*Immunology* 1984 **51** 123

# Control of immune complex and zymosan-mediated anaphylatoxin generation by proteins B and H of the alternative complement pathway

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*Accepted for publication 8 August 1983*

**Summary.** The generation of histamine releasing activity (HRA) from human basophils in fresh serum by tetanus toxoid (Te)/anti-Te complexes or by zymosan can be modulated through introduction of incremental amounts of proteins B and H of the alternative complement pathway. Serum treated at 50° in order to abolish alternative pathway-mediated haemolytic activity, lost 90% of its capacity to generate HRA upon addition of Te/aTe; such loss could be reversed through additions of purified B. Amounts of B sufficient to restore normal alternative pathway haemolytic activity also restored HRA induced by Te/aTe; as little as a 33% increase above the normal serum concentration of B increased the capacity to support Te/aTe induced HRA by a factor of 1·4. In contrast, additions of incremental doses of purified H to fresh serum reduced generation of HRA by both Te/aTe and zymosan. Total inhibition was achieved by increasing the serum H concentration by 12·5–30%; further increases of H up to 200% again permitted HRA generation induced by immune complexed aTe. H also inhibited Te/aTe induced HRA in a serum heated at 50° but only 30% inhibition of HRA could be achieved over a range of H inputs up to 187% above normal serum concentration.

Additions of H also inhibited HRA generation in fresh serum when induced with plain or C3b-coated zymosan (Z) particles. By increasing the serum concentration of H from 12·5 to 125%, dose-dependent inhibition of HRA generation was observed; the H input necessary to suppress 48% of HRA generation was ten times higher when HRA was generated by Z-C3b than by plain zymosan. Thus, the complement-dependent generation of HRA from fresh serum strongly depends on modest variations in the concentrations of the two regulatory proteins B and H of the alternative complement pathway, suggesting their direct effect on generation of anaphylatoxins C3a and C5a.

Abbreviations: C3bR, C3b receptor; $E^R$, rabbit erythrocytes; $E^{hu}$, human erythrocytes; EDTA, ethylenediamine tetraacetic acid; HRA, histamine releasing activity; NHS, normal human serum; Te, tetanus toxoid; aTe, anti-tetanus toxoid antibodies; Z, zymosan.

The nomenclature used for the protein of the alternative complement pathway conforms to Alper *et al.* (1981).

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## INTRODUCTION

Interactions of immune complexes or microbes with complement may be both beneficial and detrimental to the host. Such interactions may involve the classical as well as the alternative pathways and lead to immune complex solubilization or microbial opsonization, favouring their elimination (Nussenzweig, 1980; Ver-

brugh *et al.*, 1982), and/or the generation of activated complement components and anaphylatoxins C3a and C5a. Among the major target cells for C3a and especially C5a (Hook, Siraganian & Wahl, 1975; Siraganian & Hook, 1976) are peripheral blood basophils whose triggering results in release of the vasoactive amine histamine and other mediators. The existence of specific receptors for C5a has also been demonstrated recently on human neutrophils and on murine macrophages (Chenoweth & Hugli, 1978; Chenoweth, Goodman & Weigle, 1982) and suggested to exist on human basophils. C5a-triggering of basophils is independent of IgE-induced histamine release, but the latter can be potentiated by anaphylatoxins (Thomas & Lichtenstein, 1979; Thomas, Findlay & Lichtenstein, 1979).

The complementary fragments for C3a and C5a, that are generated during the activation of complement, are C3b and C5b; C3b can bind to various acceptor surfaces such as immune complexes, zymosan from yeast and cell membranes. Bound C3b may in turn recruit proteins B and D, components of the alternative complement pathway, assembling thereby a C3b,Bd enzyme (alternative pathway C3 convertase) that itself splits C3b from C3 (Götze & Müller-Eberhard, 1971; Fearon, Austen & Ruddy, 1973; Fearon & Austen, 1977). Further apposition of C3b to the alternative pathway C3 convertase C3b,Bb changes it into a C5 convertase, $(C3b)_nBb$, resulting in cleavage of C5 into C5a and C5b (Daha, Fearon & Austen, 1976). The function of this alternative pathway convertase is subject to positive and negative regulatory influences, involving the plasma protein P, which stabilizes the enzyme, the protein H, which decay-dissociates it (Weiler *et al.*, 1976; Whaley & Ruddy, 1976) and the endopeptidase I, which splits the alpha chain of C3b and thereby inactivates it generating C3bi (Harrison & Lachmann, 1980). The inhibitory effect of H is also operative at the formation stage of C3b,Bb, in that H competes with B for binding to C3b (Kazatchkine, Fearon & Austen, 1979). Protein H also potentiates cleavage of C4b and of C3b into C3bi by I (Pangburn, Schreiber & Müller-Eberhard, 1979) and has recently been described to also inhibit the $(C3b)_nBb$ C5 convertase (Fischer & Kazatchkine, 1982).

In view of such diverse potential levels of control of C3a and C5a generation by alternative pathway proteins, we set out to investigate their involvement in the activation of serum induced by immune complexes and zymosan, assessing anaphylatoxins from their histamine releasing activity on human basophils. A clear-cut modulation of such generation by proteins B and H is herein reported.

## MATERIALS AND METHODS

*Chemicals and serum*

HEPES (Ultrol, Calbiochem-Behring Company, La Jolla, U.S.A.) and ethylenediamine tetraacetic acid tetra sodium dihydrate (EDTA) was obtained from Merck, Darmstad FRG, Dextran T70 from Pharmacia fine chemicals, Uppsala, Sweden, and zymosan from ICN Pharmaceutical Inc., Cleveland, OH, U.S.A. HEPES buffer was used at 20 mM pH 7·4 containing 0·9% NaCl and 0·5 mM dextrose.

*Serum.* Unless otherwise stated, blood was collected from normal donors, allowed to clot at room temperature, then transferred immediately to an incubator at 37° for 2 hr. After centrifuging at +4°, the serum was divided into aliquots and stored at −70° until required.

*Complement components and activator substances*

Protein B was purified to homogeneity according to sodium dodecyl sulphate-polyacrylamide gel electrophoresis with silver staining (Pangburn *et al.*, 1979) and its biological activity was tested by its ability to restore alternative pathway (AP) haemolytic activity in a human serum heat-inactivated at 50°, as assessed by lysis of rabbit erythrocytes ($E^R$), and in a direct assay for factor B using EAC4b3b cells (Fearon & Austen, 1977). The haemolytic activity of the preparations used was 160,000 U/mg protein.

Protein H, purified according to Hunsicker, Ruddy & Austen (1973), was assessed for biological activity by its capacity to suppress the lytic activity of normal human serum (NHS) mixed with 2 mM magnesium and 8 mM ethyleneglycol tetraacetic acid (Mg EGTA) on $E^R$ (Nydegger, Fearon & Austen, 1978).

*Immune complexes*

Tetanus toxoid (Te) containing 3500 LF units/ml (Serum- and Impf-Institute, Berne, Switzerland) and human anti-tetanus toxoid (aTe) immunoglobulin containing over 95% IgG (Tetuman serum, Serum- and Impf-Institute, Berne, Switzerland) were titrated in a quantitative precipitation reaction to determine

the equivalence point. This was achieved by adding increasing amounts of antigen to a constant amount of antibody. The tubes were left 2 hr at 25° and incubated at 4° for 48 hr, then centrifuged at 1400 *g* for 30 min, and the precipitates washed twice with 0·9% NaCl. After final centrifugation, the precipitate was dissolved in 0·1 NaOH and the protein estimated according to the technique of Lowry *et al.* (1951). The equivalence point was found to be 1 mg/ml antibody and 35 LF units/ml of Te. Thereafter all immune complexes used in this study were made using these concentrations of Te and aTe in HEPES buffer. After 2 hr at 25° and 48 hr at 4° they were vacuum dialysed to a concentration between 3 and 6 mg/ml, and the amount of complexed aTe-IgG antibody calculated from the equivalence point on the precipitation curve. Zymosan particles were suspended in boiling water for 5 min and washed twice with 20 volumes of HEPES buffer. The particles were then resuspended at 10 mg/ml in NHS and incubated at 37° for 30 min, a procedure which enables them to become coated with C3b. The Z-C3b particles were washed twice with 20 volumes of HEPES buffer and finally resuspended at a concentration of 10 mg/ml in HEPES buffer.

*Activation of serum (anaphylatoxin production) and the reversed histamine release assay*

Aliquots of 0·5 ml of undiluted NHS were dispensed into 5 ml plastic tubes (Sarstedt type 55476, Federal Republic of Germany), mixed with incremental amounts of immune complexes ranging from 1 μg to 20 μg/ml aTe and incubated at 37° for 1 hr. Thereafter all tubes were incubated for an additional 30 min at 56° to prevent further complement activation. $Ca^{++}$ and $Mg^{++}$ were added to a final concentration of 600 μM each, and the mixtures were either stored for 2 hr at +4° or used immediately.

Dextran-sedimented washed human leucocytes were prepared as follows: 20 ml of venous blood (containing sufficient cells for 50–60 tests) were added to 2 ml of 100 mM EDTA, pH 7·4 and 5 ml of 6% dextran-saline in a 50 ml plastic tube (Falcon Plastic, Becton Dickinson, U.S.A.) mixed and allowed to sediment for 90 min at 22°. The upper layer containing platelets, plasma and leucocytes, was pipetted off and centrifuged at 110 *g* for 10 min at 4°. The plasma was discarded and the cells washed twice in 20 ml of HEPES buffer. Finally the cells were resuspended in HEPES buffer and adjusted to contain approximately $6 \times 10^8$ cells/ml. For assessing histamine release induced by immune complexes, 100 μl of cell suspension were added to 500 μl of immune complex-activated serum, the tubes were incubated 60 min at 37°, immediately removed to ice and centrifuged at +4° and 110 *g* for 10 min. Serum supernatants were discarded, and the cells were washed in 3 ml of cold HEPES buffer, and centrifugation carried out as above. After final resuspension in 1 ml of HEPES buffer, the cells were lysed by addition of 1 ml of 0·8 M perchloric acid and all tubes centrufuged after mixing at 700 *g* for 10 min. The residual histamine contained in the cell pellets was estimated on a Technicon AutoAnalyzer by the method of Siraganian (1975).

Histamine release had to be assessed indirectly as loss of histamine from residual cell pellets, as opposed to the more usual technique of measuring histamine present in cell supernatants, since serum constituents interfere in the fluorimetric assay of histamine.

Histamine release was therefore calculated as follows:

amount histamine released into supernatant = serum control residual histamine − test residual histamine;

$$\%\ \text{histamine release} = \left(\frac{\text{supernatant histamine}}{\text{serum control residual histamine}} - \text{spontaneous release}\right) \times 100.$$

In addition, zymosan controls were included in all experiments as a positive check of the serum's ability to produce anaphylatoxin and of the cell reactivity. Boiled and washed zymosan particles were resuspended at a concentration of 10 mg/ml in HEPES buffer, diluted into serum to a final concentration of 0·1 and 2 mg/ml respectively. When subjected to the same activation procedure as the tubes containing immune complexes followed by centrifugation to remove zymosan particles, the anaphylatoxins so produced yielded approximately 20 and 40% (±10%, ±2 SD) histamine release, respectively. These concentrations consistently produce anaphylatoxin from different sera. In addition, non-activated serum controls, that go through both incubation steps, were set up in order to assess the spontaneous release induced by non-activated serum alone. This value was compared to the residual histamine content of a serum-free control, introduced at the histamine release step only. Generally the difference between these two controls was between 5 and 10%.

Immune adherence studies to assess C3b receptor interaction of Te/aTe complexes were performed according to Miyakawa *et al.*, (1981).

## RESULTS

### Generation of histamine releasing activity by Te/aTe complexes and zymosan

Incremental amounts of Te/aTe complexes and of zymosan were added to fresh human serum, and the histamine releasing activity was estimated (Fig. 1A). A reproducible range of effective Te/aTe doses (2–20 μg of specific aTe-IgG antibody/ml) was observed with different basophil donors in three different experiments, maximal release generally occurring with 10–20 μg/ml of antibody in complexed form. Zymosan over the concentration range of 0·1–2·0 mg also generates histamine releasing activity from NHS (Fig. 1B) in a dose-dependent manner. In these experiments, the magnitude of the histamine releasing activity also decreased with dilution of the serum used to generate anaphylatoxin. When serum treated at 50° in order to abolish alternative pathway-mediated hemolytic activity was used for generation of histamine releasing activity by Te/aTe complexes, eight to 10 times higher amounts of Te/aTe complexes were necessary to generate 20–30% histamine release than by using fresh serum (Fig. 2); assessed by $E^R$ lysis test, no residual alternative complement pathway activity was detected after heat inactivation at 50° of this serum. No histamine releasing activity was observed at all, when the immune complexes were incubated in serum heated at 56°.

An absolute requirement for serum was found in these experiments as incubation of Te/aTe complexes or zymosan in buffer with basophils lead to no significant histamine release ($\leq 5\%$).

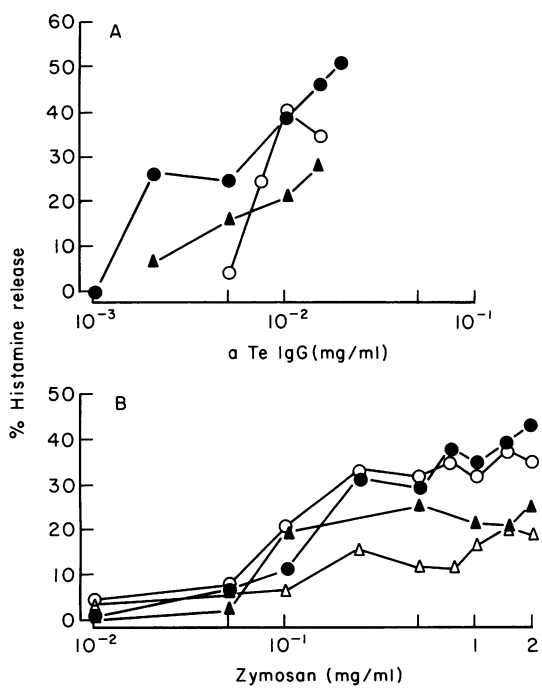


**Figure 1.** Generation of HRA by incremental amounts of Te/aTe complexes (A) or zymosan (B) in fresh human serum from human basophils. Panel A depicts results obtained in three different experiments with different donors of basophils. Panel B shows results obtained using undiluted serum (●) and serum diluted 1:2 (○), 1:4 (▲) and 1:8 (△) for HRA generation as assessed on basophils from one donor.

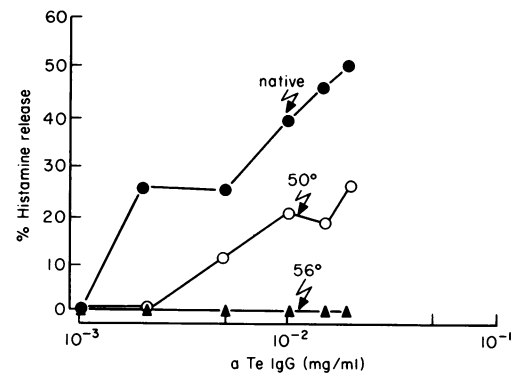


**Figure 2.** Dependency of HRA generation by Te/aTe complexes on serum complement. Serum samples were used fresh or preincubated for 30 min at the temperatures indicated.

### Modulation of anaphylatoxin generation by proteins of the alternative complement pathway

In order to investigate the role of B in immune complex-induced anaphylatoxin generation, incremental amounts of B were added to heat-inactivated (30 min at 50°) normal human serum before addition of 20 μg antibody in the form of Te/aTe complexes and histamine release from basophils was assessed (Fig. 3). Addition of amounts of purified B, sufficient to restore normal serum haemolytic activity (assayed by the $E^R$-lysis test) also restored generation of histamine releasing activity by immune complexes to the range observed with fresh human serum (Fig. 3). In this experiment, increase of B by 33% above the intrinsic serum concentration lead to a further increase in histamine releasing activity by a factor of 1·4. When incremental mounts of B were added to basophils in presence of buffer alone no release occurred.

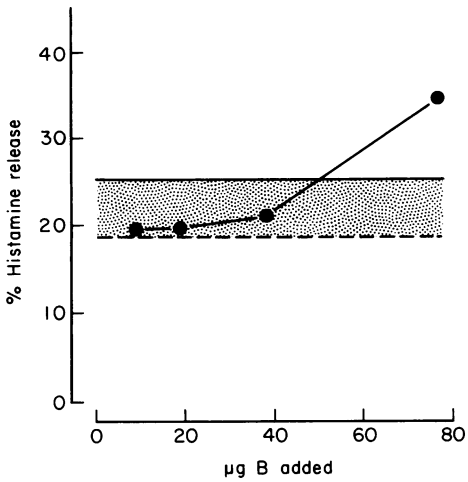


**Figure 3.** Reversal and potentiation of impaired HRA generation by Te/aTe in a serum heated for 30 min at 50° through addition of purified B. The HRA generated in heated serum alone was 18% (- - -) and in fresh serum 25% (——). HRA was generated by 20 μg of antibody in the form of Te/aTe.

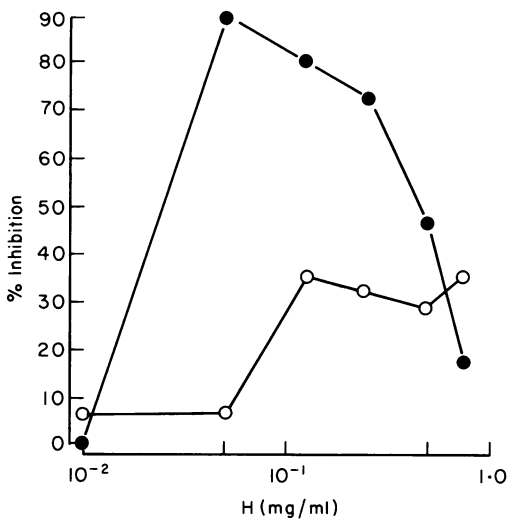


**Figure 4.** Inhibition of Te/aTe induced HRA in fresh (●) or heat-inactivated (30 min at 50°, ○) serum by H. HRA was generated by 20 μg of antibody in the form of Te/aTe.

The effect of introducing 2·5, 12·5, 31, 62·5 and 187 μg of purified H into native serum, in order to increase the concentration of this protein by 2·5%, 30%, 62% and 187% on Te/aTe-induced histamine releasing activity, was also studied. For this purpose, the incremental doses of H were preincubated with normal human serum 5 min prior to the addition of 10 μg of immune complexes, and the incubation continued for a further 55 min at 37°, before addition of the cells equilibrated at the same temperature. In the absence of H, the immune complexes alone generated histamine releasing activity of 27% in presence of native serum and 15% in presence of heated (30 min at 50°) serum respectively. Inhibition of release was achieved by increasing the intrinsic serum H concentration by 12·5% in the case of fresh serum (Fig. 4). From the curve, it is seen in the case of native serum, that excess amounts of H yielded less inhibition than those amounts, necessary for maximum inhibition: in order to exclude a direct stimulatory effect of H on basophils, incremental doses of H were added to normal serum and basophils incubated for 60 min at 37°. No release was seen neither in the native or in the heat-inactivated serum.

Since H also serves as co-factor in C4 inactivation by I, it was tested whether incremental amounts of H would inhibit the generation of histamine releasing activity by Te/aTe complexes in serum depleted of B by inactivation at 50° for 30 min but still retaining classical pathway activity. Release by such serum incubated with Te/aTe complexes was 15% and could still be inhibited to various degrees by adding various amounts of H (Fig. 4). More H was necessary to inhibit releasing activity in the 50°-inactivated serum than in the native serum, and there was no decrease of inhibitory capacity with increasing H input over the range of H concentrations tested.

Modulation of histamine releasing activity by H, when the zymosan or Z-C3b were used instead of Te/aTe for serum activation was also studied in two parallel experiments. For this purpose 12·5 μg, 62·5 μg, and 125 μg of purified H were introduced into native serum in order to increase H concentration by 12·5%, 62·5% and 125%; and 5 min later 2 mg/ml Z were added to one set of tubes and 2 mg/ml Z-C3b to the other. H was inhibitory in both experiments; however, inhibition was greater when zymosan rather than Z-C3b was the activator (Fig. 5).

### Direct role of immune complexes

Whereas the activating zymosan particles become sedimented by centrifugation at the end of incubation in fresh serum, we could not avoid the constant and simultaneous presence of solubilized Te/aTe com-

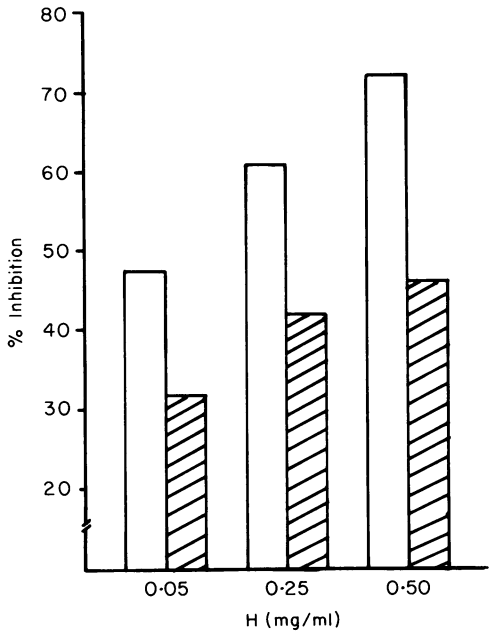


**Figure 5.** Inhibition of zymosan- (□) or zymosan-C3b- (■) induced HRA in fresh serum by three different doses of H. Note that the H input necessary to suppress 48% of HRA generation is ten times higher with zymosan C3b as the activator.

plexes along with anaphylatoxin during the incubation with the basophils. That the Te/aTe complexes were solubilized under the conditions of the test became apparent when the precipitation of $^{125}$I-Te by aTe was evaluated in presence of fresh and heat-inactivated (56°, 30 min) serum. With fresh serum, 8% of $^{125}$I-Te was found in the precipitates, whereas 62% were precipitated in presence of heat-inactivated serum. When tested by immune adherence to $E^{hu}$ under conditions where immune complex-bound C3b binds to the C3bR, 4 μg/ml of Te complexed aTe antibodies still lead to significant haemagglutination, indicating that during solubilization they became coated with C3b.

That the presence of immune complexes during the histamine releasing phase indeed could affect, at least in part, the results, is suggested by the following experiment: to constant amounts of fresh serum activated with zymosan were added various amounts of Te/aTe complexes prepared in buffer, and thereafter target cells were added (Table 1). Although it can be ruled out that these complexes were not exposed to native serum, their presence indeed lead to enhanced

**Table 1.** Effect of addition of Te/aTe immune complexes to a heat-inactivated serum, previously activated by zymosan

| Anti-Te mg/ml | % Histamine release corrected for spontaneous release |
|---|---|
| $3\times10^{-2}$ | 46 |
| $2\times10^{-2}$ | 44 |
| $10^{-2}$ | 31 |
| $5\times10^{-3}$ | 25 |
| $2{\cdot}2\times10^{-3}$ | 29 |
| $1{\cdot}1\times10^{-3}$ | 27 |

Zymosan alone (2 mg/ml) % = 23·0.
Spontaneous release % = 6·0.
After heat inactivation at 50° and addition of Te/aTe, the tubes were further incubated at 37° for 1 hr with human leucocytes.

histamine release, amounting maximally to 26% of the release achieved by zymosan-activated serum alone.

## DISCUSSION

The experiments reported here were designed to investigate whether regulatory proteins of the alternative complement pathway may affect the formation of anaphylatoxins active in inducing histamine release. Proteins enhancing (protein B) or suppressing (protein H) activity on the alternative complement pathway have indeed been shown to increase or to attenuate immune complex- or zymosan-induced anaphylatoxin generation in human serum.

In our system, histamine releasing activity from human peripheral blood basophils was shown to depend entirely on complement activation, since heat inactivation of the serum used abolished its capacity to support anaphylatoxin production induced by the two activator substances Te/aTe immune complexes or zymosan. Activation of either the classical or the alternative pathway by immune complexes (Hartmann & Glovsky, 1981) or zymosan (Hook *et al.*, 1975; Siraganian & Hook, 1976) generates histamine releasing activity from human serum, as already reported; this has been shown to be due to C5a generation (Siraganian & Hook, 1976). It has been suggested that the generation of C3a exceeds that of C5a on a molar basis, but C5a is much more potent than C3a when assessed on guinea pig smooth muscle or human basophils as target systems (Hartmann &

Glovsky, 1981; Fernandez *et al.*, 1978; Johnson, Hugli & Müller-Eberhard, 1975). For anaphylatoxin generation the presence of C3 in serum is required (Vogt *et al.*, 1975), since neither zymosan nor immune complexes were able to generate histamine releasing activity in a homozygous C3-deficient serum (Hartmann & Glovsky, 1981). The complement enzymes that cleave C5 into C5a and C5b, i.e. $(C3b)_n$ Bb and C423 are a result of activation of the alternative or the classical pathway, respectively. Therefore, immunopathological induction of the events leading to C5a-mediated allergic phenomena can hardly be controlled by the host at the step of initiation of complement activation. However, once the initial steps of complement activation have occurred, the potential role of regulatory factors becomes relatively important and may decide, whether allergic phenomena become clinically apparent.

Because of the control function of H in the alternative pathway, an inhibitory effect of this protein on induction of histamine releasing activity was to be expected, independently of whether this activity was induced by immune complexes (Fig. 4) or zymosan (Fig. 5). Less H was necessary to achieve inhibition in a native serum than in a serum made deficient in alternative pathway activity. This may be explained by the dual role of this protein in controlling both to alternative pathway C3b,Bb and the classical pathway C4b2a C3 convertases. In fresh serum, H would act on both alternative pathways allowing for initial anaphylatoxin generation and the classical pathway but only on the classical pathway in a serum inactivated at 50°. In addition, it has been observed recently, that H also controls the alternative pathway C5 convertase once it is cell-bound (Fischer & Kazatchkine, 1982). By reconstituting a serum heated at 50° with incremental amounts of purified B up to 120% of the intrinsic serum level, we observed enhancement of histamine releasing activity beyond restoration of full capacity for immune complex-induced anaphylatoxin generation by normal serum. It is well known, that H not only leads to decay-dissociation of the alternative pathway convertase, but also competes with B at the formation step of such convertase. Therefore by increasing the concentration of B over its physiological level, an advantage of the activator proteins assembling the convertase was created that favoured generation of anaphylatoxin.

Activators of the alternative pathway, such as immune complexes and zymosan, exert their effect through retardation of the actions of C3b inactivator, I, and H on bound C3b and P-stabilized, C3b,Bb respectively, thereby creating a disequilibrium that favours C3 cleavage (Fearon & Austen, 1977). The addition of H to NHS prior to its activation with immune complexes or zymosan yields a dose-dependent inhibition of anaphylatoxin generation; relatively modest increases in the concentrations of H dampened the disequilibrium induced by the activators. This is in agreement with previous reports on the regulatory effect of this protein, in which increments of H by 15–30% introduced into C2-deficient serum caused a dose-dependent suppression of zymosan-induced alternative pathway activation (Nydegger *et al.*, 1978). Beyond an optimum of H added to NHS, anaphylatoxin generation may again be facilitated (Fig. 4). This effect is not due to direct stimulation of the basophils by H, since in presence of a serum heated at 56°, H does not cause spontaneous histamine release. It has now been reported repeatedly that the inhibitory effect of H on the alternative pathway activity is observed only in a narrow range beyond which H exerts the opposite effect, i.e. again favours activation. This phenomenon has been observed with different activator substances, and it is suggested, that excess H protects fluid phase C3 before it comes available for deposition on the activating surface (Nicol & Lachmann, 1973; Thompson & Winterborn, 1981). It is likely that the Te/aTe complexes used also activated complement through the alternative pathway; although it is known that the bulk of antibodies against Te are found within the classical pathway activating subclass of IgG1 (Stevens *et al.*, 1982). At least, such complexes provided a surface for assembly of C3b,Bb since they were found to agglutinate $E^{hu}$ through the C3bR and to bind radiolabelled H (Nydegger *et al.*, 1983).

In contrast, in the experiments reported here, incremental amounts of H added to serum in order to prevent zymosan or Z-C3b-induced generation of histamine releasing activity showed dose-dependent inhibition over the whole range of H doses tested. This suggests, that the capacity of H to inhibit anaphylatoxin generation depends on the activating substance, Te/aTe on the one hand and zymosan on the other hand, providing different surfaces for the assembly of C3b,Bb and allowing different expressions of H activity.

The possibility of a contribution to the observed phenomena by cells, other than basophils in the reaction mixture (washed leucocytes), must be reckoned with. Indeed, H and C3b have been observed to activate monocytes (Schopf *et al.*, 1982), and acti-

vation products of complement induce lysosomal enzyme release from polymorph nuclear leucocytes (Showell, Glovsky & Ward, 1982). The direct effects of such bystander cells on histamine release in our system, however, would probably be restricted to release of preformed or rapidly acting mediators acting on basophils (e.g. proteolytic enzymes) since the release of other mediators such as lymphokines usually takes longer time than a few minutes. In the case of lymphokines, their action on basophils has also been shown to require several hours incubation (Needleman, Weiler & Felbush, 1981; Hernandez-Asensio *et al.*, 1979). The release of histamine is so rapid in our system, that it is unlikely that enough effector substances from bystander cells could be produced in time to affect the results (Siraganian & Hook, 1976). Another possibility is that immune complexes might, besides the generation of anaphylatoxin, also influence histamine release by binding to cellular receptor; such as the Fc receptor and the C3b receptor.

Indeed, H is likely to interfere at two different steps, as suggested by the experiments using zymosan and Z-C3b (Fig. 5): during the assembly of the convertase on the activating surface and, further on the amplifying action of the assembled convertase on C3 cleavage. At the stage of anaphylatoxin generation in serum, however, H also inhibited Z-C3b-induced anaphylatoxin production (Fig. 5). Here, excess H may bind to C3b and in conjunction with I, prevent C3b from recruiting more C3b,Bb molecules. On the other hand, Z-C3b when added to histamine releasing agents potentiates histamine release from human basophils and it was speculated that this effect would occur through a C3bR on the basophil membrane (Thomas & Lichtenstein, 1979).

As shown in Table 1, the immune complexes by themselves seem to enhance the release of histamine caused by zymosan-induced anaphylatoxin. Whether this is due to accessory effects on receptors of the basophils themselves or of some bystander cells is not yet ascertained.

In order to investigate the possible participation of bystander cells, experiments were carried out using neutrophil-depleted cell populations. Preliminary data indicate that zymosan-induced HRA appears to be not significantly different whether studied in a neutrophil rich or a neutrophil-depleted cell population. However, in the same situation but using immune complex activation, a depletion of cell populations in neutrophils leads to enhanced histamine release. The cause of this phenomenon is under further investigation.

## ACKNOWLEDGMENTS

This work was possible in part by a grant from the Swiss National Foundation.

The authors thank Prof. Haessig for support.

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