ANTIGENIC COMPETITION BETWEEN POLYPEPTIDYL DETERMINANTS IN NORMAL AND TOLERANT RABBITS*

By ISRAEL SCHECHTER, M.D.

(From the Section of Chemical Immunology, The Weizmann Institute of Science, Rehovoth, Israel)

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Inhibition of the immune response to one antigen as a result of the injection of a second antigen (1) occurs in many animal species (human, rabbit, guinea pig, mouse, rat, and chicken) under the appropriate experimental conditions (for reviews see Adler, references 2 and 3). Most investigations were conducted with complex antigenic materials. This is true not only for particulate antigens like phages, bacteria, or animal cells but for pure proteins as well. A protein molecule contains many antigenic determinants which are usually ill defined. It would therefore be advantageous to use synthetic antigens or hapten conjugates (4) in the study of antigenic competition. By choosing appropriate combinations of a number of haptens and carriers, it is possible to evaluate the role of either hapten or carrier and to find out whether their action is independent or concerted.

Competition phenomena between synthetic antigens (5, 6) as well as between hapten conjugates (3, 7–12) have been reported. Adler (3) showed that the introduction of *p*-azobenzenearsonate residues into ferritin impaired the formation of anti-ferritin antibodies in mice. Amkraut et al. (10) found, in rabbits, that immunization with doubly substituted carrier (bovine serum albumin, sheep red cell stroma, or keyhole limpet hemocyanin) enabled the dinitrophenyl groups to suppress the antibody response to *p*-azobenzenearsonate. Recently Siskind et al. (12), who also used these haptens, reported decreased antibody formation in rabbits against both determinants. The competition was observed independently of whether the two haptens were attached to one carrier molecule (rabbit IgG) or whether a mixture of singly substituted carrier was used for immunization.

Investigations of antibodies to poly-L-alanyl and poly-D-alanyl determinants showed that their formation was easily elicited in rabbits, that the potency of the immune response towards either determinant was similar, and that the anti-

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bodies were strictly stereospecific (13,14). In view of the above results, the specificity of anti-poly-DL-alanyl antibodies elicited in rabbits and goats was investigated in this laboratory (11). It was found that upon immunization of rabbits with poly-DL-alanyl, poly-DL-phenylalanyl, or poly-DL-tyrosyl protein conjugates, the antipeptidyl antibodies formed were directed mainly towards D-amino acid sequences present in the poly-DL-aminoacyl determinant. As both optical isomers are equally distributed in the peptidyl chains, the results were interpreted as antigenic competition between sequences composed of L and D residues, with the D sequence being the most efficient one.

The present study deals with immunological competition between pairs of determinants attached separately to carrier molecules. The carrier can be either identical for both determinants or different. Two pairs of determinants were investigated: (*a*) the enantiomorphic peptides, poly-L-alanine and poly-D-alanine, and (*b*) the two different peptides, poly-DL-alanine and poly-DL-phenylalanine. Only in the latter case was competition observed. This system was used to evaluate the role of the protein carrier on the capacity of one determinant to suppress the immune response against the other.

It was found that the immune response toward the poly-DL-alanyl determinant was impaired by the poly-DL-phenylalanyl determinant only when identical or similar proteins (such as human serum albumin and rabbit serum albumin) served as the protein carriers of the singly substituted antigens, and not when a mixture of poly-DL-alanyl RNase and poly-DL-phenylalanyl HSA was used. Finally, the capacity of the poly-DL-phenylalanyl determinant to inhibit the formation of anti-poly-DL-alanyl antibodies in rabbits made tolerant to poly-DL-phenylalanyl protein conjugate was studied.

Materials and Methods

Ribonuclease A (RNase), five times recrystallized, was obtained from Sigma Chemical Co., St. Louis, Mo. Human serum albumin (HSA) (fraction V, powder) was purchased from Plasma Fractionation Institute of Israel, Jaffa–Tel Aviv. Rabbit serum albumin (RSA) (fraction V, powder) was purchased from Mann Research Laboratories, New York, N. Y. Complete Freund's adjuvant was obtained from Difco Laboratories, Detroit, Mich. *N*-Carboxyanhydrides of alanine (Ala) and phenylalanine (Phe) were prepared according to procedures described previously (15) followed by recrystallization from ethyl acetate–petroleum ether at −20°C.

Polypeptidyl Proteins.—The polypeptidyl proteins used in this study are given in Table I. They were prepared by reacting the protein with the appropriate *N*-carboxyamino acid anhydride in a water-dioxane mixture according to published procedures (16). The amount of amino acid attached to a protein was calculated from the amino acid analysis (16, 17) of hydrolysates before and after peptidylation. The number of peptide chains per protein molecule was obtained by determining the lysine content after desamination of the derivative with nitrous acid (18). The designation of polypeptidyl protein is abbreviated, e.g., *p*-DL-AlaHSA means HSA molecule to which poly-DL-alanyl chains are attached.

Immunization Procedure.—Randomly bred rabbits (2.5–3.5 kg) of both sexes were used. The animals received four injections at fortnightly intervals. The first three were administered

intramuscularly into the thighs of the hind legs of the animals. Each injection (1 ml) contained both antigens (15 mg of each) incorporated in complete Freund's adjuvant (16). The last injection was administered intravenously, a mixture containing 10 mg of each antigen in 1 ml of 0.9% sodium chloride. The same schedule was followed when only one antigen was injected. The animals were bled 1 day prior to each immunizing injection and 1 wk after the intravenous injection.

Precipitin Studies.—Qualitative precipitin tests were done on all sera and quantitative studies were performed on sera obtained 1 wk after the intravenous injection. The precipitin reactions were carried out as perviously described (16). The amount of antibody is expressed as "absorbancy value" per 1 ml of serum. This is the absorbancy at 280 mμ of solutions of washed precipitate obtained at the optimal zone, in 1.1 ml of 0.1 N sodium hydroxide.

TABLE I

Characterization of Polypeptidyl Proteins

Designation and No. of derivative	*N*-Carboxyamino acid anhydride	Moles of amino acid		Moles of amino acid attached per mole protein	Moles of peptide chain per mole derivative	Average No. of amino acid residues per chain	Sedimentation coefficient*
		per mole protein	per mole derivative				
	g/g protein						
p-D-AlaHSA (574)	0.8	63	244	181	31	5.8	4.1
p-L-AlaHSA (575)	0.8	63	237	174	34	5.1	4.0
p-DL-AlaHSA (1326)	2.0	63	402	339	37	9.2	3.8
p-D-AlaRSA (570)	0.8	54	259	205	25	8.2	3.9
p-L-AlaRSA (569)	0.8	54	276	222	26	8.5	4.0
p-DL-AlaRSA (560)	1.5	54	341	287	36	8.0	4.0
p-DL-AlaRNase (596)	1.5	12	130	118	9	13.0	1.7
p-DL-PheHSA (605)	0.3	32	101	69	27	2.5	3.8 (50%); 9.9 (50%)
p-DL-PheRSA (1324)	0.3	23	75	52	20	2.6	5.0 (60%); 7.5 (40%)
p-DL-PheRNase (593)	0.3	3	18	15	7	2.1	1.8 (70%); 8.2 (30%)

* Measured in a Spinco model E ultracentrifuge on 1% solution at 20°C. Polyalanyl conjugates were dissolved in 0.1 M phosphate buffer, pH 7.0; polyphenylalanyl conjugates were dissolved in 0.1 M phosphate buffer, pH 7.8. The samples were sedimented at 59,780 rpm. When two peaks were observed, the sedimentation coefficient of each was measured and the relative areas are enclosed in brackets.

Agar diffusion studies were carried out at room temperature according to the method of Ouchterlony (19) in 1.5% gel containing 0.9% sodium chloride, 0.02 M phosphate buffer (pH 7.0), and 1:10,000 merthiolate.

Induction of Tolerance.—Rabbits were made tolerant to *p*-DL-PheRSA by injecting the tolerogen (2% solution in 0.9% sodium chloride) intraperitoneally at the following time intervals: 20 mg within 5 to 12 hr after birth, 30 mg at the age of 4, 20, and 45 days, and 40 mg at the age of 70 days. Immunization according to the regular schedule was started 20 days after the last tolerogenic injection.

RESULTS

Injection of polypeptidyl conjugates of HSA or RNase into rabbits led to the formation of antibodies specific to the polypeptidyl moiety and to the carrier. Antibodies to the protein carrier were determined by precipitation reaction with

HSA or RNase, antibodies to the polypeptidyl determinant with polypeptidyl RSA conjugate. Sera collected before immunization did not react with any of the immunogens or cross-reacting antigens.

In view of the fact that more than one type of antibody was formed in the same animal, it was necessary to show that each antibody specificity could be measured independently. Experiments demonstrating this point are summarized as follows: (*a*) Antisera of individual rabbits injected with *p*-DL-AlaRSA, *p*-DL-PheRSA, HSA, or RNase reacted with the homologous antigen but not with any of the other three antigens. Anti-*p*-DL-AlaRSA cross-reacted, as expected, with *p*-DL-AlaHSA and *p*-DL-AlaRNase. (*b*) Addition of non–cross-reacting

TABLE II

Antibody Production in Rabbits Immunized with One Antigen

Immunogen	No. of animals immunized	Precipitant			
		p-DL-AlaRSA	*p*-DL-PheRSA	HSA	RNase
		Average ± SD of absorbancy value per 1 ml serum			
p-DL-AlaRSA	17	1.10 ± 0.91	0	0	0
p-DL-AlaHSA	21	0.91 ± 0.66	0	1.0*	0
p-DL-AlaRNase	7	1.14 ± 0.48	0	0	0.5‡
p-DL-PheRSA	7	0	3.25 ± 1.70	0	0
p-DL-PheHSA	7	0	3.48 ± 2.04	6.35 ± 2.68	0
p-DL-PheRNase	8	0	0.44 ± 0.31	0	0.94 ± 0.48

* The value given was obtained from pooled serum of 12 animals. Sera of all animals reacted with HSA in the qualitative precipitin test.

‡ The value given was obtained from pooled serum of 6 animals. Sera of all animals reacted with RNase in the qualitative precipitin test.

antigen to reaction mixtures did not interfere with the quantitative determination of antibodies. (*c*) From artificial mixtures of antisera with different specificities (at various ratios), the expected amount of each antibody was precipitated by adding the appropriate precipitant.

Immune Response to Single Antigens.—In the course of immunization, each rabbit was challenged with one antigen only. In Table II the antibody content is given as the average (±) standard deviation (SD) calculated from data of individual animals of the groups. It can be seen that a good immune response to the polypeptidyl moieties was obtained, though quite a large variation was observed between individual rabbits of the same group. Antibodies to the poly-DL-phenylalanyl determinant were produced in larger amounts than those to the poly-DL-alanyl determinant when HSA or RSA served as the protein carriers. On the other hand, when RNase was the protein carrier, the immune response against the peptidyl moiety was stronger for DL-alanine than for DL-

phenylalanine. Cross-reaction between the two peptidyl determinants was not observed in any of the sera tested.

Immunodiffusion studies indicate that anti-poly-DL-alanyl antibodies formed by rabbits are identical, irrespective of whether they were elicited by the injection of *p*-DL-AlaRSA, *p*-DL-AlaHSA, or *p*-DL-AlaRNase (Fig. 1 A). Similar results were obtained with poly-DL-phenylalanyl-specific antibodies produced

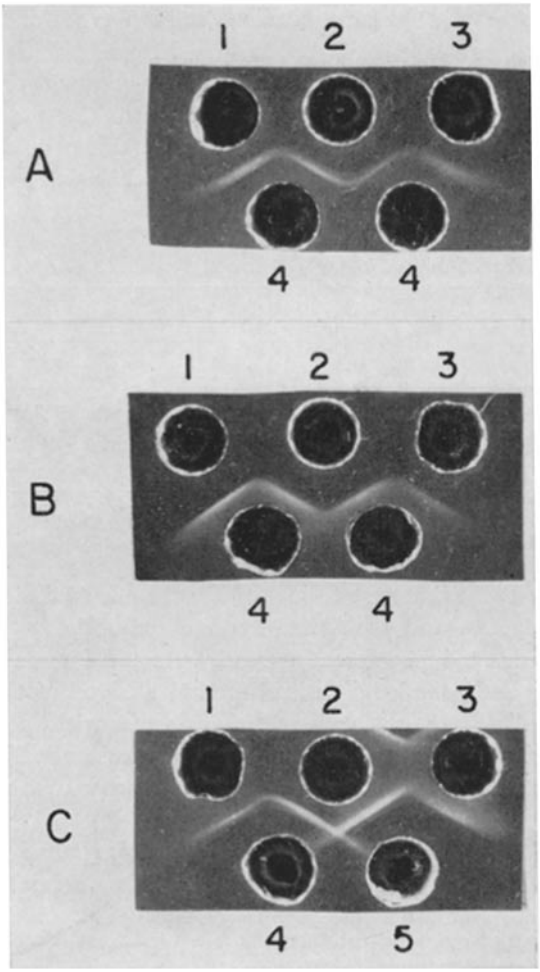

Fig. 1. Immunodiffusion study of polypeptidyl specific antibodies. A. 1, anti-*p*-DL-AlaRSA; 2, anti-*p*-DL-AlaHSA; 3, anti-*p*-DL-AlaRNase; 4, *p*-DL-AlaRSA. B. 1, anti-*p*-DL-PheRSA; 2, anti-*p*-DL-PheHSA; 3, anti-*p*-DL-PheRNase; 4, *p*-DL-PheRSA. C. 1, *p*-DL-AlaRSA; 2, *p*-DL-AlaRSA and *p*-DL-PheRSA; 3, *p*-DL-PheRSA; 4, anti-*p*-DL-AlaRSA; 5, anti-*p*-DL-PheRSA. In all plates the concentration of precipitant was 0.1 mg/ml.

by the three different poly-DL-phenylalanyl protein conjugates (Fig. 1 B). Lack of cross-reaction between the two antipeptidyl antibodies is illustrated in Fig. 1 C.

Immune Response to a Mixture of p-L-AlaHSA and p-D-AlaHSA.—It was previously shown that immunization of rabbits with *p*-L-AlaHSA or with *p*-D-AlaHSA elicited the formation of antipeptidyl antibodies which were strictly stereospecific, and that the extent of formation of antibodies to either determinant was of the same general magnitude. For both types of antibodies, the same size of the combining site was determined from inhibition studies with alanine peptide sterioisomers (14). Injection of poly-DL-alanyl proteins resulted in antibodies with specificity directed mainly towards the D-alanine sequence present in the poly-DL-alanyl determinant (11). In this case, sequences composed of L- and D-alanyl residues were distributed in chains attached to the same

TABLE III

Antibody Production in Rabbits Immunized with p-L-AlaHSA and p-D-AlaHSA

Immunogen pair	No. of animals immunized	Precipitant		
		p-L-AlaRSA	*p*-D-AlaRSA	HSA
		Average ± SD of absorbancy value per 1 ml serum		
p-L-AlaHSA + *p*-D-AlaHSA	12	0.84 ± 0.44	1.00 ± 0.68	3.02 ± 1.83*

* Value given was calculated from results of six sera. In the qualitative precipitin test sera from all animals reacted with HSA.

molecule. It was now of interest to investigate the effect of simultaneous injection of *p*-L-AlaHSA and *p*-D-AlaHSA where the optically active alanine determinants are located on different molecules. Rabbits immunized with these antigens formed anti-polyalanyl antibodies, and analysis of the sera (Table III) showed practically the same amount of antibodies with specificity toward the poly-L-alanyl and toward the poly-D-alanyl determinants. This shows that there is no interference between the two determinants when each one is attached to a separate carrier molecule.

Immune Response to Mixtures of Poly-DL-phenylalanyl and Poly-DL-alanyl Protein Conjugates.—The immune response of rabbits to injections of *p*-DL-PheRSA together with three different poly-DL-alanyl protein derivatives is summarized in Table IV. It can be seen that the response toward the poly-DL-alanyl determinant was suppressed when *p*-DL-AlaRSA or *p*-DL-AlaHSA were injected together with *p*-DL-PheRSA. On the other hand, when *p*-DL-AlaRNase and *p*-DL-PheRSA were injected, a considerable amount of anti-poly-DL-alanyl antibodies was formed (see Table II for comparison of response of animals to injection of one antigen). The lack of competition in the last case, where the protein carriers are not of a similar nature, is not due to a decrease in the amount

of anti-poly-DL-phenylalanyl antibodies formed (see Table IV). It is of interest that the simultaneous immunization with *p*-DL-PheRSA and *p*-DL-AlaHSA also led to a significant decrease in the formation of anti-HSA antibodies.

TABLE IV

Antibody Production in Rabbits Immunized with p-DL-PheRSA and Poly-DL-Alanyl Protein Derivatives

Immunogen pair	No. of animals immunized	Precipitant			
		p-DL-AlaRSA	*p*-DL-PheRSA	HSA	RNase
		Average ± SD of absorbancy value per 1 ml serum			
p-DL-PheRSA + *p*-DL-AlaRSA	12	0.05 ± 0.07	2.84 ± 2.37	0	0
p-DL-PheRSA + *p*-DL-AlaHSA	13	0.20 ± 0.17	3.07 ± 1.06	0.12 ± .0.14	0
p-DL-PheRSA + *p*-DL-AlaRNase	11	0.66 ± 0.29	5.71 ± 1.76	0	0.31 ± 0.11

TABLE V

Antibody Production in Rabbits Immunized with p-DL-PheHSA and Poly-DL-Alanyl Protein Derivatives

Immunogen pair	No. of animals immunized	Precipitant			
		p-DL-AlaRSA	*p*-DL-PheRSA	HSA	RNase
		Average ± SD of absorbancy value per 1 ml serum			
p-DL-PheHSA + *p*-DL-AlaHSA	13	0.14 ± 0.11	3.50 ± 1.38	2.17 ± 1.04*	0
p-DL-PheHSA + *p*-DL-AlaRSA	13	0.03 ± 0.03	1.93 ± 0.71	4.77 ± 1.00*	0
p-DL-PheHSA + *p*-DL-AlaRNase	10	0.75 ± 0.24	2.47 ± 0.93	4.50 ± 1.50	0.52‡

* Value given was calculated from results of seven sera. In the qualitative precipitin test, sera from all animals reacted with HSA.

‡ The value given was obtained from pooled serum of five animals. Sera of all animals reacted with RNase in the qualitative precipitin test.

Results of experiments in which *p*-DL-PheHSA was injected with poly-DL-alanyl conjugates of HSA, RSA, or RNase (see Table V) were similar to those described above. The capacity of the animal to react toward the poly-DL-alanyl determinant was impaired by injecting *p*-DL-PheHSA together with *p*-DL-Ala-

HSA or with *p*-DL-AlaRSA, but not by injection of *p*-DL-AlaRNase with *p*-DL-PheHSA.

The last two experiments of this series (Table VI) show that antibody formation against the poly-DL-alanyl determinant attached to RNase, which was not impaired by the two preceding poly-DL-phenylalanyl proteins, is efficiently suppressed by *p*-DL-PheRNase. On the other hand, *p*-DL-PheRNase does not inhibit anti-poly-DL-alanyl antibody production elicited by *p*-DL-AlaHSA. Here again, lack of competition occurs when the two protein carriers are dissimilar.

Immune Response to Poly-DL-alanyl Proteins in Animals Made Tolerant to p-DL-PheRSA.—In order to find out whether the antigenic competition described takes place only when antibodies to the poly-DL-phenylalanyl determinant are

TABLE VI

Antibody Production in Rabbits Immunized with p-DL-PheRNase and Poly-DL-Alanyl Protein Derivatives

Immunogen pair	No. of animals immunized	Precipitant			
		p-DL-AlaRSA	*p*-DL-PheRSA	HSA	RNase
		Average ± SD of absorbancy value per 1 ml serum			
p-DL-PheRNase + *p*-DL-AlaRNase	12	0.06 ± 0.06	0.40 ± 0.27	0	0.86 ± 0.52
p-DL-PheRNase + *p*-DL-AlaHSA	10	0.71 ± 0.41	0.61 ± 0.36	0.60*	0.74*

* The value given was obtained from pooled serum of eight animals. Sera of all animals reacted with HSA and RNase in the qualitative precipitin test.

produced, rabbits were made tolerant to *p*-DL-PheRSA by injection of this material from birth (see Materials and Methods). At the age of 3 months, these animals were divided into five groups which were immunized, respectively, with *p*-DL-PheRSA, *p*-DL-AlaRSA, *p*-DL-AlaHSA, a mixture of *p*-DL-PheRSA and *p*-DL-AlaRSA, and a mixture of *p*-DL-PheRSA and *p*-DL-AlaHSA.

Results given in Table VII show that the response to *p*-DL-PheRSA was markedly depressed in most of the treated animals (group 1), yet the capacity to produce antibodies with poly-DL-alanyl specificity upon infection of *p*-DL-AlaRSA or *p*-DL-AlaHSA was not affected (groups 2 and 3). Results obtained in groups 4 and 5, where *p*-DL-PheRSA was injected together with poly-DL-alanyl proteins, show that formation of antibodies toward the poly-DL-alanyl determinant was inhibited by the *p*-DL-PheRSA (in the majority of cases) even though no antibodies with poly-DL-phenylalanyl specificity were formed.

It may be noted that, in the two rabbits (of the 17 tested) which produced considerable amounts of anti-poly-DL-phenylalanyl antibodies (absorbancy

TABLE VII

Antibody Production in Animals Made Tolerant to p-DL-PheRSA

Group No.	No. of animals	Immunogen(s) injected	Precipitant	Precipitin values for individual rabbits per 1 ml serum											Average ± SD
1	11	*p*-DL-PheRSA*	*p*-DL-PheRSA	0	0	0.05	0.10	0.10	0.25	0.25	0.30	0.35	0.60	1.00	0.27 ± 0.28
2	10	*p*-DL-AlaRSA‡	*p*-DL-AlaRSA	2.10	1.50	1.40	1.10	1.10	0.50	0.45	0.30	0.25	0.20		0.89 ± 0.61
3	7	*p*-DL-AlaHSA‡	*p*-DL-AlaRSA	1.70	1.50	1.20	1.10	1.10	1.00	0.30					1.13 ± 0.41
			HSA	1.90	1.00	1.70	2.80	0.50	0.90	0.70					1.35 ± 0.75
4	8	*p*-DL-PheRSA +	*p*-DL-PheRSA	0	0	0	0	0	0	0.35	1.30				0.21 ± 0.38
		p-DL-AlaRSA	*p*-DL-AlaRSA	0	0	0	0	0	0	0.05	0.25				0.04 ± 0.07
5	9	*p*-DL-PheRSA +	*p*-DL-PheRSA	0	0	0	0	0	0	0	0.25	1.00			0.14 ± 0.31
		p-DL-AlaHSA	*p*-DL-AlaRSA	0	0.05	0.10	0.10	0.15	0.15	0.15	0.25	0.40			0.15 ± 0.11
			HSA	0	0	0	0.10	0	0.15	0.15	0.15	0.05			0.07 ± 0.06

* Cross-reaction with *p*-DL-AlaRSA was not observed with any antiserum.

‡ Cross-reaction with *p*-DL-PheRSA was not observed with any antiserum.

values 1.3 and 1.0), significant amounts of anti-poly-DL-alanyl antibodies were found.

DISCUSSION

On injection of mixtures of antigens in which polypeptidyl determinants are attached to separate carrier molecules, antigenic competition sometimes occurs, and sometimes not. Poly-L-alanine and poly-D-alanine, each on HSA, showed no competition. Poly-DL-phenylalanyl groups effectively suppress the immune response of rabbits toward poly-DL-alanyl groups when these were attached to identical or similar (RSA and HSA) protein carriers. Thus, formation of antibodies with poly-DL-alanyl specificity was impaired when *p*-DL-AlaHSA was administered together with *p*-DL-PheRSA (Table IV) or with *p*-DL-PheHSA (Table V). Similar results were obtained when the pair *p*-DL-AlaRNase and *p*-DL-PheRNase was injected (Table VI). However, suppression of the immune response against the poly-DL-alanyl moiety was not achieved when *p*-DL-AlaRNase was administered together with *p*-DL-PheRSA (Table IV) or with *p*-DL-PheHSA (Table V), and also when *p*-DL-AlaHSA was injected with *p*-DL-PheRNase (Table VI). These observations lead to conclusion that the nature of the protein carrier, to which each of the peptidyl groups is attached, strongly influences the capacity of the poly-DL-phenylalanyl determinant to inhibit the formation of anti-poly-DL-alanyl antibodies. This resembles other phenomena in which the role of carrier has been demonstrated, such as the ability of hapten-protein conjugates to stimulate a secondary response in rabbits (20), the capacity to elicit delayed hypersensitivity reaction in sensitized guineapigs (21, 22), and the influence of the carrier on some properties of antibodies formed toward the same haptenic group (23, 24).

Earlier studies indicated that the impaired immune response toward one of the antigens is not due to competition for metabolites necessary for antibody synthesis (25) or to blockade of the reticuloendothelial system (3). Another possible "nonspecific"cause for competition is that the stimulation of one clone interferes with the growth of a second clone (26). According to data given here, it is difficult to assume that proliferation of cells caused by injection of *p*-DL-PheRSA hindered the growth of cells capable of reacting to *p*-DL-AlaRSA, by imposing space limitation in the reactive tissue. The fraction of the cell population involved in the production of antibodies specific to the poly-DL-phenylalanyl determinant (as judged from the amount of circulating antibody), when the pair *p*-DL-PheRSA and *p*-DL-AlaRNase was injected, is probably larger than when the pair *p*-DL-PheRSA and *p*-DL-AlaRSA was administered (see Table IV). Yet much greater immune response to the poly-DL-alanyl moiety was elicited in the first case. Similar results obtained with other pairs of antigens, leading to the same conclusion, are given in Table V (*p*-DL-PheHSA and *p*-DL-AlaRNase vs. *p*-DL-PheHSA and *p*-DL-AlaRSA) and Table VI.

Even though not much is known today about the mechanism of immune reactions or about antigenic competition, it is of interest to discuss the results given here in terms of current ideas on antibody biosynthesis. According to the clonal selection theory, the competent cell is genetically determined to form antibodies of only one type of specificity. Antibodies to poly-DL-alanyl chains, or to poly-DL-phenylalanyl chains, or to any determinant on a protein are thus expected to be produced by different sets of cells (27). The same clone would be stimulated to produce anti-poly-DL-alanyl antibodies when *p*-DL-AlaRSA, *p*-DL-AlaHSA, or *p*-DL-AlaRNase are injected. This expectation seems corroborated by the observation that antibodies to the peptidyl moiety elicited by immunization with antigens sharing the same peptidyl chain but having different carriers are undistinguishable in agar double diffusion (Fig. 1 A, B). The natural antibodies (28) which are supposed to react with the antigen and initiate cell proliferation and formation of the clone, and the antibodies detectable later in the circulation should have the same specificity. However, serious difficulties arise when one tries to interpret our data in terms of the above hypotheses. There was no cross-reaction between antibodies to poly-DL-alanyl and antibodies to poly-DL-phenylalanyl determinants (Fig. 1 C). If one believes that a clone capable of reacting specifically with poly-DL-alanyl groups exists, on what basis would a molecule of *p*-DL-PheRSA interact and suppress the activity of this clone? Assuming that by some process the *p*-DL-PheRSA antigen can inactivate the poly-DL-alanyl clone, one must still explain why the response is inhibited toward *p*-DL-AlaRSA and *p*-DL-AlaHSA, but not toward *p*-DL-AlaRNase (see Table IV). Similar groups of data which cannot be reconciled with the concept of specific clones are given in Table V and Table VI.

The phenomenon of competition may be interpreted without too many difficulties if the notion is accepted that the antibody-forming cell is multipotent. Namely, the same cell will produce equally good antibodies with poly-DL-alanyl specificity or anti-poly-DL-phenylalanyl antibodies, when the animal is challenged with either *p*-DL-AlaRSA or with *p*-DL-PheRSA, respectively. Nevertheless, when both antigens are given simultaneously, the *p*-DL-PheRSA predominates. At the stage during which competition occurs, the integrity of the molecules of the competing antigens is probably well preserved, otherwise the role of the carrier demonstrated here would be difficult to understand. Furthermore, the preference in the immune response to a given determinant seems to be determined not only by the nature of the individual determinants, but also by the over-all properties of the competing molecules. Other evidence supporting the idea that the whole molecule, and not its degradation products, is involved in the immune processes comes from studies which show that antigenic determinants of proteins and synthetic antigens are controlled to a large extent by the secondary, tertiary, and quarternary structure of the molecule (29).

Studies by Abramoff and Wolfe indicate that there is no correlation between the inhibitory action of the competing antigen and its capacity to elicit antibody formation. They found that the production of antibodies to BSA was impaired by concurrent injection of bovine hemoglobin, though antibodies specific to bovine hemoglobin could not be detected in the chicken sera even when this antigen was injected alone (30). Since in this case the competing antigen lacks immunogenic activity, it was of interest to see whether a good immunogen behaves similarly. The experiments with rabbits made tolerant to *p*-DL-PheRSA were intended to clarify this point. Neonatal injections of *p*-DL-PheRSA induced considerable unresponsiveness toward this material without impairing the response against the *p*-DL-alanyl determinants (see Tables VII and II). However, when *p*-DL-AlaRSA or *p*-DL-AlaHSA were injected together with *p*-DL-PheRSA, the production of poly-DL-alanyl specific antibodies was effectively suppressed (as in normal rabbits, see Tables VII and IV) even though antibodies toward *p*-DL-PheRSA were practically undetectable. These experiments show that formation of antibody against the competing antigen is not the cause of its inhibitory activity. Assuming that in normal and tolerant animals the competing antigen acts similarly, it seems that interference by the competing antigen does not occur at the final stage of antibody synthesis. As far as the tolerance phenomenon is concerned, this study indicates that the material against which tolerance was induced is not recognized by the animal as an innocent "self-constituent" (28), but exhibits a definite immunological activity, namely, it effectively participates in competition of antigens.

SUMMARY

Competition between two polypeptidyl determinants was studied in normal rabbits and rabbits made tolerant to the competing antigen. The capacity of poly-DL-phenylalanyl protein conjugate to inhibit the formation of antibodies specific to the poly-DL-alanyl determinant was dependent on the nature of the protein carrier of the singly substituted antigens. Competition occurred only when the peptidyl determinants were attached to identical or similar (RSA and HSA) carriers. Thus, the immune response toward the poly-DL-alanyl determinant was impaired by injecting the pairs *p*-DL-PheRSA and *p*-DL-AlaHSA, or *p*-DL-PheRNase and *p*-DL-AlaRNase. Suppression of the formation of antibodies with poly-DL-alanyl specificity was not observed, however, upon administration of *p*-DL-PheRSA together with *p*-DL-AlaRNase or of *p*-DL-PheRNase with *p*-DL-AlaHSA.

Tolerance to *p*-DL-PheRSA was induced by injecting this material into newborn rabbits. The tolerant animals retained their capacity to produce anti-poly-DL-alanyl antibodies upon injection of *p*-DL-AlaRSA or *p*-DL-AlaHSA. However, when these poly-DL-alanyl proteins were administered together with *p*-DL-PheRSA, antibodies against the poly-DL-alanyl determinant were not formed even though no antibodies with poly-DL-phenylalanyl specificity were produced.

These results indicate that in competition experiments the preference in the immune response against a given determinant is dependent not only on the nature of the competing determinants, but it is also governed to a large extent by the over-all properties of the antigenic molecules. This suggests that at the stage at which the competition occurs the competing molecules had not undergone considerable degradation.

On the basis of experiments with tolerant animals, it is suggested that in normal animals antibody formation to the competing antigen is not the cause of its inhibitory action on the response against the other antigen.

The competition experiments described suggest that an antibody-forming cell is multipotent.

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