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BLOOD GROUP ACTIVITY OF ALLERGENIC PREPARATIONS

By

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In 1947, Rimington et al. (11) reported that their standard crude antigen SCA from house dust extracts exhibited A- and B-blood group specificity by inhibiting the agglutination of human A or B red cells by their respective natural antibodies. Blood group activity of the allergenic preparation was an order of magnitude lower than that exhibited by regular A- or B-substance. Evidence was subsequently provided by Brocteur & Versie (9) for the association of A, B, H, Le^a and P₁ activity with highly purified house dust allergen preparations; there was no evidence for M, N or D(Rho) specificity. These authors consequently considered that positive cutaneous reactions with house dust in normal and allergic people might be due to incompatible local A- anti A or B- anti B reactions. Experimental verification of this idea, using A and B group specific substance of human origin, revealed a few cases of immediate-type reactivity, but no consistency with the reactions observed with house dust allergens; nor did a relationship exist with the blood type of the test subjects (14).

Our interest in the blood group activity of allergens was stimulated by studies on the interaction of human fluid phase complement with the common allergens. The inactivation of human haemolytic complement by purified house dust fraction E was found to proceed through the classical pathway of C-consumption, i.e. by C₁ engagement (3, 7). Because this house dust fraction was prepared in identical fashion to the

product found to exhibit blood group activity by Brocteur & Versie (9), the possibility was raised that the observed C-consumption in human sera is mediated by immune complexes of allergens and human isoagglutinins. However, this paper documents the finding that allergen-induced complement consumption is not due to blood group incompatibilities.

MATERIALS AND METHODS

Human A, B, O or AB erythrocytes were obtained from volunteer blood donors; the cells were washed free of plasma and anti-coagulant and stored at $+4^{\circ}\text{C}$ in Alsever solution. Prior to use, the cells were washed and resuspended in veronal-saline buffer VSB⁺⁺, pH 7.4, containing $7.5 \times 10^{-4}\text{ M}$ CaCl_2 and $4 \times 10^{-2}\text{ M}$ MgCl_2 (Kabat & Mayer (10)).

Anti-A, anti-B and anti (A+B) test sera used for typing and for haemagglutination-inhibition were obtained from Behringwerke Diagnostics. Group specific substance A (*ex* porcine gastric mucosae) and substance B (*ex* equine gastric mucosae) were from Pfizer Diagnostics, New York. Isoagglutinin titers of sera were determined as in the previous paper (4). For establishing the blood group activity of allergenic preparations, solutions of freeze-dried preparations were made up at 5 mg/ml in VSB⁺⁺; 5 drops were mixed in doubling dilutions in 1 ml glass tubes with 5 drops of anti A- or -B test reagent. After 60 min at room temperature, 1 drop of a 2 per cent erythrocyte suspension was added. Agglutination was read by mild tapping after 30 min at room temperature, following a 2 min centrifugation period. The test sera were used in the dilution just preceding the agglutination titer. Complement inactivation by allergens was assayed as described before (4).

Allergenic preparations were obtained as described elsewhere (2). The following preparations were whole, dialyzed, lyophilized, extracts: feathers, timothy pollen, ragweed pollen, castor beans and guinea pig dander. Mould allergenic preparations were acetone-precipitated, dialyzed, culture filtrate antigens from *Micropolyspora faeni*, *Aspergillus fumigatus* and *Alternaria tenuis* grown on dialysable synthetic media. Horse dander, cat dander and dog dander (poodle) allergens were partially purified products, isolated by ammonium sulphate precipitation (C-products; 0.3–0.8 saturation). Human dandruff allergen E, house dust fractions and ipecac, fraction D, were isolated as described (2, 8). A UV-inactivated house dust allergen, 72E/UV, was prepared as reported (6).

RESULTS

Purified house dust allergen preparations were confirmed to exhibit both A and B blood group activity by inhibiting human

TABLE 1

Haemagglutination-inhibition Titers of a Number of Allergenic Preparations, Expressed as the Reciprocal of the Last of a Series of Dilutions of a 0.5 per cent Allergen Solution still Inhibiting A-anti A Agglutination (A-titer) or B-anti B Agglutination (B-titer). The Capacity to Inactivate Human Haemolytic Complement was Expressed as the Quantity of Allergen Required for a 50 per cent Reduction of Haemolytic Complement from (25 μ l of) the Serum of a Single Allergic Individual.

Allergenic preparation	A-titer	B-titer	C-inactivation, μ g
House dust, 72/E	512	256	0.5
Feathers, FEB	16	16	0.9
Human dandruff, E	16	16	0.7
Horse dander, C	16	8	5.1
Dog dander, C	512	32	2.1
Cat dander, A	16	<2	10.3
Guinea pig dander, A	8	4	17.6
Castor beans	<2	<1	N.D.
Ipecac, D	2	<1	6.3
Timothy pollen	<2	<1	>100
Ragweed pollen	<2	0	>100
<i>M. faeni</i>	512	0	18.0
<i>A. fumigatus</i>	32	<1	21.2
<i>A. tenuis</i>	32	<1	25.0
Ovomucoid	<1	<1	>100

red cell agglutination with anti A- and -B sera. Several other allergenic preparations were found to exert weak inhibiting capacity as well, albeit of relatively low potency. The majority of the allergens displayed A-specificity, less often B-specificity. Examples have been listed in Table 1. Also shown in Table 1 are the relative capacities of these different allergenic preparations to inactivate haemolytic complement in fresh human serum. Apparently, this property of allergens is not closely related to their A- or B-specific activities.

Brocteur & Versie (9) noted that the agglutination-inhibiting potential of the D-fraction of house dust (rich in human serum proteins) was lower than that of the more powerfully skin-active E-fraction. As shown in Table 2, this was con-

TABLE 2

Blood Group (A) Activity of a Number of Subfractions Obtained in the Course of House Dust Extract Fractionation (Berrens 1971); Expressed as Haemagglutination-Inhibition Titers. Also Shown is the Concentration of Test Extracts Required for Wheal and Flare Reactions on Intradermal Testing and the Human Complement-consuming Capacity (Table 1).

House dust subfraction	A-titer	Test strength, $\mu\text{g/ml}$	C-inactivation, μg
B	16	100	2.0
C	128	10	0.9
D	256	10	1.8
E	512	1	0.5
F	0	inactive	inactive

firmed for a number of different subfractions of house dust extract, isolated on the basis of a purification schedule employed both by these authors and ourselves (2, 8). Though these data indicate that the blood group activity tends to increase with the degree of purification, a correlation between A/B-activity and skin-reactivity among the total group of allergens was not apparent. For example, skin reactions to equal doses of purified house dust and human dandruff allergens very frequently run parallel, despite the difference in A/B titers, but those to house dust and dog dander do not, despite identical titers (Table 1).

The possible involvement of natural A/B-antibodies in mediating allergen-induced loss of haemolytic complement was also investigated by absorption studies, using the high-titered house dust 72/E preparation as an allergen source. In a typical experiment, recorded in Table 3, 0.2 ml of human allergic serum of an O-type donor was diluted 1:10 in veronal-saline buffer containing 0.002 M EDTA; blood group antibodies were absorbed for 30 min at 20° C by adding a suspension of 5×10^8 human AB cells and 2×10^8 A cells. After recalcification, the 72/E sensitivity of complement in the supernatant was assessed after adding 4 ml of preserved guinea pig complement (1.2 CH₅₀ units) as an extra source of C-factors. In

TABLE 3

Influence of A/B-isoagglutinin Absorption upon House Dust Allergen Activation of a Human Serum Factor Capable of Acting on Human, and Subsequently Guinea Pig, Haemolytic Complement.

	Before absorption	After absorption	
		at 20° C	at 0° C
anti A titer	240	<30	<30
anti B titer	240	<30	<30
72/E complement-sensitivity index, μ g	1.8	2.2	2.2

Human serum (0.2 ml, type O allergic donor) was diluted 1:10 in VSB-EDTA buffer; blood group antibodies were adsorbed for 30 min at 20° C with 5×10^8 human AB cells and 2×10^6 A cells. The mixture was centrifuged, the supernatant recalcified with 0.88 mg CaCl_2 , and 4 ml guinea pig complement (1.2 CH₅₀) was added; 0.5 ml was subsequently used for establishing the allergen 72/E index as described in Materials and Methods. In the absorption experiment at 0° C, no EDTA-chelating agent was employed.

a twin experiment, absorption was carried out for 20 h at 0° C in the absence of the EDTA chelating agent. The results, shown in Table 3, demonstrate that absorption of the A/B-isoagglutinins had no significant influence on the human serum factor mediating complement consumption by house dust allergen.

The non-participation of blood group antibodies was confirmed by hapten-inhibition. In these experiments, a series of tubes containing 0.5 ml 1:20 diluted human serum was kept at 37° C for 20 min in the presence of different (amine)-sugars in a dose-range of 0.5–16.0 mg. Complement-inactivation by 2 μ g of house dust allergen 72/E was then evaluated as described (4). In case of detectable inhibition of complement inactivation, dose-response curves were subsequently established, using variable allergen concentrations and a fixed sugar dose.

No inhibition of allergen-induced complement consumption was observed with: D-glucose, D-galactose, D-mannose, D-fructose, 2-deoxy-D-glucose, D-xylose, L-arabinose, L-fucose,

TABLE 4

Effect of Ultraviolet Irradiation upon the A- and B-titer of House Dust Allergen 72/E, Assessed by Haemagglutination-Inhibition. Irradiation for 45 min with Unfiltered Energy from a Hg-arc as Described (Berrens et al. 1973), using a 0.5 per cent Solution of 72/E in Saline for both Irradiation and Agglutination-Inhibition Step.

Preparation	A-titer	B-titer
Active house dust 72/E	≥ 512	128
Inactive dust 72/E-UV	8	≤ 4

L-sorbose, D-sorbitol, D-mannitol, maltose, lactose, sucrose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, α -D-galacturonic acid, or A-substance. Solutions of B-substance (A-titer 16, B-titer 1024) and of N-acetyl-D-glucosamine (A-titer 2, B-titer 16) both appeared slightly inhibitory, but this effect was entirely due to increased haemolysis in the presence of these compounds. Similarly, the amine sugars D-glucosamine and D-galactosamine augmented complement-mediated red cell lysis by 400 per cent. Correcting for this effect, only the D-glucosamine sugar, at 0.085 M concentration, completely inhibited the complement depletion induced by house dust allergens.

It has been shown that both the complement-consuming power and the skin-reactivity of purified house dust allergen

TABLE 5

Effect of Ultraviolet Irradiation upon an Aqueous Solution of Group-Specific A-substance (ex Porcine Gastric Mucosae) of the Indicated Agglutination-Inhibitory Strength.

Irradiation period (min)	A-titer
0	256 000
15	256 000
30	256 000
60	256 000

preparations are abolished by ultraviolet irradiation (6). Model experiments have made it plausible that this inactivation is largely due to the loss of "lysine-sugar" reactive sites from the allergen molecules. The data in Table 4 show that UV-irradiation under identical conditions likewise causes a sharp decline of the blood group activity of house dust allergens. This result cannot be ascribed to the destruction of regular, major or minor, A or B specific determinants, since even prolonged UV-irradiation of A-substance remained without effect on its agglutination-inhibitory power (Table 5).

DISCUSSION

Purified active house dust allergen preparations have been confirmed to exhibit A- and B-blood group specificity; the agglutination-inhibition titers of allergen product 72/E were in the same range as recorded by earlier investigators (9, 11). Moreover, a number of other allergenic preparations also display A- or B-specificity, though the inhibitory power of most products was quite weak. Comparison with the capacity of these preparations to induce consumption of human haemolytic complement *in vitro* provided no indication of positive correlation between these two properties of allergens; a relationship between blood group activity and skin-reactivity likewise appeared very unlikely. The non-participation of A/B-isoagglutinins in the phenomenon of human C consumption by allergens was confirmed by absorption experiments.

The association of A/B-activity with allergenic preparations is not surprising: polysaccharides or glycoproteins with blood group specificity are known to be widespread among plants and animals. Since the A/B-activity of allergenic products is obviously unrelated to their potency in skin tests, the property, as such, is of little consequence to allergology. The complement-inactivating power of a large number of allergens, on the other hand, is an easily reproducible *in vitro* phenomenon, which may have its *in vivo* correlate (3, 5, 6, 7). The present absorption and hapten-inhibition studies rule out

the involvement, in this respect, of blood group isoagglutinins. Neither N-acetyl-D-galactosamine (the immunodominant structure of A₁ and A₂ specificity), nor D-galactose (B-specificity) or L-fucose (Le^a and H-specificity) inhibited allergen-induced complement consumption. It also appears unlikely that P-specific antibodies are involved, because P₁ specificity is associated with α -galactosyl residues (12). Several other sugars commonly incorporated in natural heteropolysaccharides, and in allergens, likewise failed to block complement consumption, so that mediation by sub-populations within the class of blood group antibodies is equally unlikely (2, 15).

The observed inhibition of house dust allergen-induced complement consumption by D-glucosamine has some interesting consequences. It indicates that the serum protein receptor-mediating complement inactivation is sensitive to an activating site on allergens structurally resembling glucosamine. An obvious candidate is the lysine-sugar site on house dust allergens, in 2,3-enediol stabilized configuration, which is partly homologous to glucosaminyl residues (2). The homology region involves the nitrogen and C-atoms 2, 3 and 4 of glucosamine and the ϵ -nitrogen of lysine and C-atoms 1, 2 and 3 of the proposed 'allergenic site', the difference being the location of a double bond between C₂ and C₃ in the latter. It has been shown that this double bond is sensitive to ultraviolet irradiation, a procedure which abolishes both skin-reactivity and the *in vitro* complement-inactivating capacity (6). The present experiments demonstrate a rapid depletion of the agglutination-inhibitory power of house dust allergens in these conditions. As corroborated by the experiment with A-substance, constituent sugars of heteropolysaccharides can be expected to be quite resistant to UV-irradiation. It is probable, therefore, that the blood group activity of house dust allergens is due in part to lysine-sugar sites.

The above-mentioned results point to a vital role of an unsaturated sugar residue conjugated to lysine side-chains of house dust allergens. Current studies show that house dust allergens, after activating a human serum factor, fail to do so a

second time, i.e. become 'inactivated', presumably by loss of the double bond. There is little to support the idea that this serum factor represents a conventional class of naturally-occurring antibody. The participation of hypothetical (thermolabile) natural antibodies—crossreacting with house dust allergens and capable of consuming human complement only—can, however, not be excluded.

Unrefined house dust extracts may contain allergenically inactive heteropolysaccharides crossreacting with pneumococcal polysaccharides, e.g. type-specific substances VII, XII, XV and XVIII (13). Whereas natural antibodies do not appear to mediate complement consumption even with relatively crude preparations, it is a distinct possibility that such antibodies do interfere in other present-day *in vitro* techniques in allergy. For examples, they might be among the "RAST-blocking" factors found to partially prevent the uptake of house dust specific IgE (1).

SUMMARY

Haemagglutination-inhibition studies showed that several different allergenic preparations exhibit A and B blood group activity. Most products displayed A-specificity, less often B-specificity; the inhibition titers were usually low. No correlation was evident between isoagglutination-inhibition titers and the complement-consuming capacity or skin-reactivity of the allergens. Absorption of A- and B-isoagglutinins from human serum did not affect the complement consumption by house dust allergens. Hapten-inhibition studies confirmed the non-participation of blood group antibodies in allergen-induced complement consumption. The observed inhibition by D-glucosamine is attributed to a partial structural homology with the lysine-sugar site on house dust allergens.

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