

Serum IgD concentrations in children with atopic diseases

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When serum IgD was measured by electroimmunodiffusion (EID) in 60 children with atopy and in 55 normal children, the children with atopy had a mean serum IgD level of 2.47 mg. per 100 ml. and a standard error of the mean of 0.32 mg. per 100 ml. In contrast, the control group of normal children had a mean level of $3.59 \pm (\text{S.E.M.}) 0.48$ mg. per 100 ml. This difference was not statistically significant. IgD levels in the two groups could not be correlated with sex or diagnosis, but there was a significant ($p < 0.05$) correlation with age in the group with atopy. Further statistical analysis indicated that the lower mean IgD level in the atopic group was related to the lower mean age in this group. The study also demonstrated that EID is more sensitive than single radial diffusion (SRD) for the measurement of IgD in the serum of normal and atopic children.

Immunoglobulin D, discovered in 1965 by Rowe and Fahey,¹ exists in small quantities in the serum of normal persons. Its role in the human immune response has not been clearly defined. Several cases of IgD myeloma have provided large amounts of this protein for investigative work,² and results of two studies have indicated that serum IgD is present in higher than normal concentrations in the sera of persons with atopic diseases.^{3, 4} The reaginic antibody that mediates Type I hypersensitivity reactions appears to reside in the IgE fraction of serum immune globulins.⁵ However, participation of IgD in the immunologic process that mediates atopic disease has not been demonstrated.

Attempts to quantitate serum IgD by single radial diffusion have encountered decreased sensitivity in the lower range of values. In their series, Berg and Johansson⁴ found that serum IgD was absent in 39 per cent of normal children between the ages of 4 and 6 years.

This study was designed to apply electroimmunodiffusion (EID) to quantitate and study the concentration of IgD in the serum of children with atopic diseases.

MATERIALS

A total of 60 children with atopic diseases, 37 boys and 23 girls, aged 6 months to 16 years, was included in this survey. The control group consisted of 55 children, 28 boys and 27 girls, aged 10 months to 16 years, who were being seen in the pediatric clinic for general

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TABLE I. Serum IgD concentration

Group	No.	Milligrams per 100 ml.		
		Mean	S.D.	S.E.M.
Children with atopy	60	2.47	2.49	0.32
Normal children	55	3.59	3.55	0.48

p = N.S.

examinations, psychosomatic complaints, or minor pediatric problems. None had a history of atopic disease, acute or recurrent respiratory infection, hepatic disease, diabetes, collagen-vascular or chronic inflammatory disease. The mean age of the children with atopy was 6.48 years while the mean age of the control group was 8.01 years.

METHODS

In most instances, serum was collected during the initial allergic evaluation in conjunction with protein electrophoresis and immunoglobulin quantitation. Sera were stored at -20°C . until examined.

Goat antiserum specific for IgD was obtained commercially.* Immunoelectrophoresis showed a single precipitin band against normal human serum.

The IgD concentration of the sera in the two population groups was quantitated by the method of EID that utilizes the principle of antigen diffusion in an electrical field through a layer of agar containing specific antiserum.⁶ The length of the immunoprecipitate formed was proportional to antigen concentration and duration of electrophoresis.

For each slide, 0.05 ml. of antiserum was dispersed in 3.0 ml. of 2 per cent Bacto agar solution in a veronal buffer, pH 8.6. Three 50×75 mm. glass slides were utilized per run. Seven wells were punched in a line along the short axis of the slides. Three standards and four unknowns were measured on each slide. Electrophoresis was performed at 30 ma. for 75 minutes. Following electrophoresis, the slides were washed in saline for 24 hours to remove unprecipitated protein, washed for one hour in water, dried, and stained with 0.1 per cent thiazine red R in one per cent acetic acid to enhance the visibility of the precipitates before measurement.

The standards for the study were prepared by dilution of pooled adult sera containing a known amount of IgD. The IgD concentration of the pooled adult sera was measured at the NCI Immunoglobulin Reference Center, Bethesda, Maryland.

RESULTS

When EID was employed, IgD was detected in the serum of each of the 115 children in this survey. The IgD levels ranged from 0.74 to 14.4 mg. per 100 ml. The children with atopic diseases had a mean level of 2.47 mg. per 100 ml. and a standard error of the mean of 0.32 mg. per 100 ml. The control group had a mean level of $3.59 \pm (\text{S.E.M.}) 0.48$ per 100 ml. (Table I). The serum IgD concentrations did not follow a gaussian distribution. Therefore, a nonparametric method (distribution-free) was utilized for statistical analysis. A Wilcoxon (Mann-Whitney) test showed no significant difference between the two populations.

The differences in IgD measurements in each of the various subgroups (e.g., boys with atopy, girls with atopy; normal boys, normal girls) were not significant (Table II). Correlation with age was significant in the allergic group ($p < 0.05$) but not in the normal group.

*Meloy Laboratories, Springfield, Va.

TABLE II. Serum IgD concentration in subgroups

Group	No.	Milligrams per 100 ml.		
		Mean	S.D.	S.E.M.
Boys with atopy	37	2.51	2.51	0.41
Girls with atopy	23	2.41	2.51	0.52
Normal boys	28	3.76	3.95	0.75
Normal girls	27	3.42	3.15	0.61

 $\bar{p}$ = N.S.

A review of the diagnoses recorded for the children with atopy failed to reveal a significant pattern. Those with allergic rhinitis who did not have a history of allergic bronchial disease (e.g., asthma) had a mean IgD level of 2.64 mg. per 100 ml. with a standard error of the mean of 0.57 mg. Children who had involvement of both the upper and lower respiratory tracts (e.g., allergic rhinitis and asthma) had a mean level of 2.40 ± 0.39 mg. per 100 ml. This difference in the two populations was not statistically significant.

The reproducibility of the method was tested by replicate measurements of two unknown samples on different days. The coefficients of variation were 2.4 per cent and 6.0 per cent. This compares favorably with the studies of Merrill, Hartley, and Claman⁶ and Salvaggio, Arquembourg, and Sylvester.⁷

For comparative purposes, the IgD standards and appropriate dilutions were quantitated by both EID and single radial diffusion (SRD) in agar gel. With EID there was a sharp cutoff between detectable and nondetectable levels of IgD. The lowest detectable IgD by EID was 0.74 mg. per 100 ml. With SRD the precipitin rings in the lower concentrations were faint and difficult to measure accurately. The lowest detectable precipitin ring by SRD was 1.85 mg. per 100 ml. This is comparable to the findings of Merrill, Hartley, and Claman.⁶

DISCUSSION

The results of surveys recorded in the literature disagree regarding the serum levels of IgD in allergic individuals. In a study dealing primarily with adults, Kohler and Farr³ reported that IgD levels were higher in persons with atopy (mean 5.5 mg. per 100 ml.) than they were in nonatopic persons (mean 3.2 mg. per 100 ml.). Berg and Johansson⁴ reported that children with allergic diseases had a similar increase in serum IgD. However, Johansson,⁸ in a previous study, had reported IgD to be lower in adults with asthma (4.3 mg. per 100 ml.) than in normal adults (11.7 mg. per 100 ml.).

Immunoglobulin D remains an enigma in the human immune mechanism despite some evidence that antibody activity may be present.⁹⁻¹¹ One study postulated the fundamental defect in persons with atopy to be the propensity to produce IgD and IgE following antigen exposure.¹² However, IgD has not been implicated in the immune response of persons with atopy. On the contrary, studies have indicated that IgD does not participate in Type I hypersensitivity reactions.^{13, 14} Thus, any such relationship must remain speculative at the present time. Both children and adults with atopic diseases have increased levels of IgE, a protein known to play a fundamental role in the atopic reaction.^{8, 15}

One of the major problems encountered in IgD investigation relates to the small concentration of this immunoglobulin in the serum. In adults, the generally accepted mean level of 3.0 mg. per 100 ml. is in the lower range of detectability by the SRD technique. In their series, Berg and Johansson⁴ did not detect IgD in the serum of 39 per cent of the children who were between the ages of 4 and 6 years. IgD was not detectable in 20 per cent of the healthy adults tested by Johansson, Hogman, and Killander.¹⁶ This inability to measure minute amounts of IgD seriously limits statistical analysis in the various population groups.

The present study utilized EID, a sensitive and reproducible method for the demonstration of minute concentrations of immunoglobulins in body fluids. This technique has been used successfully for the measurement of immunoglobulins in spinal fluid,¹⁷ umbilical cord serum,¹⁸ and salivary secretions.^{19, 20} In this laboratory, levels of serum IgD as low as 0.74 mg. per 100 ml. can be detected by employing EID, whereas the lowest detectable level by SRD was 1.85 mg. per 100 ml. IgD was detectable in the serum of each of the 115 children.

In this study, the level of serum IgD was not significantly lower in children with atopy than it was in the normal children (2.47 ± 0.32 mg. per 100 ml. vs. 3.59 ± 0.48 mg. per 100 ml.). In addition, there was no relationship between serum IgD and sex in the four subgroups (Table II). Regression analysis showed a significant correlation of serum IgD with age in the group with atopy ($p < 0.05$), but IgD and age could not be related in the control group. This significant correlation was maintained when both groups were considered together ($p < 0.05$) in a manner similar to that employed in the study by Berg and Johansson.⁴ Further statistical analysis indicated that the lower IgD concentration in children with atopy was related to the lower mean age of this group as compared to the control group.

The data were examined for differences in the types of allergic diseases. Children with upper respiratory tract disease (e.g., allergic rhinitis) and children with both upper and lower respiratory tract disease (e.g., allergic rhinitis and asthma) did not have significant differences in serum IgD concentration. Berg and Johansson¹⁵ found that serum IgE levels were higher in children with asthma than in children with hay fever. They speculated that the differences were due to the nature and the degree of antigenic exposure. In any event, organ specificity does not seem related to serum IgD concentration in children with atopy.

Finally, this study confirms the work of Merrill, Hartley, and Claman,⁶ who found EID to be more sensitive than SRD in the measurement of small amounts of immunoglobulins in various body fluids.

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