Brief Definitive Report

THE IMMUNE RESPONSE OF ALLOPHENIC MICE TO THE SYNTHETIC POLYMER L-GLUTAMIC ACID, L-LYSINE, L-PHENYLALANINE*

II. Lack of Gene Complementation in Two Nonresponder Strains

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The genetic control of the immune response of inbred strains of mice to certain antigens has been demonstrated to be governed by a set of *Ir* genes linked to the major histocompatibility complex (*H-2*) of mice (1, 2). Until recently, the control was thought to be governed by single, dominant genes, located within the *I* region of the *H-2* complex. Merryman et al. (3) originally demonstrated that the immune response to the synthetic terpolymer L-glutamic acid, L-lysine, L-phenylalanine (GLϕ) is under dominant, *H-2*-linked *Ir* gene control. It has now been demonstrated that the response to GLϕ is at least under dual *Ir* gene control (4–7). This was shown both by crossing two nonresponder parental strains to produce responder offspring in the F_1 generation, and by the analysis of appropriate recombinant strains of mice. The two complementing genes have been mapped in the *IA* and *IC* regions of the *H-2* complex, and have been termed β and α, respectively (5, 6). Thus, any strain of mouse may contain neither, one, or both genes Only mice containing both genes are capable of responding to GLϕ It has been shown using F_1 hybrid and recombinant strains of mice, that the α- and β-genes can complement each other in either the *cis* (on the same chromosome) or in the *trans* (on different chromosomes) position (8).

In this paper we report the results of studies aimed at answering the question of whether or not the α- and β-genes can complement each other when they are present in different lymphoid cells. To this end we have constructed allophenic mice composed of two nonresponder strains (A and C57BL/6), which show gene complementation in the F_1 generation. Allophenic mice are chimeras containing two cell types coexisting in a "normal" environment. The mice were tested for the specific cellular composition of the two parental cell types and were found to possess a complete range in the relative proportion of the two cell types. This report demonstrates that regardless of the mixture of cell types present in the allophenic mice, none of them were responders to GLϕ. Thus, no complementation of the α- and β-genes is seen when the two genes are present in different cells.

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Materials and Methods

Preparation of Allophenic Mice. The allophenic mice were produced by the fusion of an eight-cell C57BL/6 embryo with an eight-cell A embryo by the method described previously (9). The resulting mice are designated C57BL/6 ↔ A. The inbred strains of mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. The F_1 hybrid mice were produced in our laboratory

Characterization of Allophenic Mice. The composition of the peripheral leukocytes of the allophenic mice was determined as described previously (10) Briefly, the Ficoll-Hypaque-isolated cells were analyzed by a trypan blue dye exclusion cytotoxicity test. The percent C57BL/6 cells was determined by treating the unknown cell mixture from each mouse with the appropriate antiserum plus complement

Antigen and Immunization Schedule. Poly(Glu^{56}Lys^{35}Phe^{9}) (GLϕ^9), mol wt 35,000, was polymerized starting with the alpha *N* carboxyanhydrides of the L amino acids (11) An aqueous solution of the polymer was dialyzed free of HBr for a week against two daily changes of distilled water, lyophilized, and stored at −20°C Before use, the polymer was dissolved in saline and the concentration determined by micro-Kjeldahl analysis (12).

The mice were immunized with 100 μg of the GLϕ polymer which was emulsified in complete Freund's adjuvant and injected into the hind foot pads. 3 wk later, an aqueous booster injection of the same polymer concentration was given intraperitoneally. Bleedings from the retro-orbital plexus were obtained on day 10 (1° response) and on day 31 (2° response). All mice were 6- to 12-mo old at the time of immunization.

Antigen-Binding Assay. The antibody activity against the immunizing polymer was measured as described previously (10). Iodinated glutamic acid^{60}alanine^{30}tyrosine^{15} (GLT^{15}), which has been found to cross-react very highly with mouse anti-GLϕ sera (5, 7), was used in the antigen-binding assay The results are reported as the percentage of antigen bound by 25 μl of a 1.2 dilution of the mouse antisera Controls included normal mouse serum and both rabbit and mouse antisera containing high titer antibody Binding values of not more than 10% were observed with the nonimmune normal mouse sera. Therefore, for discussion purposes, binding values of less than 15% were considered to reflect "nonresponsiveness" (7)

Results

The results of the immunization of C57BL/6, A, and (C57BL/6 × A)F_1 mice with GLϕ is shown in Table I. It is seen that both the α- and the β-genes are necessary for antibody production to GLϕ. The genes complement each other in the *trans* position, as is demonstrated by the F_1 mice. The primary response of the F_1 mice (data not shown on Table I) was 36 ± 11% antigen bound at a 1:2 serum dilution. Table II shows the composition and immune response of 17 allophenic mice to GLϕ. It is seen that the mice cover the whole range of possible cellular compositions. It is clear from these results that none of these mice produced antibody to GLϕ at either the primary or the secondary bleedings.

Discussion

The recent findings that the immune response to the synthetic polymer GLϕ is under dual *Ir* gene control (4–7), has prompted several investigations into the types of cells in which each of the genes is expressed. In addition, other studies performed on the synthetic polymer poly(Tyr,Glu)-poly D,L Ala--polyLys[(T,G)-A--L] have also led to the speculation that dual *Ir* gene control of the response to this polymer is governed by one gene, which is expressed in T cells, and the other gene that is expressed in B cells (13). Thus, the genetic defect in nonresponder strains to (T,G)-A--L has been demonstrated to be in B cells in some strains and in both T and B cells in other strains (14–16).

TABLE I

Characterization of the Immune Response of Inbred and F_1 Mice to GLϕ

Strain	*H-2* haplotype	*H-2* regions*						Ir-Glϕ genes‡		Response to GLϕ§
		K	*IA*	*IB*	*IC*	*S*	*D*	β	α	
C57BL/6	*b*	*b*	*b*	*b*	*b*	*b*	*b*	+	−	5 ± 7
A	*a*	*k*	*k*	*k*	*d*	*d*	*d*	−	+	11 ± 9
(C57BL/6 × A)F_1	*b* × *a*	*b/k*	*b/k*	*b/k*	*b/d*	*b/d*	*b/d*	+/−	−/+	64 ± 16

* Based on Shreffler and David (19)

‡ Based on Dorf et al (6)

§ The data is the average percent antigen bound by a 1 2 serum dilution, ± standard deviation, at the secondary response Each group consisted of five mice

TABLE II

Characterization of the Immune Response of Allophenic Mice to GLϕ

Mouse no	% C57BL/6 peripheral white blood cells at the 2° response*	Response to GLϕ‡	
		1° Response	2° Response
170	3	10	6
171	29	11	11
159	32	15	12
191	41	10	11
172	50	9	7
162	59	12	8
169	60	4	3
190	69	4	15
173	72	3	6
167	80	5	3
160	90	11	4
157	92	7	4
187	97	4	8
158	98	5	10
168	102	11	4
189	109	0	14
188	119	10	12

* The data are the percent of the control, so that some values are >100%

‡ The data are the percent antigen bound by a 1 2 serum dilution

A similar hypothesis governing the immune response to GLϕ has been tested by Schwartz et al. (17) and by Katz et al. (18). In the first study, it was found that strains of mice bearing responder alleles at only the α- or the β-locus were nonresponders, as assessed by a T-lymphocyte proliferation assay. Thus, the presence of both genes was necessary for T-cell proliferation to occur in response to stimulation with GLϕ. Consistent with the data presented by Schwartz et al. (17) is the hypothesis that neither gene is expressed in the B cells, but that either both genes are expressed in the T cells, or else either one or both *Ir* genes are expressed in macrophages.

In the second study, Katz et al. (18), using an adoptive transfer system, imply, but do not fully prove, that both the α-gene and the β-gene must be expressed in B cells as well as T cells for a full response to GLϕ. For one thing, Katz et al. (18) found the surprising result that the two genes in the B cells had to be in the *cis* configuration (e.g., 5R mice) and not in the *trans* configuration [e.g., (C57BL/6 × A)F_1 mice] for effective cell cooperation to occur. They state that this result might be explained in terms of a gene dosage effect. In view of this fact, it also seems possible that a gene dosage effect could account for the lack of stimulation of nonresponder parental cells (e.g , B10.A or C57BL/10) by carrier-primed F_1 T cells [e.g., (B10 × A)F_1]. Thus, whereas the Schwartz et al. (17) experiments seem to show unequivocally that both the α-gene and β-gene must be

expressed in the T-cell population, the Katz et al. (18) study does not show unequivocally that the genes must be expressed in the B-cell population.

We have approached the question of cellular expression of the two GLϕ genes from a different point of view. It is suggested from the fact that the α-gene and β-gene can interact in the *cis* position as well as in the *trans* position, that at least one of these genes may produce a soluble gene product. The question we sought to answer is whether this product (or factor) is an intracellular product or an extracellular product. Thus, if two cell types, each possessing one of the two *Ir-Gl*ϕ genes, were allowed to interact, the successful interaction would be strong evidence for an extracellular product. If, on the other hand, no such interaction was observed, the evidence would be in favor of an intracellular product. An extracellular product could either be membrane bound or secreted into the fluid surrounding the cell.

A unique way in which to allow histoincompatible cells to interact without any apparent allogeneic effect is to produce allophenic (tetraparental) mice between two different parental strains. Only in this way is it possible to allow two nonresponder strains, with complementing α- and β-genes, to coexist in a normal environment. As is seen in Table II, the complementing nonresponder $\leftrightarrow$ nonresponder allophenic mice produced in this experiment cover a complete range of parental cell mixtures. It is apparent from Table II that none of the mice, regardless of their cellular composition, were able to respond to GLϕ. This finding may be interpreted in several ways. First, it may be necessary to have both the α- and β-genes in a single cell to get an immune response to GLϕ. Second, cell cooperation may not be able to occur between the histoincompatible cells in the allophenic mice, thus precluding an immune response. However, in a previous study from our laboratories, we showed that allophenic mice produced from the combination of a responder and a nonresponder strain do exhibit a normal GLϕ response in direct proportion to the percentage of responder cells present in a given mouse (10). Thus, it seems highly unlikely that it is the allophenic mouse environment itself that leads to the lack of response seen in the present study.

We would like to propose a tentative model, amenable to experimentation, to account for our results and the other reported results on the immune response of mice to GLϕ. We propose that the product of the β-gene may be a T-cell extracellular product, which allows the T cell to recognize antigen. The product could be loosely bound to the T-cell surface and could dissociate from the membrane upon interaction with antigen. The antigen-T-cell product complex could then stimulate the appropriate B cell to produce antibody directly, or else indirectly via a secondary product secreted by the T cell upon removal of the β-gene product by antigen.

The unique feature of this model is the postulate that the α-gene product is a molecule that allows the β-gene product to be externalized. The α-gene product itself may or may not also be externalized when β-gene product secretion occurs. Without the α-gene product, the β-gene product would remain inside the cell in a nonfunctional state. The main features of this model as applied to the C57BL/6 $\leftrightarrow$ A allophenic mice, are shown in Fig. 1

This model predicts several points. First, it predicts that in the GLϕ system both the α- and β-genes are necessarily expressed in T cells (or macrophages), but not necessarily in B cells at all. Second, it predicts that two α-genes could never complement each other in the absence of a functional β-gene. On the other hand, two β-genes might complement each other by a gene dosage effect, which could lead to leakage of the β-gene product out of the T cells in the absence of a functional α-gene. These predictions are completely consistent with the lack of response of the allophenic mice studied herein. They are also consistent with many other observed experimental facts (4–8, 17). The model is especially appealing since it could explain how C57BL/6 and SJL mice, each with a functional β-

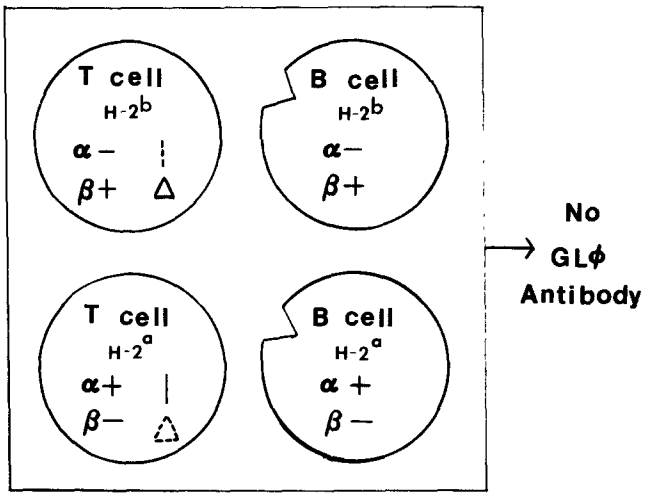

FIG 1 The four cell types of C57BL/6 ↔ A allophenic mice △, β-gene product; ∴, absence of β-gene product, |, α-gene product; ⋮, absence of α-gene product

gene, but no functional α-gene, complement each other in the F_1 generation (5, 7). This model is currently being further tested in our labortories.

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