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THE ORIGIN OF SERUM PROTEIN, A, B AND H BLOOD GROUP, AND Gm AND Inv ANTIGENS IN HOUSE DUST

By

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Several workers have examined the biochemical constitution of house dust, and have found it to contain a number of antigenic compounds, including some which react with antibodies to human serum and red cell antigens. Thus, the serum proteins, albumin, IgG and IgA, and the IgG allotype, Gm(1), have been identified immunologically and are considered to be of human origin (1, 5, 9). The red cell antigens A, B, H, Le^a and P₁ have also been detected (6). Their origin is not certain, since there are several known nonhuman sources of these antigens, and since the P₁ antigen has not been identified in saliva or tissues, though it is found on blood cells.

In the work to be reported here an extract of house dust was examined for the presence of human serum proteins by immunoelectrophoresis and immunodiffusion; for Gm and Inv antigens by haemagglutination inhibition; and for A, B and H blood group antigens also by haemagglutination inhibition. The possible human origin of these antigens has been examined and the implications of the results are discussed.

MATERIALS AND METHODS

House dust extract: Six kg of house dust were collected during the summer from a vacuum cleaner in a hotel in Bern (Switzerland). An aqueous extract was prepared by a previously reported method (2). The house dust was mixed with 60 l water containing 0.1 per cent sodium azide. The mixture was left at room temperature for 2 weeks, and was stirred daily. It was then filtered through a series of sieves with different mesh sizes and, finally, through cloth. The volume of the liquid was reduced to 1/6 of the original, by removing water through a dialysis membrane. This liquid was then centrifuged at 2500 g to remove particulate matter. A single fraction was precipitated from the extract by 100 per cent saturation with ammonium sulphate. The precipitate was extensively dialyzed against 0.15 M sodium chloride, and was then lyophilized. This is referred to as the house dust extract.

Immunoelectrophoresis: Microscopic slides were covered with 3 ml of a warm solution of 2 per cent agar dissolved in sodium barbital buffer 0.05 M at pH 8.2. Electrophoresis was performed at 100 V during 50 min. To the slide was applied 2 μ l of a solution made by mixing 200 mg of the lyophilized house dust extract in 1 ml of physiological (0.87 per cent) saline. After applying the antisera, the slides were incubated during 24 h at room temperature, washed during 48 h in a 0.87 per cent solution of NaCl, then water, and finally stained with Ponceau-S.

Immunodiffusion: Microscopic slides were covered with 3 ml of a warm solution of 1 per cent agar dissolved in physiological saline. To each hole there were applied 5 μ l of the antisera of the same house dust solution as used in the immunoelectrophoresis. The rest of the technique is the same as that used for the diffusion step in immunoelectrophoresis.

Antisera: The following commercial antisera against human serum proteins from Behringwerke were used: anti-whole human serum, anti-IgG, anti-IgG/Fc, anti-IgG/Fab, anti-IgG/Fd, anti-IgA, anti-IgM, anti-free Kappa chain, anti-free lambda chain, anti-prealbumin, anti-albumin, anti- α_1 acid glycoprotein, anti- α_1 lipoprotein, anti- α_1 antitrypsin, anti- α_1 antichymotrypsin, anti-Gc globulin, anti-ceruloplasmin, anti- α_2 HS glycoprotein, anti-haptoglobin, anti- α_2 macroglobulin, anti- β lipoprotein, anti-hemopexin, anti-C₃/C₃c, anti-C₄, anti-transferrin, anti- β_2 glycoprotein I, anti- β_2 glycoprotein III, and anti-C reactive protein.

Gm and Inv antigens: The Gm and Inv antigens were determined in 1970 with the reagents shown in Table 1, by methods previously published (7).

A, B and H blood group antigens: Anti-A and anti-B were pooled human sera, chosen for their low haemolytic activity and lack of suggestion of "boosting". The anti-A had agglutination titres of 256 with A₁ and 128 with A₂ cells. The anti-B had an agglutination titre of 256 with B cells. *Ulex europaeus* (anti-H) extract was prepared as a 0.87 per cent saline extract of a powder prepared by crushing *Ulex europaeus* in a porcelain ball mill. The clear, pale-yellow liquid was used as the anti-H reagent, and had agglutination titres of 128 with O, 64 with A₂, 16 with B and 8 with A₁ red cells.

TABLE 1

Reagents Used to Determine the Gm and Inv Allotypes in House Dust Extract.

Allotype nomenclature			
Numerical	Alphabetic	Anti-allotype	Anti-D
Gm 1	a	Wee	251
2	x	Tef	Ham
3	f	Goh	Mil
5	b	Ben	3419
6	c	Eil	Key
21	g	Monkey E	Ham
Inv 1	l	Mat	Roe

To test the house dust extract for the presence of ABO antigens, 0.25 g of the lyophilized extract was dissolved in 2 ml of physiological (0.87 per cent) saline. This solution was then examined for antigens by an agglutination inhibition technique using glass blood-spotting tiles. The procedures and the agglutinins used were those which had been used previously for studies of blood group substances in human salivas, and which have been described in detail elsewhere (4). Thus, the results from the present study, and those on the salivas, may be compared.

RESULTS

Immunoelectrophoresis of house dust extract against anti-human serum produced a single line of precipitation which appeared to be due to the presence of albumin, and this was confirmed by immunodiffusion using specific antiserum.

Besides albumin, the house dust extract was tested in immunodiffusion with specific antisera against 21 other proteins (see Materials and Methods) being positive for α_1 acid glycoprotein (Fig. 1) and IgG (Fig. 2). However, immunodiffusion of the extract against specific antisera to the Fab and Fc portions of IgG precipitated only with anti-Fc antiserum, although pooled human serum reacted most strongly with anti-Fab (Fig. 2). It was also negative for IgG/Fd, IgA, IgM and for free light chains.

The extract was examined for the Gm antigens 1, 2, 3, 5, 6

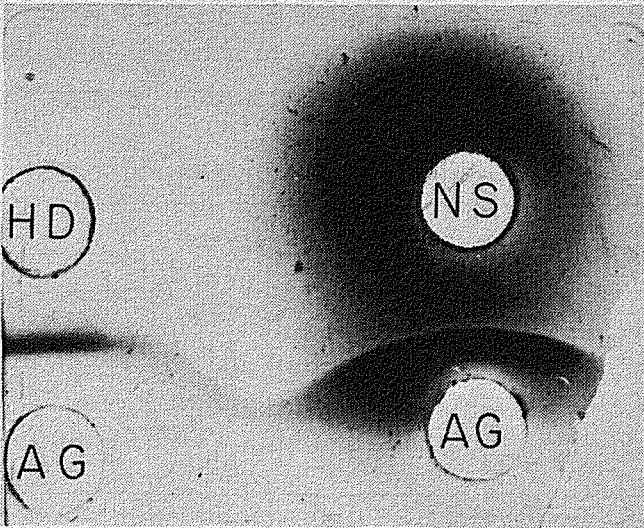


Fig. 1.

Double diffusion between house dust extract (HD) and a pool of 60 normal sera (NS) against anti- α_1 acid glycoprotein (AG).

and 21, and for Inv (1), and all were found to be present (Table 2). These titres are remarkably lower than those found for normal serum, which commonly are equal to or greater than 1/1024. Tests of isolated of myeloma proteins and of Bence Jones proteins indicate that the Gm and Inv allotypes may be detected at concentrations of 0.01 mg/ml or

TABLE 2
Gm and Inv Antigens in House Dust Extract.

Antigens	Reciprocal of the last positive titre
Gm (1)	undiluted
Gm (2)	N.T.*
Gm (3)	N.T.
Gm (5)	undiluted
Gm (6)	4
Gm (21)	N.T.
Inv (1)	N.T.

* N.T. = Not titrated.

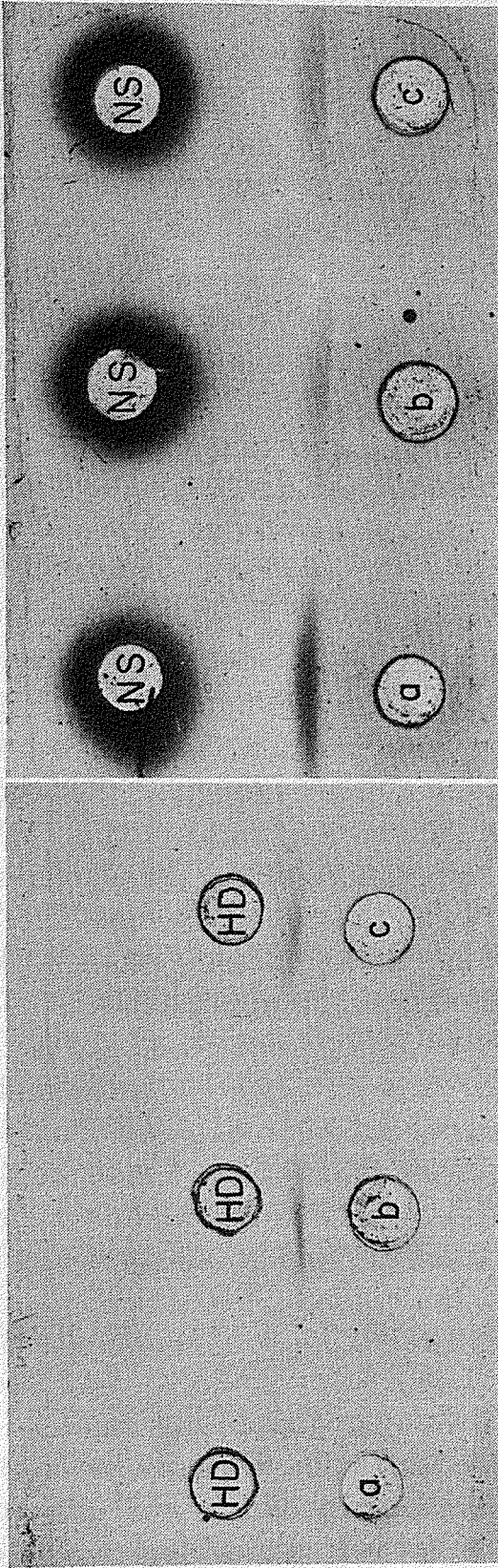


Fig. 2.

Double diffusion between house dust extract (HD) and a pool of 60 normal sera (NS) against anti-Fab (a), anti-IgG (b) and anti-Fc (c).

TABLE 3

Titres of A, B and H Activities in House Dust Extract and Human Salivas.*

Activity	House dust extract	Saliva † (average)	Ratio of house dust : saliva titres
A	4	A ₁ (79) - 0.78	1 : 24
		A ₂ (25) - 0.52	1 : 14
B	1	B (35) - 0.71	1 : 3
H	4	O (127) - 0.88	1 : 24
		A ₁ (79) - 0.57	1 : 12
		A ₂ (25) - 0.08	1 : 16
		B (35) - 1.91	1 : 4

* Titres are expressed as reciprocal of $\log_2$. Thus, a titre of $1/4$ is expressed here as 2, and a titre of 4 as -2 .

† The numbers in parentheses are the numbers of salivas examined, and the A₁, A₂, B and O blood groups of the donors are given.

less. The low titres of the allotypes in the house dust combined with the fact that positive reactions were found for all the allotypes tested, including Gm(6), suggest that the reactions are artefacts and not indicative of the allotypes.

The blood group antigens A, B and H were readily identified in the extract (Table 3). The amounts detected, compared with amounts in saliva, deserve comment. The anti-A inhibition titre was found to be approximately $1/14$ of the mean salivary titre for salivas from group A₂ secretors, and about $1/24$ of that for A₁ secretors. The anti-B inhibition titre was about $1/3$ of that for saliva from group B secretors. The anti-H inhibition titre was about $1/24$ of that for saliva from group O secretors, or $1/16$ of that for A₂, or $1/12$ of that for A₁, or $1/4$ of that for saliva from group B secretors.

DISCUSSION

As previously shown (1, 5), house dust appears to contain serum proteins, at least albumin. Our results from immunoelectrophoresis and from immunodiffusion indicate that albumin is present, and that it is immunologically indistinguish-

able from human serum albumin. There is no suggestion that it is other than this. Consequently, it seems certain that serum albumin of human origin is present.

The presence of IgA in house dust has been reported (9), but we were unable to demonstrate it. However, we detected the presence of α_1 acid glycoprotein (Fig. 1), which at this stage must be considered of human origin.

The results of immunodiffusion of the house dust extract against antiserum to human IgG indicate that human IgG is present. However, some doubt is cast on this conclusion, since specific antisera to the Fab portion of IgG did not react with the house dust extract (Fig. 2). If human IgG had been present, reaction with anti-Fab as well as anti-Fc should have been detected, since human serum reacts more strongly with the former reagent. Further, doubt as to whether the reaction with the anti-IgG is in fact due to human IgG arises from the results of testing for Gm antigens.

If human IgG was present, it would be expected that Gm and Inv antigens should be detectable, especially since the technique used for detecting Gm and Inv antigens (haemagglutination inhibition) is at least 2 orders of magnitude more sensitive than that used for the detection of IgG, (immunodiffusion). However, the detection of Gm(6) activity, at a strength at least comparable with those of the other Gm activities detected, was quite unexpected. This is an antigen found most commonly in Negroes (8) and which is virtually absent in Swiss people. Since the activity of only the Fc portion of IgG was detected, the expectation would be not to find Gm (3) and Inv (1) antigens, since they are located in the Fab portion (8), which seems to be missing in the house dust extract.

Consequently, the absence of Fab and the unexpected presence of Gm(6) activity are inconsistent with what would be expected if human IgG was present, and it should be concluded that the apparent presence of IgG is due to the presence not of human IgG, but of some substance(s) that cross-reacts with anti-Fc. Of course, it cannot be excluded that some human

IgG is present. However, due to the failure to detect Fab activity, much of the apparent IgG activity seems to be due to cross-reaction.

If the A, B and H blood group activities detected in the house dust extract were solely of human origin, it would be expected that the relative expressions of these activities would be highest for H, since approximately half of the population is of blood group O, and further, individuals of the other blood groups express this activity. If a sample was taken from a mixture of equal quantities of secretions from a group of individuals, chosen at random with regard to their ABO blood groups, it would be expected that the H expression would be more than half of its average expression in the group O individuals. Similarly, the expression of B would be expected to be only about $1/10$ of its average expression in group B individuals (since only about 10 per cent express the B antigen).

However, in the house dust extract, the expression of B activity was, relatively, the highest (Table 3). It was present in the house dust extract sample tested at about $1/3$ the average concentration in salivas from group B secretors, whereas the expression of H in the extract sample was only about $1/24$ of the average concentration in salivas from group O secretors. If it should be proposed that the A, B and H activities in the house dust extract are of human tissue origin, rather than from human secretions, and that the H antigen is expressed relatively more weakly on cells than it is in secretions in comparison with A and B antigens (3), a similar argument for the unexpectedly high expression of B can be advanced. In the extract, the expression of B relative to A is higher than would be anticipated in a mixture taken from a population where there are about four times as many group A individuals as there are group B. The anti-A and the anti-B antisera had similar titres with A and B red cells, respectively.

It thus seems clear that the A, B and H activities detected in the house dust extract are not in proportions to be expected if they were of human origin, either from secretions or from

tissues. It would be surprising if A, B and H antigens of human origin were not present in house dust, since the antigens are present in epidermal cells, hair, sweat, etc., and since they are not labile. Consequently, it would seem appropriate to conclude that the A, B and H activities detected are from both human and non-human sources.

House dust is an ubiquitous material and, as discussed, contains materials which react with antisera to human antigenic compounds. However, material of non-human origin appears to be involved in the detection of IgG, Gm and also A, B and H activities. It seems not unlikely that all of these materials could, under the appropriate conditions, stimulate the production of the type of antibodies with which they have been shown to react here. Thus, some of these materials might be the source of stimuli which lead to the production of "natural" antibodies where the source of the antigenic stimulus has not been apparent. However, it would be difficult to ascertain whether house dust *per se*, or the ultimate source of the antigenic materials, is the actual stimulating antigen.

SUMMARY

Materials precipitated from an aqueous extract of house dust by saturation with ammonium sulphate showed immunological reaction with antisera to human serum albumin, human α_1 -acid glycoprotein, human IgG, Gm and Inv antigens and to A, B and H antigens. It is concluded that the albumin and α_1 -acid glycoprotein are of human origin. It seems that the apparent IgG activity is due to cross-reactivity since, from the anticipated specificities of the Fab and Fc fragments of human IgG, the former could not be detected, and since Gm(6) activity was present, unexpectedly, in Swiss house dust.

The A, B and H blood group antigenic activities were detected in relative concentration different from those expected if they were solely of human origin. It is concluded that they are from both human and non-human sources.

REFERENCES

1. Berrens, L., Morris, J. H. & Versie, R. (1965): The complexity of house dust, with special reference to the presence of human dandruff allergen. *Int. Arch. Allergy* 27, 129-144.
2. Berrens, L. & Young, E. (1961): Purification and properties of house dust allergen. *Int. Arch. Allergy* 19, 341-359.
3. Boettcher, B. (1965): Genetical control of blood group substances in body secretions. Ph. D. Thesis, University of Adelaide.
4. Boettcher, B. (1967): Correlation between inhibition titres of blood group substances in salivas from A₁, A₂ and B secretors. *Austr. J. exp. Biol. med. Sci.* 45, 495-506.
5. Brocteur, J. & Versie, R. (1965): Présence de γ -globulines humaines dans des préparations d'allergènes de poussière des maison. *C. R. Soc. Biol. (Paris)* 159, 2090-2093.
6. Brocteur, J. & Versie, R. (1967): Mise en evidence de divers facteurs de groupes sanguins dans des extraits allergeniques de poussières de maison. *Acta allergol.* 22, 118-130.
7. Steinberg, A. G. (1962): Progress in the study of genetically determined human gamma globulin types (the Gm and Inv groups). *Progr. med. Genet.* vol. 2, p. 1-33. Grune & Stratton, New York, London.
8. Steinberg, A. G. (1969): Globulin polymorphisms in man. *Ann. Rev. Genet.* 3, 25-52.
9. Versie, R. & Brocteur, J. (1967): Identification de proteines humaines dans de extraits allergeniques de poussières de maison. *Acta allergol.* 22, 11-27.

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