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Effects of histamine on monocyte complement production

I. INHIBITION OF C2 PRODUCTION MEDIATED BY ITS ACTION ON H2 RECEPTORS

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SUMMARY

Histamine produced dose-dependent inhibition of the production of the second complement component (C2) by monocytes in tissue culture. The effect was not associated with either cell death, as ascertained by trypan blue exclusion, or loss of cells from the monolayer, as determined by measuring their DNA content. The specificity of the response was shown by the failure of histidine or histamine metabolites to inhibit C2 production. Preincubation of histamine with histaminase also abrogated the histamine effect. The kinetics of the effect were extremely rapid and irreversible, most of the reduction being achieved during a 5-min exposure to histamine. The H2 receptor antagonist cimetidine was able to prevent the histamine response, whereas chlorpheniramine, the H1 receptor antagonist, had no effect. Dimaprit and 4-methyl histamine, H2 receptor agonists, simulated the effect of histamine whereas the H1 receptor agonist 2-(2-aminoethylthiazole) was ineffective, confirming that the effect of histamine on C2 production by monocytes is mediated by the H2 receptors. Thus histamine, released from basophils or mast cells by the C3 and C5 cleavage products C3a and C5a respectively, may exert a negative feedback on further C3 and C5 cleavage by limiting the formation of the C3 ($C\overline{42}$) and C5 ($C\overline{423}b$) convertases.

INTRODUCTION

Human leucocytes possess specific receptors for a number of hormones including histamine, norepinephrine, prostaglandin E2, vasopressin, growth hormone and insulin (Melmon *et al.*, 1972; Weinstein *et al.*, 1973; Bourne *et al.*, 1974). Using purified subpopulations of leucocytes histamine has been shown to inhibit the secretion of histamine from basophils (Bourne, Melmon & Lichtenstein, 1971), and lysosomal enzymes from neutrophils (Weissmann, Zurier & Hoffstein, 1974). Histamine has also been found to exert profound effects on different lymphocyte functions including the production or secretion of antibody (Shearer *et al.*, 1972), T lymphocyte cytotoxicity (Henney, Bourne & Lichtenstein, 1972), antigen-induced production of macrophage migration inhibitory factor (MIF) and mitogen- and antigen-induced lymphocyte proliferation (Ballett & Merler, 1976; Rocklin, 1976). These two latter effects have been shown to be mediated by a subpopulation of lymphocytes, which when stimulated by histamine release a factor, histamine-induced suppressor factor (HSF), which abrogates the production of MIF and lymphocyte proliferation (Rocklin, Greineder & Melmon, 1979; Rocklin *et al.*, 1978). All these effects are considered to be mediated by histamine-type 2 (H2) receptors as competitive antagonists reverse the histamine

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effects at lower concentrations than H1 receptor antagonists (Weissmann *et al.*, 1974; Rocklin, 1976; Lichtenstein & Gillespie, 1973; Plaut, Lichtenstein & Henney, 1973).

Using a rosette assay, human monocytes have recently been shown to possess histamine receptors (Saxon, Morledge & Bonavida, 1977) although the specificity of the receptors was not identified.

The present report details the effects of histamine on the production or secretion of the second component of complement (C2) by human monocytes, and defines the specificity of the receptors involved.

MATERIALS AND METHODS

Reagents. The compounds used in this study were obtained as follows. Histamine dihydrochloride, *N*-acetyl histamine and L-histidine (Sigma); imidazole acetic acid (IAA), 1-methyl histamine and 3-methyl histamine (Calbiochem); cimetidine, 4-methyl histamine dihydrochloride, S(3-(N, *N*-dimethylaminopropyl) isothiourea (dimaprit) and 2-(2-aminoethylthiazole dihydrochloride (2-2-AET) were a gift from Smith, Kline and French Laboratories.

Tissue culture materials. Linbro tissue culture plates and foetal calf serum (FCS) (Flow Laboratories), circular glass coverslips (McQuilken Ltd), RPMI 1640, sodium bicarbonate, antibiotic–antimycotic solution (GIBCO BIOCULT) were obtained from the sources shown. Cell suspensions were washed in RPMI 1640 and monocyte monolayers were cultured in RPMI 1640 containing 20% heat-inactivated (2 hr, 56°C) FCS, 9 mM $NaHCO_3$, 100 μg/ml streptomycin, 100 u/ml penicillin, 0·25 μg/ml fungizone and 4 mM L-glutamine (RPMI-FCS).

Complement components and assays. Human C1 (Lachmann, Hobart & Aston, 1973) and C2 (Ruddy & Austen, 1967) were purified as described. C2 concentrations in culture supernatants were assayed using a standard stoichiometric heamolytic assay (Rapp & Borsos, 1970).

Isolation of mononuclear leucocytes and preparation of monocyte monolayers. Mononuclear leucocytes were isolated from venous blood by Ficoll–Hypaque centrifugation (Böyum, 1968). The washed mononuclear leucocytes were resuspended to 1×10^7/ml in RPMI FCS and monolayers formed by incubating 0·5 ml of the cell suspensions on the surfaces on which they were to be cultured (Whaley, 1980). Following washing, the adherent cells were over 95% monocytes.

Monolayers were usually prepared by incubating mononuclear cell suspensions in wells of Linbro plates, and were cultured in 1 ml RPMI–FCS in a humidified 5% CO_2/air atmosphere. One hundred microlitres of culture supernatant were removed daily for C2 assay and replaced by an equal volume of fresh RPMI–FCS. The samples of culture supernatants were stored at −70°C until used. On the last day of culture the total supernatant was removed and stored at −70°C, the monolayer washed and then lysed in 0·5 ml 2% SDS solution at 37°C. The lysates were stored at −20°C for DNA determinations (Einstein, Schneeberger & Colten, 1976). For the studies on cell viability monolayers were prepared on coverslips which were then placed in the wells of Linbro plates for culture.

The effects of the addition of histamine to monocyte cultures prior to the onset of C2 synthesis (day 0) and when C2 synthesis was established (day 5) were studied. For ease of presentation these monocyte cultures will be called 'naïve' and 'established' respectively.

Effect of histamine on C2 production by monocytes. The supernatants of naïve and established monocyte cultures were removed and replaced with fresh RPMI–FCS containing histamine (10^{-4}–10^{-6} mol/l) and their culture continued as described above. Samples of culture supernatants were removed daily and assayed for their C2 content. Naïve cultures were studied for 10 days (days 1–10) and established cultures for 7 days (days 6–12).

To assess the effect of histamine on viability, monocytes were cultured on coverslips and exposed to normal RPMI–FCS or RPMI–FCS containing histamine (10^{-4}–10^{-6} mol/l). Naïve monocytes were maintained in culture until day 7 and established monocytes were cultured until day 9. Coverslips were washed in warm RPMI 1640, stained with 0·5% trypan blue, and studied by light microscopy.

The effect of histamine on monocyte adherence was investigated by exposing replicate sets of cultures to normal RPMI–FCS or RPMI–FCS containing histamine (10^{-4}–10^{-6} mol/l). Naïve

cultures were removed on days 1, 3, and 7, and established cultures were removed on days 6 and 8. Following washing the monolayers were lysed in SDS for DNA determination.

Histamine dose-response curves. Dose–response curves for the effect of histamine on C2 production were constructed by incubating histamine with a set of naïve and a set of established cultures from each of four individual donors. To construct dose–response curves we used the C2 concentrations on day 7 of naïve cultures and day 8 of established cultures. The results were expressed as per cent inhibition of C2 production:

$$\% \text{ inhibition} = \frac{\text{C2 conc. in control} - \text{C2 conc. in treated culture}}{\text{C2 conc. in control}} \times 100$$

Specificity of the histamine effect. Monocytes were cultured in normal RPMI–FCS or in the presence of histidine, or the histamine metabolites IAA, 1-methyl histamine, 3-methyl histamine, or *N*-acetyl histamine (10^{-3}–10^{-6} mol/l).

The specificity of the histamine response was further evaluated by incubating histamine (10^{-2} mol/l) with histamine decarboxylase (histaminase, 10^{-3} mol/l) for 1 hr at 37°C, prior to its addition to the culture medium. Controls included histamine alone (10^{-2} mol/l), histaminase alone (10^{-3} mol/l), IAA, the reaction product of histaminase on histamine (10^{-2} mol/l), and histamine (10^{-2} mol/l) in 20% heat-inactivated FCS which contained histaminase (H. R. Colten, personal communication).

Identification of the histamine receptor involved in the effect on C2 production. (a) *The use of histamine antagonists:* Naïve monocytes were cultured in RPMI–FCS alone, or RPMI–FCS containing histamine (10^{-4} mol/l), histamine + chlorpheniramine (10^{-3} mol/l), histamine + cimetidine (10^{-3} mol/l), chlorpheniramine alone (10^{-3} mol/l) or cimetidine (10^{-3} mol/l) alone.

(b) *The use of histamine agonists:* Naïve monocytes were cultured in RPMI–FCS alone, or in RPMI–FCS containing dimaprit, 4-methyl histamine or 2-2-AET at 10^{-3}–10^{-7} mol/l.

Kinetics of the histamine effect. Histamine (10^{-5} mol/l) in RPMI–FCS was added to a series of replicate naïve and established monocyte cultures. At 5, 30 and 60 min and 16 hr one supernatant was removed, fresh RPMI–FCS substituted, and the cultures incubated at 37°C as described earlier. Controls included one culture which had never been exposed to histamine, and one which had histamine present continuously. The supernatants taken at 5, 30 and 60 min and 16 hr were then added to a second set of monocyte monolayers from the same donor and incubated under identical conditions.

RESULTS

Effect of histamine on C2 production by monocytes

The addition of histamine to both naïve and established monocyte cultures produced a dose-dependent reduction in the concentration of C2 in all the samples of supernatants taken daily (Fig. 1). The reduction in C2 concentration in the supernatants of histamine-treated cultures was not due to a cytotoxic effect; the percentage of cells staining with trypan blue was never greater than 5% in the histamine-treated cultures. Neither was the fall in C2 concentration due to loss of monocytes from the monolayers, as the DNA concentrations in the lysates of the histamine-treated cultures did not differ from the controls when the drug was used at concentrations of 10^{-5} and 10^{-6} mol/l. However, there was a slight decrease (approximately 15%) in the DNA content of 10^{-4} M histamine-treated cultures (Fig. 2). This decrease was statistically significant only in naïve monocyte cultures after 7 days of incubation at a histamine concentration of 10^{-4} mol/l ($P < 0{\cdot}05$). Histamine in concentrations of 10^{-4} and 5×10^{-5} also produced a slight but statistically insignificant fall in the DNA content of established monocyte monolayers. Thus the histamine effect is not due to monocyte death or loss of cells from the monolayers.

The dose–response curves showed that histamine produced a dose-dependent inhibition of C2 production (Fig. 3) and that established cultures were more sensitive to histamine than were naïve cultures.

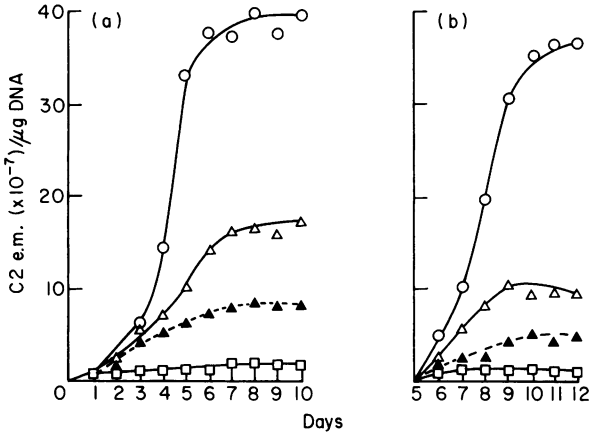

Fig. 1. Kinetics of C2 production by cultured human monocytes, in normal medium (o—o) or medium containing histamine (Δ—Δ, 10^{-6} mol/l; ▲—▲, 10^{-5} mol/l; or □—□, 10^{-4} mol/l). (a) Naïve monocyte cultures, (b) established monocyte cultures.

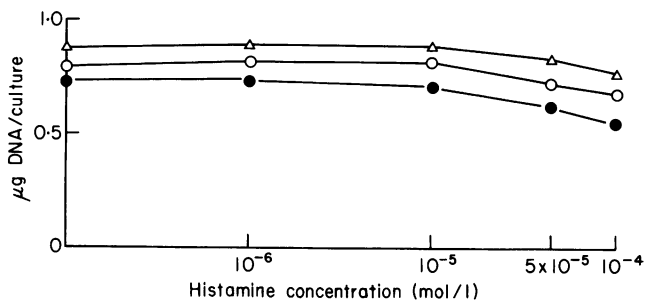

Fig. 2. Effect of histamine on the cell content of monocyte monolayers; 1 day after exposure to histamine (Δ—Δ), 3 days after exposure (o—o), 7 days after exposure (●—●).

Specificity of the histamine effect

Neither histidine, 1-methyl histamine, 3-methyl histamine or *N*-acetyl histamine had any effect on C2 production although IAA appeared to enhance C2 production at high concentrations (10^{-3} mol/l) (Fig. 4). Preincubation of histamine with histaminase or foetal calf serum completely abrogated the inhibition of C2 production, and was associated with a slight enhancement of C2 production, as was the addition of histaminase alone to the monocyte culture fluid (Fig. 4).

Identification of histamine receptor involved in inhibition of C2

Use of histamine antagonists. It was found that in the presence of cimetidine, histamine did not inhibit C2 production, whereas chlorpheniramine could not reverse the histamine effect (Fig. 5). Neither cimetidine (10^{-3} mol/l) nor chlorpheniramine maleate (10^{-3} mol/l) alone had any significant effect on C2 production.

Use of histamine agonists. Both the H2 receptor agonists, dimaprit and 4-methyl histamine, produced a dose-dependent inhibition of C2 production by monocytes, whereas the H1 receptor agonists, 2-2-AET, did not affect C2 production (Fig. 6).

The kinetics of the histamine effect

The kinetics of the histamine effect on C2 production show that its onset is extremely rapid, and

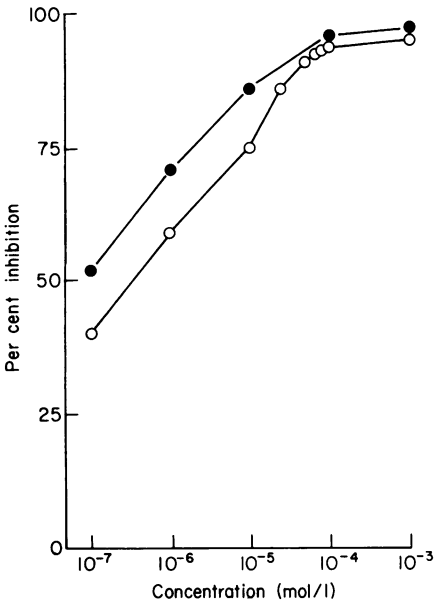

Fig. 3

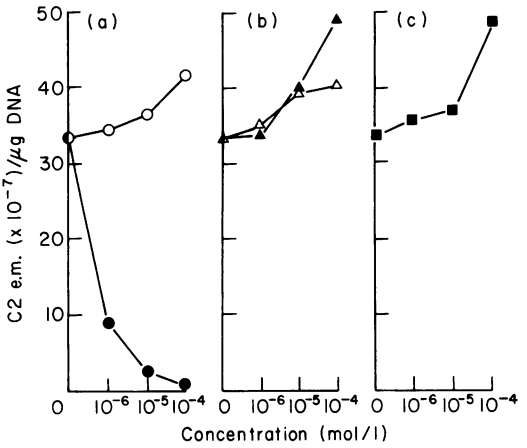

Fig. 4

Fig. 3. Dose–response curves for histamine-mediated inhibition of C2 production by monocytes. Naïve monocyte cultures (o—o); established monocyte cultures (•—•). Each point represents the mean of four cultures.

Fig. 4. The specificity of the histamine effect on monocyte C2 production. (a) Comparison of the effects of histamine (•—•) and imidazole acetic acid (o—o) on C2 production. (b) Effect of preincubating histamine with histaminase (▲—▲) on C2 production. The effect of histaminase alone (△—△). (c) Effect of preincubating histamine with 20% foetal calf serum on C2 production (■—■). Each point represents a single determination.

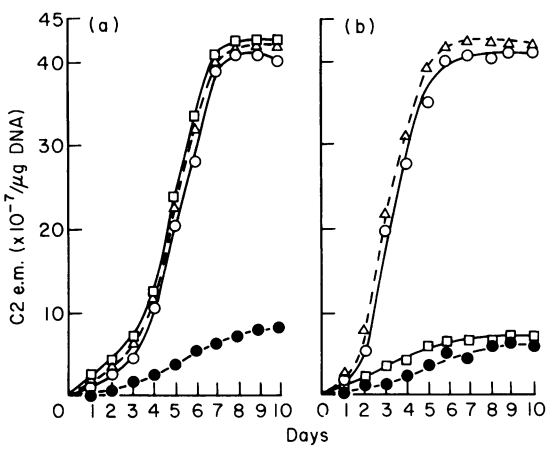

Fig. 5. Effect of histamine antagonists on histamine-mediated inhibition of C2 production. (a) Kinetics of C2 production in control cultures (o—o), culture containing histamine (•—•, 10^{-4} mol/l), chlorpheniramine (□—□, 10^{-3} mol/l) and cimetidine (△—△, 10^{-3} mol/l). (b) Kinetics of C2 production in control cultures (o—o), cultures containing histamine (•—•, 10^{-4} mol/l), histamine+chlorpheniramine (□—□, 10^{-3} mol/l), and histamine+cimetidine (△—△, 10^{-3} mol/l).

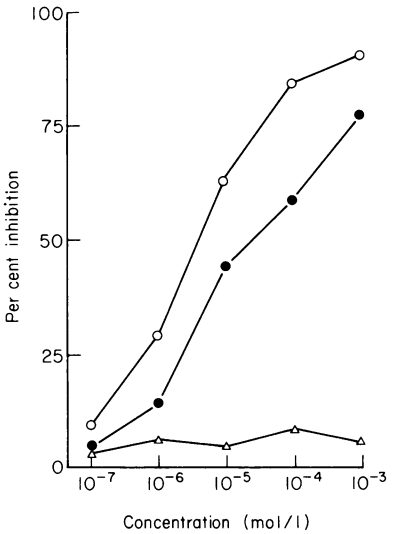

Fig. 6

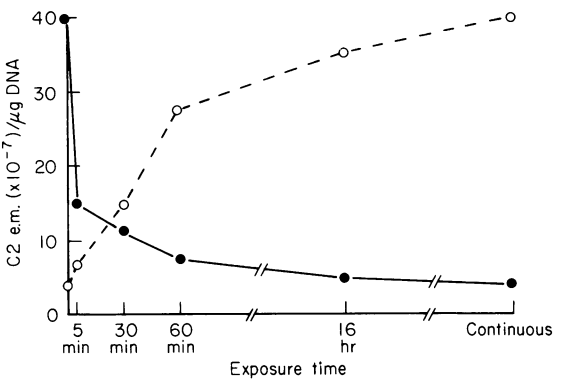

Fig. 7

Fig. 6. Effects of histamine receptor agonists on monocyte C2 production. Dimaprit (o—o), 4-methyl histamine (•—•) and 2-2-AET (Δ—Δ).

Fig. 7. Kinetics and irreversibility of histamine effect on C2 production. C2 concentrations in monocyte culture supernatants exposed to histamine (10^{-5} mol/l) for different time intervals. At the times shown, the supernatant was removed and added to monocyte monolayers from the same donor to examine the rate of loss of the histamine activity in the culture medium (o—o). Naïve monocyte cultures: C2 concentrations measured on day 7.

irreversible (Fig. 7). Both the initial rate and the final extent of this effect were more marked in established than naïve cultures. There was a progressive decrease with time in the ability of the culture supernatants to reduce C2 concentrations when added to fresh monocyte monolayers. This loss of activity was probably due to the degradation of histamine by histaminase in foetal calf serum.

DISCUSSION

Histamine, a mediator of inflammation, may be released from mast cells or basophils by the action of anaphylotoxins. In this study we have shown that histamine inhibits the production of C2 by human monocytes in culture. The effect was seen in cultures of naïve monocytes before they started to produce C2, and also on established cultures which were already producing C2. The inhibition of C2 production was found to be dose-dependent with almost complete inhibition of C2 production occurring at histamine concentrations of 10^{-4} mol/l and over, and progressively less inhibition with decreasing concentrations of the compound. The sensitivity of the monocyte to histamine is shown by the observation that concentrations as low as 10^{-7} mol/l caused between 40 and 50% reduction in the C2 concentration (Fig. 3). The inhibitory property of histamine was not due to a cytotoxic action or to a reduction in the number of monocytes in the monolayers (Fig. 2). Although at histamine concentrations of 5×10^{-5} and 10^{-4} mol/l there was a decrease in the number of monocytes in the monolayers as shown by their reduced DNA content, this was never less than 85% of the control.

The specificity of the histamine reaction was established by the failure of histidine, or histamine metabolites to reduce C2 production, and by the abrogation of the histamine effect by preincubating histamine with the enzyme histaminase, either in the isolated form or in the foetal calf serum

(Fig. 4). The ability of IAA to stimulate C2 production by monocytes is curious but it is not the first biological activity ascribed to this molecule; Turnbull & Kay (1976) have shown that IAA is selectively chemotactic for eosinophils, and Herman *et al.* (1979) have shown that IAA inhibits C3b-mediated histaminase release from neutrophils.

The binding of histamine to monocyte cell membranes appears to be by H2 receptors as cimetidine but not chlorpheniramine inhibited the effect (Fig. 5). These results were supported by the observation that the H2 receptor agonists dimaprit and 4-methyl histamine exerted a similar effect to histamine, whereas the H1 agonist 2-2-AET was inactive (Fig. 6). Ox erythrocytes coated with a histamine–rabbit serum albumin conjugate rosette with monocytes via H1 receptors (Kay, personal communication). Weinstein and his colleagues (Weinstein *et al.*, 1973) found histamine rosettes on the majority of leucocytes and these were formed via H1 receptors, whereas all the known actions of histamine on these cell types are mediated via H2 receptors (Weissmann *et al.*, 1974; Rocklin, 1976; Lichtenstein & Gillespie, 1976; Plaut *et al.*, 1973). The biological significance of the H1 receptors demonstrated by rosetting is obscure, as histamine–protein complexes conjugated to beads were incapable of increasing intracellular cyclic AMP levels, which is thought to be the mechanism by which histamine exerts its effects on leucocytes (Weinstein *et al.*, 1973).

The increased sensitivity to histamine and the more rapid onset of the histamine effect in established compared with naive monocyte cultures (Fig. 7) could be due to the presence of increased numbers of histamine receptors on monocytes which have been cultured for 5 days. It has previously been shown that H2 receptors on lymphocytes increase during the maturation of these cells (Plaut *et al.*, 1973; Ballet & Merler, 1976).

The mechanism whereby histamine inhibits the production of C2 by monocytes has not yet been elucidated. It could be simply inhibiting the release of C2 from the monocyte following synthesis of the molecule. Alternatively histamine may inhibit synthesis of C2. Histamine is known to inhibit secretion of histamine from mast cells (Bourne *et al.*, 1971), and lysosomal enzymes from neutrophils (Weissmann *et al.*, 1974), so it is therefore probable that histamine inhibits secretion of C2 by monocytes. The mechanism of histamine inhibition of C2 production is currently under investigation.

The biological importance of this phenomenon is that histamine, released by the actions of C3a and C5a on mast cells or basophils, can then limit further anaphylotoxin formation as the reduced C2 production of monocytes will restrict formation of the C3 and C5 convertases, $C\overline{42}$ and $C\overline{423}b$.

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