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Immune Responses of Inbred Guinea Pigs to the Random Terpolymers Poly ($Glu^{57}Lys^{38}Tyr^{5}$) and Poly ($Glu^{51}Lys^{34}Tyr^{15}$)[1,2] (38405)

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During the past few years considerable evidence has been provided dealing with the genetic control of the immune response in a number of species employing synthetic polymers of amino acids and proteins (1, 2). The studies of Benacerraf and coworkers have established the presence of immune response (Ir) genes in guinea pigs determining responses against the polymers poly L lysine (PLL), poly (glutamic acid^{60} alanine^{40}) (GA) and poly (glutamic acid^{50} tyrosine^{50}) (GT) (1, 2). It was established that strain two guinea pigs have the PLL and GA genes but not the GT gene, whereas strain 13 guinea pigs have the GT gene but not the other two genes. The presence of these Ir genes also controlled the responses against hapten conjugates of the specific polymers.

In addition, although guinea pigs of strains 2 and 13 responded against the complex terpolymer of glutamic acid, alanine and tyrosine ($G^{60}A^{30}T^{10}$), the recognition of the polymer was via different determinants, *i.e.*, strain 13 guinea pigs via GT determinants, and strain 2 guinea pigs via GA determinants. The responses were monitored both by measuring stimulation by antigen of uptake of ^{3}H thymidine into lymphoid cells (cellular response) and measurement of antibody in the sera (3).

The purpose of the present investigation was to determine whether the above observations would pertain to other polymers containing both "immunogenic" and "nonimmunogenic" determinants within the same molecule.

The studies with the random terpolymers of glutamic acid, lysine and tyrosine, GLT^{5} and GLT^{15}, will show that GLT^{5} is indeed immunogenic only in strain 2 guinea pigs, but GLT^{15}, which has considerably more tyrosine present, is immunogenic in both strains 2 and 13 guinea pigs. These findings are related to the fact that these polymers consist of both "carrier" and "haptenic" determinants within the same molecule, and that the recognition of the carrier determinants determines whether a response will ensue or not.

Materials and Methods. Polymers. The various random polymers containing different percentages of glutamic acid, lysine, alanine and tyrosine were prepared by the polymerization of the *N* carboxy anhydrides of the specific amino acids (4). Table I lists the polymers used for immunization of the guinea pigs as well as those used for measuring the various cross reactions.

The polymers were iodinated with ^{125}I according to the chloramine-T procedure (5). However, in order to iodinate the GLA^{5} polymer, it was necessary to first tyrosylate it. GLA^{5} was reacted with *N*- hydroxysuccinimide- L- tert-butyloxycarbonyl tyrosinate according to our reported procedure (6). In all iodination reactions, carrier iodide was added to the carrier-free Na ^{125}I solution in a quantity equivalent to the amount involved in diiodination of 10% of the available tyrosine residues in the polymer. Unreacted ^{125}I was removed by extensive dialysis against distilled water until no ^{125}I was detected in the dialyzing medium. The ^{125}I polypeptide

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[2] Nomenclature of polymers is based upon recommendation of IUPAC-IUB Commission on Nomenclature, October, 1967 (Biochem. J. **106**, 577 (1968)). Abbreviations for amino acids used in the text are as follows: tyrosine = T; glutamic acid = G; alanine = A; lysine = L; poly L lysine = PLL.

TABLE I. Synthetic Polymers of Amino Acids Used in Present Study.

Nomenclature and composition of polymers	Abbreviation employed in text	Average molecular weight
Poly(Glu[57]Lys[38]Tyr[5])	GLT[5]	50,000
Poly(Glu[51]Lys[34]Tyr[15])	GLT[15]	70,000
Poly(Glu[57]Lys[38]Ala[5])	GLA[5]	68,000
Poly(Glu[60]Ala[30]Tyr[10])	GAT[10]	55,000
Poly(Glu[58]Ala[38]Tyr[4])	GAT[4]	50,000
Poly(Glu[90]Tyr[10])	GT	70,000

solutions were then lyophilized and stored at −20° for future use.

Specific activity of polymers. The ^{125}I content and specific activity of all iodinated polymers were determined on an aliquot of a solution in which the nitrogen content was previously analyzed by the Markham modification of the Kjeldahl procedure (7). The specific activity of the ^{125}I labeled polymers varied from 0.4 to 0.8 μCi/μg. At least 90% of the polymer-associated ^{125}I was precipitable with homologous rabbit antisera prepared against the noniodinated polymer.

Animals. Guinea pigs of strain 2 and strain 13 were either bred in our animal facility from a breeding stock received from NIH, or obtained from the National Jewish Hospital, Denver, COL.[3] Animals were immunized in the foot pads with 100 or 500 μg of polymer in complete Freund's adjuvant containing 0.5 mg per ml Mycobacterium butyricum (Difco Labs.). Ten to 14 days later, the guinea pigs were bled and injected intradermally with 100 μg of the polymer in 0.1 ml of solution. After reading the delayed skin reactions, the animals were boosted intracutaneously with the same amount of polymer in adjuvant. Two weeks later, they were bled and skin tested as above. Non specific irritation of up to 6 mm was occasionally observed. Erythema and induration greater than 10 mm in diameter was considered a positive skin reaction and the intensity of the reactions was graded as follows: −, 0–8 mm diameter; +, 8–15 mm diameter; ++, 15–25 mm diameter; +++, 25 mm diameter.

[3] We wish to acknowledge the cooperation of Dr. Ira Green and Dr. William Paul for supplying the initial breeding stock of these animals, and the Division of Research of the National Jewish Hospital, Denver, Colorado, for supplying additional inbred guinea pigs.

Antibody assay. Sera were assayed for antibody activity using a modified Farr-type antigen binding assay employing the ^{125}I labeled polymer (8). A mixture of 0.050 ml of polymer containing 0.001 μg *N* in phosphate buffered saline and 0.025 ml of a 1:5 dilution of the guinea pig sera in 1% bovine serum albumin was placed at 4°. After 60 min, about 0.25 ml of sheep anti guinea pig globulin was added to precipitate all the guinea pig gamma globulin. The rest of the assay was as described (9). The percent antigen bound was determined by counting the supernatant in a Nuclear Chicago Co. gamma well counter. Appropriate controls of normal guinea pig serum and known guinea pig antisera were always included. Cross reactions with "related" polymers were also performed as above.

Results and Discussion. The data presented in Table II show that strain 13 guinea pigs do not respond to immunization with either 100 or 500 μg of the polymer GLT[5], whereas strain 2 guinea pigs respond well. However, the responses against the polymer GLT[15] were different and dose dependent. Although the strain 2 guinea pigs responded against both doses of polymer, the strain 13 guinea pigs responded only when immunized with 500 μg of the polymer.

The nature of the determinants, *i.e.*, specificity, against which the responses were generated are also presented in Table II. The anti GLT[5] serum (strain 2) reacted with GLT[5] (90% binding), GLT[15] (85%) and GLA[5] (75%), but not with GT, indicating that the specificity was directed predominantly against GL determinants. This is in agreement with the findings that strain 2 guinea pigs have the PLL or GL gene, and therefore can respond well against the GL polymers (10). However, because of the considerable interaction of glutamyl and lysyl residues in the polymer GLT[5], the availability of GT determinants of G[50]T[50] composition is very limited and not sufficient to trigger the B cells having receptors for the haptenic groups. This might also explain why strain 13 guinea pigs did not respond against the GLT[5] polymer, even though they have the GT gene and the appropriate receptors on T and B lymphocytes necessary for responding against sequences in G[50]T[50].

However, when the amount of tyrosine in the GL polymer was increased to 15 mole%, and the amount injected increased to 500 μg of polymer,

TABLE II. Immune Response of Strain 2 and 13 Guinea Pigs to Poly(Glu Lys Tyr) Terpolymers.

Guinea pig Strain	Polymer injected	Dose injected (μg)	No. of animals	No. of responders	Intensity of skin reaction[a]	% Antigen bound[b] 2° response			
						(GLT[5])	(GLT[15])	(G[90]T[10])	(GLA[5])
2	$G^{57}L^{38}T^{5}$	100	10	10	+++	60 ± 10	58 ± 9	0 ± 0	55 ± 8
		500	3	3	+++	91 ± 10	85 ± 2	14 ± 0	71 ± 9
13		100	10	0	–				
		500	4	0	–	15 ± 5	0 ± 0	12 ± 4	17 ± 3
2	$G^{51}L^{34}T^{15}$	100	3	3	+++	56 ± 1	76 ± 2	81 ± 1	59 ± 1
		500	8	8	+++	84 ± 1	85 ± 1	68 ± 9	79 ± 1
13		100	5	0	–		4 ± 1	7 ± 0	
		500	10	10	++	38 ± 8	54 ± 5	52 ± 6	16 ± 8

[a] Delayed skin reactions read 24 hr after injection are graded according to text.

[b] The amount of ^{125}I polymers bound by 1:5 dilution of sera compared to normal sera. (See text for assay.)

responses were obtained in the strain 13 guinea pigs. This could be explained by the need to present the lymphocyte receptors with an appropriate concentration of the specific GT determinants to initiate the immune reaction. The interesting finding is that the strain 13 anti GLT[15] sera exhibited good binding reactions with GLT[15], GLT[5] and GT, and much poorer reactions with GLA[5], indicating that the specificity of the response was predominantly against GT and not GL determinants.

In contrast to the above are the findings with the strain 2 guinea pig sera. The sera obtained following immunization with 100 μg GLT[15] reacted well with GLT[15], GLT[5], GT and GLA[5], indicating that specificity was directed against both GL and GT determinants. The reactions with the antisera obtained from 500 μg were similar.

These findings may be explained as follows: The strain 2 guinea pigs, which have the PLL gene and therefore respond against GL polymers, have produced antibody reacting not only with GL but also GT, as the GT sequences act as haptenic determinants within the immunogenic carrier polymer GL.

However, although the strain 13 guinea pigs respond against GLT[15] because they have the GT Ir gene, in this situation (GLT[15]) the GT determinants do not act as the carrier for GL sequences, even though these guinea pigs can produce anti GL antibody. This was accomplished when the carrier was phosphorylated bovine serum albumin to which the GL was complexed. The anti GL response (% antigen bound) was 40 ± 9 (Unpublished observations).

These observations are analogous to those of Bluestein *et al.* with the GAT[10] system in guinea pigs (3). Strain 2 guinea pigs which have the GA gene produced both anti GA and anti GT antibodies when immunized with GAT[10]. The strain 13 guinea pigs produced only anti GT antibodies, indicating that GA could act as a carrier for GT haptenic determinant but not vice versa.

The need for a significant concentration of GT determinants to be present in the GLT polymer in order for strain 13 guinea pigs to respond, *i.e.*, GLT[15] was immunogenic but not GLT[5], was noted in another analogous situation. Whereas the polymer GAT[10] produces responses in both strain 2 and strain 13 guinea pigs, the polymer GAT[4], which contains less GT determinants, is

immunogenic only in strain 2 guinea pigs (11).

Although the explanation for these analogous findings is not clear, it may be related to the requirement for a specific concentration of "GL" determinants (or GA determinants) to trigger receptors on B cells. If one assumes the need for an approximate equivalent concentration of glutamyl and tyrosyl residues to form the GT determinants to interact at the T cell (and B cell) level, then possibly the alteration in "available" GL determinants has been sufficient to eliminate interaction with receptors on B cells for those determinants. As mentioned before, the strain 13 guinea pigs can produce anti GL (and anti-GA) antibody and therefore their B cells do have receptors for these determinants.

Summary. The immune response patterns (cellular and humoral) or inbred guinea pigs of strains 2 and 13 have been studied employing the two random polymers poly($glu^{57}lys^{38}tyr^{5}$) and poly($glu^{51}lys^{34}tyr^{15}$). Strain 2 guinea pigs responded against both polymers, whereas strain 13 guinea pigs responded only against the latter polymer. Cross reaction studies with GL and GT polymers indicated that in GLT^{15}, GL sequences could function as carrier for GT determinants (strain 2 response pattern), but GT sequences could not function as carrier for GL determinants (strain 13 response pattern). A possible explanation is offered to account for these findings which are analogous to similar results in guinea pigs with GAT^{10}.

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