

Demonstration of human reagin in monkey tissues

V. In vitro passive sensitization of monkey ileum with absorbed globulin fractions from sera of patients sensitive to ragweed

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In the previous studies it was shown that the following serum fractions of ragweed-sensitive patients are able to sensitize the monkey ileum when examined by the Schultz-Dale (S-D) test: euglobulin (IgM), zinc precipitate at 22° C. (IgG), and zinc supernatant (IgA). In the reported studies, these fractions were examined by the S-D test after the immunoglobulin contents were absorbed. The euglobulin (IgM) and zinc precipitate (IgG) fractions were negative after absorption, but the zinc supernatant (IgA) fraction not only remained positive but the tissue-sensitizing ability increased. This activity could be due to the IgE globulin which may be present in the zinc supernatant fractions, as shown by Ishizaka. Also, chromatographed serum fractions containing IgE, IgG, and IgA antibodies to ragweed extract (Antigen E) were examined by the S-D and Prausnitz-Küstner tests. Only such fractions which contained IgE antibody were positive in both tests. The absorption of these positive fractions with rabbit monospecific antisera to human IgA or IgG did not influence the reaginic activity, while the antisera to IgE globulin eliminated the activity completely.

It has been reported that monkey ileum can be used for detecting antibodies in the sera of allergic patients.^{1, 2} In a previous article,³ it was shown that serum fractions (IgM, IgG, and IgA) of allergic individuals can passively sensitize the monkey ileum in vitro. The greatest sensitizing activity was found in the fraction composed chiefly of IgA globulin. However, Ishizaka and colleagues⁴ reported that IgE antibody in sera of allergic patients has an electrophoretic

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mobility of γ_1 globulin, which is similar to the fraction containing IgA globulin. Therefore, the question arose as to whether the tissue-sensitizing activity in the serum fraction is associated with the IgA globulin or with some other component present in the fraction, such as IgE.

To clarify this point, experiments were performed with serum fractions containing chiefly IgM, IgG, and IgA globulins, from which the immunoglobulin content was absorbed. In addition, studies with protein components which contained IgE, IgG, and IgA antibody to ragweed extract were done.

All samples were examined by means of the Schultz-Dale test with monkey ileum.

MATERIALS AND METHODS

Fraction of sera

The fractions originated from sera of a patient (J. K.) sensitive to ragweed who had had hyposensitization therapy. The control fractions were obtained from the serum of an individual without any clinical history of allergic diseases and with negative skin reactions to ragweed. The fractionation procedure and the details of these fractions were described previously.³ The fractions were: euglobulin, which contained mostly IgM but also small amounts of IgG globulin; zinc precipitate (ZnPpt₂₂), which contained primarily IgG but also a trace of IgA globulin; and zinc supernatant (ZnSup), which consisted essentially of the IgA globulin, although some IgG protein remained.

Serum fractions were prepared from another patient (E. K.) sensitive to ragweed who had not received any previous hyposensitization therapy. These fractions were prepared in Denver by methods previously described.^{5, 6} Briefly, the serum was precipitated with half saturation of ammonium sulfate, followed by DEAE cellulose chromatography with stepwise elution of phosphate buffers from 0.005M to 0.05M. The fractions were designated I to IV. The fraction eluted by 0.025M phosphate buffer (fraction II) was further fractionated by gel filtration with Sephadex G-200. These fractions were coded and sent to Buffalo for study.

Absorption

The euglobulin, ZnPpt₂₂, and ZnSup fractions from Serum J. K. were absorbed with monospecific goat antisera* to human IgA, IgG, and IgM globulins. Before absorption the protein concentration of each serum fraction was determined with the Beckman Spectrophotometer DU at 280 m μ , with a factor of 1.45 O.D. U. per milligram of protein per milliliter.

The absorption was carried out as follows: 1 volume of euglobulin fraction (2 mg. per milliliter) was mixed with 3 volumes of goat anti-IgM and 3 volumes of anti-IgG globulin; 1 volume of ZnPpt₂₂ fraction (3 mg. per milliliter) was mixed with 4 volumes of goat anti-IgG and 2 volumes of anti-IgA globulin; and 1 volume of ZnSup fraction (2 mg. per milliliter) was mixed with 4 volumes of goat anti-IgA and 2 volumes of anti-IgG globulin.

*Obtained from Hyland Laboratories, Los Angeles, Calif.

The mixtures were shaken for 3 to 4 hours at room temperature and left overnight in a cold room. The precipitate formed was removed by centrifugation at 20,000 r.p.m. for 20 minutes, and the supernatant was examined for globulin content. Fractions which still contained some immunoglobulins were submitted to additional absorption.

The fraction II₁₁ of Serum E. K.⁶ (one of those termed I to IV) and whole serum were absorbed with monospecific rabbit antisera to human IgA, IgG, and IgE globulin as described previously.⁵

Immunodiffusion method

The absorbed and unabsorbed euglobulin, ZnPpt₂₂, and ZnSup fractions were examined in gel precipitation with the Ouchterlony method as described in a previous article.³

The immunoglobulin content of fractions I to IV was determined by antibody-agar plates.⁶ The presence of the IgG, IgA, or IgE antibodies to purified ragweed component-Antigen E (prepared according to the method of King and colleagues⁷) was determined by radioimmunodiffusion. This procedure was described earlier.⁵

In vitro sensitization of monkey ileum

For the in vitro sensitization of the monkey ileum, only those absorbed euglobulin, ZnPpt₂₂, and ZnSup fractions which did not show any precipitation reaction with goat antiserum to human IgA, IgG, or IgM globulins were used. The unabsorbed and absorbed fractions prepared in Denver were coded and only the total protein concentration of these fractions was known. All tested fractions were diluted in isotonic buffered glucose (IBG)³ and used for titration with the Schultz-Dale (S-D) method. The details concerning this procedure were described in previous publications.^{1, 2}

Passive transfer to human skin

The Prausnitz-Küstner (P-K) test was carried out only with the fractions marked I to IV, with Antigen E. The technique of the P-K test was described in a previous publication.⁵

RESULTS

The qualitative immunoglobulin content of the euglobulin, ZnPpt₂₂, and ZnSup fractions before and after absorption are presented in Table I. After absorption, no immunoglobulin could be detected by the double-diffusion method

Table I. *Immunoglobulin content of serum fractions before and after absorption*

Fractions	Before absorption			Absorbed with mono-specific goat antisera to human globulins		After absorption		
	IgA	IgG	IgM			IgA	IgG	IgM
Euglobulins	—	+	++	IgM	IgG	—	—	—
ZnPpt ₂₂	+	+++	—	IgG	IgA	—	—	—
ZnSup	++	+	—	IgA	IgG	—	—	—

of Ouchterlony. When the absorption of some samples was not complete, absorption was repeated a second time.

The results of the S-D titration of the unabsorbed and absorbed serum fractions are shown in Table II. The lowest concentration of a tested sample which still produced a definite contraction of the monkey ileum when challenged with ragweed extract was defined as the end-point activity.

The end-point activity of the unabsorbed euglobulin fraction was obtained at a protein concentration of 0.1 μg per milliliter and of the ZnPpt₂₂ fractions at 1 μg per milliliter. (The liberation of histamine of an equivalent of 3 ng. obtained with the concentration of 0.1 μg per milliliter cannot be recognized as a positive reaction.) At these protein concentrations, the absorbed fractions were negative. The end-point activity of the unabsorbed ZnSup fraction was obtained at a protein concentration of 0.005 μg per milliliter, whereas 0.0005 μg per milliliter of the absorbed fraction was still able to sensitize the monkey gut.

A control ZnSup fraction from a serum of nonallergic individual was also

Table II. *Schultz-Dale reaction with monkey ileum unabsorbed and absorbed serum fractions*

Fraction	Protein concentration ($\mu\text{g}/\text{ml.}$)	Unabsorbed	Absorbed
		Histamine equivalents (ng.)	
Euglobulin	1.0	8	<1
	0.1	5	<1
	0.05	<1	<1
ZnPpt ₂₂	1.0	14	<1
	0.1	3	<1
	0.05	<1	<1
ZnSup	0.005	8	10.0
	0.001	<1	5.0
	0.0005	<1	5.0
	0.0001	<1	<1

Table III. *Antibody content in fractions obtained by DEAE cellulose chromatography and gel filtration with Sephadex G-200*

Fraction	Phosphate buffer used for elution	Presence of antibodies to ragweed-Antigen E* in:		
		IgG	IgA	IgE
I	0.005M	++	—	—
II ₁₁	0.025M†	+	—	+++
II ₂₂	0.025M	+	—	—
III	0.035M	±	+	+
IV	0.05M	—	—	—

*The presence of IgG, IgA, or IgE antibodies against purified ragweed fraction-Antigen E was determined by radioimmunodiffusion method. The allergen-binding activity of the fractions was reported by Ishizaka and colleagues.⁶

†The protein sample eluted by the phosphate buffer of 0.025M was submitted to gel filtration by Sephadex G-200. Fraction II₁₁ represents the portions of the ascending part of the peak and fraction II₂₂ the portions of the descending part of the peak.

tested. Both the unabsorbed and absorbed fractions failed to passively sensitize the monkey tissue.

The goat antisera used for absorption were also examined by the S-D test. All these antisera were negative.

The description of fractions I to IV obtained by DEAE cellulose chromatography and gel filtration are given in Table III.

The protein samples eluted by the phosphate buffer of 0.025M were pooled, concentrated, and submitted to gel filtration with Sephadex G-200. One peak was obtained, but the skin-sensitizing activity could be found only in the protein portions of the ascending part of this peak. The ascending and descending halves of the peak were pooled separately (fractions II₁ and II₂). For further purification, each fraction was rechromatographed on the Sephadex column. The ascending half of fraction II₁ (fraction II₁₁) and the descending half of fraction II₂ (fraction II₂₂) were used in subsequent experiments.⁶

The fractions were examined by the S-D method. The results of the S-D tests and the P-K tests are compared in Table IV.

Antibody associated only with IgG globulin was detected in fractions I and II₂₂ by radioimmunodiffusion. Neither P-K nor S-D activity could be demonstrated in fraction I at a protein concentration of 0.018 mgN per milliliter in the P-K test and at 0.053 mgN per milliliter in the S-D test. Similar lack of activity was observed in the fraction II₂₂, in which 0.036 mgN per milliliter of this fraction gave a negative P-K test, and 0.012 mgN per milliliter gave a negative S-D test. It was found in previous experiments⁶ that fraction II₁₁ contained the highest concentration of IgE antibody and had the highest reaginic activity. As little as 0.0002 mgN per milliliter of fraction II₁₁ still sensitized the monkey ileum and human skin. Fraction III, which contains other active immunoglobulins in addition to IgE antibody, was also active in both tests. The end-point activity of the P-K test was reached at a protein concentration of 0.008 mgN per milliliter and of the S-D test at 0.006 mgN per milliliter. Fraction IV, which did not contain demonstrable antibodies, gave negative results in the P-K and S-D tests.

The fraction II₁₁, which had the highest tissue-sensitizing activity, and the serum from which this fraction was obtained were absorbed with monospecific

Table IV. *Tissue-sensitizing activity in fractions obtained by DEAE cellulose chromatography and gel filtration with Sephadex G-200*

Fraction	Presence of antibodies in:			Protein concentration (mgN/ml.)	Titers*	
	IgG	IgA	IgE		S-D	P-K
I	++	-	-	1.6	< 30	< 90
II ₁₁	+	-	+++	0.55	> 2500	2760
II ₂₂	+	-	-	0.36	< 30	< 10
III	±	+	+	1.83	300	230
IV	-	-	-	2.17	< 30	< 70

S-D = Schultz-Dale monkey ileum technique; P-K = passive transfer to human skin.

*The titers indicate the dilution factor of the protein concentrations listed in the Table.

rabbit antisera to the human IgG, IgA, and IgE globulins. The results of the P-K and S-D tests are compared in Table V. The absorption of the whole serum with rabbit antiserum to human IgA globulin did not influence the ability to sensitize human skin or monkey gut. But the antiserum to IgE globulin eliminated these activities. Similar results were obtained with the fraction II₁₁. Only the rabbit antiserum to IgE globulin removed the activity almost completely, while the antiserum to IgG globulin did not influence the activity at all. The absorption with antiserum to IgA globulin slightly diminished the P-K activity, but in the S-D test the activity remained unchanged.

DISCUSSION

It is still not known which immunoglobulin classes are involved in the passive sensitization of monkey ileum *in vitro*. In the previous report³ it was shown that serum fractions which contained IgM, IgG, or IgA globulins from patients with sensitivity to ragweed are able to sensitize the monkey ileum. Further, IgG globulin fractions after being heated at 56° C. for 4 hours or treatment with 2-mercaptoethanol were still able to sensitize the monkey ileum. This would indicate that antibodies other than reagins can participate in the S-D reaction with monkey ileum.

This observation is strengthened by the negative S-D reaction obtained with absorbed euglobulin and ZnPpt₂₂. Both fractions, before absorption of the immunoglobulin content, gave positive S-D reactions, but after the immunoglobulins were removed, both fractions were negative in the S-D test. This would mean that both fractions possess their own capacity to sensitize the monkey ileum.

There is the possibility that the unabsorbed euglobulin and ZnPpt₂₂^o fractions might contain a trace of IgE antibody which was responsible for the positive S-D reaction and that this antibody could be removed by absorption with the monospecific goat antisera. One would then have to assume that the monospecific goat antisera to human globulins contains some anti-IgE activity. However, against this thesis is the finding that the absorbed ZnSup fraction still could passively sensitize the monkey ileum. It should be emphasized that the ZnSup contained the highest concentration of the reaginic activity among the discussed fractions; therefore, even if the monospecific goat antisera used for

Table V. *Tissue-sensitizing activity of serum and purified fractions after absorption with monospecific rabbit antisera*

Sample	Test	Unabsorbed	Absorbed with antiserum to:		
			IgG	IgA	IgE
Undiluted whole serum	P-K	1,600*	N.D.	1,500	< 265
	S-D	> 1,000	N.D.	1,500	< 75
Fraction II ₁₁ (0.55 mgN/ml.)	P-K	2,760	2,750	1,380	< 340
	S-D	2,500	2,500	2,500	< 50

*The numerical values indicates the titer of the whole serum or the dilution factor of the protein concentration (0.55 mgN/ml.) of the fraction II₁₁.

absorption of the ZnSup had some anti-IgE activity, it was not enough to abolish the tissue-sensitizing activity of the ZnSup fraction.

The results of the S-D test obtained with absorbed ZnSup fraction provided evidence that the tissue-sensitizing antibody in the examined fraction was not associated either with IgA or with IgG globulin. It was shown that when the immunoglobulin content was removed, the tissue-sensitizing activity increased 10 times, and in ZnSup fractions obtained from other allergic sera the activity was increased a hundredfold (not listed in any of the tables). Ishizaka and colleagues⁴ showed that the electrophoretic mobility of the IgE globulin is similar to that of the IgA globulin and that zinc supernatant contains IgE globulin. Hence it is assumed that the active component in the ZnSup after absorption could be the IgE antibody.

The results obtained with fractions I to IV, which were prepared in Denver, seem to support the deduction mentioned previously. It was shown by means of S-D and P-K tests that only those fractions which contained IgE antibody are able to sensitize the human skin or monkey ileum. The sensitizing activity observed in the S-D or P-K tests could not be removed by absorption with monospecific rabbit antiserum to human IgG or IgA globulin. Absorption with rabbit antiserum to IgE globulin removed the activity in whole serum and in isolated serum fractions almost completely. Comparison of the results of the P-K tests performed in Denver and the results of the S-D tests performed in Buffalo with the isolated serum fractions showed a very close correlation in the activity obtained by both methods. Therefore, it could be concluded that the reaginic antibody in the P-K test is probably the same as the antibody which participates in the sensitization of the monkey ileum.

It is already known that the reaginic activity correlates with the IgE antibody⁶ and that this antibody can passively sensitize the human skin⁵ and induce passive cutaneous anaphylaxis in monkey skin.⁸ The close correlation of the passive sensitization of human skin and the passive sensitization of monkey ileum is additional evidence that the IgE antibody is the reaginic antibody in those sera of patients sensitive to ragweed which were under study.

Nevertheless, some conflicting results were obtained. The euglobulin and the ZnPpt₂₂ fractions (J. K.) which contained IgM or IgG immunoglobulins did sensitize passively the monkey ileum in contrast to the IgG fraction (E. K.) produced by DEAE cellulose chromatography and gel filtration, which did not give positive S-D reactions. These discrepancies could conceivably be explained in different ways:

1. The euglobulin and the ZnPpt₂₂ fractions could contain IgE antibody whereas the chromatographic IgG fraction does not. It also seems feasible that the S-D positive fractions (except IgE globulin) could contain an unknown immunoglobulin or subfraction of an immunoglobulin which would be responsible for the tissue-sensitizing activity.

2. Patient J. K. had received specific hyposensitization therapy. In all probability, such treatment produced a higher IgG ragweed antibody content than would be found in the IgG fraction obtained from E. K., who had not had previous hyposensitization therapy.

3. It is entirely conceivable that sera from various patients, whether previously treated with antigen or not, may differ and that reagin to ragweed pollen could be present in other globulin fractions.

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