

ORIGINAL ARTICLES

Hemagglutinating antibodies to grass in maternal and cord sera

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Twelve of 24 sera from nonimmunized atopic mothers at delivery contained hemagglutinating antibody to ryegrass, but no cord sera contained this. Naturally occurring hemagglutinating antibody did not pass through the placenta in demonstrable quantities. In two nonatopic and two atopic mothers immunized with mixed grass during pregnancy, maternal and cord sera contained hemagglutinating activity in equal titers. Artificially induced hemagglutinating antibody passed freely through the placenta. None of the sera obtained from 58 nonatopic mother-cord pairs contained hemagglutinating antibody. Since hemagglutinating antibody to ryegrass in the nonimmunized individual does not cross the placenta, it is probably associated with γ_{1A} - or γ_{1M} -globulin.

The human placenta presents a barrier to a number of maternal serum proteins.^{1, 2} Of the three immunoglobulins, γ_2 -globulin freely passes into the cord serum, while γ_{1A} - and γ_{1M} -globulins do not.¹⁻⁴ Passage of maternal antibody activity into cord serum appears to depend on the immunoglobulin involved. Conventional γ_2 -globulin antibodies are found in equal titer in maternal and cord sera,^{5, 6} whereas γ_{1A} - and γ_{1M} -globulin antibodies are found only in the maternal circulation.^{2, 5, 6} Tracer studies have revealed that other proteins, widely variant in physicochemical properties (acid glycoprotein, albumin, iron-binding globulin, fibrinogen, α_2 -macroglobulin) do not pass the placenta to any significant degree.¹

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Atopic reagin has recently been shown to be a γ_{1A} -globulin^{2, 7, 8, 9} and shows a distribution identical to that of γ_{1A} -globulin in maternal and cord sera.^{2, 10} In contrast, the bulk of hemagglutinating^{11, 12} antibody (HA) activity to pollens, particularly that found in individuals subsequent to immunization with pollen antigen, appears to be γ_2 -globulin.^{13, 14} However, skin-sensitizing and hemagglutinating activities have not been completely separated, since some of the latter activity has always been found in those serum fractions containing reagin.^{13, 14}

Since the placenta serves as a natural barrier to γ_{1A} and γ_{1M} -globulins, we believed that some further knowledge about the globulin nature of hemagglutinating antibody could be gained by comparing titers in maternal and cord sera. In individuals with low titers such as those seen in subjects with sensitivity to grass pollen, IIA activity of γ_{1A} or γ_{1M} type would not likely be masked by an overabundance of γ_2 IIA antibody.

This study is concerned with comparing hemagglutinating antibody in maternal and cord sera from mothers sensitive to grass pollen. Both treated and nontreated patients were studied. A number of normal mothers were also studied, including two who received grass pollen immunization before delivery. The results indicate the existence of naturally occurring hemagglutinating antibody which does not cross the placenta and therefore is probably associated with γ_{1A} - or γ_{1M} -globulin.

Table 1. *Hemagglutination titers of maternal-cord serum pairs*

			Titer	
			Maternal	Cord
1.	L. A.	Atopic, nonimmunized	8	0
2.	P. A.	Atopic, nonimmunized	32	0
3.	S. B.	Atopic, nonimmunized	0	0
4.	P. B.	Atopic, nonimmunized	0	0
5.	D. B.	Atopic, nonimmunized	0	0
6.	L. C.	Atopic, nonimmunized	16	0
7.	E. C.	Atopic, nonimmunized	128	0
8.	M. D.	Atopic, nonimmunized	0	0
9.	M. G.	Atopic, nonimmunized	0	0
10.	R. G.	Atopic, nonimmunized	8	0
11.	M. G.	Atopic, nonimmunized	0	0
12.	M. J.	Atopic, nonimmunized	16	0
13.	P. H.	Atopic, nonimmunized	0	0
14.	S. N.	Atopic, nonimmunized	0	0
15.	C. P.	Atopic, nonimmunized	8	0
16.	D. S.	Atopic, nonimmunized	64	0
17.	A. S.	Atopic, nonimmunized	32	0
18.	F. S.	Atopic, nonimmunized	32	0
19.	K. S.	Atopic, nonimmunized	0	0
20.	V. W.	Atopic, nonimmunized	0	0
21.	E. W.	Atopic, nonimmunized	32	0
22.	M. W.	Atopic, nonimmunized	0	0
23.	G. W.	Atopic, nonimmunized	0	0
24a.	A. M.	Atopic, nonimmunized, first pregnancy	16	0
24b.	A. M.	Atopic, immunized, second pregnancy	32	32
25.	D. H.	Atopic, immunized	16	16
26.	A. H.	Normal, immunized	64	64
27.	M. K.	Normal, immunized	8	8
28-85.		Normal nonimmunized mothers	0	0

MATERIALS AND METHODS

Sera

Paired maternal and cord sera were obtained from mothers on the obstetrical service of the Palo Alto-Stanford Hospital. Sera were stored frozen until used. Twenty-five mothers with positive skin tests to mixed grass* and a clinical history of spring hayfever were designated "atopic." These mothers produced 26 babies included in the series. Sixty mothers with negative skin tests and no history of atopic allergy were designated "normal." Each normal mother produced one baby included in the series.

Two of the atopic mothers (Nos. 24 and 25) had received therapeutic injections of mixed grass antigen before and during pregnancy. Mother No. 24 produced one baby before immunization and another baby after one year of treatment. Two of the normal mothers were immunized with mixed grass, 345 and 1,209 pollen units,† respectively, from the eighteenth to the thirty-fifth week of gestation.

These same maternal-cord serum pairs were examined for the presence of the three immunoglobulins and reaginic antibody to mixed grass. These studies, reported previously,² showed that reagin and γ_{1A} -globulin could usually be detected only in the maternal circulation.

Hemagglutination tests

Bisdiazotized benzidine hemagglutination tests were performed as described by Frick¹² using as antigen ryegrass (*Lolium perenne*) which is one component of the mixed grass antigen mixture.

RESULTS

Atopic mothers

The sera of 12 of the 24 nonimmunized allergic mothers (including the first pregnancy of mother No. 24) contained hemagglutinating antibody to ryegrass (Table I), while none of the respective cord sera contained this. Thus, the placenta seemed to be a barrier to the passage of naturally induced hemagglutinating antibody. The cord sera of the two immunized allergic mothers (No. 25 and second pregnancy of No. 24), as well as the cord sera of two immunized normal mothers (Nos. 26 and 27), contained hemagglutinating antibody, and maternal and cord titers were identical. Thus, induced hemagglutinating antibody appeared to pass through the placenta. Neither maternal nor cord sera of 58 nonimmunized normal mothers contained hemagglutinating activity.

DISCUSSION

The human placenta shows a differential permeability to two types of hemagglutinating antibody found in the sera of these mothers. The naturally occurring HA in nonimmunized atopic mothers did not cross into the fetal circulation. HA induced artificially by injection of grass pollens in both atopic

*Mixture of equal parts of 10 grass pollens causing spring hayfever.

†One pollen unit translated for this study as that amount of antigen contained in 1 c.c. of a 1/1,000,000 weight per volume solution dilution.

and nonatopic mothers freely passed the placenta and was found in cord blood. Artificially induced HA to both ragweed¹³ and grass¹⁴ has been shown to be associated with γ_2 -globulin, and would thus be expected to pass through the placenta.

Naturally occurring HA varies quantitatively with different antigenic systems. Ragweed-sensitive patients may have high HA titers¹³ and even precipitins as the result of natural exposure alone.¹⁵ The bulk of such activity is associated with γ_2 -globulin.¹³ However, after column fractionation, HA activity is also found in fractions containing the other two immunoglobulins along with most of the skin-sensitizing activity.¹³ Ultracentrifugal analysis of sera from untreated ragweed-sensitive individuals¹⁶ led to the conclusion that two classes of hemagglutinating activity were present: one with a sedimentation coefficient of approximately 19S, the other with a sedimentation coefficient of about 7S, indicating again a heterogeneity of hemagglutinating antibody in untreated individuals.

Grass pollen seems to be a less potent antigenic stimulus than ragweed. Nonimmunized grass-sensitive patients have low titers of hemagglutinating antibody,^{12, 17, 18} which have not been clearly separated by DEAE-cellulose column chromatography. Attempts at such fractionation of maternal sera in our laboratory have been unsuccessful. Augustin¹⁴ reported finding traces of HA in many fractions derived from the sera of untreated (grass-sensitive) hay-fever patients, including those fractions containing reaginic activity. It appears that some of the naturally occurring HA activity may reside in γ_{1A} - and/or γ_{1M} -globulins, and these would not be expected to cross the placenta.

Recent studies show that γ_{1A} and γ_{1M} antibodies may occur more frequently as part of the immune response than was previously suspected. Yagi and associates,¹⁹ by using the radioimmuno-electrophoretic technique, demonstrated the presence of ragweed-binding antibodies in the γ_{1A} -, γ_{1M} -, and γ_2 -globulins of sensitive individuals. Heremans and co-workers²⁰ showed that antibody activity was present in γ_2 -, γ_{1A} -, and γ_{1M} -globulins in a subject immunized with diphtheria toxoid, and in patients recovering from infection with *Brucella abortus*. Animal studies have shown that earliest responses to immunization may consist of γ_{1M} antibody; this later is succeeded by γ_2 antibody.²¹ Other workers have shown that variations in the route of immunization cause some alteration in distribution of antibody activity among the three gamma globulin components.²²

The seemingly different immunologic responses on natural exposure to ragweed and grass could be explained by assuming ragweed is a stronger stimulus than grass (by virtue of greater immunogenicity and/or greater abundance) and that only a strong stimulus can produce γ_2 hemagglutinating antibody in addition to reagin and non- γ_2 HA. A less strong stimulus would produce only reagin and non- γ_2 HA.

It has been postulated and not disproved that the reagin molecule itself may contain HA activity since attempts to isolate pure skin-sensitizing antibody free of HA activity have been unsuccessful.^{14, 15, 17} An equally tenable explanation for the presence of non- γ_2 HA activity occurring with fractions containing reagin would be the presence of hemagglutinating and reaginic ac-

tivities on different γ_{1A} molecules. Both possibilities are consistent with the results reported in the present study. Both reagin and the low levels of HA failed to cross the placenta.

The ability to produce heat-labile skin-sensitizing antibody is not exclusively a property of atopic individuals. Reagin has been produced by nonatopic subjects following immunization with *Ascaris* extract²³ or as the result of natural infestation,²⁴ after injection of pollens in water-oil emulsion²⁵ or of diphtheria toxoid,²⁶ and in penicillin sensitivity²⁷ and serum sickness.²⁸ What is peculiar about individuals with atopic disease is the production of reagins following natural exposure to environmental allergens. They also form reagin more readily than do normal subjects on artificial exposure to *Ascaris* extract²³ or antigens in water-oil emulsion.²⁵

We conclude that grass-sensitive patients produce reagin and non- γ_2 hemagglutinating antibody on natural exposure to allergens, and these antibodies do not cross the placenta. Deliberate immunization leads to production of γ_2 antibody which does cross the placenta and is demonstrable in cord blood.

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