THE NATURE OF THE ANTIGENIC DETERMINANT IN A GENETIC CONTROL OF THE ANTIBODY RESPONSE*

By EDNA MOZES,‡ PH.D., HUGH O. McDEVITT,§ M.D., JEAN-CLAUDE JATON,‖ PH.D., AND MICHAEL SELA, PH.D.

(From the Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305 and the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel)

(Received for publication 29 April 1969)

The immune response of inbred mice to a related series of multichain, synthetic polypeptides is under direct genetic control (1, 2). The studies leading to this conclusion were carried out with a series of synthetic polypeptides built on multi-poly-D,L-alanyl--poly-L-lysine (denoted A--L) which is not immunogenic in mice. When short, random sequences of tyrosine and glutamic acid are added to A--L, yielding poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [denoted (T,G)-A--L] (Fig. 1 *a*), C57 mice respond to immunization with about ten times more antigen-binding capacity than CBA mice. When the tyrosine in (T,G)-A--L is replaced with histidine, the resulting poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys [(H,G)-A--L] elicits a poor response in C57 mice, while CBA mice respond well. Both strains respond well to a third branched polymer, poly-L-(Phe,Glu)-poly-D,L-Ala--poly-L-Lys [(Phe,G)-A--L]. The F_1 hybrid (C57 × CBA) responds well to all three antigens. Backcross progeny segregate in response to (T,G)-A--L and (H,G)-A--L as a 1:1 mixture of the F_1 and the respective homozygous parent animals.

These results indicate that antibody responses of mice to these polypeptides are quantitative traits which are under a dominant, determinant-specific type of genetic control. The gene(s) responsible for this control has been named Ir-1 (Immune Response-1).

There is no correlation between Ir-1 and the immunoglobulin class of the resultant antibody (3). In a segregating backcross population, no linkage was found between Ir-1 and the γG_{2a} allotype of the responding animal, indicating that Ir-1 is not associated with the known structural genes coding for the F_c fragments of mouse immunoglobulin heavy chains (3). Recent results have shown that the ability to respond well to these polypeptides can be transferred with high-responder spleen cells and is linked to the major histocompatibility (H-2) locus in the IXth mouse linkage group (4, 5).

* This research was supported by United States Public Health Service Grants AI-07757 and AI-04715.

‡ Recipient of Dernham Junior Fellowship, J-119, from the American Cancer Society, California Division.

§ Senior Investigator of the Arthritis Foundation.

‖ Present address: The Cardiac Unit, Massachusetts General Hospital, Boston, Massachusetts 02114.

From these results obtained with the A--L series of antigens, it appeared that the antigenic determinants required to activate the Ir-1 alleles were restricted to the amino acids (tyrosine, histidine or phenylalanine plus glutamic acid) attached to the alanyl side chains of the polypeptide, while A--L itself seemed to play the role of a nonspecific carrier. This consideration is pertinent because the work of Levine,

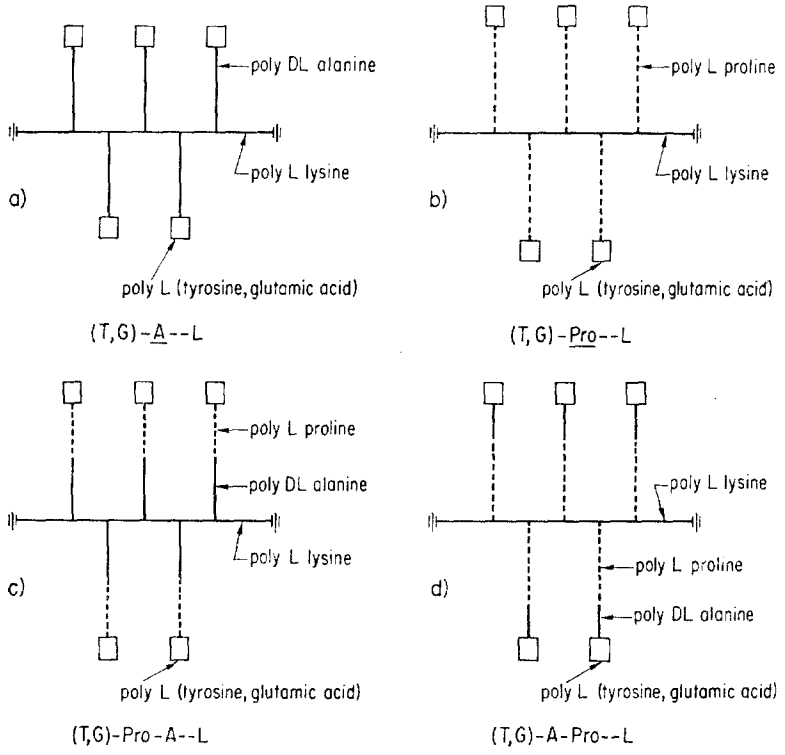

FIG. 1. A schematic diagram of a portion of the structural patterns of: (a) (T,G)-A--L; (b) (T,G)-Pro--L; (c) (T,G)-Pro-A--L; (d) (T,G)-A-Pro--L.

Ojeda, and Benacerraf (6, 7, 8) has shown that the response of guinea pigs to different haptens conjugated to poly-L-lysine (PLL) is under genetic control, but the specificity of the genetic difference is for the "carrier" (PLL) and not for the hapten determinants against which the antibodies are produced. However, the recent demonstration that PLL responder guinea pigs (but not PLL nonresponders) do develop delayed hypersensitivity to PLL alone, raises the possibility that the response to or recognition of PLL itself may be an important part of the mechanism of action of the PLL gene (9).

In the present study, we have tested the immune response of several inbred mouse strains to synthetic polypeptides derived from multichain poly-

prolines: poly-L-(Tyr,Glu)-poly-L-Pro--poly-L-Lys, denoted [(T,G)-Pro--L] (Fig. 1 *b*) and poly-L-(Phe,Glu)-poly-L-Pro--poly-L-Lys, [(Phe,G)-Pro--L]. The results show that the prolyl and alanyl side chains of the multichain polymers also play an important role in their immunogenicity. Substitution of proline for alanine in the side chains of the antigenic molecule, without changing the amino-terminal tyrosine and glutamic acid residues, leads to a different pattern of response, indicating that (T,G)-Pro as an antigenic determinant is under a genetic control distinct from that operating for (T,G)-A).

Materials and Methods

CBA and C57 mice were bred from strains originally obtained from the National Institute for Medical Research, Mill Hill, London. C3H.SW and C3H/DiSn mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. All the above strains are maintained at Stanford. Mice of the following strains were purchased from the Jackson Laboratories: A, DBA/2, C3H/He, DBA/1, SJL, and A.SW.

The following polypeptides were used in this study:

(*a*) Poly-L-(Tyr,Glu)-poly-L-Pro--poly-L-Lys 701, denoted (T,G)-Pro--L (Fig. 1 *b*), and poly-L-(Phe,Glu)-poly-L-Pro--poly-L-Lys 702, denoted (Phe,G)-Pro--L, are two branched synthetic polymers built on a multi-poly-L-prolyl--poly-L-lysine to which short, random peptides of tyrosine and glutamic acid or phenylalanine and glutamic acid are attached. Their synthesis and characterization have been described previously (10).

(*b*) Two samples of poly-L-(Tyr,Glu)-poly-L-Pro-poly-D,L-Ala--poly-L-Lys 717 and 718 [denoted (T,G)-Pro-A--L 717 and 718] (Fig. 1 *c*), were synthesized in two steps as follows: (i) 100 mg of poly-D,L-Ala--poly-L-Lys (A--L) synthesized as described previously (11) were dissolved in 1.5 ml water. After addition of 90 ml dimethylsulfoxide, (DMSO, anhydrous), 100 mg of N-carboxy-L-proline anhydride dissolved in 10 ml DMSO were added to the reaction mixture. After stirring for 20 hr at 20°C, the clear reaction mixture was dialyzed for 48 hr against distilled water. Chromatography of the polymer on Sephadex G-150 (Uppsala, Sweden) in 0.05 M ammonium bicarbonate (NH_4HCO_3) yielded a fraction containing the purified poly-L-Pro-poly-D,L-Ala--poly-L-Lys (Pro-A--L) which was dialyzed against water and lyophilized. (ii) Synthesis of (T,G)-Pro-A--L 717: 180 mg of Pro-A--L (residue molar ratio of 7.7:13.5:1) were reacted with 550 mg N-carboxy-L-tyrosine-anhydride (Tyr-NCA) and 900 mg γ-Benzyl-L-Glu-NCA. Polymerization was performed in aqueous dioxane (2:1 mixture of 0.05 M phosphate buffer, pH 7.0, and dioxane), essentially according to the technique described by Sela and Fuchs (12). After gel filtration on Sephadex G-150 equilibriated with 0.05 M ammonium bicarbonate, (T,G)-Pro-A--L 717 was isolated with a residue molar ratio of Tyr:Glu:Pro:Ala:Lys of 2.5:9:7.8:13:1, respectively. (iii) The synthesis of (T,G)-Pro-A--L 718 was similar to that of (T,G)-Pro-A--L 717, except that a new batch of Pro-A--L was used with amino acid composition of Pro:Ala:Lys as 13:13:1, and the amino acid analysis of the polypeptide gave the following results: Tyr:Glu:Pro:Ala:Lys as 2.6:7.3:13:13:1, respectively.

(*c*) The synthesis of two samples of poly-L-(Tyr,Glu)-poly-D,L-Ala-poly-L-Pro--poly-L-Lys 719 and 721 [denoted (T,G)-A-Pro--L 719 and 721] (Fig. 1 *d*) was as follows: (i) To 300 mg of poly-L-Pro--poly-L-Lys (Pro--L) synthesized as described before (10) and dissolved in 40 ml 0.05 M phosphate buffer, pH 7.0, 3.5 g of Ala-NCA dissolved in 15 ml dioxane were added. After stirring at 4°C for 24 hr, anhydrous formic acid was added to clear the turbid reaction mixture, followed by dialysis for 3 days against distilled water. Gel filtration on Sephadex-150 was performed, yielding poly-D,L-Ala-poly-L-Pro--poly-L-Lys (A-Pro--L) with a residue molar ratio of Ala:Pro:Lys as 9.7:25.2:1. (ii) The synthesis of (T,G)-A-Pro--L 719 and 721 was

performed as described previously (12) using in the two cases different ratios of A-Pro--L and the appropriate N-carboxy-α-amino acid anhydrides. The amino acid analysis of the two polymers gave the following results: (T,G)-A-Pro--L 719 contains Tyr:Glu:Ala:Pro:Lys in the ratio of 3.9:2.3:9.5:25.3:1; in (T,G)-A-Pro--L 721 the amino acid ratio of Tyr:Glu:Ala:Pro:Lys is as 2.6:1.4:10.5:25:1.

(*d*) Poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys 509 [denoted (T,G)-A--L] (1) and poly-L-(Phe,Glu)-poly-D,L-Ala--poly-L--Lys 223 [denoted (Phe,G)-A--L] (13) were described previously.

Different inbred mouse strains (10 mice per strain, one-half males and females, approximately 2 months old) were immunized with a primary stimulus of 10 μg of the antigens in complete Freund's adjuvant (1 part antigen:1 part lanolin:2 parts liquid paraffin with 4 mg Mycobacterium tuberculosis H37Ra per ml (the latter kindly supplied by Dr. Sidney Raffel) in the hind footpads. 3 wk later, the mice received another dose of 10 μg of the polypeptides in aqueous solution. The animals were bled 10 days after the secondary stimulus. Groups of mice were immunized with different amounts of (T,G)-Pro--L (2 μg, 10 μg, 100 μg per mouse). No differences in response were noted with these doses and, therefore, for the other immunogens 10 μg were used for each mouse.

Antibody response was measured by an antigen-binding capacity assay, using iodinated (with ^{125}I [14]) or tritium-labeled (with acetic anhydride-^{3}H [15]) polypeptides. The antibody assay was a modification of that previously described (1) based on a method described by Herzenberg et al. (16). 50 μl of (T,G)-Pro--L, 0.05 μg per ml (or an approximately equimolar amount of any of the other iodinated or tritiated antigens) in 1% bovine serum albumin (crystallized, Sigma Chemical Company, St. Louis, Mo.) in phosphate buffered saline (0.15 M, pH 7.0) was mixed with 25 μl of the appropriate dilution of mouse antiserum (1/10 through 1/25,000) and incubated for 1 hr at 37°C. After incubation 25 μl of the appropriate dilution of polyvalent rabbit anti-mouse-γ-globulin antiserum (½, ¼, ⅛) was added, followed by a second 2 hr period of incubation at 37°C. After centrifugation at 10,000 g at 4°C in a Sorvall/GSA rotor, one-half (50 μl) of the supernatant was removed and counted, either in a well-type gamma scintillation counter or, where appropriate, by liquid scintillation counting in a polyether scintillator solution. The results were expressed as the per cent antigen bound in the sedimented complexes of antigen, antibody, and rabbit antibodies to the antibody globulin.

The properties of the labeled antigen preparations used for antibody assays are given in Table I. All the labeled polypeptides built on multipoly-prolines tend to aggregate in dilute solutions and to stick to glass. Therefore, siliconized glass tubes and vials were used for storage and diluting the labeled antigens, and new dilutions of the labeled antigens were prepared daily before use to minimize the effects of aggregation. Siliconization of glassware and the use of fresh dilutions of labeled antigen in each titration did not prevent aggregation of (Phe,G)-Pro--L (Ac)-^{3}H. Efforts to prevent aggregation of the labeled polypeptide with 1–8 M urea, 6 M guanidine hydrochloride, and 0.2–1% sodium dodecyl sulfate were unsuccessful. Therefore, antibodies to (Phe,G)-Pro--L were assayed with two cross-reacting polypeptides, (T,G)-Pro--L and (Phe, G)-A--L, in addition to titering the antibody with the homologous antigen.

RESULTS

Antibody Response to (T,G)-Pro--L.—Table II presents the antibody response of nine mouse strains to (T,G)-Pro--L, titered with the iodinated homologous antigen. The results are given as average per cent of antigen bound at three antiserum dilutions.

The results are significantly different from those obtained with (T,G)-

A--L (Table II). There is no difference in response between C3H and CBA, low responders to (T,G)-A--L, and C3H.SW and C57 mice, the high responders to (T,G)-A--L. The four strains are medium responders to (T,G)-Pro--L. A/J, which are poor responders to (T,G)-A--L, responded well to (T,G)-Pro--L. The DBA/1 strain is a low responder to both (T,G)-A--L and (T,G)-Pro--L. It is striking that SJL mice, which respond very poorly to all the antigens derived from multichain polyalanine, are the best responders to (T,G)-Pro--L. The A.SW strain, also a poor responder to (T,G)-A--L, re-

TABLE I

Properties of the Radio-Labeled Polypeptides

Antigen	Specific activity	Per cent precipitability by TCA*	Per cent bound by excess specific antibody
	μc/μg		
(T,G)-Pro--L-701-^{125}I	3.5–7.2	90–96	75–83
(T,G)-Pro-A--L 717-^{125}I	6.3	97	82
(T,G)-A-Pro--L 721-^{125}I	5	95	35
(Phe,G)-A--L-223-(Ac)-^{3}H	0.17	93	80
(Phe,G)-Pro--L-702-(Ac)-^{3}H	0.114	90	64

* Trichloroacetic acid.

TABLE II

Antibody Response of Inbred Mouse Strains to (T,G)-Pro--L, (T,G)-A--L and (Phe, G)-A--L

Strain	(T,G)-Pro--L 701						(T,G)-A--L 509*		(Phe,G)-A--L 223*	
	Antiserum dilution						Antiserum dilution		Antiserum dilution	
	1/50		1/100		1/500		1/500		1/500	
	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range
A/J	45	23–67	39	19–67	24	14–47	10	5–15	75	73–76
C3H.SW	35	18–56	33	13–47	20	11–29	79	52–91	73	70–74
C57	28	15–36	18	8–25	7	0–15	69	53–82	69	67–71
DBA/2J	16	10–25	14	3–23	5	0–12	34	11–53	65	53–74
C3H.HeJ	30	17–46	29	18–50	17	6–28	17	9–26	74	72–75
CBA	35	23–50	26	15–50	8	0–20	12	0–27	71	69–72
DBA/1J	10	0–29	11	9–19	3	0–7	6	4–12	74	69–76
SJL/J	**65**	**48–81**	**44**	**35–67**	**33**	**12–60**	5	3–7	13	0–39
A.SW	40	34–45	38	28–45	13	7–16	0	—	15	6–22

Antigen used is equimolar to amount of (T,G)-A--L 509 used in standard assay (1), where sera are usually titered at 1/500 dilution.

* From McDevitt and Chinitz (5).

sponds well to (T,G)-Pro--L. Assay of antisera to (T,G)-Pro--L with iodinated (T,G)-A--L showed that there is almost no cross-reaction between antisera to (T,G)-Pro--L and (T,G)-A--L. Anti-(T,G)-Pro--L sera taken from SJL mice bind (T,G)-A--L to the extent of 3% and the average of (T,G)-A--L bound to anti-(T,G)-Pro--L taken from C57 and CBA mice was 2%.

It should be noted that (T,G)-Pro--L appears to be a weak immunogen and that the antigen-binding capacity (ABC) of the high responder to (T,G)-Pro--L is lower than the ABC of high responders to (T,G)-A--L (Table II).

Antibody Response to (T,G)-A-Pro--L and to (T,G)-Pro-A--L.—Since the above results showed the importance of side chain composition in the amount

TABLE III

Antibody Response of Inbred Mouse Strains to (T,G)-A-Pro--L 719 and 721 (Antiserum Dilution 1/500)*

Strain	Immunizing antigen			
	(T,G)-A-Pro--L 719		(T,G)-A-Pro--L 721	
	Average per cent antigen bound‡	Range	Average per cent antigen bound‡	Range
A/J	4	1–7	2	0–7
C3H.SW	**13**	**8–25**	**16**	**9–27**
C3H/DiSn	**4**	**0–8**	**4**	**0–6**
DBA/1J	1	0–3	3	0–10
SJL/J	7	5–12	5	0–6

* Titered with (T,G)-A-Pro--L 721-^{125}I.

‡ (T,G)-A-Pro--L 721-^{125}I is only 35% precipitable by excess specific antibody (Table I). This implies that the majority of this antigen, although soluble, is in a state in which it is unable to react with antibody.

and specificity of the antibody produced, we tried to determine which parts of the polypeptides participate in the antigenic determinant by testing the immune response of mice to ***(T,G)-A***-Pro--L 719 and 721 and to ***(T,G)-Pro***-A--L 717 and 718. If, in (T,G)-A--L and (T,G)-Pro--L, the antigenic determinants consist of (T,G) and a short peptide of D,L-alanine or L-proline, respectively, then the mice should respond to (T,G)-A-Pro--L as they respond to (T,G)-A--L, and their response to (T,G)-Pro-A--L should be similar to their response to (T,G)-Pro--L.

Table III illustrates the average per cent antigen bound values for several mouse strains immunized with the two samples of (T,G)-A-Pro--L, 719 and 721, and titered with iodinated (T,G)-A-Pro--L 721. The two polypeptides are poor immunogens and the antibody response shown in the table is very low. The poor immunogenicity of (T,G)-A-Pro--L may be due to the small amounts of glutamic acid in comparison with the amount of tyrosine in these two an-

tigens. C3H.SW, the high responders to (T,G)-A--L, are the highest responders to (T,G)-A-Pro--L in both cases, while the response of all the other strains is very poor, as it is to (T,G)-A--L. The responses to (T,G)-Pro-A--L 717 and 718 (assayed with (T,G)-Pro-A--L 717-^{125}I) are given in Table IV. Both polypeptides are good immunogens and the per cent of antigen bound to the various antisera tested is high even at 1/2500 or 1/5000 dilution of antiserum. (The molar amount of antigen is always the same, as described in Materials and Methods.) The response to (T,G)-Pro-A--L 718 at 1/2500 dilution is qualitatively similar to the response of the same mouse strains to (T,G)-Pro--L. The SJL mice are the best responders to this immunogen, while C3H.SW and

TABLE IV

*Antibody Response of Inbred Mouse Strains to (T,G)-Pro-A--L 717 and 718**

Strain	Immunizing antigen: (T,G)-Pro-A--L 717						(T,G)-Pro-A--L 718			
	Antiserum dilution: 1/500		1/2500		1/5000		1/500		1/2500	
	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range
A/J	46	28–58	17	7–28	11	6–16	—	—	—	—
C3H.SW	68	66–71	59	44–67	48	17–70	55	33–66	26	14–47
C3H/DiSn	62	35–68	44	5–63	28	0–58	44	21–64	19	5–35
DBA/1J	43	25–58	8	0–16	7	0–12	16	5–32	3	0–11
SJL/J	67	63–67	57	39–67	50	28–62	67	54–71	48	29–58

* Titered with (T,G)-Pro-A--L 717-^{125}I.

C3H/DiSn are weaker responders and DBA/1 antisera almost did not bind the antigen at this dilution. We did not obtain the same results by injecting (T,G)-Pro-A--L 717 since, even at 1/5000 dilution of antiserum, there is no significant difference in the capacity to bind antigen between C3H.SW and SJL mice. C3H/DiSn mice are intermediate responders and DBA/1 are low responders. Amino acid analysis of the two polymers revealed differences in their residue molar ratios which may explain the variation in response to them. as will be discussed below.

Antibody Response to (Phe,G)-Pro--L.—The immune responses of inbred mice to this antigen, assayed with the homologous (^{3}H) acetylated antigen, are given in Table V. The values of average per cent antigen bound are low and uniform, but similar to those obtained by immunizing mice with (T,G)-Pro--L (Table II). The major difference between response to (T,G)-Pro--L and to (Phe,G)-Pro--L is the response of the DBA/1 strain, which is a poor responder to (T,G)-Pro--L and a good responder to (Phe,G)-Pro--L. (Phe,G)-

Pro--L tends to aggregate in dilute solutions (Materials and Methods) and, since the antibody assay requires centrifugation, it was immediately apparent that 60–70% of the labeled antigen precipitates spontaneously, while only 64% of the antigen remaining in the supernatant is precipitable by antibody.

TABLE V

Antibody Response of Inbred Mouse Strains to (Phe,G)-Pro--L 702 (Antiserum Dilution 1/50)

Strain	Average per cent antigen bound	Range
A/J	38	25–43
C3H.SW	31	25–37
C57	22	15–28
DBA/2J	13	4–32
C3H.HeJ	32	25–40
CBA	31	25–42
DBA/1J	42	35–48
SJL/J	42	34–48

TABLE VI

Antibody Response to (Phe,G)-Pro--L Assayed With (T,G)-Pro--L and (Phe,G)-A--L

Strain	Titering antigen: (T,G)-Pro--L 701						(Phe,G)-A--L 223							
	Antiserum dilution: 1/50		1/100		1/500		1/100		1/500		1/5000		1/25000	
	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range	Average per cent antigen bound	Range
A/J	41	20–50	35	12–51	19	9–32	14	6–21	5	0–10				
C3H.SW	34	24–42	35	29–43	22	16–28	5	0–12	3	0–8				
C57	26	15–37	25	16–33	9	3–21	4	0–9	4	0–10				
DBA/2J	17	0–23	14	8–26	7	0–15	5	0–9	4	0–9				
C3H.HeJ	30	19–42	30	7–41	17	11–22	10	5–17	2	0–7				
CBA	39	26–49	37	26–46	17	9–27	4	1–11	6	0–12				
DBA/1J	35	23–50	41	28–51	22	14–30	69	59–74	68	63–72	72	65–76	64	53–70
SJL/J	53	43–58	52	33–60	43	26–54	16	2–26	12	3–25	19	7–28	5	0–14

Therefore, we assayed antibody to (Phe,G)-Pro--L with two cross-reacting polypeptides: (T,G)-Pro--L and (Phe,G)-A--L. The results are given in Table VI.

The results of titering anti-(Phe,G)-Pro--L antisera with (T,G)-Pro--L are similar to the results obtained with the immunizing antigen, but the titers are slightly higher. SJL mice again show the highest titer, and the results parallel those obtained after immunization with (T,G)-Pro--L, *except* that DBA/1 mice show a high response to (Phe,G)-Pro--L; however, DBA/1

anti-(Phe,G)-Pro--L binds (T,G)-Pro--L less than SJL anti-(Phe,G)-Pro--L does. The results of titering the same anti-(Phe,G)-Pro--L antisera with (Phe,G)-A--L were striking. SJL anti-(Phe,G)-Pro--L (which binds (T,G)-Pro--L quite well) shows very poor binding of (Phe,G)-A--L. In contrast, DBA/1 anti-(Phe,G)-Pro--L binds (Phe,G)-A--L very well. The titers of DBA/1 mice with (Phe,G)-A--L are remarkably high even at a 1/25,000 dilution of serum. Thus, DBA/1 mice make antibodies which react primarily with the determinant, (Phe,G), while SJL antisera appear to be specific primarily for the polyproline moiety of the polypeptide.

DISCUSSION

The results obtained by immunizing inbred mice with (T,G)-Pro--L are very different from those given by the same mouse strains immunized with (T,G)-A--L (1, 3, 5). Since the major difference between these two polypeptides is the polyproline in (T,G)-Pro--L which replaces the poly-D,L-alanine in (T,G)-A--L, it seems probable that the polyprolyl side chains are also part of the specific determinants. Indeed, antisera to (T,G)-Pro--L cross-react very poorly with (T,G)-A--L, indicating that (T,G)-A--L and (T,G)-Pro--L possess different antigenic determinants. Since inbred mouse strains respond to (T,G)-Pro--L differently than to (T,G)-A--L, it implies that (T,G)-Pro is an antigenic determinant which is under a genetic control distinct from that operating for (T,G)-A.

The response of inbred mice to the two preparations of (T,G)-A-Pro--L (719 and 721) is similar to the response of the same strains to (T,G)-A--L, except for the fact that (T,G)-A--L is a good immunogen and (T,G)-A-Pro--L 719 and 721 appear to elicit a much lower response.

The response to (T,G)-Pro-A--L 718 resembles the response of the same mouse strains to (T,G)-Pro--L (Tables II and IV), while the response to (T,G)-Pro-A--L 717 is different, as C3H.SW and SJL mice are high responders. Amino acid analysis shows less proline and more glutamic acid in (T,G)-Pro-A--L 717 than in (T,G)-Pro-A--L 718. It may be that in some areas on this polypeptide, peptides of alanine are attached almost directly (or with only a few intervening prolyl residues) to glutamic acid or tyrosine, and may thus resemble (T,G)-A. This may be the cause for the high response of C3H.SW mice to (T,G)-Pro-A--L 717. The main conclusion that can be drawn from the response to (T,G)-Pro-A--L and (T,G)-A-Pro--L is that residues in and adjacent to the amino terminal sequences in the polypeptide side chain play a major role in the antigenic determinant, although the contribution of all the *other* amino acids in the polypeptide, their sequence, and the tertiary structure of the immunogen may also be important. It is not possible from the present studies to determine precisely the role of the latter factors.

The reaction of anti-(Phe,G)-Pro--L antisera with (T,G)-Pro--L and (Phe,G)

A--L indicates that different mouse strains appear to synthesize antibody specific for different parts of the same polypeptide antigen. DBA/1 mice make antibody specific mainly for the (Phe,G) part of the immunogen. This strain responds well only to (Phe,G)-A--L in the A--L series of polypeptides (Table II, ref. 5) and to (Phe,G)-Pro--L in the series of immunogens derived from multichain polyprolines, while SJL antisera seem to react primarily to the Pro--L part of the (Phe,G)-Pro--L immunogen and do not respond to or react with any of the polymers in the A--L series (Table II, ref. 5).

Different inbred strains of mice may produce similar amounts of antibodies against the same protein, but this may be due to the complexity of the multideterminant antigen, i.e., the specificity of the antibodies formed may differ. In agreement with this hypothesis, in this study, two different mouse strains (DBA/1 and SJL), immunized with the same antigen [(Phe,G)-Pro--L], responded equally well, but with the production of antisera of markedly different specificity, implying either that the specificity of the antibodies produced, or the recognition of antigenic determinants, is under direct genetic control. The genetic segregation of the ability of anti-(Phe,G)-Pro--L sera from (DBA/1 × SJL) F_1 × DBA/1 and (DBA/1 × SJL) F_1 × SJL mice to bind (T,G)-Pro--L and (Phe,G)-A--L will be reported in a subsequent publication.[1] Evidence leading to a similar conclusion has been reported by Arquilla and Finn (17) and Maurer and Pinchuck (18).

As already noted above [for (T,G)-Pro--L], it appears that the genetic control of the immune response to polypeptides derived from A--L is qualitatively different from that for polypeptides built on multichain polyprolines.

SUMMARY

The response of inbred mouse strains to two polypeptides derived from multichain polyprolines, (T,G)-Pro--L and (Phe,G)-Pro--L, is different from the response of the same mouse strains to a similar series of polymers built on multi-poly-D,L-alanyl--poly-L-lysine, although the same short sequences of amino acids are attached to the side chains of the polypeptides in the two series. These results indicate that a portion of the side chain (e.g. polyalanine or polyproline) participates in the antigenic determinant. This was confirmed by studying the response of different mouse strains to two kinds of polypeptides: (T,G)-Pro-A--L 717 and 718 and (T,G)-A-Pro--L 719 and 721.

Antibody assay of antisera to (Phe,G)-Pro--L with the cross-reacting antigens (T,G)-Pro--L and (Phe,G)-A--L indicates that different inbred mouse strains make antibodies specific for different parts of the same polypeptide. Thus, antibody from DBA/1 mice reacts almost exclusively with the (Phe,G) sequence, while SJL antisera bind only (T,G)-Pro--L and fail to bind (Phe,G)-A--L.

[1] Data to be published.

The immune responses to the same amino acids on two different polypeptides (i.e. A--L and Pro--L) appear to be under separate genetic control.

The authors would like to express their thanks to Mrs. Geraldine Nelson and to Miss Linda Siri for their excellent technical assistance throughout this work.

BIBLIOGRAPHY

1. McDevitt, H. O., and M. Sela. 1965. Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. *J. Exp. Med.* **122**:517.
2. McDevitt, H. O., and M. Sela. 1967. 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. *J. Exp. Med.* **126**:969.
3. McDevitt, H. O. 1968. Genetic control of the antibody response. III. Qualitative and quantitative characterization of the antibody response to (T,G)-A--L in CBA and C57 mice. *J. Immunol.* **100**:485.
4. McDevitt, H. O., and M. L. Tyan. 1968. Genetic control of the antibody response in inbred mice. Transfer of response by spleen cells and linkage to the major histocompatibility (H-2) locus. *J. Exp. Med.* **128**:1.
5. McDevitt, H. O., and A. Chinitz. 1969. Genetic control of the antibody response: Relationship between immune response and histocompatibility (H-2) type. *Science.* **163**:1207.
6. Levine, B. B., A. Ojeda, and B. Benacerraf. 1963. Studies on artificial antigens. III. The genetic control of the immune response to hapten-poly-L-lysine conjugates in guinea pigs. *J. Exp. Med.* **118**:953.
7. Levine, B. B., A. Ojeda, and B. Benacerraf. 1963. Basis for the antigenicity of hapten-poly-L-lysine conjugates in random bred guinea pigs. *Nature.* **200**:544.
8. Levine, B. B., and B. Benacerraf. 1965. Genetic control in guinea pigs of immune response to conjugates of haptens and poly-L-lysine. *Science.* **147**:517.
9. Green, I., W. E. Paul, and B. Benacerraf. 1967. A study of the passive transfer of delayed hypersensitivity to DNP-poly-L-lysine and DNP-GL in responder and non-responder guinea pigs. *J. Exp. Med.* **126**:959.
10. Jaton, J-C., and M. Sela. 1968. Role of optical configuration in the immunogenicity and specificity of synthetic antigens derived from multichain polyproline. *J. Biol. Chem.* **243**:5616.
11. Sela, M., E. Katchalski, and M. Gehatia. 1956. Multichain polyamino acids. *J. Amer. Chem. Soc.* **78**:746.
12. Sela, M., and S. Fuchs. 1967. Preparation of synthetic polypeptides as antigens. *In* Methods in Immunology and Immunochemistry. C. A. Williams and M. W. Chase, editors. Academic Press Inc., New York. Vol. 1, p. 167.
13. Fuchs, S., and M. Sela. 1964. Antigenicity of some new synthetic polypeptides and polypeptidyl gelatins. *Biochem. J.* **93**:566.
14. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**:114.

15. Vratsanos, S. M. 1960. On the mechanism of enzyme action. LXXI. Acylations of trypsin in organic solvents. *Arch. Biochem. Biophys.* **90**:132.
16. Herzenberg, L. A., N. L. Warner, and L. A. Herzenberg. 1965. Immunoglobulin isoantigens (allotypes) in the mouse. I. Genetics and cross-reactions of the 7S γ_{2a}-isoantigens controlled by alleles at the Ig-1 locus. *J. Exp. Med.* **121**:415.
17. Arquilla, E. R., and J. Finn. 1965. Genetic control of combining sites of insulin antibodies produced by guinea pigs. *J. Exp. Med.* **122**:771.
18. Maurer, P. H., and P. Pinchuck. 1967. Immunogenicity of synthetic polymers of amino acids; role of carrier and genetic background. *In* Immunity, Cancer, and Chemotherapy. E. Mihich, editor. Academic Press Inc., New York, p. 319.