GENETIC CONTROL OF THE ANTIBODY RESPONSE

II. Further Analysis of the Specificity of Determinant-Specific Control, and Genetic Analysis of the Response to (H,G)-A--L in CBA and C57 Mice*

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The genetic difference between CBA and C57 mice in their antibody response to the branched, multichain synthetic polypeptide, poly(tyr,glu)-poly-DL-ala--polylys, (T,G)-A--L, has been shown to be specific for the (tyrosine, glutamic acid) antigenic determinant (1) and to involve more than one genetic factor.[1] Although this trait is polygenic, it is possible that most of the difference is due to a single major genetic factor.[1] Whether or not this is so, the genetic difference shows a sharp specificity for the nature of the antigenic determinant on the multipolyalanyl--polylysine (A--L) backbone—CBA's responding poorly to the (tyrosyl, glutamyl) peptide determinants and well to the (histidyl, glutamyl) peptide determinants, while C57's do just the opposite.

The present study was designed to explore further the chemical nature and uniformity of this determinant specificity through the use of a series of related synthetic polypeptide antigens, and to determine the type of genetic control involved in the antibody response to multipoly(histidyl, glutamyl)-poly-DL-alanyl--polylysine, (H,G)-A--L. The results show that this determinant-specific control discriminates sharply between peptides of glutamic acid, tyrosine and glutamic acid, histidine and glutamic acid, and phenylalanine and glutamic acid on A--L; it does not discriminate between different preparations of polymers of the "poly(tyrosine, glutamic acid)" or "poly(histidine, glutamic acid)"

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[1] McDevitt, H. O., and W. G. Cochran. Genetic control of the antibody response: An estimate of the number of genetic factors responsible for the different response of CBA and C57 mice to the synthetic polypeptide (T,G)-A--L. Unpublished data.

type which have been synthesized in a similar manner. In addition, the genetic control of the antibody response to (H,G)-A--L appears to be dominant and polygenic, just as it is for (T,G)-A--L.

Materials and Method

Synthetic Polypeptide Antigens.—The properties of the antigens used in this study are summarized in Table I, and further details of preparation are given in the references cited. The

TABLE I
Properties of Synthetic Polypeptide Antigens

Synthetic polypeptide	Molecular weight	Residue molar ratio	Reference
Linear polymers			
T,G,A 253*	23,000	Tyr :Glu :Ala 1 : 5 : 4	—
Branched polymers			
G-A--L 233	43,500	Glu :Ala Lys 2.9 :25 : 1	(2)
(T,G)-A--L 509	232,000	Tyr :Glu :Ala :Lys 2.14:4.1 :19.6: 1	(1)
(T,G)-A--L 210	33,200	Tyr :Glu :Ala :Lys 1.8 :2.4 :22 : 1	(2)
T-G-A--L 415	160,000	Tyr :Glu :Ala :Lys 0.6 :2.6 :14 : 1	—
(H,G)-A--L 1201‡	15,000	His :Glu :Ala :Lys 1.2 : 4 :18 : 1	—
(P,G)-A--L 223	36,600	Phe :Glu :Ala :Lys 2.4 :4.0 :19.6: 1	(3)

* This compound was prepared and characterized by Dr. Sara Fuchs.
‡ This compound was prepared and characterized by Dr. Asher Frendsdorff.

linear polymer poly (tyr,glu,ala) 253 was prepared in a single polymerization step by Dr. Sara Fuchs. With the exception of T-G-A--L 415, all the multichain synthetic polypeptides were prepared in a similar manner by a three step synthesis. In the first step, the poly-L-lysine backbone was polymerized. In the second step, side chains of poly-DL-alanine were polymerized on the ϵ-amino groups of the poly-L-lysine. In the final step, short, mixed, random sequences of different L-amino acids were added to the amino termini of these poly-DL-alanine side chains. The final result is shown schematically in Fig. 1. T-G-A--L 415 was synthesized by adding only glutamic acid to the poly-DL-alanine side chains, after which short stretches of tyrosine were added onto the glutamic acid amino termini in a fourth step.

Immunological Methods.—The animals used, the method of immunization, and the method

of serum antibody determinations have all been described (1). In general, 10–16 wk old mice were given 10–800 μg of antigen in 0.06 ml complete Freund's adjuvant in the hind footpads, followed 5 wk later by a similar dose of the same antigen in 0.15 N NaCl, pH 7.0. The mice were bled 10 days after the second injection of antigen, and individual sera were tested for their ability to bind the antigen. Any deviations from this standard procedure are noted where appropriate.

In the usual procedure, 5 μl of mouse antiserum was tested for its ability to bind 0.8 μg of (T,G)-A–L 509-^{125}I (1) or an approximately *equimolar* amount of any of the other antigens (labeled with ^{125}I) listed in Table I, and the results are expressed as "% antigen bound." For those antigens containing tyrosine, the procedure was identical with that already described (1) using rabbit anti-mouse γ-globulin to precipitate the mouse antigen-antibody complexes. However, for (H,G)-A–L 1201 and (P,G)-A–L 223, an alternative method of radioactive labeling was employed. These two antigens were trace-labeled by acetylation with acetic

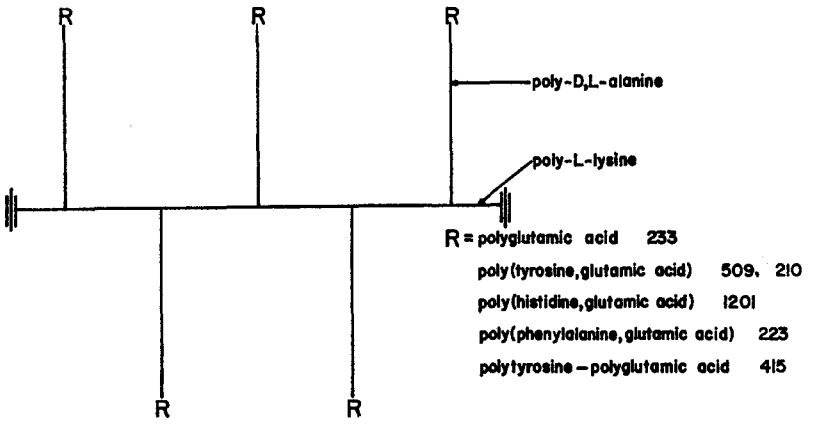

FIG. 1. A schematic diagram of the structural pattern of the multichain synthetic polypeptide antigens used in this study.

anhydride-^{3}H, (Ac)^{3}H, (498 mc/mM, Nuclear-Chicago Corp., Des Plaines, Ill.) by the method of Vratsanos (4). The specific acitivity of (H,G)-A–L 1201-(Ac)^{3}H was 6 μc/mg and this preparation was 98% bound by excess specific antibody. The specific activity of (P,G)-A–L 223-(Ac)^{3}H was 4.2 μc/mg and this preparation was 86-90% bound by excess specific antibody. The binding of these two preparations by normal mouse serum did not exceed 5%. The determination of "% antigen bound" values for these two antigens was done in the same way as that already described (1), except that only the radioactivity bound to mouse globulin and precipitated by the rabbit anti-mouse γ-globulin was counted. This was done by dissolving the precipitates in 0.3 ml of 0.1 N NaOH, after which they were transferred to a dioxane-polyether scintillator solution and counted in a Nuclear-Chicago Mark I liquid scintillation counter.

All the mice used in these studies were bred from the original CBA and C57 inbred lines (1).

RESULTS

The Specificity of Determinant-Specific Control.—The immune response of CBA and C57 mice to a series of related synthetic polypeptide antigens is summarized in Table II.

TABLE II

Antibody Response of CBA and C57 Mice to a Related Series of Linear and Multichain Synthetic Polypeptide Polymers

Synthetic polypeptide antigen	Immunizing dose*	Strain and No. of mice‡	Average % antigen bound§	Range	Average % antigen bound 509-^{125}I‖
				%	
Linear polymers					
T,G,A 253	100	CBA (4)	53	49–67	34
¶ p (tyr, glu, ala), mol wt 23,000		C57 (5)	42	39–52	40
Branched polymers					
G-A--L 233	10,100	CBA (10)	Neg.	precipitin	<5
pGlu-pDL-ala--plys, mol wt 43,500		C57 (10)	Neg.	precipitin	<5 (1/10 = 7)
(T,G)-A--L 509	10	CBA (46)	28	5–60	—
p(tyr, glu)-pDL-ala--plys mol wt 232,000		C57 (37)	78	55–95	—
(T,G)-A--L 210	100	CBA (10)	2**	1–3	7
p(tyr, glu)-pDL-ala--plys mol wt 33,200		C57 (8)	18	10–35	60
T-G-A--L 415	100	CBA (10)	8‡‡	5–15	15‡‡
ptyr-pglu-pDL-ala--plys mol wt 160,000		C57 (10)	41	19–62	56
(H,G)-A--L 1201	100	CBA (16)	68	48–89	55
p(his, glu)-pDL-ala--plys mol wt 15,000		C57 (22)	<5	1/22 = 16	4
(P,G)-A--L 223	100	CBA (5)	65	55–72	51
p(phe, glu)-pDL-ala--plys mol wt 36,600		C57 (4)	62	55–67	63

* Results are given only for that immunizing dose which elicited a maximal response when given in complete Freund's adjuvant, followed 5 wk later by an identical booster dose in aqueous solution.

‡ The numbers of mice are made up of roughly equal numbers of males and females in all cases.

§ The results are expressed as per cent antigen bound of the immunizing antigen, labeled with ^{125}I or ^{3}H, and added in amounts equimolar with that amount of (T,G)-A--L 509-^{125}I used in our standard assay (1).

‖ Per cent of (T,G)-A--L 509-^{125}I bound in our standard assay (1) by antisera against the series of antigens listed.

¶ Lower case p throughout table stands for poly.

** These titers have subsequently been shown to be falsely low due to a low molecular weight, tyrosine-containing contaminant.

‡‡ In titering the anti-(T,G)-A--L 415 antisera, one-tenth as much antigen was used per 5 μl of antiserum as is usual. The actual amounts used were 0.1 μg (T,G)-A--L 415-^{125}I and 0.08 μg (T,G)-A--L 509-^{125}I. In short, these antisera were 10-fold lower in antigen-binding than anti-(T,G)-A--L antisera.

The linear polymer T,G,A 253 was employed because it contains—in a random linear sequence—those amino acids present in the main antigenic determinant of (T,G)-A--L 509 (1). Although a small number of mice were used, the results show that CBA's and C57's overlap completely in their immune response to this linear polymer. This result is compatible with either of two main interpretations: (*a*) that linear polymers are "processed" in a different manner from branched polymers such as (T,G)-A--L, or (*b*) that a linear polymer of tyrosine, glutamic acid, and alanine contains a much greater variety of antigenic determinants than is found in the short terminal sequences of tyrosine and glutamic acid in (T,G)-A--L; and this greater variety of antigenic determinants obscures the difference between CBA and C57 mice which is brought out by (T,G)-A--L.

The results obtained using a series of related multichain, synthetic polypeptides are much more conclusive. The main points can be summarized briefly as follows:

(*a*) G-A--L (polyglu-poly-DL-ala--polylys) is not antigenic. One C57 anti–G-A--L antiserum did show a very slight cross-reaction with (T,G)-A--L 509–^{125}I. Otherwise, no response was detectable.

(*b*) Antigens of the same type, e.g. (T,G)-A--L [poly(tyr,glu)-poly-DL-ala--polylys], but of different molecular weights and synthesized separately, give the same *pattern* of response at that dose level which elicits a maximal response. This is true for (T,G)-A--L 509 and (T,G)-A--L 210, and is also true for (H,G)-A--L 1201 (see Table II) and (H,G)-A--L 220. [The results for (H,G)-A--L 220, similar to those with (H,G)-A--L 1201, are given in reference 1.]

(*c*) The pattern of response of CBA and C57 mice to this class of antigens is determined almost entirely by the amino acid composition of the side chain termini. With poly(tyr,glu), CBA's are low and C57's are about 10-fold higher (Table II and reference 1); with poly(his,glu), CBA's are high and C57's are very low; and, with poly(phe,glu), the two strains respond well and equally. [In addition to the small numbers of anti–(P,G)-A--L sera in Table II titered with both (P,G)-A--L-223–(Ac)^{3}H and (T,G)-A--L 509–^{125}I, 6 more CBA and 6 more C57 anti–(P,G)-A--L antisera titered only against (T,G)-A--L 509–^{125}I gave results identical with those seen in Table II.] Since (T,G)-A--L 509 and (P,G)-A--L 223 have similar residue molar ratios, it is likely that they have similar charge distributions and a similar distribution of hydrophobic residues. Therefore, the possibility that these different patterns of response reflect differences in response to charge or to hydrophobic areas on the antigen molecule seems unlikely. On the other hand, it seems clear that the different patterns of response are due to the different amino acids (tyrosine, histidine, or phenylalanine) in the antigenic determinant.

(*d*) For a single branched antigen in which the sequence of residues in the side chain is polytyr-polyglu-poly-DL-ala-, the pattern of response is the same

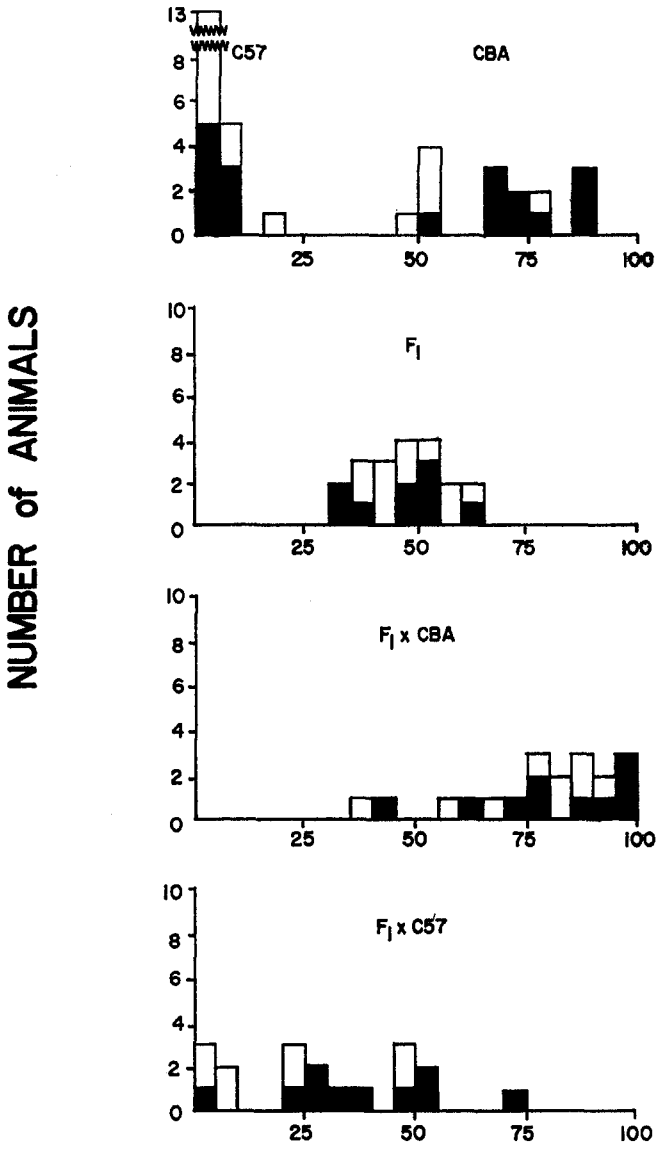

FIG. 2. Immune response of mice given 100 μg (H,G)-A–L 1201 in complete Freund's adjuvant and boosted with 100 μg of the same antigen in aqueous solution. The open squares represent males and the shaded squares represent females.

as that seen with (T,G)-A--L, although 10-fold lower. The very weak response to this antigen may be due to its low tyrosine content, or to the possibility that this sequence is a "weak" antigenic determinant.

Genetic Analysis of the Response to (H,G)-A--L in CBA and C57 Mice.—Fig. 2 shows the results of immunizing CBA, C57, (CBA × C57) F_1 and F_1 × CBA

TABLE III

Cross-Reactions between a Related Series of Multichain Synthetic Polypeptide Antigens and Their Respective Antisera

Antiserum	Immunizing antigen	% antigen-bound*		
		(T,G)-A--L 509-^{125}I	(H,G)-A--L 1201-(Ac)^{3}H	(P,G)-A--L 223-(Ac)^{3}H
C57 ♂ No. 1	(T,G)-A--L 509	78	40	44
♂ 5	"	78	36	58
♀ 2	"	84	53	73
♀ 3	"	67	48	81
CBA ♂ No. 1	(H,G)-A--L 1201	59	77	70
♂ 3	"	44	51	56
♀ 2	"	52	70	55
♀ 5	"	77	67	53
CBA ♀ No. 1	(P,G)-A--L 223	40	14	60
♀ 2	"	59	20	70
♀ 3	"	24	16	55
♀ 4	"	56	28	72
C57 ♀ 1	"	74	69	67
♀ 2	"	57	52	63
♀ 3	"	52	38	55

* These determinations were done using 5 μl of mouse antiserum and 0.8 μg (T,G)-A--L 509-^{125}I, or 0.1 μg (H,G)-A--L 1201-(Ac)^{3}H, or 0.1 μg (P,G)-A--L 223-(Ac)^{3}H. This is an excess of (H,G)-A--L on an equimolar basis, but was used due to this preparation's low specific activity.

and F_1 × C57 mice with 100 μg (H,G)-A--L 1201 in complete Freund's adjuvant, followed 5 weeks later by an identical booster dose in aqueous solution. The mice were bled 10 days after receiving the booster dose, and individual sera were tested for their ability to bind (H,G)-A--L 1201-(Ac)^{3}H and (T,G)-A--L 509-^{125}I. The results given in Fig. 2 are with (H,G)-A--L-(Ac)^{3}H; the results obtained with (T,G)-A--L 509-^{125}I are similar but slightly lower.

From these results, it would appear that the differing ability of CBA and C57 mice to make an antibody response to (H,G)-A--L is under genetic control, and that this control is dominant and in all likelihood due to more than one genetic factor, or to one major genetic factor plus an unknown number of modi-

fiers. This tentative conclusion is based on the observation that in the $F_1 \times$ C57 backcross there is not a clear 1:1 Mendelian segregation of C57 and F_1 phenotypes. Thus, the genetic control of the immune response to (H,G)-A--L is of the same type as that already described for (T,G)-A--L (1).[1]

Cross-Reaction Versus Cross-Stimulation.—Table II shows extensive cross-reactions between anti-(H,G)-A--L and anti-(P,G)-A--L and (T,G)-A--L 509–^{125}I. A more systematic study of these cross-reactions is seen in Table III, where the ability of individual antisera to bind each of these related antigens is shown (only a few typical antisera of each type are shown). With only a few exceptions, each antiserum had a higher "% antigen bound" value with its immunizing antigen than with the two cross-reacting antigens. The amount of cross-reaction usually exceeded 50% in all combinations except CBA anti-

TABLE IV

Attempted Cross-Stimulation of the Secondary Response in (CBA × C57) F_1 Mice

Immunizing antigen	Average antigen bound* prior to boosting	Secondary stimulus, aqueous	Average antigen bound* 10 days after secondary stimulus
	%		%
10 μg (T,G)-A–L 509 in complete Freund's adjuvant	16 (12 mice)	10 μg (T,G)-A--L 509	55 (5 mice)
		100 μg (H,G)-A--L 1201	16 (7 mice)
100 μg (H,G)-A--L 1201 in complete Freund's adjuvant	33 (20 mice)	10 μg (T,G)-A--L 509	31 (10 mice)
		100 μg (H,G)-A--L 1201	40 (10 mice)

* All antisera were titered against (T,G)-A--L 509–^{125}I.

(P,G)-A--L versus (H,G)-A--L 1201. With this exception, extensive cross-reactions were shown in all combinations tested. However, it should be pointed out that the antigen-binding type of assay is one which would be expected to maximize detectable cross-reactions.

Since these antigens and their respective antisera do cross-react so extensively, an attempt to demonstrate cross-stimulation of antibody formation was made. (CBA × C57) F_1 mice respond well to both (T,G)-A--L 509 and (H,G)-A--L 1201. The results of an attempt to cross-stimulate a secondary response in F_1 mice are seen in Table IV. The results with (H,G)-A--L 1201 as the primary stimulus are inconclusive since neither antigen gave a definite secondary response. However, when (T,G)-A--L 509 is the primary stimulus, a strong secondary response occurs after boosting with the same antigen, while no secondary response occurs after boosting with (H,G)-A--L. The results would be more clearcut if we could be sure that (H,G)-A--L elicits a definite secondary response in (H,G)-A--L–primed animals. With this reservation, it appears that these antigens cross-react extensively, but are unable to cross-stimulate a secondary response.

DISCUSSION

The most striking fact to emerge from these studies is the existence of separate systems of genetic control of the antibody response to different antigenic determinants carried on the same type of carrier molecule. An integral part of this finding is the very sharp degree of discrimination between different types of antigenic determinant. In short, the genetic factors controlling the ability to respond to a given antigenic determinant can discriminate clearly between tyrosine, histidine, and phenylalanine in the antigenic determinant. This is all the more remarkable in view of the extensive cross-reactions which occur between these antigens and their various antisera, although cross-stimulation of the antibody response does not seem to occur.

The genetic differences affecting the antibody response to (T,G)-A--L and (H,G)-A--L in CBA and C57 mice appear to be of the same type, i.e., dominant, polygenic, quantitative, and determinant-specific. This does not prove that the genetic factors controlling the two immune responses operate via the same mechanism(s), but it is compatible with this view. As has already been noted, these genetic factors appear to recognize different amino acid compositions, a degree of stereospecificity that is most commonly found in enzymes and in antibodies. It is not possible at this point to know whether the genetic differences in response found between CBA and C57 mice are due to genetic differences in enzymes which "process" the antigen, to genetic differences in the structure of antibodies made against these antigens, or to some alternative mechanism.

SUMMARY

CBA and C57 mice were tested for their ability to make an immune response to a related series of branched, multichain synthetic polypeptide antigens in which the antigenic determinants on the amino termini of the branched side chains were systematically varied. Neither strain responded to the polyglutamic acid determinant. Both strains responded well and equally to the poly(phenylalanine, glutamic acid) determinants. CBA mice responded poorly, and C57 mice responded well to two different antigens bearing poly(tyrosine, glutamic acid) determinants. CBA mice responded well, and C57 mice responded poorly to two different antigens bearing poly(histidine, glutamic acid) determinants.

The genetic control of the immune response to (H,G)-A--L appears to be dominant and polygenic, as it has been shown to be for (T,G)-A--L.

The related antigens used in this study show extensive cross-reactions with antisera against other members of the related series.

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