GENE COMPLEMENTATION IN THE T-LYMPHOCYTE PROLIFERATIVE RESPONSE TO POLY(Glu^{55}Lys^{36}Phe^{9})$_n$

A Demonstration that Both Immune Response Gene Products Must Be Expressed in the Same Antigen-Presenting Cell

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The immune response to poly (Glu^{53}Lys^{36}Phe^{11})n (GLϕ^{11})[1] has been shown to be under the control of two major histocompatibility complex (MHC) - linked immune response (*Ir*) genes at both the antibody and T-lymphocyte proliferative levels (1–3). The two genes map to different subregions of *I*, one in *I-A*, the other in *I-E/I-C*. Recently we have shown that antibodies directed against Ia antigens coded for by genes in either of the two subregions could inhibit the T-lymphocyte proliferative response to GLϕ (4). This result suggested that both *Ir* gene products were expressed at the cell surface (or in the supernate as factors) and that both were essential participants in this secondary response. In the present paper, we continue to explore the biological basis of *Ir* gene complementation by addressing the question of whether both gene products have to be expressed in the same or different cell types to generate an immune response to GLϕ. It will be demonstrated that both *Ir* gene products must be expressed in the same antigen-presenting cell.

Materials and Methods

Animals. C57BL/10Sn(B10), B10.A/SgSn, B10.A(5R)/SgSn[5R], (B6A)F$_1$, A/J, C57BL/6J, and B10.A(2R)/SgSn[2R] mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.A(3R)/Sg[3R], B10.A(18R)/Sg[18R] and all F$_1$ hybrids derived from these and the Jackson strains were bred in our own laboratories. Mice of both sexes were entered into the experiments between 8 and 20 wk of age.

Antigens. Two preparations of GLϕ were utilized during the course of these studies. GLϕ^{11} was the generous gift of Dr. Elkan Blout, Department of Biological Chemistry, Harvard Medical School, Boston, Mass. Poly (Glu^{56}Lys^{35}Phe^{9})$_n$ (Glϕ^{9}) was purchased from Miles-Yeda, Rehovot, Israel. Both were synthesized from the *N*-carboxyanhydrides of the amino acids, although the former was polymerized in benzene using sodium methoxide as an initiator, while

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[1] *Abbreviations used in this paper:* B, bone marrow-derived; CFA, complete Freund's adjuvant; DNA-OVA, dinitrophenyl conjugated ovalbumin; EHAA, Eagle's-Hanks'-amino acid medium; FCS, fetal calf serum; H-2, histocompatibility 2 locus of the mouse; GLϕ, the terpolymer of L-glutamic acid, L-lysine and L-phenylalanine; (H,G)-A—L, poly(His,Glu)-poly D,L-Ala—poly Lys; Ir, immune response; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; PETLES, peritoneal exudate T-lymphocyte-enriched subpopulation; PPD, purified protein derivative of tuberculin; T, thymus-derived; TEPC-15, an IgA myeloma protein with binding specificity for phosphorylcholine; (T,G)-A—L, poly(Tyr,Glu)-poly D,L-Ala—poly Lys; α, Ir-GLϕ-α gene; β, Ir-GLϕ-β gene.

the latter was polymerized in dioxane using triethylamine as an initiator (5). Both polymers were dissolved in 1% Na_2CO_3 in normal saline and immediately neutralized to pH 7.2 with 1 N HCl. They were then diluted to 2 mg/ml with phosphate-buffered saline (PBS) and stored at −20°C. The branched chain synthetic copolymers, poly (Tyr,Glu)-poly D,L-Ala—poly Lys [(T,G)-A—L] (lot no. 958 and 1383) and poly (His,Glu)-poly D,L-Ala—poly Lys [(H,G)-A—L], were synthesized from the *N*-carboxyanhydrides as previously described (6) and were the generous gift of Dr. Edna Mozes, the Weizmann Institute, Rehovot, Israel. The polymers were dissolved in water, adjusted to 2 mg/ml with 10 × PBS and stored at −20°C. The IgA myeloma protein, TEPC 15, was obtained from an ascites tumor (Litton Bionetics, Rockville, Md.) and purified by affinity chromatography on a phosphorylcholine column according to the procedure of Chesebro and Metzger (7). The *p*-nitrophenyl phosphorylcholine reagent was kindly provided by Dr. H. Metzger, National Institutes of Health, Bethesda, Md. and the organic coupling reactions were performed with the help of Dr. J. K. Inman, National Institutes of Health. Pigeon cytochrome *c* was prepared from breast muscle according to the procedure of Brautigen et al. (8) and was the generous gift of Doctors M. Ultee and E. Margoliash, Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Ill. It was dissolved directly in PBS at 2 mg/ml and stored at −20°C. In some experiments, T lymphocyte cultures from mice immunized with pigeon cytochrome *c* were stimulated with the cytochrome *c* from tobacco hornworm moth because the latter elicits a heteroclitic response with a twofold higher maximum stimulation (A. Solinger, M. Ultee, E. Margoliash, and R. H. Schwartz, unpublished observations). Purified protein derivative of tuberculin (PPD) was purchased from Connaught Medical Research Laboratory, Willowdale, Ontario, as a 2 mg/ml solution and stored at −20°C. The 2,4-dinitrophenyl derivative of ovalbumin ($DNP_{7.5}OVA$) was prepared by reacting the protein (200 mg in 5 ml of borate buffer, pH 8) with fluorodinitrobenzene (10 μl) for 6 h and dialyzing against PBS. The conjugation ratio was determined by measuring the optical density at 360 nm. All antigens were diluted with culture medium to appropriate concentrations (20 μg/ml for PPD, 100–200 μg/ml for all the others) just before use. The ability of the various mouse strains used in this study to mount T-lymphocyte proliferative responses to each of these antigens is summarized in Table I.

Immunizations. Mice were immunized with 30 μg of $DNP_{7.5}OVA$, 20–50 μg of GLϕ, 20–100 μg of pigeon cytochrome *c* or 50 μg of (T,G)-A—L, (H,G)-A—L, and TEPC 15, emulsified 1:1 in complete Freund's adjuvant containing 1 mg/ml of *Mycobacterium tuberculosis*, strain H37Ra (Difco Laboratories, Detroit, Mich.). ½0th ml of emulsion was injected in either of the two hind footpads or in the base of the tail. When all five antigens were administered together, two were given in each footpad and one in the tail.

Cell Cultures. The preparation of peritoneal exudate, T-lymphocyte-enriched cells (PETLES) and their in vitro culture with soluble antigen has been previously described (9–11). In one series of experiments the lymph node proliferation assay of Corradin et al. (12) was utilized except that 2×10^5 cells were cultured in round bottom microtiter plates instead of 4×10^5 cells in flat bottom plates. The use of antigen-pulsed nonimmune spleen cells to present antigen to immune PETLES has recently been discussed in detail (13). In the present experiments, 1×10^7 spleen cells were exposed to 100 μg of GLϕ or 50 μg of PPD or DNP-OVA in the presence of 50 μg of mitomycin C in 1 ml of RPMI-1640 + 10% fetal calf serum (FCS) for 1 h at 37°C. The cells were then washed five times with cold RPMI-1640 without FCS to remove unbound antigen and mitomycin C. Finally, the cells were counted, mixed with the immune PETLES or lymph node cells, and cultured for 5 days. Stimulation was assessed by measuring the incorporation of a 1 μCi pulse of (tritiated-methyl)-thymidine 16 h before harvesting the cells. The data are expressed as cpm ± the arithmetic standard error of the mean (SEM) for triplicate or occasionally duplicate determiniations.

Positive Selection of Antigen-Specific T-Cell Populations. 1×10^5 PETLES from $GL\phi^9$-primed $(B6A)F_1$ donors were cultured in the presence of 1×10^5 $GL\phi^9$- or PPD-pulsed $(B6A)F_1$ spleen cells in Eagle's Hanks' amino acid (EHAA) medium + 10% FCS for 10 days. The remaining cells were harvested, pooled, counted, and replated in fresh medium at 2×10^4 cells per U bottom microtiter well. They were restimulated with 1×10^5 fresh antigen-pulsed nonimmune spleen cells for 3 days. Stimulation was assessed by measuring the incorporation of a 1 μCi pulse of thymidine added to the wells 16 h before the cells were harvested.

Preparation of Bone Marrow, Radiation Chimeras. F_1 mice of both sexes, 10 wk or older, were

TABLE I

*Capability of the B10 Congenic Strains Utilized to Respond to the Antigens Administered in the Chimera Experiments**

Strain	Ability to mount a T-lymphocyte proliferative response to					
	(T,G)-A—L	TEPC-15	Pigeon cytochrome *c*	(H,G)-A—L	GLϕ	DNP-OVA or PPD
B10.A (or A/J)	−	+	+	+	−	+
B10 (or B6)	+	−	−	−	−	+
B10.A(2R)	−	+	+	+	−	+
B10.A(3R)	+	−	−	−	+	+
B10.A(5R)	+	−	−	−	+	+
B10.A(18R)	+	−	−	−	−	+
(B10 × B10.A)F_1	+	+	+	+	+	+
(2R × B10)F_1	+	+	+	+	+	+
(3R × B10.A)F_1	+	+	+	+	+	+
(5R × B10.A)F_1	+	+	+	+	+	+
(18R × B10.A)F_1	+	+	+	+	+	+

* This data is a summary of experiments published in references 10, 11, and 16 as well as unpublished observations of the authors. Plus indicates a responder, minus a nonresponder.

lethally irradiated with 850–900 rads (250 kV, 15 mamp dual X-ray source with 0.25 cm Cu and 0.55-cm Al filters; dose rate 130 rads/min) and within 24 h received an intravenous injection of a mixture of 5–7.5 × 10^6 bone marrow cells from each parent according to the method of Von Boehmer et al. (14). Mice were treated intraperitoneally with 25 U of heparin 30 min before injection of cells. The bone marrow cells were obtained from male donors and pretreated with anti-Thy 1 (AKR anti-θC3H) serum plus guinea pig complement. The effectiveness of the anti-Thy 1 treatment was monitored by demonstrating that the small response of bone marrow cells to the T-cell mitogen, concanavalin A, was eliminated. Recipient mice were kept in laminar flow hoods and maintained on drinking water containing neomycin and tetracycline (Neo-Terramycin, Pfizer Inc., New York; 1 teaspoon per liter of water). Mortality varied with the strain combination but was generally 60–70%. Most of the deaths occurred during the first 2 wk after irradiation. The surviving mice were used between 2 mo and 1 yr after reconstitution. At sacrifice, spleen cells or PETLES from the mice were typed with anti-*H-2* sera to insure that they were chimeras. An (A/J × B10.A)F_1 anti-B10.A(5R) serum was used to detect cells bearing K^bI^b and a [B10.A(5R) × A.BY]F_1 anti-B10.A serum was used to detect cells bearing K^aI^a. Both sera were raised by multiple injections of the appropriate spleen cells according to the method of David et al. (15). The assay was a two stage dye exclusion cytotoxicity one using rabbit complement screened for low background cytotoxicity (16). The same sera were used for the mass kill experiments involving lymph node cells (Table III). In initial experiments, all mice were typed individually using spleen cells. After it became clear that every mouse tested was a balanced chimera (between 25 and 75% of the cells were of each parental type), we assayed the PETLES to be sure that the responding cell population was in fact chimeric. The data are presented in Table II as the percent of cells expressing a given histocompatibility type, which was calculated as follows:

$$1 - \frac{\text{(live cells after antiserum treatment)}}{\text{(live cells after normal mouse serum treatment)}} \times 100.$$

In the case of those experiments using spleen cells, the numbers presented represent the averages for all the mice used in the experiment. Generally three to five chimeras were pooled for each experiment.

Results

Radiation Chimeras. To address the question of whether separate cells, each expressing one of the *Ir-GLϕ* genes, can collaborate and thus render an animal responsive to

GLϕ, radiation chimeras were created which allowed complementation at the cellular level but not the genomic level. Responder F_1 hybrids, consisting of crosses between the two types of complementing GLϕ nonresponder strains (*Ir-GL*ϕ-α^+,β^- and *Ir-GL*ϕ-α^-,β^+), were lethally irradiated (850–900 rads) and reconstituted with a mixture of anti-Thy 1-treated bone marrow cells from both parental strains. After 2–12 mo the chimeras were immunized and the PETLES proliferative response assayed. The basic conception in this type of experiment was that if both *Ir-GL*ϕ gene products must be expressed in the same cell(s), then chimeras, which possess the *Ir-GL*ϕ-α^+ and *Ir-GL*ϕ-β^+ genes in separate cells, should not respond to GLϕ. On the other hand, if the two gene products function in separate cells, which interact in the immune system to generate a response (e.g. T cells and antigen-presenting cells), then the chimeras should respond to GLϕ. The data are shown in Table II and Fig. 1. The experimental results for GLϕ were unequivocal. In all chimeras composed of a mixture of parental cells from the two nonresponders (2R ↔ B10 and 18R ↔ B10.A) there was no significant proliferative response to GLϕ. This suggests that complementation at the cellular level had not occurred.

However, proving that this is the correct interpretation of the negative result required a large number of controls which were more difficult to firmly establish. First, it was necessary to demonstrate that the procedure for making radiation chimeras did not produce a generally hyporesponsive immune system which could not respond to GLϕ. This was tested by transferring bone marrow cells from B10.A(5R) or (2R × B10)F_1 mice, which are responders to GLϕ, into lethally irradiated syngeneic recipients. As shown in exp. 1–4 in Table II, these mice all responded to GLϕ, and in most cases quite substantial proliferative responses were observed. These mice also responded well to (T,G)-A—L and PPD. However, the F_1 mice failed to give a proliferative response to the IgA myeloma protein, TEPC-15, an antigen to which the normal (2R × B10)F_1 is capable of responding. The reasons for this failure to respond are not clear. However, it should be pointed out that the immunopotency of antigens such as TEPC-15, (H,G)-A—L and pigeon cytochrome *c* is less than that of GLϕ or (T,G)-A—L. Thus, when the chimeras are slightly hyporesponsive, this appears to be expressed in a failure to respond only to the weaker antigens and never to GLϕ.

A second possible cause for the failure to respond to GLϕ was an unbalanced chimeric state, i.e. the existence in the chimeras of lymphoid cells composed predominantly of one or the other parental cell type. H-2 typing of spleen or lymph node cells (Table II) revealed that every individual chimera derived 25% or more of its cells from each parental haplotype. The group averages for the three to five mice used in each experiment generally ranged from 40 to 60% for both haplotypes (Table II). This indicates the presence of lymphocytes of both parental types. However, the more difficult question to answer was whether the cells in the chimera were functionally balanced, i.e. were there cells of both parental haplotypes capable of proliferating by themselves in response to antigenic challenge. To test this, we immunized the chimeras with antigens, the response to which was controlled by *Ir* genes present in one, but not the other, parent (Table I). Thus, we evaluated the functional capacity of B10 and of 18R cells by immunizing chimeras with (T,G)-A—L. B10 and 18R mice can respond to this antigen but B10.A and 2R mice (their respective partners in the radiation chimeras) can not (10). As shown in Table II a significant proliferative response to (T,G)-A—L in PETLES from both 2R ↔ B10 or 18R ↔ B10.A chimeras was

TABLE II

The T-Cell Proliferative Response to GLϕ of Petles from Bone Marrow Radiation Chimeras

Exp.	Chimera*	%K^{b}I^b	%K^{a}I^a	Cell (× 10^{-5})	Proliferative Response (cpm ± SEM) to‡§						
					Medium	GLϕ	(T,G)-A—L	TEPC-15	Pigeon cytochrome *c*	(H,G)-A—L	PPD
1	B10.A(5R)	—	—	2.0	3,700 ± 1,000	79,700 ± 2,600	39,600 ± 1,700	—‖	—	—	46,900 ± 400
2	B10.A(5R)	—	—	2.0	4,900 ± 800	74,100 ± 700	59,200 ± 10,500	—	—	—	52,700 ± 10,300
3	(B10.A(2R) × B10)F$_1$	—	—	2.0	1,600 ± 200	8,200 ± 300	6,700 ± 600	1,900 ± 200	—	—	21,900 ± 1,200
4	(B10.A(2R) × B10)F$_1$	—	—	2.0	2,100 ± 500	38,200 ± 2,600	23,900 ± 2,200	2,900 ± 400	—	—	55,200 ± 6,000
5	2R ↔ B10	—	—	2.0	2,600 ± 800	3,100 ± 800	9,900 ± 300	7,700 ± 800	—	—	—
6	2R ↔ B10	46	44	2.0	1,800 ± 1,100	1,200 ± 140	8,000 ± 350	2,200 ± 900	3,000 ± 1,000	9,700 ± 4,700	25,000 ± 1,700
7	2R ↔ B10	61	47	2.6	3,300 ± 1,700	2,300 ± 100	18,300 ± 5,000	7,100 ± 900	7,900 ± 400	2,200 ± 800	17,400 ± 3,500
				2.0	2,000 ± 700	2,100 ± 900	20,500 ± 900	2,700 ± 20	6,600 ± 800	1,000 ± 450	16,000 ± 1,400
8	2R ↔ B10	40	67	2.0	67,000 ± 1,600	60,800 ± 8,700	55,700 ± 7,800	59,400 ± 3,500	—	—	86,900 ± 7,800
9	18R ↔ B10.A	50	52	2.0	700 ± 25	1,000 ± 250	15,800 ± 1,600	—	9,300 ± 2,300	—	32,600 ± 6,700
10	18R ↔ B10.A	27	31	2.0	7,500 ± 400	11,600 ± 1,400	31,200 ± 2,700	23,300 ± 2,300	—	12,100 ± 2,600	31,900 ± 500
11	18R ↔ B10.A	40	44	2.0	4,600 ± 200	4,300 ± 400	37,600 ± 2,800	6,900 ± 200	31,100 ± 3,800	3,900 ± 300	42,000 ± 6,700
12	5R ↔ B10.A	50	47	2.0	15,500 ± 5,000	64,500 ± 6,700	38,900 ± 2,700	41,500 ± 5,000	—	—	99,800 ± 5,000
13	5R ↔ B10.A	—	—	2.0	6,300 ± 1,100	40,400 ± 1,200	21,000 ± 800	18,800 ± 6,100	6,000 ± 300	—	—
14	3R ↔ B10.A	39	47	2.0	2,400 ± 800	32,200 ± 200	—	—	10,400 ± 1,500	—	61,900 ± 2,900
15	3R ↔ B10.A	54	64	2.6	1,000 ± 70	16,700 ± 3,500	5,300 ± 700	—	4,600 ± 300	—	—

* The first four experiments are controls in which B10.A(5R) or [B10.A(2R) × B10]F$_1$ mice were lethally irradiated (850 rads) and reconstituted with syngeneic bone marrow. Experiments 5–11 and 12–15 are the true chimeras in which the irradiated (850–900 rads)F$_1$ mice were reconstituted with a mixture of 5–7.5 × 10^6 anti-Thy 1 plus complement-treated bone marrow cells from each parent.

‡ Chimeras were immunized 2–12 mo after reconstitution and 3 wk later their PETLES harvested. 2–2.6 × 10^5 cells were cultured in microtiter wells for 5 days in the presence of 100 μg/ml GLϕ^9 or GLϕ^{11}, 100 μg/ml (T,G)-A—L, 100 μg/ml (H,G)-A—L, 20 μg/ml PPD or 100 μg/ml of pigeon cytochrome *c*. In experiments 11 and 15 30 μg/ml of Tobacco Hornworm Moth cytochrome *c* was used instead of pigeon cytochrome *c*. Stimulation was assessed by measuring the incorporation of a 1 μCi pulse of tritiated thymidine 16 h before harvesting the cultures.

§ Underlined values were significantly different ($P < 0.05$) from the medium control using a Student's *t* test.

‖ A dashed line indicates that that particular antigen was not tested in the experiment.

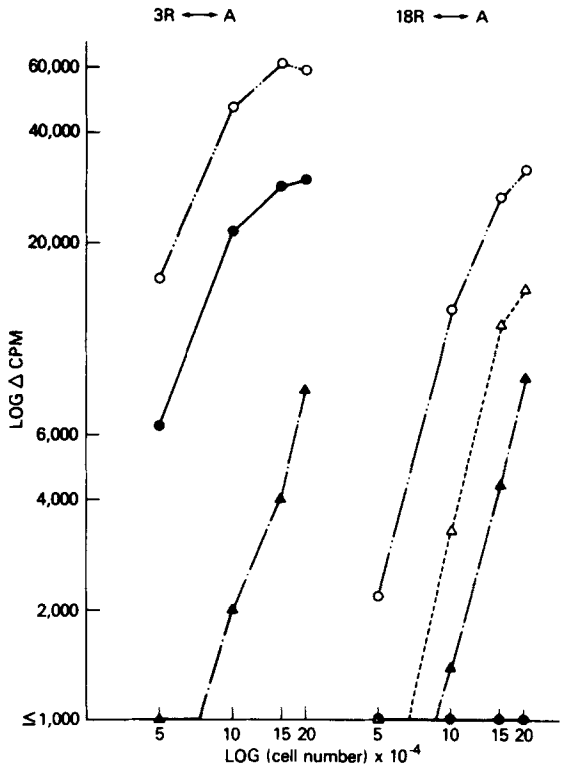

FIG. 1. The T-lymphocyte proliferative response of PETLES from 3R ↔ B10.A and 18R ↔ B10.A chimeras. Chimeras were immunized 6 mo after irradiation and bone marrow reconstitution. 3 wk later PETLES were harvested, and cultured at various cell numbers per well in the presence of 20 μg/ml of PPD (○), 100 μg/ml of GLϕ^9 (●), 100 μg/ml of (T,G)-A—L (△), 100 μg/ml of pigeon cytochrome *c* (▲) or only medium. Stimulation was assessed 5 days later by measuring the incorporation of titriated-thymidine. The responses are expressed as the difference between cultures containing antigen and those with medium alone (Δcpm). Both the cells per well and the responses are presented as the actual numbers but plotted on a log scale.

observed, indicating that the B10 and 18R cells were functionally present. To test the functional capacity of B10.A and 2R cells, the chimeras were immunized with (H,G)-A—L, TEPC-15 and/or pigeon cytochrome *c*. B10.A and 2R mice can respond to these antigens while B10 and 18R mice can not (10, 17). The reason for using a panel of antigens to test responsiveness in this case was because of the relatively weak immunogenicity of each of them. This approach increased the probability of detecting at least one significant proliferative response by cells of the B10.A or 2R type. As shown in Table II one of these three antigens did stimulate a significant proliferative response in all but one of the experiments, suggesting the presence of functional B10.A and 2R cells. Despite this difficulty in uniformly eliciting B10.A and 2R cell responses with all three antigens all of the time, we think the most striking aspect of the data is the failure to elicit any response in PETLES from these chimeras with the much more potent antigen, GLϕ.

The data discussed so far strongly suggest that the chimeras possess functional cells of both parental haplotypes. However, one could now raise the possibility that the failure of these mice to respond to GLϕ was caused by suppressive influences from a

potential mixed lymphocyte reaction occurring between cells of the two parental haplotypes. In setting up the chimeras, the bone marrow cells from both parents were treated with anti-Thy 1 serum plus complement to eliminate any mature T cells the population might contain. Although we attempted to document the complete elimination of functional T cells by demonstrating the disappearance of the small concanavalin A response of bone marrow cells, and although Von Boehmer et al. failed to find evidence of an MLR in spleen cells from such chimeras (14), the findings of Stutman (18) and of Gorczynski and Macrae (19) that there exists a rabbit anti-mouse brain resistant, postthymic T cell in bone marrow, which quickly (16 days) develops functional T-cell properties on transfer into lethally irradiated recipients, makes it necessary to consider the possibility of a cryptic mixed lymphocyte reaction (MLR). That this might be a serious problem in some bone marrow, radiation chimeras is suggested in exp. 8 of Table II. In this group of mice the spontaneous proliferative response of the PETLES population in the absence of antigen was 67,000 cpm, a value never seen with PETLES from normal B10 or B10.A mice, nor from most chimeras (Table II). Only two other chimera experiments showed a comparable result (data not shown) but in all cases there was little or no proliferative response detected over this high background to any antigen. Thus, the response to (T,G)-A—L, which in all the other chimera experiments was significant, either did not occur or was masked by the large antigen-independent proliferation. Whether this spontaneous proliferation is actually an ongoing mixed lymphocyte reaction has not been formally tested. However, such data do raise this possibility and thus demand an experimental demonstration that, even in the case of low medium controls, the failure of chimeras to respond to GLϕ is not due to a suppressive MLR.

To eliminate this possibility we created chimeras which have a similar potential for an MLR but in which one of the parental bone marrow donors is a responder to GLϕ. In this case, both *Ir-GL*ϕ gene products would be expressed in a single cell(s), unlike the previous chimeras. Therefore, these mice should respond to GLϕ, unless suppressed by the MLR. The chimeras set up were 5R ↔ B10.A and 3R ↔ B10.A. These combinations maintain the potential *K,I-A,I-B,* and, in one case, the *I-J* incompatibilities existing in the 2R ↔ B10 and 18R ↔ B10.A chimeras, thus keeping the major potential MLR stimulating differences (20). The only changes in the two sets of chimeras lie in the *I-E,I-C, S,* and *G* regions which supply the 5R and 3R with the *Ir-GL*ϕ-α responder allele missing in the B10 and 18R. Since these regions code for very weak MLR stimulating determinants (20, 21), making the parental cells compatible at these loci should not substantially reduce the total MLR. PETLES from 5R ↔ B10.A and 3R ↔ B10.A chimeras immunized with GLϕ gave a substantial proliferative response to GLϕ, (exp. 12–15 in Table II, and Fig. 1). The response of PETLES from these chimeras to GLϕ was greater than responses to (T,G,)-A—L, TEPC-15 or pigeon cytochrome *c*. The striking difference between the 3R ↔ B10.A and 18R ↔ B10.A chimeras is best seen in the dose-response curves shown in Fig. 1. These animals were immunized at the same time and assayed 2 days apart. PETLES from both chimeras responded to the relatively weak antigen, pigeon cytochrome *c*, with a small but significant proliferative response. In contrast, the 3R ↔ B10.A PETLES gave a strong proliferative response to GLϕ while the 18R ↔ B10.A PETLES showed no response at all. These results demonstrate that the existence in radiation chimeras of a potential suppressive MLR resulting from histoincompatibility at the *K* region and

the *I-A,I-B,* and *I-J* subregions is not sufficient to account for the failure of 18R ↔ B10.A and 2R ↔ B10 chimeras to respond to GLϕ.

However, there still remained another possible reason why 2R ↔ B10 and 18R ↔ B10.A chimeras would not be capable of responding to GLϕ, while the 3R ↔ B10.A and 5R ↔ B10.A chimeras would respond. The in vitro proliferative response depends upon antigen-presentation by an adherent radioresistant cell (22–24). When primed T lymphocytes from normal (i.e. nonchimeric) T lymphocytes are used, they can only be activated by antigen associated with cells which derive from donors possessing common alleles of genes encoded within the *I-A* subregion (13). In other assay systems, e.g. T-B collaboration (25) and expression of delayed-type hypersensitivity (26), it has been shown that chimeric cells can collaborate across such *I-A* barriers. However, if such collaboration were not possible utilizing our assay system or our particular chimeras, then $\alpha^-\beta^+ \leftrightarrow \alpha^+\beta^-$ chimeras, such as the 2R ↔ B10, might fail to respond to GLϕ because the relevant cells could not collaborate with each other to generate a proliferative response to any antigen. In other words, the GLϕ response of $\alpha^-\beta^+ \leftrightarrow \alpha^+\beta^-$ chimeras would depend on collaboration across an *I-A* barrier, while responses to other antigens (e.g. (T,G)-A—L) by these chimeras could be obtained by syngeneic collaboration. In contrast, the GLϕ response of $\alpha^+\beta^+ \leftrightarrow \alpha^-\beta^+$ chimeras, such as the 5R ↔ B10.A, does not have to result from collaboration across a histocompatibility barrier as the responder $\alpha^+\beta^+$ parental cells could generate the GLϕ response with syngeneic collaboration. Thus, to prove that the two *Ir-GL*ϕ genes can not complement at the cellular level, it was essential to demonstrate in our assay system that collaboration across an *I-A* barrier could be achieved.

To address this problem required a more complex protocol than that used in the previous experiments. Lymphoid cells from chimeras immunized with antigens to which both parents respond, e.g. DNP-OVA, have to be treated with anti-*H-2* sera plus complement to eliminate cells of one or the other parental haplotype. One then has to show that the remaining lymphoid cells respond to DNP-OVA presented on antigen-presenting cells from the opposite parental haplotype. These experiments proved technically difficult with the PETLES assay system because of the low cell yield. However, this problem was overcome by turning to the lymph node proliferation assay recently described by Corradin et al. (12). 5R ↔ B10.A chimeras were immunized with $DNP_{7.5}$-OVA and 8 days later the draining lymph nodes excised. A lymphoid cell suspension was prepared and treated with an (A/J × B10.A)F_1 anti-B10.A(5R) serum to remove the B10.A(5R) parental cells and leave the B10.A parental cells, or treated with a [B10.A(5R) × A.BY]F_1 anti-B10.A serum to remove the B10.A parental cells and leave the B10.A(5R) parental cells. The remaining immune, parental-type lymphocytes were then challenged with $DNP_{7.5}$-OVA-pulsed nonimmune spleen cells from either parent. As shown in Table III, B10.A-type lymphoid cells obtained from the chimera were capable of proliferating in response to DNP-OVA bound to either B10.A or B10.A(5R) parental spleen cells. The magnitude of the response was similar for both presenting populations. The same was true for the B10.A(5R)-type lymphoid cells obtained from the chimeras (exp. 2). These results demonstrate that the chimeric cells can collaborate with each other across a histocompatibility barrier to generate a proliferative response to DNP-OVA. Therefore, we feel confident in concluding that the failure of chimeras, composed of mixtures of the complementing nonresponder parental cells, to respond to GLϕ cannot be the result

TABLE III

Histoincompatible Cells Maturing in a Chimeric Environment Can Collaborate to Generate a Proliferative Response

Exp.	Antiserum	Antigen-pulsed spleen cells	Proliferative response (cpm ± SEM) to:	
			Nonpulsed	DNP-OVA-pulsed
1	Anti-K^aI^a	B10.A	3,300 ± 1,300	13,600 ± 2,400
		B10.A(5R)	3,000 ± 400	9,700 ± 3,000
2	Anti-K^aI^a	B10.A	1,800 ± 500	6,300 ± 400
		B10.A(5R)	2,500 ± 800	7,700 ± 1,400
	Anti-K^bI^b	B10.A	2,300 ± 450	10,200 ± 3,200
		B10.A(5R)	2,100 ± 200	12,500 ± 2,700

For each experiment three 5R ↔ B10.A chimeras were immunized with 30 μg of $DNP_{7.5}OVA$ in CFA at the base of the tail. 8 days later the inguinal and periaortic lymph nodes were excised and the cells treated with either an (A/J × B10.A)F_1 anti-B10.A (5R) serum (anti-K^bI^b) or a [B10.A (5R) × A.BY]F_1 anti-B10.A serum (anti-K^aI^a) plus rabbit complement. 2 × 10^5-treated lymphoid cells were then cultured in round bottom microtiter plates with 1 × 10^5 mitomycin C inactivated, nonimmune spleen cells previously pulsed with 50 μg/ml $DNP_{7.5}OVA$, or similarly handled nonpulsed spleen cells. Stimulation was assessed 5 days later by measuring the incorporation of tritiated thymidine using standard liquid scintillation counting techniques.

of a general failure of cells from the $\alpha^-\beta^+$ donor to collaborate with cells from the $\alpha^+\beta^-$ donor. Rather, it must reflect a need for at least one cell type involved in the proliferative response to GLϕ to derive from an $\alpha^+\beta^+$ donor.

Antigen-Presentation of GLϕ. Most proliferation assays appear to involve the interaction of at least two cell types, one a primed, antigen-specific, T-lymphocyte, the other a nonimmune antigen-presenting cell (22, 23). In the mouse, the best sources of the latter cell type are PETLES and spleen (13). We have previously demonstrated for antigen responses under the control of single immune response genes (e.g. poly[Glu^{60},Ala^{30}, Tyr^{10}]$_n$), that the antigen-presenting cells must be derived from a strain with a responder haplotype to obtain a proliferative response (27). In the case of (high responder × low responder)F_1 PETLES, only spleen cells from the F_1 or the high responder parent could present antigen; spleen cells from the low responder parent were ineffective. These results suggested that *Ir* gene products had to be expressed in antigen-presenting cells.

The GLϕ system represents a more complex case. With two genes operating, it was possible that only one was functioning in the antigen-presenting cell. The chimera data argue that at least one cell type must express both gene products, but this cell could be the T-lymphocyte. On the other hand, both gene products might be expressed in both cell types or, according to some models (28, 29), only in the antigen-presenting cell. To investigate the *Ir* gene requirements for antigen-presentation, complementing (nonresponder × nonresponder)F_1 mice were immunized with GLϕ and their PETLES stimulated in vitro with nonimmune, GLϕ-pulsed spleen cells from F_1 responders or from either type of parental nonresponder. The results from a typical experiment are shown in Table IV (exp. 1). PETLES from (B10.A × B10)F_1 mice immunized with $GL\phi^9$ in CFA proliferated in vitro when stimulated with nonimmune (B10.A × B10)F_1 spleen cells that had been pulsed with either GLϕ or PPD. In contrast, GLϕ-pulsed B10 and B10.A parental spleen cells did not elicit responses to GLϕ, although these same parental spleen cells when pulsed with PPD could elicit significant PPD responses. The magnitude of the PPD response obtained

with either of the two PPD-pulsed parental spleen cell populations, although quite significant, was less than the response obtained with PPD-pulsed F_1 spleen cells. Thus, one could argue that the failure of the parental spleen cells to present GLϕ was just an extreme form of the poorer presentation of all antigens to F_1 PETLES by parental cells as compared to F_1 spleen cells. However, the last two experimental groups presented in each experiment in Table IV make this an unlikely possibility. First, mixtures of equal numbers of spleen cells from the B10 and B10.A parents did not present GLϕ any better than either parental cell type alone; whereas, in most cases, the mixture did result in a larger proliferative response to PPD. Second, when B10.A(5R) spleen cells were used for antigen presentation, they presented GLϕ as well as the (B10.A × B10)F_1 spleen cells, although their presentation of PPD was only as good as that of the B10 and B10.A parental strains. B10.A(5R) is a responder to GLϕ, the haplotype being an intra-*H-2* recombinant derived from the nonresponder B10 and B10.A parental strains in such a way as to acquire the responder alleles at both *Ir-GL*ϕ loci. Thus, possession of both *Ir* gene products in the same antigen-presenting cell is clearly required for the optimal presentation of GLϕ.

In some experiments, (exp. 2, Table IV) the nonresponder parental spleen cells did stimulate modest proliferative responses to GLϕ, although never as great as the stimulation achieved with spleen cells from responder strains. We suspected that this low level of stimulation was caused by the transfer of antigen from the pulsed parental spleen cells to the residual antigen-presenting cells in the population of F_1 PETLES used as a source of responding T lymphocytes. To test this, however, we had to turn to in vitro-positive selection procedures. In this technique, PETLES from primed donors are cultured for 10 days in the presence of antigen bound to syngeneic spleen cells. When the harvested cells are recultured in the presence of soluble antigen, no proliferative response is observed. However, if antigen-pulsed syngeneic spleen cells are added instead of soluble antigen, specific proliferation is seen. Thus, this tertiary proliferative response is completely dependent on the addition of fresh antigen-presenting cells, presumably because antigen-presenting cells in the PETLES population are lost during the initial 10 day culture. Thus, problems of antigen transfer should be eliminated. When PETLES from (B6 × A/J)F_1 mice primed to GLϕ in CFA were selected with GLϕ-pulsed (B6A)F_1 spleen cells, the recultured cells gave a significant proliferative response when restimulated with GLϕ-pulsed (B6A)F_1 spleen cells but no response at all when stimulated with GLϕ-pulsed B6 or A/J parental spleen cells (Table V). In contrast, if the same PETLES were selected with PPD-pulsed (B6A)F_1 spleen cells, then the recultured cells could be stimulated with either PPD-pulsed (B6A)F_1 cells or PPD-pulsed spleen cells from both parental strains. Mixtures of the two parental strains did not produce a response to GLϕ in GLϕ selected cells, although it did increase the proliferation to PPD in PPD selected cells. These results indicate that it is essential to have both *Ir-GL*ϕ gene products expressed in the same antigen-presenting cell to stimulate a primed T-lymphocyte proliferative response to GLϕ.

Discussion

The discovery that the immune response to several synthetic polypeptides (1, 30, 31) and protein antigens (17, 32, 33) requires responder alleles at two distinct *Ir* loci, opened up the possibility of dissecting such *Ir* gene control into its component parts.

We chose to work with the antigen GLϕ because of the clear difference between the proliferative responses of responder and nonresponder strains (34). If each *Ir* gene involved in this immune response expressed its function in a distinct cell and these cells had to interact to generate an immune response, then cell mixing experiments should be able to demonstrate cooperative interactions between $\alpha^-\beta^+$ and $\alpha^+\beta^-$ cells. To study such collaborations in the absence of alloreactions, bone marrow, radiation chimeras were established. In most cases a histocompatible environment was achieved (Table II). The results clearly showed that chimeras composed of the two types of nonresponder parental cells failed to mount an immune response to GLϕ. This failure to respond has also been observed in allophenic mice (35); however, in those studies no rigorous attempt was made to rule out other explanations for the negative results. In our studies both parental cell types were shown to be present and to be biologically functional since the chimeras were capable of responding to the much weaker antigens, (T,G)-A—L, TEPC-15, pigeon cytochrome *c* and occasionally (H,G)-A—L. Furthermore, the possibility of a cryptic mixed lymphocyte reaction stemming from an H-2^a/H-2^b incompatibility at the *K* region and the *I-A* through *I-J* subregions was eliminated by showing that the 3R $\leftrightarrow$ B10.A and 5R $\leftrightarrow$ B10.A chimeras could respond to GLϕ. Finally the failure to cooperate was shown to be a unique property of complementing *Ir* gene systems in that cell cooperation across a potential histocompatibility barrier could be demonstrated for the antigen, DNP-OVA.

It should be pointed out that the recent studies of Zinkernagel et al. (36, 37) and Bevan (38), which demonstrate the importance of the thymus in determining the recognition capabilities of T cells, do not cloud the interpretation of the present chimera experiments. In our chimeras, the T cells mature in an F_1 thymus and therefore should learn to recognize both H-2^a and H-2^b histocompatibility structures as well as any F_1 specific structures that might be present on radioresistant cells. The failure of such chimeras to respond to GLϕ is consistent with and represents an extension of the recent observations of von Boehmer et al. (39) on the transfer of *I*-region controlled low responder parental bone marrow cells into lethally irradiated (high responder $\times$ low responder)F_1 recipients. These chimeras remained low responders, suggesting a requirement for a radiosensitive, high responder cell (or a cell with a radiosensitive precursor) either in the peripheral lymphoid system or in the thymus of the chimera. In the case of the two gene GLϕ system, it is the high responder F_1 cells which are lacking. Moreover, the two gene system is actually a somewhat cleaner demonstration of this phenomenon in that parent $\rightarrow$ F_1 chimeras often are not fully tolerant to the opposite parent (40), thus leaving any results obtained with such chimeras open to the possibility of allogeneic effect artifacts.

In the second series of experiments in this paper (Tables IV and V), one of the critical F_1 cells required for responsiveness to GLϕ was shown to be the antigen-presenting cell. Only spleen cells from responder mice were capable of presenting GLϕ to GLϕ-primed responder F_1 cells. Neither nonresponder parental spleen cell population was effective. In fact, mixtures of the $\alpha^+\beta^-$ and $\alpha^-\beta^+$ parental cell types also failed to present GLϕ. This last experiment is formally analogous to the situation in the chimeras where all the genetic material required for an immune response was present in the system but was segregated into separate cells. The failure to achieve cooperation in both the chimeric and presentation experiments argues strongly that both *Ir-GLϕ* gene products must be expressed in the same antigen-presenting cell to generate a T-cell-immune response to this antigen.

TABLE IV

Proliferative Response of GLϕ Primed F_1 Petles to GLϕ and PPD Presented on Parental or F_1 Spleen Cells

Exp.	Antigen-presenting* nonimmune spleen cells	Responder status‡ to GLϕ	Proliferative response (cpm ± SEM) to spleen cells§		
			Nonpulsed	GLϕ-pulsed	PPD-pulsed
1.	(B10.A × B10)F_1	High (α^+, β^+)	1,500 ± 300	18,100 ± 2,900	28,700 ± 500
	B10	Low (α^-, β^+)	1,150 ± 300	2,500 ± 500	13,400 ± 800
	B10.A	Low (α^+, β^-)	800 ± 200	2,200 ± 200	9,600 ± 900
	B10 + B10.A	Low + low	1,550 ± 250	3,300 ± 850	20,000 ± 300
	B10.A(5R)	High (α^+, β^+)	600 ± 200	16,200 ± 1,100	12,400 ± 950
2.	(B10.A × B10)F_1	High (α^+, β^+)	1,300 ± 500	20,400 ± 1,200	30,700 ± 4,400
	B10	Low (α^-, β^+)	1,600 ± 300	6,000 ± 900	17,600 ± 4,300
	B10.A	Low (α^+, β^-)	1,100 ± 300	8,500 ± 2,200	19,900 ± 1,500
	B10 + B10.A	Low + low	2,100 ± 500	5,600 ± 700	23,900 ± 4,300
	B10.A(5R)	High (α^+, β^+)	900 ± 200	20,700 ± 3,000	19,800 ± 1,500

* 1×10^7 nonimmune spleen cells were pulsed with 20 μg/ml of PPD or 100 μg/ml of GLϕ^9 or GLϕ^{11} in the presence of 50 μg/ml of mitomycin C for 1 h at 37°C. The cells were washed five times and then used as a source of antigen-presenting cells.

‡ Responder status refers to the ability of the spleen cell donors to mount an immune response to GLϕ. The letters in parentheses refer to the *Ir-GLϕ-α* and *-β* alleles possessed by these mice.

§ 3 wk after immunization with 30 μg of GLϕ in CFA, 1.5×10^5 PETLES from (B10.A × B10) mice were cultured in the presence of 1×10^5 antigen-pulsed spleen cells for 5 days. Mixtures consisted of 1×10^5 spleen cells of each type. Stimulation was measured as described in Table II.

TABLE V

Tertiary Response of Positively Selected (B6A)F_1 T Lymphocytes to GLϕ and PPD Presented on Parental or F_1 Spleen Cells

Selecting* antigen	Spleen cells	Proliferative response (cpm ± SEM) to spleen cells*		
		Nonpulsed	GLϕ-pulsed	PPD-pulsed
GLϕ	(B6A)F_1	2,400 ± 300	11,000 ± 200	3,400 ± 160
	B6	1,100 ± 200	1,700 ± 400	ND‡
	A/J	900 ± 150	1,600 ± 20	ND
	1/2 B6 + 1/2 A/J	3,000 ± 50	3,100 ± 500	ND
PPD	(B6A)F_1	4,500 ± 600	5,600 ± 400	11,800 ± 1,000
	B6	4,100 ± 250	ND	12,400 ± 1,600
	A/J	3,900 ± 900	ND	8,800 ± 1,100
	1/2 B6 + 1/2 A/J	6,400 ± 600	ND	19,100 ± 1,000

* (B6A)F_1 PETLES immune to GLϕ^9 and PPD were cultured for 10 days in the presence of (B6A)F_1 nonimmune spleen cells pulsed with either GLϕ^9 or PPD (selecting antigen). The in vitro selected T lymphocytes were then recultured at 2×10^4 cells/well and stimulated for 3 days with 1×10^5 antigen-pulsed spleen cells from (B6A)F_1, B6 or A/J mice. Mixtures consisted of 5×10^4 spleen cells from both parents. Cultures were pulsed with 1 μCi of tritiated thymidine on day 2 and the incorporation measured 16–18 h later by standard liquid scintillation counting techniques.

‡ ND, not determined.

This conclusion is incompatible with the original model proposed to explain two gene complementation in the immune response to (T,G)-A—L (31). That model suggested that one gene product was expressed in T lymphocytes, the other in B lymphocytes (or alternatively in the antigen-presenting cell). Complementation in the F_1 involved the cooperation between the functional T cell contributed by one parent and the functional B cell contributed by the other parent. Both the chimera data and the antigen-presentation data presented in this papr indicate that this is not the explanation for *Ir* gene complementation in the T-lymphocyte proliferative response

to GLϕ as at least one of the cell types must express both gene products. The present data are also incompatible with a more recent two gene model proposed by Keck (41) in which each *Ir* gene was responsible for the immune response to a separate determinant. Recognition of either determinant alone was insufficient to generate a detectable immune response; but the cooperative interaction in an F_1 between the two responding T cells, each still recognizing its own determinant on the antigen, would lead to a strong response. Again, one would have expected the chimeras to respond to GLϕ if such a model were correct. Also recent experiments carried out by A. M. Solinger from our laboratory in collaboration with M. Ultee and E. Margoliash at Northwestern University, Evanston, Ill., have demonstrated that the dual *Ir* gene-controlled proliferative response to pigeon cytochrome *c* in B10.A mice involves the recognition of a single antigenic determinant and not two separate determinants as would be predicted by the Keck model.

The requirement for both gene products to be expressed in a single cell would thus seem to necessitate that any model for *Ir* gene complementation postulate an interaction at the molecular level. This could occur in a number of ways. In the model proposed by Warner et al. (35), the *Ir-GLϕ-α* product is postulated to allow the *Ir-GLϕ-β* gene product to be externalized, presumably by some sort of enzymatic modification or biologic transport mechanism. This idea is compatible with the present data; however, it is not readily compatible with our previously published observations that the proliferative response to GLϕ could be blocked with anti-Ia antisera directed against products of the *I-E/I-C* subregion, which codes for the *Ir-GLϕ-α* gene (4). This result suggested that the *Ir-GLϕ-α* gene product is expressed on the cell surface and that its function can be inhibited there. In its simplest form the Warner model predicts that, if expressed on the surface at all (e.g. in transport), the *Ir-GLϕ-α* gene product's function would have been completed before the in vitro test and, therefore, no inhibition should have occurred.

The model which we currently favor is based on the conclusion from the blocking studies that *both Ir-GLϕ* gene products are expressed on the cell surface and function there. We would postulate that the *I*-region products exist on the surface of the antigen-presenting cell, where they are present as dimers or tetramers of the original gene products. *I-A* and *I-E/I-C* gene products would be structurally equivalent and, therefore, hybrid molecules would exist containing elements of both gene products. If the immune response to GLϕ could only occur via these hybrid molecules, then they would constitute the unique F_1 intermolecular structures required by the results presented in this paper. As shown in Fig. 2, the mechanism by which they function could involve either dual recognition or associative-recognition type models (24, 28, 42–45) although the latter is somewhat simpler to reconcile with the two gene model (for a discussion of this point see reference 24). Support for such an *I*-region hybrid product model is provided by the recent work of Fathman and Nabholz (46). They demonstrated that in an A/J anti-(B6A)F_1 mixed lymphocyte reaction, some responding T lymphocytes were specific for F_1 determinants. Mapping studies suggested that at least two and possibly more *I*-region gene products were required to form these F_1 stimulating determinants on the cell surface. Thus, the requirement that GLϕ be recognized in association with such F_1 structures seems to us only a logical extension of this biological situation.

Very recently, a variation on our model has been suggested by Jones et al. (47)

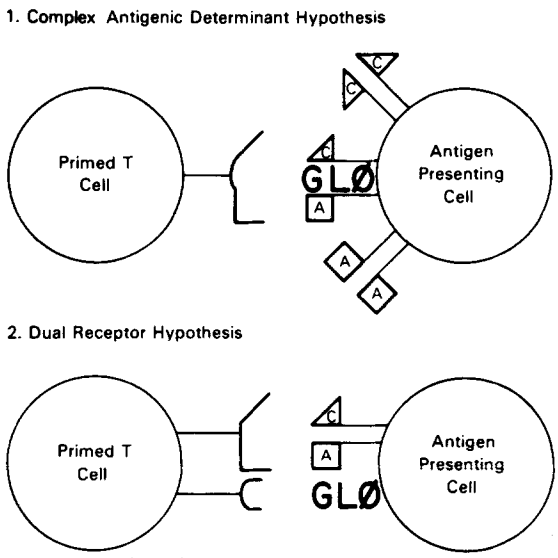

FIG. 2. A schematic representation of the two types of hybrid molecule models for the antigen-presenting cell, T-cell interaction in the immune response to GLϕ. In the complex antigenic determinant model the GLϕ associates with the F_1-specific *I* region product (A/C) on the surface of the antigen-presenting cell and is recognized by a single receptor on the T cell with specificity for both the antigen and the *I* region product. In the dual recognition model, GLϕ and the unique F_1 *I* region product are recognized by two separate T-cell receptors.

based on the biochemical characterization of unique Ia-bearing molecules found only in certain F_1 hybrids and recombinants and not in the parental haplotypes from which they were derived. These molecules appeared to contain Ia specificities coded for by the *I-E/I-C* subregion but in addition contained some product(s) of the *I-A* subregion. Such potential chain mixtures of Ia-bearing components could explain most of the GLϕ results, if recognition and response to GLϕ required these unique hybrid molecules. It could even explain the phenomenon of β-β complementation (2), if unique *I-A, I-A* hybrid molecules could also be found. However, certain aspects of the blocking studies are difficult to reconcile with such a model. In this case blocking with antisera directed against the *I-A* subregion products might not be expected, because such antisera failed to precipitate the unique hybrid molecules described by Jones et al. (47). On the other hand, her studies were conducted with anti-$I\text{-}A^k$ and $I\text{-}A^b$ sera whereas ours were done with anti-$I\text{-}A^d$ sera. Furthermore, we found the blocking of the proliferative response to GLϕ with anti-$I\text{-}A^d$ sera quite difficult in that only a few sera out of many tested worked, and adsorption to remove the anti-$I\text{-}A^d$ antibodies from those sera only partially eliminated the blocking activity (4). Thus, further studies are required to definitely decide whether the F_1 hybrid molecules are formed in the cytoplasm as suggested by Jones et al. (47) or on the cell surface as we have suggested (Fig. 2).

Summary

The immune response (*Ir*) to the random copolymer GLϕ depends upon the function of two *Ir* genes, *Ir-GLϕ-β[β]* and *Ir-GLϕ-α[α]*, mapped to the *I-A* and *I-E/C* subregions of the major histocompatibility complex, respectively. In this paper, the

site(s) of expression of the products of these two *Ir* genes was examined by evaluating T-lymphocyte proliferative responses of bone marrow radiation chimeras. Chimeras were created in $[\alpha^+\beta^- \times \alpha^-\beta^+]F_1$ responder mice by lethal irradiation and reconstitution with a mixture of bone marrow cells from both parental strains. These chimeras failed to respond to GLϕ, although they were capable of responding to the much weaker antigens, (T,G)-A—L, TEPC-15, pigeon cytochrome *c*, and (H,G)-A—L. This failure to respond to GLϕ was shown not to be the result of a cryptic mixed lymphocyte reaction, as similar chimeras created in $(\alpha^+\beta^+ \times \alpha^-\beta^+)F_1$ mice responded well to GLϕ, although they possessed almost the same potential histoincompatibility. Furthermore, the lack of response to GLϕ could not be attributed to a general failure of the two parental cell types in the chimeras to collaborate with each other, as each chimeric parental cell type could respond to dinitrophenyl conjugated ovalbumin presented on nonimmune spleen cells from the other parent. Thus, the failure of low responder parental into F_1 high responder chimeras to generate an immune response to GLϕ suggests that immune competence for this antigen requires at least one cell type in the immune system to express gene products of both the *Ir-GLϕ-α* and *-β* genes, i.e. one cell must be of high responder genotype.

That the antigen-presenting cell is one such cell type was shown by experiments in which GLϕ-primed T lymphocytes from responder F_1 mice were stimulated with antigen bound to nonimmune spleen cells. Only spleen cells from responder F_1 and recombinant mice could present GLϕ. Neither of the two complementing nonresponder parental spleen cell populations, either alone or mixed together, could present GLϕ, although both could present purified protein derivative of tuberculin. This was shown to be the case for T cells positively selected in vitro as well as freshly explanted T cells. Thus, both *Ir-GLϕ-α* and *Ir-GLϕ-β* gene products must be expressed in the same antigen-presenting cell to generate a T-lymphocyte proliferative response to GLϕ. The implications of these findings for models of two gene complementation are discussed.

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