The Role of Complement in Allergen–Reagin Mediated Histamine Release from Monkey Lung Tissue[1] (35260)

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The role that serum factors, particularly the complement (C) system, play in allergic reactions has been somewhat uncertain (1, 2). Lichtenstein and Osler (3) showed that inactivation of C1, C4 and C2 had no effect on the *in vitro* release of histamine from leukocytes obtained from patients allergic to ragweed pollen. The partial inhibition of Prausnitz-Kustner reactions by a C3 inactivator isolated from human serum (4) suggested a role of C3 in human allergic reactions. A relationship between C3 and anaphylatoxin has been reported by several investigators (5–7), and the increases in vascular permeability resulting from the formation of anaphylatoxin was discussed by Lepow *et al.* (8). With these observations in mind, we felt that the role of C3 in allergic reactions should be reexamined using the *in vitro* release of histamine from monkey lung tissue passively adsorbed with human reagin (9). The results of these studies demonstrate that both the C3 inactivator and the rabbit anti-C3 sera markedly inhibit allergen induced histamine release from monkey lung tissue indicating a role of C3 in human allergic reactions.

Material and Methods. The nine purified human complement components (C1–C9), C1 and C3 inactivators, stabilized in gelatin, were obtained from Cordis Laboratories (Miami, Florida). Rabbit anti-C3 was prepared by subcutaneous immunization of rabbits at weekly intervals for 4 weeks with 2000 units of purified human C3 in complete Freund's adjuvant. Rabbits were bled 2 weeks later, and the antisera was tested for its specificity by immunoelectrophoresis and gel diffusion against the nine purified complement components and C1 and C3 inactivators. The rabbit anti-C3 sera showed a precipitin band only against the purified human C3 complement component. The reaginic antibody used to passively sensitize monkey lung tissue was obtained from timothy grass pollen sensitive patients. A typical experiment involved the sensitization of monkey lung tissue with reagin containing human serum by incubation at 37° for 2 hr. The tissue was washed with 75–100 ml of fresh Tyrode's solution, incubated with varying concentrations of either the C3 inactivator or rabbit anti-C3 for 10 min at 37° prior to the addition of timothy allergens (WST) (10). The reaction mixture was incubated for an additional 10–15 min at 37°, and the histamine released was measured spectrofluorometrically as previously described (9). Control experiments were also performed where either gelatin or normal rabbit sera were added to the reaction mixture rather than the C3 inactivator or rabbit anti-C3 sera.

Results and Discussion. Figure 1 shows the effect of C3 inactivator on the release of histamine from monkey lung tissue. A direct relationship between the concentration of C3 inactivator and the degree of inhibition of histamine release is apparent. On the other hand, neither heat inactivation, 56° for 20 min (inactivates C1 and C2, but had no effect on reagin titer), of the human sera used to passively sensitize the monkey lung tissue nor the addition of 400 units of human C1 inactivator had any effect on allergen induced histamine release. These results would suggest that the reaction mechanism of allergic histamine release involves C3 without the prior activation of C1, C4, and C2.

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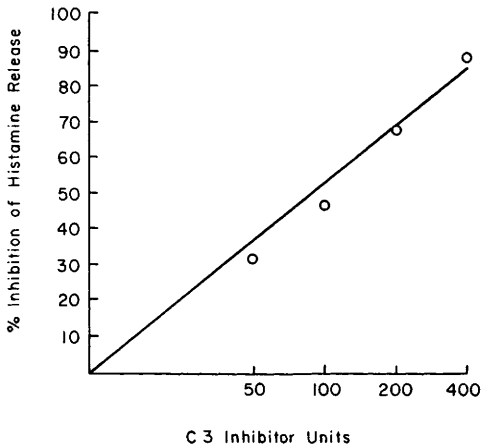

FIG. 1. Effect of C3 inactivator from human sera on the release of histamine from monkey lung tissue passively sensitized with human reagin.

The depletion of C3 from sera used to passively sensitize monkey lung tissue was accomplished by incubation with rabbit antihuman C3 sera at 37° for 30 min, and an additional 48 hr at 4°. The supernatant was carefully removed, tested for the removal of C3, and then used to passively sensitize monkey lung tissue. Depletion of C3 from human allergic sera had no effect upon the histamine releasing capacity of the sera. This observation suggested that the C3 participating in allergic histamine release was already bound to the monkey lung tissue. A series of experiments utilizing the rabbit antihuman C3 sera were designed (i) to determine if C3 is bound to monkey lung tissue, and (ii) to provide additional information regarding a functional role of C3 in allergic histamine release from monkey lung tissue. The effect of rabbit antihuman C3 sera upon addition to monkey lung tissue sensitized at 37° for 2 hr with normal human sera, saline, or serum from a timothy sensitive patient is shown in Table I. Further evidence for a functional role of C3 in the allergic histamine release is indicated by the 40% inhibition observed upon addition to the reaction mixture of rabbit antihuman C3 sera (0.2 ml). The incubation of tissue sensitized with either normal human sera or saline with rabbit antihuman C3 sera for 10 min at 37° showed no histamine release. On the other hand, tissue washed thoroughly with Tyrode's solution (100–120 ml) and incubated with rabbit antihuman C3 sera for 2 hr at 37° shows a significant level of histamine release (3 μg/ml). Tissue similarly incubated with normal rabbit sera shows no release of histamine.

The results of these experiments would suggest that C3 is not only involved in allergic histamine release reactions but also is functionally bound to the tissue prior to passive sensitization of the tissue with human

TABLE I. Effect of Rabbit Antihuman C3 Sera on Histamine Release from Monkey Lung Tissue.

Tissue[a] sensitization	Treatment[b]	Antigen challenge[c]	Histamine release[d]	Inhibition (%)
Normal sera	0.2 ml antihuman C3	WST	—	—
Allergic sera	0.2 ml NRS	WST	4.72	—
	0.1 ml antihuman C3	WST	3.88	18
	0.2 ml antihuman C3	WST	2.82	40
Saline	0.2 ml antihuman C3[e]	—[f]	3	—
	0.2 ml NRS	—	—	—

[a] Tissue incubated with normal human sera or human allergic sera at 37° for 2 hr, then washed with 150 ml of Tyrode's solution.

[b] Sensitized tissue incubated with normal rabbit sera (NRS) or rabbit antihuman C3 for 10 min at 37°.

[c] Tissue incubated additional 10 min at 37° with WST.

[d] Histamine (μg/ml sera/g of tissue).

[e] Tissue incubated at 37° for 2 hr and reaction mixture assayed for histamine.

[f] (—) represents none or zero.

reagin. Huber *et al.* (10) previously demonstrated that human monocytes contain two distinct receptor sites, one specific for C3 and the other for immunoglobulin G (IgG). It would appear from these studies that monkey lung tissue similarly has distinct receptor sites for both human reagin and the C3 component of complement. The histamine release due to the interaction of rabbit antihuman C3 sera and tissue bound C3 may be due to a reaction mechanism similar to that described by Austin and Valentine (11) involving all nine complement (C1–C9) components. On the other hand, the reaction mechanism that releases histamine from monkey lung tissue due to allergen–reagin interaction involves C3 without the participation of complement components C1, C4, or C2. Recently Sanberg *et al.* (12) reported that histamine released from rabbit platelets with guinea pig homocytotropic antibody (γ-1 globulin) involves the participation of complement components C3, 5–9 but not Cl, C4, and C2. The reaction mechanisms of histamine release from monkey lung tissue due to allergen–reagin interaction also may involve additional complement components. Experiments are in preparation to evaluate this possibility. Preliminary experiments with leukocytes from timothy sensitive patients indicates that the C3 component of complement also plays a functional role in the histamine released from these cells.

Summary. Both C3 inactivator isolated from human sera and rabbit antihuman C3 sera markedly inhibit allergen induced histamine release from monkey lung tissue passively sensitized with human reagin. These studies suggest that C3 participates in allergic reactions without the prior action of C1, C4, and C2 and that monkey lung tissue has distinct receptor sites for both C3 and human reagin.

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