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STUDIES ON THE ANTIBODIES IN RABBIT ANTISERA RESPONSIBLE FOR SENSITIZATION OF HUMAN SKIN

II. CHARACTERISTICS OF ANTISERA TO EGG WHITE AND TO CONALBUMIN*

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IN A previous study¹ it was demonstrated that the ability of certain rabbit antisera to recrystallized egg albumin (Ea) to induce in human skin a wheal and erythema type of sensitivity was due to the presence in these sera of antibody to egg white constituents other than Ea. Evidence was presented to show that these antibodies resulted from trace impurities in the highly purified crystalline Ea used for immunization.

Previous studies by several groups of workers had already demonstrated that some, but not all, rabbit or guinea pig antisera to antigens such as egg white,² ragweed pollen,³⁻⁶ horse serum,⁷ or crystalline egg albumin⁸ possessed the capacity passively to confer wheal and erythema sensitivity to human skin; no correlation between sensitizing capacity and precipitin content was noted. Sherman, Menzel, and Seeborn⁸ also demonstrated that the original skin-sensitizing capacity of rabbit anti-Ea sera was retained after serial absorption with Ea so as to leave nonprecipitating anti-Ea in solution and inferred that nonprecipitating anti-Ea was skin sensitizing. In our recent study,¹ however, precipitating antibodies to several antigenic impurities were demonstrated in such preparations of nonprecipitating anti-Ea, and the sensitizing capacities were shown to be caused by antibodies as yet unidentified.

In the present report the characteristics of rabbit antisera to another purified egg white protein, crystalline conalbumin (Ca), and to whole egg white (EW) are described. It has been found that there are two distinct types of skin-sensitizing anti-Ca and anti-EW sera. These types correspond to similar types of skin-sensitizing anti-Ea sera previously described.¹ This general similarity in the skin-sensitizing properties of anti-Ea, anti-Ca, and anti-EW sera is taken as a further indication that there are in EW a limited

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number of antigens capable of inducing in the rabbit the production of skin-sensitizing antibody and that these antigens are present as impurities in crystalline egg albumin and crystalline conalbumin.

MATERIALS AND METHODS

The materials, techniques, and procedures used in this study have been described in detail elsewhere.¹ The antigens used were recrystallized egg albumin, conalbumin, and lysozyme, and highly purified ovomucoid.* Whole egg white thoroughly dialyzed against saline was kept frozen and portions were thawed when needed.

All animals were immunized with intravenous alum-precipitated antigen,^{1, 9} conalbumin being given as a series of 16 injections totaling 20 mg. per rabbit during the first month and 10 mg. per rabbit the second month. EW was given in a dosage of 20 mg. per rabbit during both months of immunization. Animals were bled on the fifth to seventh days after the last injection.

Analyses for antibody to the major antigenic component were performed by the quantitative precipitin technique,^{9, 10} analyses for antibodies to the trace contaminants being carried out by the modification recently described.¹ Agar diffusion analyses,¹¹⁻¹³ as described in detail,¹ were used for estimating the number of antibodies in given antisera.

All skin tests were done on normal human subjects with negative allergic histories; 0.05 ml. of serum or serum dilution was used for sensitization and forty-eight hours later 0.01 to 0.02 ml. of antigen for eliciting the reactions, which were read at fifteen to twenty minutes.

RESULTS

In Table I are recorded the antibody analyses and skin test results on the sera from 9 rabbits immunized with conalbumin and 8 rabbits immunized with whole egg white. The preponderance of the antibody response in the anti-Ca sera was to Ca, but some anti-Ea was also regularly seen. In only 2 sera, R224₄ and R226₄, were significant amounts of anti-Lyso found and in none did anti-Ovo occur. The major antibody response in the anti-EW sera, on the other hand, was to Ea, lesser responses occurring to Ca, Ovo, and Lyso roughly paralleling the amounts of these antigens in EW.¹⁴

As had been previously observed in anti-Ea sera,^{1, 8} there was no parallelism between the skin-sensitizing capacities of the various sera and their content of any of the precipitating antibodies measured. Furthermore, serum samples taken after only one month of immunization showed much lower skin-sensitizing capacity than those taken after two months. The sera in Table I are from the latter bleeding. As had also been noted for anti-Ea sera,¹ the present antisera could be divided into two distinct types on the basis of their patterns of response when the various purified antigens were used to elicit the reactions in sensitized skin sites. Thus, with 7 of the 9 anti-Ca sera, EW and Ca were approximately equivalent in their capacities to elicit the reactions, Ea being

*We are grateful to Dr. Robert C. Warner of New York University for the conalbumin, lysozyme, and ovomucoid.

TABLE I. ANTIBODY CONTENT AND SKIN-SENSITIZING CAPACITY OF RABBIT ANTISERA

	SERUM DESIG- NATION	ANTIBODY CONTENTS				REACTIONS ELICITED IN SKIN SITES SENSITIZED WITH 0.05 ML. OF SERUM AND TESTED WITH 10 μ G N OF				
		ANTI- EA	ANTI- CA	ANTI- OVO	ANTI- LYSO	EW	EA	CA	OVO	LYSO*
		μ g sp. ppt.N/ ml.	μ g AbN/ ml.	μ g sp. ppt.N/ ml.	μ g sp. ppt.N/ ml.					
<i>Anti-Ca Sera</i>										
Type I	R218 ₄	9	262	0	0	++ $\pm$	$\pm$	++ $\pm$	-	
	R219 ₄	16	377	4†	0	++	$\pm$	+	-	
	R220 ₄	17	328	0	0	$\pm$	-	$\pm$	$\pm$	
	R223 ₄	16	468	2†	0	++	$\pm$	+++	-	
	R224 ₄	20	557	2†	12	++ $\pm$	$\pm$	++	+	
	R225 ₄	13	157	0	3†	$\pm$	-	$\pm$	-	
R227 ₄	10	412	0	0	$\pm$	-	$\pm$	-	-	
Type II	R221 ₄	11	66	0	4†	++ $\pm$	++	$\pm$	-	
	R226 ₄	20	536	2†	25	++	+	+	$\pm$	
<i>Anti-EW sera</i>										
Type I	R236 ₄	0	0	0	0	++	+	++	+	-
	R238 ₄	218	31	35†	0	++	+	++	+	
Type II	R233 ₄	50	28§	6†	0	++	$\pm$	$\pm$	-	
	R234 ₄	792	45	40	0	++	+	$\pm$	-	-
	R240 ₄	66	52	26	20	++	++	+	-	
	R241 ₄	916	68	56	13	+	$\pm$	-	-	
	R242 ₄	926	81	46	25	+	$\pm$	-	-	
	R243 ₄	36	38	17	0	-	-	-	-	-

*Lyso used only in 1 μ G N quantity, as 10 μ G N produced nonspecific irritation.

†Values not significant.

‡AbN.

§Spec. ppt. N.

distinctly less potent (Type I). With the other 2 sera, EW was the most reactive antigen, Ea being next, and Ca relatively unreactive (Type II). Reactions with Ovo were inconstant. Lysozyme could not be adequately evaluated, as in the high concentration (1.0 mg. N/ml.) of antigen required to elicit reactions with these relatively weak sera lysozyme was a primary irritant, giving wheal and erythema responses in control sites as well as in sensitized sites. When used at a concentration of 0.1 mg. N/ml., it gave no reactions in either sensitized or control sites. Among the 8 anti-EW sera, there were 2 of Type I and 5 of Type II. One serum failed to develop a sensitizing capacity.

The striking difference in the incidence of the 2 types of sensitizing sera among the anti-Ca and anti-EW groups is interesting. Though a larger number of such sera would have to be studied to establish definitely the statistical significance of this difference, the finding seems logical and consistent with the assumption that crytalline Ca is contaminated to a relatively greater degree with the EW antigen reacting with the skin-sensitizing antibody of the Type I sera than with the antigen specific for the skin-sensitizing antibody in the Type II sera, as indicated by the relative potencies of Ca in eliciting reactions with the two types of sera.

Although there was no apparent parallelism between the sensitizing capacities of the anti-Ca sera and their content of anti-ca and although it was shown by absorption studies¹ that neither precipitating nor nonprecipitating anti-Ca was responsible for the passive transfer capacity of anti-Ea sera, it seemed wise to repeat such studies in these anti-Ca sera, in which anti-Ca was the major antibody. Since the sensitizing capacities of these sera were relatively weak, it was necessary to study partially as well as completely absorbed sera to obtain the critical data. The results are tabulated in Table II, from

TABLE II. EFFECT OF ABSORPTION OF ANTICONALBUMIN SERA WITH CONALBUMIN

SERUM DESIG- NATION	Ca N ADDED TO 1.0 ML. SERUM	TOTAL N PPTD.	ANTI-Ca N PPTD.	SUPERNATANT TEST FOR EXCESS		REACTION ELICITED BY 10 μ G EW PROT. N IN SITES SENSITIZED WITH 0.05 ML. OF SUPERNATANT IN SKIN OF	
				AB	AG	J. M.	P. A.
R221 ₄	μ G	μ G	μ G.				
	0	0	0			+±	++
	4.6	37	33	++	0	+±	++
	7.0	66	59	+	0	+	+±
	10.0	81	71	0	0	+	+±
	14	105	101	0	+	±	+±
	18	109	91	0	+	±	+±
R223 ₄	0	0	0			+	+
	23	364	341	+++	0	±	+
	46	514	468	tr	0	-	+
	69	592	523	0	0	-	+
	92	622	530	0	+	-	+
	115	636	521	0	++	-	+

which it can be seen that the disappearance of passive transfer capacity in the two sera did not coincide with the point at which anti-Ca was completely absorbed. The variability of the reactivity of the sera in different recipients is consistent with similar previous findings by the present authors,¹ as well as others using antisera from laboratory animals²⁻⁸ or naturally sensitive patients.¹⁵

When anti-EW sera of both types were absorbed with increasing amounts of EW (Table III), it was clear that the amount of EW required for neutralization of skin-sensitizing ability was primarily related to the potency of the original serum and was definitely greater than the amount necessary for neutralization of all anti-Ea, anti-Ca, anti-Ovo, and anti-Lyso in the sera. For serum R241₄, for instance, the latter was calculated to be 50 μ G EW prot. N/0.5 ml.¹⁶ whereas more than 163 μ G EW prot. N/0.5 ml. was required to neutralize the passive transfer activity (Table III) for R241₄. For serum R236₄, the figures were 10 μ G N and > 4,090 μ G N, respectively. Furthermore, separate preliminary absorption of these antibodies with purified preparations of Ea, Ca, Ovo, and Lyso from sera R234₄ and R238₄ reduced sensitizing capacity only slightly and in proportion to what might be expected on the basis of impurities in the absorbing antigens.

TABLE III. EFFECT OF ADDITION OF INCREASING AMOUNTS OF EGG WHITE TO ANTI-EGG WHITE SERA

SERUM DESIGNATION	EW PROT. N ADDED TO 0.5 ML. SERUM	SPECIFIC PPT. N	REACTION ELICITED BY 10 μ G EW PROT. N IN SITES SENSITIZED WITH 0.05 ML. OF SUPERNATANT (SERUM 1:3)
R241 ₄	μ G	μ G	
	0	0	±±
	163	341	±
	409	110	-
	818	40	-
	1,636	40	-
	4,090	21	-
R236 ₄	0	0	++++
	818	21	+++±
	2,454	22	++
	4,090	18	++
R234 ₄			±±
R234 ₄ A*	0	0	+
	409	24	
	818	24	+
	1,227	22	
R238 ₄			++
R238 ₄ A*	0	0	±±
	163	9	+
	409	21	+
	818	27	±
	1,636	28	-
	4,090	23	-

*Sera absorbed successively with Ea, Ca, Ovo, and Lyso.

DISCUSSION

The failure of rabbit anti-Ca or anti-EW sera to manifest any characteristic feature that would allow the differentiation of their sensitizing properties from those of anti-Ea sera may be taken as further evidence in support of the previously expressed hypothesis¹ that there are in EW a limited number of antigens, possibly only two, that are capable of eliciting skin-sensitizing antibody in the rabbit. These antigens appear to contaminate crystalline preparations of both Ea and Ca, and they are minor and otherwise undefined components of EW. Quantitatively the great preponderance of antibodies in these anti-Ca and anti-EW sera is antibody to the four major EW constituents, Ea, Ca, Ovo, and Lyso,¹⁴ but none of these major rabbit antibodies appear to have skin sensitizing capacity in human beings¹ (Table III).

The concept that only certain antigens may have significance in human skin sensitivity is supported by clinical experience,¹⁷ and in recent years several studies on the fractionation of various allergen extracts have provided data to indicate that, with a given allergen extract, only some of the antigens present can react in sensitized skin.¹⁸⁻²¹ The recent report by Miller and Campbell²² that there are in EW "perhaps materials not yet described" to which egg-sensitive patients may react also is significant, and one may hypothesize that the activities of the purified protein preparations used by these workers, just as those in the present study, were due not to the major antigens but to materials present as contaminants. There are as yet no definitive data,

however, to tell whether or not the EW antigens productive of skin-sensitizing antibody in the rabbit are the same as those producing skin-sensitizing antibody in man. Likewise there is no reason to believe that the active ingredient of any particular allergenic mixture is necessarily a minor constituent.

No information is provided by this study on the question of the precipitability of skin-sensitizing antibody. Although the medical literature is replete with the idea that skin-sensitizing antibody is nonprecipitating and often implies that this behavior in some mysterious way confers upon the antibody the capacity to sensitize skin, there is as yet no convincing evidence on this point. Defined immunochemically, nonprecipitating antibody is antibody which by itself will not precipitate with antigen, but which may be carried down in aggregates of antigen with precipitating antibody to the corresponding antigen. That antiragweed skin-sensitizing antibody, for instance, has not been precipitated by ragweed extract cannot be taken as proof that this antibody is nonprecipitating, for it is clear that if either the antigen or the antibody is present in sufficiently low concentration it is impossible to form visible precipitates for quantitative reasons alone. In the same fashion, it would be improper to consider a given complement-fixing antibody to be nonprecipitating simply because no antigen-antiserum combination had been found which would produce a precipitate, though such failure of precipitation is frequent in human convalescent sera with high complement fixation titers. Indeed, human nonprecipitating diphtheria antitoxin has been found to fix complement much more poorly per unit of antitoxin than its precipitating counterpart.²³ Waksman's recent assertion²⁴ that rabbit anti-Ea sera contain a nonprecipitating skin-sensitizing antibody makes this very error, by the apparent assumption that because a precipitating skin-sensitizing antibody could not be demonstrated, the antibody was nonprecipitating.

The only report in the literature which clearly demonstrates the presence of a truly nonprecipitating antibody in a skin-sensitizing serum is that of Kuhns and Pappenheimer²³ who used a diphtheria toxoid-antitoxin system in man and believed that the nonprecipitating antitoxin was the skin sensitizing antibody. However, a more recent report by Kuhns²⁵ on a rather striking separation of the two types of antibody by alcohol fractionation adds further to doubts already expressed by us¹ as to the identity of the nonprecipitating and skin-sensitizing antibodies. Kuhns'²⁶ interpretation that this apparent separation was an artifact probably due to antibody denaturation during the separation procedures requires independent experimental substantiation.

In contradistinction to the prevalent concept that antibody formed in a given individual to a given antigen may or may not be skin sensitizing in accordance with certain characteristics of the antibody made by that individual, the present findings emphasize the importance of the antigen as a major determinant. It is not known why certain antigens are productive of skin-sensitizing antibody while others apparently are not.

SUMMARY AND CONCLUSIONS

The skin-sensitizing capacities of rabbit antisera to egg white and to conalbumin are identical in their essential features with those previously shown for antisera to egg albumin. Two types of antisera are seen within each group, apparently on the basis of two different skin-sensitizing antibodies not corresponding to anti-Ea, anti-Ca, anti-Ovo, or anti-Lyso. The limited number of egg white antigens capable of eliciting skin-sensitizing antibody in the rabbit is also pointed out, and the presence of these antigens as impurities in even the highly purified preparations of the egg white proteins used in this study is indicated.

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