is unlikely (cf. Ishizaka and Ishizaka, 1969). Another indication that the anti-IgE reaction is not cytotoxic is shown in Fig. 6. Here we are contrasting the rate of K^+ efflux, relative to control, and the rate of histamine release when the latter is induced by

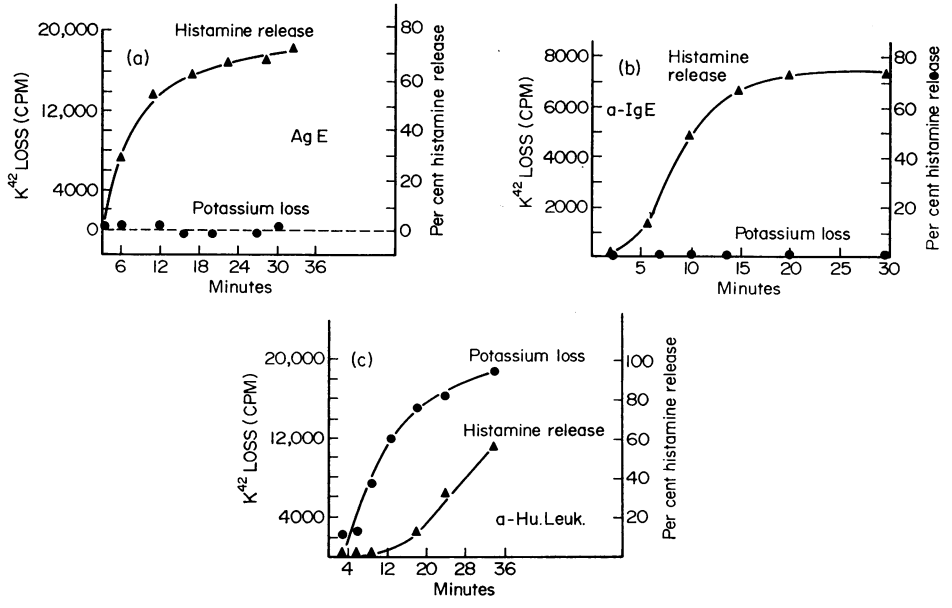

FIG. 6. Histamine release (▲) and potassium efflux (●) from leucocytes challenged with (a) antigen E, (b) anti-IgE or (c) an anti-human leucocyte serum.

ragweed antigen E, anti-IgE and an anti-human leucocyte serum which serves as a cytotoxic control. The leakage of intracellular K^+ is taken as a sensitive indicator of membrane damage (Green, Barrow and Goldberg, 1957). The anti-IgE and antigen E reactions are similar in showing histamine release in the absence of K^+ efflux and stand in contrast to the cytotoxic, anti-leucocyte reaction in which histamine release only occurs after the development of a membrane lesion as indicated by K^+ loss.

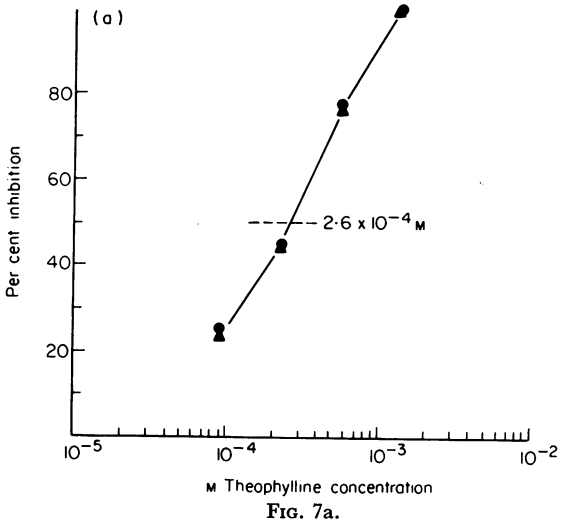

FIG. 7a.

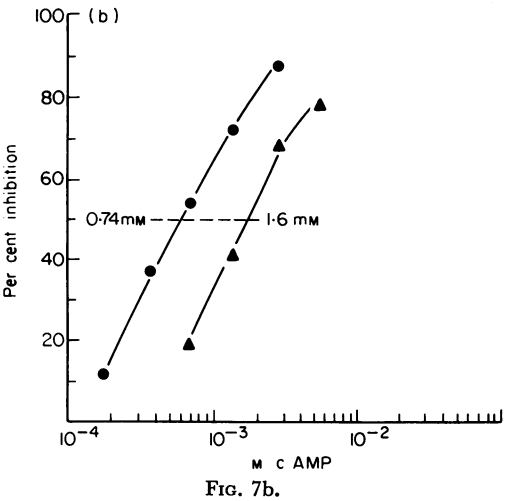

FIG. 7b.

FIG. 7. Inhibition of the histamine release caused by antigen E (●) and anti-IgE (▲) by (a) theophylline, and (b) dibutyryl cyclic AMP.

Another approach to studying the relationship between the direct and reversed reactions is through the use of inhibitors. We have recently shown that antigen mediated histamine release is inhibited by the methylxanthines and catecholamines, presumably through their action on the cyclic AMP system (Lichtenstein and Margolis, 1968). The action of

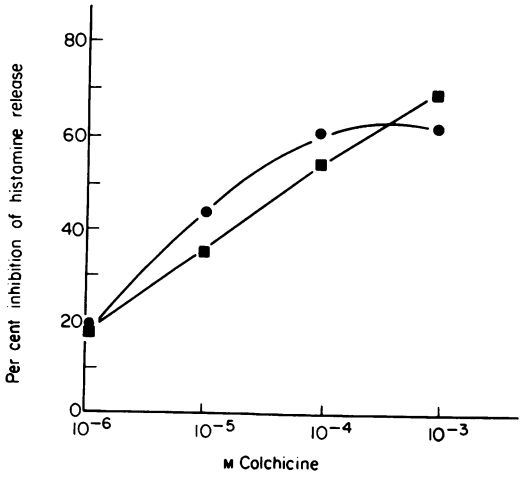

FIG. 8. Inhibition of antigen E (■) and anti-IgE (●) caused histamine release by colchicine.

theophylline (a methylxanthine) and of the dibutyryl derivative of cyclic AMP on the two reactions is shown in Fig. 7. The inhibition dose–response curves are similar. In the case of theophylline, inhibition curves on different donors showed that the two types of histamine release were inhibited by essentially the same concentrations of drug; in the case of cyclic AMP slightly higher concentrations appear to be required for anti-IgE inhibition. The inhibition of these two reactions by theophylline stand in contrast to the cytotoxic reaction caused by anti-leucocyte antibody which is inhibited only slightly by the theophylline (Lichtenstein, 1969).

Another inhibitor, colchicine, which distorts the microtubular structure of a variety of cell types has been shown to block antigenically induced histamine release (Levy and Carlton, 1969). The action of this drug on anti-IgE and antigen E induced histamine release is compared in Fig. 8. Again the curves are quite similar. The anti-leucocyte response is not inhibited by colchicine.

DISCUSSION

The data presented above serve to confirm and extend the report of Ishizaka *et al.* (1969) that antibodies against IgE release histamine from human leucocytes. It was also found that antisera against IgG, even when carefully absorbed to remove both anti-light chain and anti-IgE antibodies, still can cause some histamine release. Whether this is a function of imperfect absorption or a true anti-IgG response remains to be seen. However this may be, it is clear that it takes lower concentrations of anti-IgE than of anti-IgG to cause histamine release. Moreover, anti-IgE causes the release of a greater portion of the total histamine in most cell preparations than does anti-IgG. Inasmuch as there is an inverse relationship between the amount of IgE on a cell and the concentration of either anti-IgE or antigen required for histamine release (cf. Fig. 2), it is reasonable to conclude that with reference to the anaphylactic response, IgE is the major cell-bound immunoglobulin.

That IgE fixation is associated with the ability of the cells to respond to antigen is shown by the passive sensitization experiments. It is not clear, however, why it is not possible to show an increased sensitivity to anti-IgE in all cell preparations rendered responsive to antigen E by passive sensitization. The variation in the ability of cells from normal donors to become sensitized is not, as might have been expected, due to the pre-sensitization level of IgE on the cells. Moreover, even among the good 'acceptor' cells there is no relationship between the relative ability to become sensitized and the shift in the response curve to anti-IgE.

Allergic individuals have, on the average, higher levels of serum IgE than normals (Johansson, 1967; Berg and Johansson, 1969). We found that there was a significant correlation between the serum IgE level and the sensitivity of the leucocytes to anti-IgE, although there are numerous exceptions; these exceptions are perhaps more important than the rough relationship observed. Ishizaka *et al.* (1969) have also reported that wheal-and-flare skin tests with anti-IgE parallel the histamine release studies but do not necessarily reflect the serum IgE level. The correlation between the sensitivity to anti-IgE and to antigen E, using the cells of ragweed-sensitive donors, adds a dimension to this picture. It suggests that a considerable portion of the total IgE, in a ragweed sensitive donor, is antibody against antigen E. It is also possible, however, that the cells of allergic donors simply release histamine with less provocation than that required by the cells of

normal donors. Clarification of this area now depends upon being able to measure the serum levels of specific antibody of the IgE class in relation to the total serum concentration of IgE and contrasting the serum antibody level to the biological capability of cells to respond to challenge with antigen (or anti-IgE).

The mechanism of anti-IgE mediated histamine release is, by the criteria available, identical to the antigen mediated response. Both are non-cytotoxic reactions in that serum factors are not required and that histamine release occurs without an increase in the rate of K^+ leakage from cells. The cellular response to both antigen and anti-IgE is inhibited by theophylline and cyclic AMP, suggesting a role for this membrane associated enzyme system in both reactions. Finally, both are blocked when the microtubular subcellular architecture is injured by colchicine. In each case the extracellular appearance of histamine can best be described as the result of an active, energy requiring process akin to secretion. The seeming identity between the antigen and anti-IgE mediated release of histamine suggests that anything which 'triggers' the cell-bound IgE activates a series of reaction steps which are similar. A practical consequence of this conclusion is that mechanistic studies carried out with anti-IgE should have direct bearing on the mechanism of the antigen-induced anaphylactic response.

ACKNOWLEDGMENTS

This work was supported by grants Nos AI 07290, AI 08270, AI 08104 and AI 04985 from U.S. Public Health Service and by a John A. Hartford Foundation Inc. grant. Part of this work was presented at the meeting on Cellular and Humoral Mechanisms in Anaphylaxis and Allergy sponsored by the Canadian Society for Immunology, October 1968. One of the authors (L.M.L.) was supported by a Research Career Development Award from the National Institute of Allergy and Infectious Diseases.

REFERENCES

BERG, T. and JOHANSSON, S. G. O. (1969). 'IgE concentrations in children with atopic diseases.' *Int. Arch. Allergy*, **36**, 219.

GREEN, H., BARROW, P. and GOLDBERG, B. (1957). 'Effect of antibody and complement on permeability control in ascites tumor cells and erythrocytes.' *J. exp. Med.*, **109**, 511.

HUMPHREY, J. H., AUSTEN, K. F. and RAPP, H. J. (1963). '*In vitro* studies of reversed anaphylaxis with rat cells.' *Immunology*, **6**, 226.

ISHIZAKA, K. and ISHIZAKA, T. (1967). 'Identification of γE antibodies as a carrier of reaginic activity.' *J. Immunol.*, **99**, 1187.

ISHIZAKA, K. and ISHIZAKA, T. (1968). 'Reversed type allergic skin reactions by anti-γE globulin antibodies in humans and monkeys.' *J. Immunol.*, **100**, 554.

ISHIZAKA, K. and ISHIZAKA, T. (1969). 'Immune mechanisms of reversed type reaginic hypersensitivity.' *J. Immunol.*, **103**, 588.

ISHIZAKA, T., ISHIZAKA, K., JOHANSSON, S. G. O. and BENNICH, H. (1969). 'Histamine release from human leukocytes by anti-γE antibodies.' *J. Immunol.*, **102**, 884.

JOHANSSON, S. G. O. (1967). 'Raised levels of a new immunoglobulin class (IgND) in asthma.' *Lancet*, **ii**, 951.

KELLER, R. (1962). 'Effect of antibodies against rat gamma globulin or rat albumin on isolated rat mast cells.' *Nature (Lond.)*, **193**, 282.

LEVY, D. A. and CARLTON, J. A. (1969). 'Influence of temperature on the inhibition by colchicine of allergic histamine release.' *Proc. Soc. exp. Biol. (N.Y.)*, **130**, 1333.

LEVY, D. A. and OSLER, A. G. (1966). 'Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization *in vitro of* human leukocytes to ragweed pollen antigen.' *J. Immunol.*, **97**, 203.

LEVY, D. A. and OSLER, A. G. (1967a). 'Studies on the mechanisms of hypersensitivity phenomena. XV. Enhancement of passive sensitization of human leukocytes by heparin.' *J. Immunol.*, **99**, 1062.

LEVY, D. A. and OSLER, A. G. (1967b). 'Studies on the mechanisms of hypersensitivity phenomena. XVI. *In vitro* assays of reaginic activity in human sera: Effect of therapeutic immunization on seasonal titer changes.' *J. Immunol.*, **99**, 1068.

LICHTENSTEIN, L. M. (1969). 'Characteristics of leukocyte histamine release by antigen and by anti-immunoglobulin and anti-cellular antibodies.' *Cellular and Humoral Mechanisms in Anaphylaxis and Allergy* (Ed. by H. Z. Movat). Karger, Basel.

LICHTENSTEIN, L. M. and MARGOLIS, S. (1968). 'Histamine release *in vitro:* Inhibition by catecholamines and methylxanthines.' *Science*, **161**, 902.

LICHTENSTEIN, L. M. and OSLER, A. G. (1964). 'Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen.' *J. exp. Med.*, **120**, 507.

LICHTENSTEIN, L. M. and OSLER, A. G. (1966). 'Comparative studies of histamine release and potassium efflux from human leukocytes.' *Proc. Soc. exp. Biol. (N.Y.)*, **121**, 808.

MCCONAHEY, P. J. and DIXON, F. J. (1966). 'A method of trace iodination of proteins for immunologic studies.' *Int. Arch. Allergy*, **29**, 185.

OSLER, A. G., LICHTENSTEIN, L. M. and LEVY, D. A. (1964). 'Immunologic aspects of human reaginic allergy: An *in vitro* method and some applications.' *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **250**, 111.

OVARY, Z. (1960). 'Reverse passive cutaneous anaphylaxis.' *Immunology*, **3**, 119.

PORTER, R. R. (1959). 'The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain.' *Biochem. J.*, **73**, 119.

ROWE, D. S. (1962). 'Antigen analysis of rheumatoid factor.' *Immunology*, **5**, 553.

ROWE, D. S. (1969). 'Radioactive single radial diffusion: A method for increasing the sensitivity of immunochemical quantification of proteins in agar gel.' *Bull. Wld. Hlth Org.*, **40**, 613.

SADAN, N., RHYNE, M. B., MELLITS, D., GOLDSTEIN, E. A., LEVY, D. A. and LICHTENSTEIN, L. M. (1969). 'Immunotherapy of pollinosis in children.' *New Engl. J. Med.*, **280**, 623.

SHORE, P. A., BURKHALTER, A. and COHEN, V. H. (1959). 'A method for the fluorometric assay of histamine in tissues.' *J. Pharmacol.*, **127**, 182.