SPECIFIC IMMUNE RESPONSE GENES OF THE GUINEA PIG

V. INFLUENCE OF THE GA AND GT IMMUNE RESPONSE GENES ON THE SPECIFICITY OF CELLULAR AND HUMORAL IMMUNE RESPONSES TO A TERPOLYMER OF L-GLUTAMIC ACID, L-ALANINE, AND L-TYROSINE*

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Histocompatibility-linked specific immune response (Ir)[1] genes, controlling the ability to develop cellular and humoral immune responses, have been identified in guinea pigs and mice (1). Two experimental approaches used to identify Ir genes and to investigate their function have been the study of immunogenicity of synthetic polypeptides of limited structural diversity, and the study of the immunogenicity of native protein antigens injected in limiting immunizing doses. In earlier reports we have identified four Ir genes in inbred and random-bred guinea pigs, the "PLL," "GA," "GT," and "L-BSA" genes controlling, respectively, immune responsiveness to hapten-poly-L-lysine conjugates, to random copolymers of L-glutamic acid and L-alanine (GA), and to L-glutamic acid and L-tyrosine (GT), and to limiting immunizing doses of bovine serum albumin (BSA) (2–4).

Inbred strain 2 guinea pigs are homozygous for the PLL, GA, and L-BSA genes which were shown to be very closely linked to the major locus controlling strain 2 histocompatibility antigens (4–7). The GT gene is found in strain 13 guinea pigs and is similarly linked to a major strain 13 histocompatibility specificity (7). Furthermore, in most random-bred guinea pigs the GA and PLL genes remain linked and behave as alleles or pseudoalleles to the GT gene (8).

The successful transfer of immune responsiveness controlled by the PLL gene to

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DNP, dinitrophenyl; GA, poly-α-[L-glutamic acid (60%), L-alanine (40%)]; GAT, poly-α-[L-glutamic acid (60%), L-alanine (30%), L-tyrosine (10%)]; GL, poly-α-[L-glutamic acid (60%), L-lysine (40%)]; GT, poly-α-[L-glutamic acid (50%), L-tyrosine (50%)]; HSA, human serum albumin; Ir, immune response genes, PBS, phosphate-buffered saline; PLL, poly-L-lysine.

irradiated strain 13 nonresponder recipients with lymph node and spleen cells from $(2 \times 13)F_1$ responder donors and the observation that the responding cells in the chimeric animals were of donor origin demonstrate the expression of Ir genes in immunocompetent cells (9, 10). There is, in addition, considerable evidence for the expression of histocompatibility-linked Ir genes in cells concerned with cellular immunity and with "helper" or "carrier" function in antibody responses. Thus, delayed hypersensitivity and in vitro correlates of cellular immunity are observed only in animals possessing the specific Ir gene required to respond to the corresponding antigen (2, 3). Further, a specific Ir gene, controlling responsiveness to a carrier molecule, controls also anti-hapten antibody responses to all hapten conjugates of this molecule (2). Correspondingly, nonresponder animals lacking the PLL gene can nevertheless be induced to form antibodies but not cellular immunity against DNP-PLL, when immunized with DNP-PLL complexed with an immunogenic albumin (12).

The mechanism of action of specific Ir genes in immunocompetent cells is not clear. In addition to their expression in cells mediating cellular immunity, specific Ir genes may affect the specificity of the antibody population produced in some systems. This puzzling effect is observed in spite of the fact that the antibodies produced in response to antigens under unigenic control are markedly heterogeneous with regard to class, specificity, and affinity. In the present study we have taken advantage of the immunogenicity of a random terpolymer of L-glutamic acid, L-alanine, and L-tyrosine (GAT) for all strain 2, strain 13, and random-bred Hartley guinea pigs (3) to study the effect of the GA and GT genes on the specificity of immune responses to a more complex antigen. Since GAT presumably contains GA and GT determinants in addition to antigenic determinants unique to itself, we felt that a comparative study of the specificities of cellular and humoral responses to GAT made by genetic responder and nonresponder animals may clarify the role of Ir genes in immune responses to complex antigens.

Materials and Methods

Polymers.—Poly-α-[L-glutamic acid (60%), L-alanine (40%)], GA, mol wt 43,000; poly-α-[L-glutamic acid (60%), L-alanine (30%), L-tyrosine (10%)], GAT, mol wt 25,000; poly-α-[L-glutamic acid (60%), L-lysine (40%)], GL, mol wt 115,000; and poly-L-lysine, PLL, mol wt 110,000 were obtained from Pilot Chemical Division of New England Nuclear Corp., Boston, Mass.

Poly-α-[L-glutamic acid (50%), L-tyrosine (50%)], GT, mol wt 14,500 was obtained from Miles Laboratories, Kankakee, Ill.

DNP_{37}-PLL was made by reacting poly-L-lysine with 2,4-dinitrofluorobenzene as previously described (13). The subscript refers to the average number of dinitrophenyl (DNP) groups per molecule.

Animals.—The inbred strain 2 and strain 13 guinea pigs and some of the random-bred Hartley guinea pigs used in this study were obtained from the Animal Production Unit, National Institutes of Health, Bethesda, Md. Other Hartley guinea pigs used were obtained from Camm Research Laboratories, Inc., Wayne, N. J. Camm Hartley guinea pigs have a lower GA and PLL and a higher GT gene frequency than the NIH Hartley animals. All animals used in this study weighed between 250 and 450 g when immunized.

Immunization Procedures.—Antigen solutions in 0.015 M phosphate buffer, pH 7.5, containing 0.15 M NaCl (PBS) were emulsified with an equal volume of complete Freund's adjuvant containing 0.5 mg/ml *Myobacterium butyricum* (Difco Laboratories, Inc., Detroit, Mich.). The immunizing dose was 500 μg of GAT, GA, or GT administered in 0.4 ml of the emulsion distributed in the four foot pads. The guinea pigs that were immunized with GAT and DNP-PLL simultaneously received 500 μg of GAT and 100 μg of DNP-PLL, each contained in 0.2 ml of the adjuvant emulsion distributed in a front and rear foot pad.

Skin Tests.—3 wk after immunization the guinea pigs were challenged with intradermal injections of the appropriate antigens. The concentrations were adjusted so that the animals received 50 μg of the glutamic acid containing polypeptides or 10 μg of DNP-PLL in 0.1 ml of normal saline. The injection sites were examined 24 hr later. Erythema and induration significantly greater than observed in unimmunized control animals was considered a positive skin test.

Antibody Assay.—The guinea pigs were bled from the retro-orbital venous plexus just before skin testing and the sera were assayed for antibody against the immunizing polymer as well as for antibodies cross-reacting with other synthetic antigens, using the modified Farr-type assay described previously (3). Radioactive ligands, made by iodinating GT and GAT polymers with ^{125}I, using the chloramine-T method (14), were used to detect antibodies specific for those antigens. A ligand capable of detecting anti-GA antibodies in sera containing anti-GAT antibodies was prepared by binding covalently GA with human serum albumin (HSA) and subsequently iodinating the complex with ^{125}I. The complex was formed by reacting GA (10 mg), HSA (10 mg), and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (200 mg) in 0.2 M carbonate buffer, pH 9.8, in a total volume of 1.2 ml. The reaction mixture was stirred continuously at room temperature for 30 min and then dialyzed against 2000 vol of PBS overnight. The GA-HSA complex was separated from free GA and free albumin on the basis of their different electrophoretic mobility using Geon block preparative electrophoresis. The Geon resin was obtained from B. F. Goodrich Chemical Company, Louisville, Ky. The complex was eluted from the Geon with normal saline, concentrated by vacuum dialysis, and iodinated by the chloramine-T method (14). A maximum 70% of the complex, GA-HSA-^{125}I, was bound by strong specific anti-GA guinea pig antisera.

Culture Conditions.—Axillary, inguinal, and popliteal lymph nodes were removed under sterile conditions 3–6 wk after immunization and teased into a single cell suspension in Eagle's minimal essential medium. 5 million cells were placed in 12 × 75 mm plastic culture tubes with a final volume of 1.5 ml containing 5% fetal bovine serum and various concentrations of the test antigens as indicated. Each experimental group consisted of triplicate cultures. They were incubated at 37°C in a 5% CO_2 in air atmosphere. After 48 hr 1 μCi of thymidine-methyl-^{3}H (1.9 Ci/mmole) was added to each tube and incubation continued another 24 hr. The cultures were harvested by trapping the cells on Millipore filters, washing twice with 5 ml of PBS containing 0.001 M thymidine, twice with 5 ml 5% trichloroacetic acid (TCA) containing 0.001 M thymidine, and once with 5 ml 95% ethanol. The wet filters were placed directly in the scintillation fluid (Aquasol, New England Nuclear Corp., Boston, Mass.) and counted in a liquid scintillation spectrometer.

RESULTS

Immunological Specificity of Anti-GA and Anti-GT Responses.—No cross-reactivity between delayed hypersensitivity skin reactions specific for GA and GT could be detected (Table I). After immunization with GA, 9 of 10 NIH Hartley guinea pigs demonstrated delayed skin reactivity to GA. All nine GA responders were also sensitive to GAT, but none of the animals displayed delayed hypersensitivity to GT or GL. The considerable similarity in the

amino acid composition of GA and GAT, which is GA with 10% added L-tyrosine, probably accounts for their high degree of cross-reactivity in GA responder animals. After GT immunization, 8 of 10 Camm Hartley guinea pigs developed delayed skin reactivity. None of these animals showed sensitivity to either GA or GL. However, only two of the eight GT responders also responded to intradermal challenge with GAT. GAT probably does contain GT determinants, but this must represent a small part of the molecule, which accounts for the limited cross-reactivity observed with GT.

The lack of cross-reactive delayed skin reactions between GA and GT is mirrored in vitro (Table II). Cultured lymph node cells from GA responder Hartley guinea pigs respond to GA stimulation with increased tritiated thymidine (TdR-^{3}H) incorporation. The increase was fourfold on the average over the tritiated thymidine incorporation of the same cells not stimulated by GA. GA does not stimulate cells from genetic nonresponders nor does GT induce increased TdR-^{3}H incorporation in the cells from GA responders. Similarly, lymph node cells from GT responder guinea pigs, but not from GT nonresponders, are stimulated in vitro by GT. The responder cells are stimulated by GT in vitro also with a fourfold average increase in TdR-^{3}H incorporation, but do not respond at all to GA.

TABLE I

The Specificity of Delayed Hypersensitivity Reactions in Hartley Guinea Pigs Immunized with GA or GT

Immunizing polymer	Skin testing polymer	Animals with delayed sensitivity/animals immunized
100 μg	*50 μg*	
GA	GA	9/10*
	GT	0/10
	GL	0/10
	GAT	9/10*
GT	GT	8/10†
	GA	0/10
	GL	0/10
	GAT	2/10‡

* The same nine animals reacted to GA and to GAT.

‡ Two of the GT-sensitive animals reacted to GAT.

There is no detectable cross-reactivity between anti-GA and anti-GT antibodies produced by genetic responder Hartley guinea pigs (Table III). The anti-GA antibodies in sera obtained 3 wk after immunization with GA bind the GA-HSA-^{125}I and GAT-^{125}I ligands but do not bind GT-^{125}I. The GAT is consistently a more sensitive ligand for the detection of anti-GA antibody than is the GA-albumin complex perhaps because the tertiary structure of GA is

altered by coupling it to the protein. Similarly, anti-GT antibody binds GT-^{125}I and GAT-^{125}I but does not bind the GA-HSA-^{125}I ligand. The GAT and GT ligands are approximately equally sensitive for the detection of anti-GT antibody.

TABLE II

The Specificity of Antigen-Stimulated Tritiated Thymidine Incorporation in Lymph Node Cell Cultures from Hartley Guinea Pigs Immunized with GA or GT

Immunizing polymer	Responder status*	Challenging polymer in vitro‡	No. of animals	TdR-^{3}H incorporation (E/C)§	
500 μg/animal				*mean*	*(range)*
GA	−	GA	3	0.92	(0.85–1.0)
	+	GA	4	4.0	(3.2 –5.1)
	+	GT	4	1.0	(0.89–1.2)
GT	−	GT	5	0.88	(0.76–1.1)
	+	GT	6	4.4	(2.7 –6.0)
	+	GA	5	1.1	(0.94–1.2)

* Responders (+) demonstrated both delayed skin reactivity and serum antibody directed against the immunizing polymer. Nonresponders (−) made no detectable immune responses to the immunizing polymer.

‡ GA was added to the cultures at a concentration of 100 μg/ml. GT was added to the cultures at a concentration of 10 μg/ml. These doses elicited the maximal responses in cultures of cells from responder animals.

§ $E/C = \frac{\text{cpm incorporated in stimulated cultures}}{\text{cpm incorporated in cultures receiving no antigen}}$.

TABLE III

The Specificity of Serum Antibody Produced by Responder Hartley Guinea Pigs Immunized with GA or GT

No. of animals	Immunizing polymer	Per cent binding*		
		GAT-^{125}I	GA-HSA-^{125}I	GT-^{125}I
	500 μg/animal			
9	GA	71.3 (53–86)‡	47.2 (34–62)	1.9 (0–5)
7	GT	39.9 (28–61)	1.9 (0–4)	48.9 (17–63)

* The per cent GAT-^{125}I (5 mμg), GA-HSA-^{125}I (0.4 μg), and GT-^{125}I (3 mμg) bound by the globulin fraction of 0.05 ml of a 1:10 dilution of the test serum using the assay procedure described previously (3). Excess antibody can bind over 90% of the GAT and GT ligands, but a maximum of only 70% of the GA-HSA-^{125}I ligand.

‡ The data is expressed as the arithmetic mean with the range in parentheses.

Cross-Reactivity of Anti-GAT Responses for GA.—In order to assess the influence of a specific immune response gene on the immune responses to a more complex antigen, we have examined the specificity of both cellular and humoral immune responses to GAT for cross-reactivity directed toward GA. After immunization with GAT, all inbred strain 2 and strain 13 guinea pigs

develop delayed hypersensitivity and circulating antibody directed against GAT (3). However, when tested for cross-reactivity directed against GA, the anti-GAT sera from strain 2 guinea pigs which possess the GA gene were able to bind significantly the GA-HSA-^{125}I ligand while none of the anti-GAT sera from strain 13 guinea pigs (GA nonresponders) had antibodies capable of binding GA (Table IV). Since the strain 2 guinea pigs develop higher anti-GAT antibody levels than do the strain 13 animals, we have expressed the data as a ratio of the per cent GA bound to the per cent GAT bound. Clearly, the presence of the GA gene influences the specificity of the anti-GAT antibody population produced.

In most random-bred Hartley guinea pigs the GA gene is linked to the PLL gene (8). We can, therefore, identify most Hartley animals possessing the GA

TABLE IV
The Effect of GA Responder Status on the Specificity for GA of Anti-GAT Antibodies Produced by Strain 2 and Strain 13 Guinea Pigs

Strain	No. of animals	No. with anti-GA specificity*	Anti-GA specificity per cent binding‡		Per cent anti-GA binding / Per cent anti-GAT binding	
			Mean	(SE)	*Mean*	(SE)
2	7	7	30.8	(4.41)	0.41	(0.051)
13	5	0	2.2	(1.39)	0.03	(0.020)

* Greater than 10% antigen binding is required to demonstrate the presence of specific antibody in this assay based on the highest nonspecific binding of GA-HSA-^{125}I observed with a control panel of normal sera.

‡ The per cent binding of GA-HSA-^{125}I was determined as described in the legend to Table III.

gene by testing for PLL responsiveness. After simultaneous immunization of Camm Hartley guinea pigs with GAT and DNP-PLL, all of the animals developed delayed skin reactivity to GAT and circulating anti-GAT antibody, as expected. However, when skin tested for cross-reactivity to GA, with few exceptions, only those animals expected to possess the GA gene, on the basis of their responsiveness to DNP-PLL, demonstrated delayed hypersensitivity to GA (Table V). Positive GA skin tests developed in all but one of the eight PLL responders, and in only two of eleven PLL nonresponders. The two PLL nonresponders demonstrating cross-reactivity with GA and the one PLL responder not cross-reacting with GA are consistent with the number of animals expected in this population in which the GA and PLL immune response genes are not linked (8). Thus, the GA immune response gene also determines the specificity of delayed hypersensitivity responses induced by GAT immunization.

The specificity of the antibodies produced by these GAT-immunized Hartley guinea pigs is also influenced by the presence of the GA gene (Table VI).

Although the amount of circulating anti-GAT antibody produced by GA responders and nonresponders is similar, antibodies capable of binding GA specifically were detected only in the antisera from GAT-immunized animals showing delayed hypersensitivity to GA, and thus possessing the GA gene.

Cross-Reactivity of Anti-GAT Responses for GT.—A study of the specificity of cellular and humoral anti-GAT responses for GT was made in strain 2 and

TABLE V

The Relationship between PLL Responder Status and Delayed Hypersensitivity to GA in Hartley Guinea Pigs Immunized with GAT

PLL status*	No. of animals with D.H. to GAT‡	No. of animals with D.H. to GA‡
Responder	8	7
Nonresponder	11	2

* Responder status to DNP-PLL based on delayed skin reactivity to DNP-PLL and circulating anti-DNP antibody 3 wk after simultaneous immunization with DNP-PLL and GAT as described in Materials and Methods.

‡ Delayed hypersensitivity (D.H.) to GAT and GA was determined by skin test with 50 μg of those polypeptides 3 wk after immunization.

TABLE VI

The Relationship between Delayed Hypersensitivity to GA and Anti-GA Antibodies in Sera of Hartley Guinea Pigs Immunized to GAT

GA responder status*	No. of animals	Anti-GA specificity per cent binding‡		Anti-GAT antibodies per cent binding‡	
		Mean	(SE)	*Mean*	(SE)
+	9	22.9	(6.8)	79.2	(2.8)
−	10	4.7	(1.7)	74.0	(3.2)

* Those GAT-immunized animals showing cross-reactive delayed hypersensitivity to GA are indicated (+), those not cross-reacting to GA (−).

‡ The per cent binding of GA-HSA-^{125}I and GAT-^{125}I was determined as described in the legend to Table III.

in strain 13 guinea pigs immunized with the terpolymer. The five strain 2 and 10 strain 13 guinea pigs immunized with 500 μg of GAT developed strong delayed hypersensitivity reactions against the immunizing polypeptide and produced normal anti-GAT antibody responses. None of these animals, however, showed delayed sensitivity cross-reactions to GT. No difference was observed in this respect between strain 2 guinea pigs lacking and strain 13 possessing the GT gene. The ability of the anti-GAT sera from the two groups to bind GT-^{125}I is shown in Table VII. In sharp contrast with the demonstrated dependence of anti-GA cross-reacting antibodies on the presence of the GA gene in anti-GAT responses, both strain 13 and strain 2 guinea pigs produced

antibodies capable of binding GT in response to GAT without any detectable influence of the GT gene. It was not ascertained, however, if these antibodies are identical or different from anti-GT antibodies produced by strain 13 guinea pigs immunized with GT itself.

TABLE VII

The Effect of GT Responder Status on the Specificity for GT of Anti-GAT Antibodies Produced by Strain 2 and Strain 13 Guinea Pigs

Strain	No. of animals	Anti-GAT antibodies per cent binding*		Anti-GT specificities per cent binding*	
		Mean	(SE)	*Mean*	(SE)
2	5	77.3	(3.00)	54.3	(2.54)
13	10	53.0	(5.48)	26.1	(3.72)

* The per cent binding of GAT-^{125}I and GT-^{125}I were determined as described in the legend to Table III.

DISCUSSION

After immunization with GA or GT, guinea pigs possessing the appropriate specific immune response genes develop both delayed hypersensitivity and antibody directed against the immunizing antigen, but despite the fact that both GA and GT are composed of at least 50% randomly distributed glutamic acid residues, the immune responses induced by these synthetic antigens do not cross-react. Responder guinea pigs immunized with GA do not react either in vivo or in vitro to challenge with GT, nor do their antisera bind GT. Similarly, anti-GT responses do not cross-react with GA. A high degree of cross-reactivity was observed, however, between delayed hypersensitivity responses to GAT and GA. All genetic responder guinea pigs immunized with GA displayed strong reactivity to GAT, whereas delayed hypersensitivity to GA in GAT-immunized animals depended upon the presence of the GA gene.

In contrast, the cross-reactivity between cellular immunity to GT and GAT was very poor. A small fraction of GT responder guinea pigs immunized with this antigen showed weak cross-reactive sensitivity to GAT, and none of the animals immunized with GAT displayed reactivity to GT, irrespective of their responder status to this antigen. The difference in the cross-reactivity of cellular immunity to GAT in GA- and GT-immunized animals probably reflects the considerably greater similarity of GAT with GA than with GT. The GAT preparation used in this study contains L-glutamic acid and L-alanine in approximately the same ratio as in GA, with only 10% L-tyrosine, while GT is composed of 50% L-glutamic acid and 50% L-tyrosine residues.

The data demonstrate that the activity of a specific Ir gene, the GA gene, is required for the development of GA-specific cellular immunity in animals immunized with a highly cross-reactive antigen, GAT. They illustrate, also,

how the specificity of delayed hypersensitivity against a relatively complex antigen is determined by the genetic ability of the animal to respond to defined sequences in the molecule. These results are evidence that the cellular immune response to complex antigens is under multiple gene control and that the specificity of these responses is determined by the Ir gene constitution of the individual. The absolute dependence of the expression of cellular immunity on the possession of the relevant Ir gene is consistent with the expression of histocompatibility-linked specific Ir genes in thymus-derived cells.

In addition to determining, in part, the specificity of cellular immune responses to GAT, the GA gene is also able to affect the specificity of the anti-GAT antibody population. In both inbred and random-bred guinea pigs after immunization with GAT, antisera obtained from only those animals possessing the GA gene were able to bind GA significantly. The anti-GAT sera obtained from animals lacking the GA gene, on the other hand, had no detectable cross-reactivity directed against GA. Similar genetic influences on the specificity of the antibody populations produced has been observed in the response to other synthetic polypeptides. Pinchuck and Maurer (15) demonstrated in mice a genetic influence in the cross-reactions to a synthetic antigen. They used a terpolymer of L-glutamic acid, L-lysine, and L-alanine containing 40% alanine residues (GLA_{40}) which is immunogenic in all mice tested. When the anti-GLA_{40} sera produced by three different inbred mouse strains were tested for cross-reactivity, anti-GL specificity was found in the sera from C3H/HeJ mice but not in the sera of C57BL/6J or 129/J mice. While clearly indicating a genetic influence on the specificity of the antibody produced to GLA_{40}, these results do not relate the specificity differences to specific immune response genes, since GL itself has been shown to be nonimmunogenic in mice. Mozes et al. (16) studying antibody responses to synthetic polypeptides built on multichain polyprolines, have demonstrated the effect of Ir genes in determining the specificity of antibodies produced by different mouse strains to the same antigen. Two inbred mouse strains, DBA/1 and SJL, immunized with (Phe,G)-Pro--L produce similar amounts of anti-(Phe,G)-Pro--L antibody, but the specificity of the antisera toward the cross-reactive antigen (Phe,G)-A--L is markedly different. The ability of anti-(Phe,G)-Pro--L sera from the progeny of the reciprocal backcross mating of $(DBA/1 \times SJL)F_1$ animals with SJL mice to bind (Phe,G)-A--L segregates with *H-2* genotype. The ability of these mice to bind (Phe,G)-A--L is linked to the $H\text{-}2^q$ locus (17), as is their ability to respond to immunization with this antigen. These findings are quite analogous to the results we have presented in this report. Thus, in both mice and guinea pigs, specific immune response genes may affect the specificity of the antibody population produced against certain complex polypeptide antigens.

However, this effect of histocompatibility-linked Ir genes on the specificity of humoral antibody is not universally observed with all determinants. For

instance, anti-hapten responses are generally not affected in this way (11, 18, 19). The response to immunogenic carrier molecules under unigenic control is accompanied with strong antibody production against any hapten associated with them, illustrating the specific cooperation between thymus-derived cells and antibody-secreting cells with different specificity. Furthermore in our study, strain 2 guinea pigs lacking the GT gene produced anti-GAT antibodies capable of binding GT as well as those produced by GT responder strain 13 guinea pigs. There is no obvious explanation for our inability to demonstrate a GT gene effect on the anti-GAT antibody population in the inbred strain other than the possibility that GT may be efficiently bound by antibodies elicited by unique GAT determinants.

It is also difficult to explain the process by which a specific Ir gene expressed in thymus-derived cells can affect in some systems the specificity of the antibody population produced to more complex antigens as shown in our experiments for the GA gene and also in the reports discussed above (15, 16). Several possibilities must be considered: The activity of the gene in thymus-derived cells may determine the mode of presentation of the antigen to the antibody-producing cell precursors and thereby affect the nature of the active determinants recognized. Alternatively the gene may determine, directly or indirectly, as suggested by Jerne (20) the structure and specificity of the antibody combining site. We tend to favor the first possibility based upon the predominant effects of specific Ir genes on cellular immunity and carrier function.

SUMMARY

The ability of guinea pigs to make immune responses to the random linear copolymer of L-glutamic acid and L-alanine, GA, and to L-glutamic acid and L-tyrosine, GT, is each controlled by a different immune response gene. On the other hand, the random linear terpolymer of L-glutamic acid, L-alanine, and L-tyrosine, GAT, which contains both GA and GT antigenic determinants, is immunogenic in all guinea pigs. After GAT immunization, all animals develop delayed hypersensitivity and serum antibody specific for GAT. However, only those guinea pigs possessing the GA immune response gene demonstrate cross-reactive delayed hypersensitivity when challenged with GA. In addition, the anti-GAT antisera produced by those animals having the GA gene contain cross-reacting anti-GA antibodies. The sera from guinea pigs lacking the GA gene have no anti-GA antibody activity. Thus, we have demonstrated that a specific immune response gene controlling responsiveness to a "simple" antigen can determine the specificity of both cellular and humoral immune responses to a more complex antigen.

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