CONTRIBUTION OF DIFFERENT CELL TYPES TO THE GENETIC CONTROL OF IMMUNE RESPONSES AS A FUNCTION OF THE CHEMICAL NATURE OF THE POLYMERIC SIDE CHAINS (POLY-L-PROLYL AND POLY-DL-ALANYL) OF SYNTHETIC IMMUNOGENS*

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Cellular aspects of genetic regulation of immune responsiveness in mice for two synthetic polypeptide immunogens built on multichain polyproline have recently been investigated by a limiting dilution analysis of lymphoid cell populations in high and low responder mouse strains (1–3). These studies demonstrated a direct correlation between the numbers of immunogen-stimulated antigen-sensitive units detected in the spleens of immunized and unimmunized donors and the genetically determined immune response potential of a given mouse strain (1, 2). Furthermore, in the case of poly-L-(Phe, Glu)-poly-L-Pro--poly-L-Lys, denoted (Phe, G)-Pro--L, the strain-dependent cell frequency differences observed in the spleens were also reflected in the bone marrow, but not in the thymus or thymus-derived populations of relevant immunocytes (3). This was the case irrespective of whether the cellular analysis of immune responsiveness was determined for the specificity whose response potential is *H-2* linked, i.e. (Phe, G), or for the Pro--L specificity, whose response potential is not linked to the *H-2* region (3–5).

As (Phe, G)-Pro--L contains two immunopotent regions which elicit antibodies of distinct specificities and the immune response potentials of these two regions are controlled by separate genes (5), it could be argued that cellular studies using this polypeptide might represent a more complex situation than experiments in which poly-L-(Tyr, Glu)-poly-L-Pro--poly-L-Lys, denoted (T, G)-Pro--L, or poly-L-(Phe, Glu)-poly-DL-Ala--poly-L-Lys, denoted (Phe, G)-A--L, were investigated. Furthermore, the fact that (Phe, G)-A--L is built on a backbone of multichain poly-DL-alanine, whereas the branched polymeric side chains of (T, G)-Pro--L consist of poly-L-proline, provides an opportunity for comparing cellular aspects of genetic control of immune response as a function of the chemical structure of the immunogen. The genes controlling immune responsiveness to the A--L series of polypeptides and to the Pro--L specificity have been designated *Ir-1* and *Ir-3*, respectively (6).

The present report describes experiments in which spleen, marrow, and

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thymus cell–limiting dilutions were compared between high and low responder mouse strains for (Phe, G)-A--L. It also extends the previously reported finding that genetic control of immune responsiveness to (T, G)-Pro--L could be accounted for by the numbers of splenic antigen-sensitive units stimulated in SJL and DBA/1 mice, to thymus and marrow cell dilution studies. For (Phe, G)-A--L, eighteen times as many units of immune response were detected in the spleens of immunized DBA/1 high responder as in those of SJL low responder donor mice. Limiting dilution studies using thymocytes and marrow cells indicated that the genetic control of immune response for (T, G)-Pro--L (which is expressed mainly in the formation of anti–Pro--L antibodies and is not linked to *H-2*) is reflected only in the number of relevant marrow cells stimulated, but apparently not in the thymocyte population. In contrast, for the *H-2*–linked (Phe, G)-A--L response (essentially only anti–[Phe, G]), both bone marrow and thymus cells were demonstrated to be important in expression of the genetic regulation of immune response potential.

Materials and Methods

Mice.—Inbred SJL and DBA/1 mice of both sexes, 9–11 wk of age were used as recipients, whereas females of the same age were used as donors. Both mouse strains were obtained from the Experimental Animal Unit, The Weizmann Institute of Science.

Immunization and Assay.—The immunogens used were poly-L-(Tyr, Glu)-poly-L-Pro--poly-L-Lys, abbreviated (T, G)-Pro--L 706, and poly-L-(Phe, Glu)-poly-DL-Ala--poly-L-Lys 223, abbreviated (Phe, G)-A--L. The description, synthesis, characterization, and immunogenicity of these polypeptides have been reported (4, 5, 7–9). The methods for immunization of donor and recipient animals have been published elsewhere (1–3). The same polypeptides used for immunization were also used for assaying the sera of recipients. Sheep erythrocytes were formalinized, tanned, and coated either with (T, G)-Pro--L or with (Phe, G)-A--L (10), and passive microhemagglutination tests were performed using twofold dilutions of antisera as described elsewhere (1–3, 11). The hemagglutination assays were performed on the sera from individual recipient mice 12–14 days after immunization, at the time of maximum antibody titers (1).

Irradiation and Cell Transfers.—Recipient mice to be injected with lymphoid cells were exposed to 800 R of whole body X-irradiation (250 kv peak, 15 ma, with 0.5 mm of Cu and 1.0 mm of Al added filtration, source-to-target distance 50 cm, and exposure of 60 R/min) in a rotating Lucite chamber. Spleen, bone marrow, and thymus cell suspensions were prepared, counted, diluted, and injected as previously described (1–3).

Experimental Design and Statistical Methods.—The Poisson model was used to describe the theoretical probability that a given inoculum of donor-derived cells would generate a detectable titer (at a serum dilution greater than that obtained in irradiated control mice) of anti-(T, G)-Pro--L or anti-(Phe, G)-A--L antibodies in the sera of irradiated recipients (3). Limiting inocula of marrow cells were injected along with an excess of 10^8 or 2×10^8 thymocytes, whereas limiting numbers of thymus cells were mixed with a nonlimiting number of 2×10^7 marrow cells before transplantation. This was done to ensure that the frequencies of immune responses obtained would depend mainly on the number of limiting cells injected.

RESULTS

Frequency of Immune Responses to (Phe, G)-A--L in Syngeneic Recipients Injected with Graded Numbers of Spleen Cells from Immunized or Unimmunized

DBA/1 and SJL Donors.—In repeated experiments a total of 69 DBA/1 and 106 SJL irradiated recipient mice were injected with graded numbers ($5 \times 10^5 - 4 \times 10^7$) of spleen cells pooled from immunized syngeneic donors and were simultaneously immunized with (Phe, G)-A--L. The results presented in Table I and Fig. 1 indicate an increase in the fraction of sera positive for (Phe, G)-A--L as the number of spleen cells injected was increased. The experimental frequency distribution of responses followed the expected Poisson pattern for both mouse strains. The probability values (based on the Poisson model and maximum likelihood method [12]) that 10^6 spleen cells would contain one unit of immune

TABLE I

Percentage of Positive Sera in Irradiated DBA/1 and SJL Recipients 12 Days after Injection of (Phe, G)-A--L and Graded Numbers of Syngeneic Spleen Cells from Immunized Donors

Mouse strain	No. of cells transplanted ($\times 10^6$)	Fraction of positive sera*	Percentage of positive sera	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times 10^{-6}$)‡
			%		
DBA/1	0.5	3/14	21		
	1	6/14	43	0.63	1/1.6
	2	11/15	73	(0.56–0.71)§	(1/1.4–1/1.8)§
	4	15/16	93		
	8	10/10	100		
SJL	1	0/14	0		
	2	2/10	20	0.036	1/28
	4	5/21	24		
	8	6/19	32	(0.032–0.041)§	(1/24–1/31)§
	20	11/22	50		
	40	12/20	60		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.

‡ Probability value estimates were based on the Poisson model.

§ 95% confidence intervals shown in parentheses.

response potential were estimated to be 0.63 and 0.036 for high responder DBA/1 and low responder SJL spleen cells, respectively. The corresponding limiting precursor cell frequencies were estimated at one per 1.6×10^6 and one per 28×10^6 spleen cells. Thus, a significant 18-fold difference was observed in the relative abilities of spleen cells from immunized SJL and DBA/1 donors to generate an immune response to (Phe, G)-A--L.

Differences in the frequencies of splenic antigen-sensitive units associated with genetically controlled immune responsiveness have also been reported for spleen cells from unimmunized donors using the Pro--L series of immunogens, although these differences were usually less pronounced than those observed using immunized donors (1, 2). It was of interest to establish whether the differences observed between the secondary responses of SJL and DBA/1

mice to (Phe, G)-A--L would also be seen in the primary response. Groups of DBA/1 and SJL recipients were irradiated and injected with graded inocula (1×10^6–4×10^7) of cells harvested from pooled spleens of unimmunized

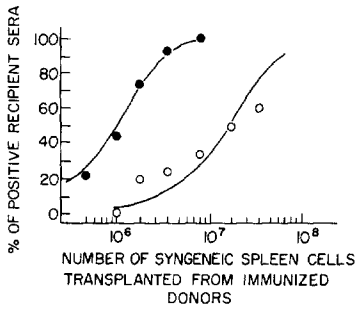

FIG. 1. Percentage of anti-(Phe, G)-A--L-positive sera in SJL (symbol ○) and DBA/1 (symbol ●) recipients after irradiation and injection of (Phe, G)-A--L and graded numbers of spleen cells from immunized syngeneic donors.

TABLE II

Percentage of Positive Sera in Irradiated DBA/1 and SJL Recipients 14 Days after Injection of (Phe, G)-A--L and Graded Numbers of Syngeneic Spleen Cells from Unimmunized Donors

Mouse strain	No. of cells transplanted ($\times 10^6$)	Fraction of positive sera*	Percentage of positive sera*	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times 10^{-6}$)‡
			%		
DBA/1	1	2/8	25		
	2	6/12	50	0.25	1/4.0
	4	8/13	62		
	8	10/13	77	(0.17–0.37)§	(1/2.7–1/5.9)§
	20	20/23	87		
SJL	1	1/11	9		
	2	2/7	29		
	4	5/12	42	0.046	1/29
	8	6/17	35		
	20	11/18	61	(0.032–0.066)§	(1/15–1/31)§
	40	9/13	69		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.
‡ Probability value estimates were based on the Poisson model.
§ 95% confidence intervals shown in parentheses.

syngeneic donors. The results of these dilution experiments are shown in Table II and Fig. 2. The probability values and their corresponding limiting cell frequencies were 0.25 and one per 4×10^6 for the DBA/1 spleen cells, and 0.046 and one per 29×10^6 for SJL spleens, respectively. These differences were

statistically significant ($P = 0.05$), although less pronounced than those obtained for a secondary response. Thus, expression of the genetic control of immune responsiveness at the cellular level to an immunogen built on multi-

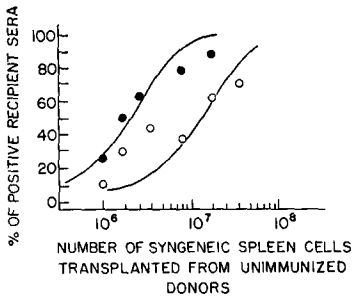

FIG. 2. Percentage of anti-(Phe,G)-A--L-positive sera in SJL (symbol ○) and DBA/1 (symbol ●) recipients after irradiation and injection of (Phe,G)-A--L and graded numbers of spleen cells from unimmunized syngeneic donors.

TABLE III

Cell-to-Cell Intraction for (T, G)-Pro--L and (Phe, G)-A--L Immune Responses in SJL and DBA/1 Mice

Immunogen	Mouse strain	Thymocytes injected	Marrow cells injected	Fraction of positive sera*	Percentage of positive sera*
					%
(T, G)-Pro--L	SJL	7.5×10^7	0	0/9	0
		0	1.5×10^7	2/24	8
		7.5×10^7	1.5×10^7	16/16	100
(T, G)-Pro--L	DBA/1	7.5×10^7	0	0/9	0
		0	1.5×10^7	2/11	18
		7.5×10^7	1.5×10^7	8/11	73
(Phe, G)-A--L	SJL	1×10^8	0	1/6	16
		0	2×10^7	3/16	19
		1×10^8	2×10^7	9/11	82
(Phe, G)-A--L	DBA/1	1×10^8	0	0/4	0
		0	2×10^7	4/24	17
		1×10^8	2×10^7	14/14	100

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.

chain poly-DL-alanine, i.e. (Phe, G)-A--L, is correlated with the relative numbers of immune response units detected in the spleens of unimmunized and immunized high and low responder mice.

Thymus-Bone Marrow Cell Cooperation for (T, G)-Pro--L and (Phe, G)-A--L

Immune Responses.—In order to determine whether the genetic controls of immune responsiveness to (T, G)-Pro--L or (Phe, G)-A--L are expressed in a population of thymic helper cells or in marrow precursors of immunocytes, thymus–marrow cell cooperation experiments were performed for both immunogens in SJL and DBA/1 mice. As shown in Table III, responses to (T, G)-Pro--L were detected in a high proportion of recipients only when both thymus and marrow cells were injected. Responses were observed in a small fraction (8–18%) of recipients injected with marrow cells alone. Immune

TABLE IV

Percentage of Positive Sera in Irradiated SJL and DBA/1 Recipients 14 Days after Injection of (T, G)-Pro--L, 10^8 Thymocytes, and Graded Numbers of Marrow Cells from Syngeneic Donors

Mouse strain	No. marrow cells transplanted ($\times 10^6$)	Fraction of positive sera*	Percentage of positive sera*	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times 10^{-6}$)‡
			%		
SJL	0.1	2/13	15		
	0.25	5/13	38		
	0.5	6/12	50	0.85	1/1.2
	1	8/12	67		
	2	7/10	70	(0.54–1.35)§	(1/0.74–1/1.8)§
	8	7/8	88		
	16	16/16	100		
DBA/1	1	3/13	23		
	2	8/25	32	0.10	1/10
	4	5/14	36		
	8	6/13	46	(0.065–0.15)§	(1/6.7–1/15)§
	16	8/11	73		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.
‡ Probability value estimates were based on the Poisson model.
§ 95% confidence intervals shown in parentheses.

responses to (Phe, G)-A--L were also most frequent (82–100%) in animals injected with thymocytes and marrow cells, although a small percentage of responses (16–19%) was obtained when only one cell type was injected. These low response frequencies could have been due to the contamination of bone marrow or thymus tissue with the complementary cell type. The addition of thymocytes markedly enhanced the responses of marrow cells in both high and low responder strains to (T, G)-Pro--L and (Phe, G)-A--L. The immune responses of antigens considered to be thymus-independent (by presently accepted criteria in the mouse) should not be lower in the absence of thymocytes or thymus-derived cells than when these helper cells have been added to the marrow-derived component of the immune system (13). Therefore, the responses to both synthetic polypeptides appear to be thymus-dependent in the

sense that cell-to-cell interaction between thymocytes and marrow cells was demonstrated.

Frequency of Anti-(T, G)-Pro--L Responses in SJL and DBA/1 Recipients Injected with Dilutions of Syngeneic Bone Marrow or Thymus Cells.—Irradiated SJL and DBA/1 mice were injected with graded inocula of marrow cells ($1 \times 10^5 - 1.6 \times 10^7$) mixed with 10^8 thymocytes from syngeneic donors and immunized with 10 μg of (T, G)-Pro--L 24 hr later. The frequency of immune responses detected in the sera of recipients are shown in Table IV and Fig. 3 A, along with the probability values and estimated precursor cell frequencies. The data followed the expected Poisson pattern, although some divergence was observed at the lower cell inocula. An eightfold greater number of marrow precursors was estimated for the SJL high responder than for the DBA/1 low responder donors. This finding is similar to the results reported for spleen cells

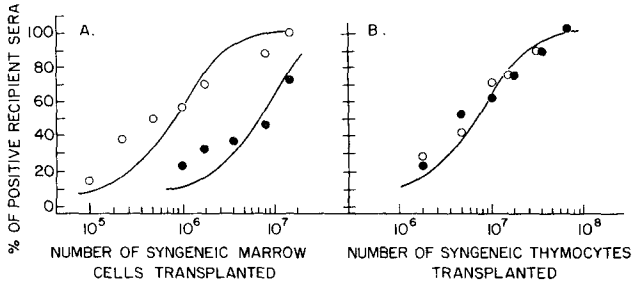

FIG. 3. Percentage of anti-(T,G)-Pro--L-positive sera in SJL (symbol ○) and DBA/1 (symbol ●) recipients after irradiation and injection of (T,G)-Pro--L and: (*A*) 10^8 thymocytes with graded numbers of marrow cells; or (*B*) 2×10^7 marrow cells with graded numbers of thymocytes. All cell combinations were injected into syngeneic hosts.

using this immunogen (1), and for spleen and marrow cells using (Phe, G)-Pro--L (2).

The limiting dilution results obtained for thymocytes in the presence of a nonlimiting number of 2×10^7 syngeneic marrow cells are summarized in Table V and Fig. 3 B. The frequency data obtained conformed to the expected Poisson model for both mouse strains. Identical frequencies of helper cells were detected (one per 13×10^6 thymocytes) in high and low responder donors, in contrast to the spleen and marrow cell dilution studies. This finding is similar to the results reported for thymocytes responsive to (Phe, G)-Pro--L (3), and indicates that the cellular lesion associated with the *Ir-3* genetic defect is not expressed in the relative number of thymocytes responding to (T, G)-Pro--L.

Frequency of Anti-(Phe, G)-A--L Responses in SJL and DBA/1 Recipients Injected with Dilutions of Syngeneic Bone Marrow or Thymus Cells.—The results obtained from transfer of graded inocula of marrow cells along with 10^8 thymocytes in irradiated syngeneic recipients are shown in Table VI and Fig.

4 A. About five times as much immunocompetent activity was detected per 10^6 cells in the marrow of DBA/1 high responders than in the marrow of SJL low responders. This is the reverse of the situation seen for (T, G)-Pro--L (see above section), for which SJL are high responders and DBA/1 are the low responders.

Limiting dilution experiments using graded numbers of thymocytes in the presence of 2×10^7 syngeneic marrow cells are summarized in Table VII and Fig. 4 B. In contrast to the results of thymus cell dilutions which were obtained

TABLE V

Percentage of Positive Sera in Irradiated SJL and DBA/1 Recipients 14 Days after Injection of (T, G)-Pro--L, 2×10^7 Marrow Cells, and Graded Numbers of Thymocytes from Syngeneic Donors

Mouse strain	No. thymocytes transplanted ($\times 10^6$)	Fraction of positive sera*	Percentage of positive sera*	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times 10^{-6}$)‡
			%		
SJL	2	4/14	29		
	5	5/12	42	0.078	1/13
	10	12/17	71		
	20	14/19	74	(0.054–0.11)§	(1/9.1–1/19)§
	40	15/17	88		
DBA/1	2	3/13	23		
	5	7/13	54		
	10	6/10	60	0.078	1/13
	20	11/15	73	(0.056–0.11)§	(1/9.1–1/18)§
	40	15/17	88		
	60	7/7	100		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.

‡ Probability value estimates were based on the Poisson model.

§ 95% confidence intervals shown in parentheses.

for (T, G)-Pro--L (see above) and for (Phe, G)-Pro--L (3), clear differences were seen between the response frequencies detected for DBA/1 and SJL donors. From 6–8 $\times 10^7$ SJL low responder thymocytes had to be injected in order to obtain detectable responses in 2/3 of the recipients. The proportion of responses detected as a function of thymus cell number generally followed the Poisson pattern, and the estimated helper cell frequency was one per 6×10^7 thymocytes. These values are considerably below the frequency of thymocytes relevant for generating responses to (T, G)-Pro--L and to (Phe, G)-Pro--L from high or low responder donors. Results of limiting dilutions of thymocytes from DBA/1 high responder donors to (Phe, G)-A--L did not conform to the prediction of the Poisson model (see Table VII and Fig. 4 B). The percentage of positive responses detected varied from 20% to 90% over a 500-fold range of

TABLE VI

Percentage of Positive Sera in Irradiated DBA/1 and SJL Recipients 14 Days after Injection of (Phe, G)-A--L, 10^8 Thymocytes, and Graded Numbers of Marrow Cells from Syngeneic Donors

Mouse strain	No. marrow cells transplanted ($\times 10^6$)	Fraction of positive sera*	Percentage of positive sera*	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times 10^{-6}$)‡
			%		
DBA/1	0.25	1/6	17		
	0.5	6/15	40	0.50	1/2.0
	1	7/15	47		
	2	13/21	62	(0.43–0.59)§	(1/1.7–1/2.3)§
	4	8/12	75		
	8	10/11	91		
SJL	1	1/11	9		
	2	11/33	33	0.11	1/9.1
	4	11/26	42		
	8	6/11	54	(0.087–0.14)§	(1/7.1–1/11)§
	12	8/15	53		
	20	21/24	87		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.
‡ Probability value estimates were based on the Poisson model.
§ 95% confidence intervals shown in parentheses.

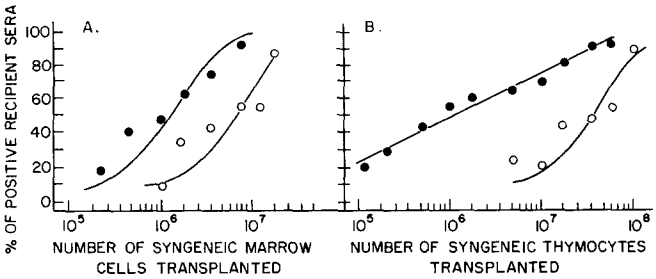

FIG. 4. Percentage of anti-(Phe,G)-A--L-positive sera in SJL (symbol ○) and DBA/1 (symbol ●) recipients after irradiation and injection of (Phe,G)-A--L and: (*A*) 10^8 thymocytes with graded numbers of marrow cells; or (*B*) 2×10^7 marrow cells with graded numbers of thymocytes. All cell combinations were injected into syngeneic hosts.

thymus cell inocula. Therefore, the DBA/1 data shown in Fig. 4 B has been eye fitted to a straight line rather than to a curve describing the Poisson model. The probability value and the corresponding precursor cell frequency cannot be accurately estimated using the Poisson model and the maximum likelihood method. Nevertheless, by considering the frequency data obtained from 10^5 to 2×10^6 injected thymus cells and from 5×10^6 to 6×10^7 transferred thymo-

cytes independently, it is possible to obtain an upper and lower extreme within which the frequency of relevant helper cells should be found. This range was estimated to be one per 1.5 to 5 $\times$ 10^6 thymocytes, and suggests that DBA/1 high responder thymocytes are between 12 and 40 times as effective in generating a response to (Phe, G)-A--L as an equal number of thymus cells from SJL low responders.

TABLE VII

Percentage of Positive Sera in Irradiated DBA/1 and SJL Recipients 14 Days after Injection of (Phe, G)-A--L, 2 $\times$ 10^7 Marrow Cells, and Graded Numbers of Thymocytes from Syngeneic Donors

Mouse strain	No. thymocytes transplanted ($\times$ 10^6)	Fraction of positive sera*	Percentage of positive sera*	Probability of positive sera per 10^6 cells‡	Precursor cell frequency ($\times$ 10^{-6})‡
			%		
DBA/1	0.12	3/15	20		
	0.25	4/14	29		
	0.5	5/12	42		
	1	12/22	55		
	2	12/20	60		
	5	9/14	64	0.20–0.67§	1/1.5–1/5§
	10	23/33	70		
	20	8/10	80		
	40	8/9	89		
	60	9/10	90		
SJL	5	4/17	23		
	10	2/10	20		
	20	8/18	44	0.017	1/60
	40	7/15	57	(0.012–0.030)‖	(1/34–1/88)‖
	60	8/15	53		
	100	21/24	87		

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera.

‡ Probability value estimates were based on the Poisson model.

§ Since it was not possible to calculate these parameters for DBA/1 thymocytes due to the lack of adherence to Poisson statistics, the values shown indicate upper and lower extremes.

‖ 95% confidence intervals shown in parentheses.

Since the frequency of SJL thymocytes was found to be only one per 6 $\times$ 10^7 the marrow cell dilution experiments for this strain may not have included an excess of helper cell activity. Therefore, a dilution experiment of SJL marrow cells in the presence of 2 $\times$ 10^8 syngeneic thymocytes was made to verify that the marrow dilutions with 10^8 low responder thymocytes were correct. Dilutions of 2 $\times$ 10^6 and 4 $\times$ 10^6 SJL marrow cells with 2 $\times$ 10^8 syngeneic thymocytes generated anti-(Phe, G)-A--L responses in 28% and 40% of the recipients,

respectively. This is comparable to the results obtained for equal numbers of marrow cells transferred with 10^8 thymocytes.

Limiting Dilutions using Allogeneic Thymus and Marrow Cell Mixtures from High and Low Responder Donors.—The dilution experiments described above and in preceding reports (1–3) have involved only syngeneic cell transfers in order to avoid possible histoincompatibility difficulties. However, the transfer of a cell mixture containing thymocytes and marrow cells from donors differing in their immune response potential to (Phe, G)-A--L would serve as an important verification of the syngeneic data, provided the SJL and DBA/1 cell combinations are shown to be functionally immunocompetent. The functional interaction of SJL marrow cells and DBA/1 thymocytes in irradiated DBA/1 hosts has been demonstrated for (Phe, G)-Pro--L, since the response to the

TABLE VIII

Percentage of Positive Sera in Irradiated DBA/1 Recipients 14 Days after Injection of (Phe, G)-A--L and Thymus and Marrow Cells from SJL and/or DBA/1 Donors

Thymocytes injected	Marrow cells injected	Fraction of positive sera*	Percentage of positive sera*
			%
7×10^7 DBA/1	4×10^6 DBA/1	6/9	67
7×10^7 DBA/1	4×10^6 SJL	4/11	36
7×10^7 DBA/1	1×10^6 SJL	0/5	0
1×10^7 DBA/1	2×10^7 DBA/1	13/16	81
1×10^7 SJL	2×10^7 SJL	11/24	46

* Donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera

Pro--L specificity, to which SJL is a high responder, was equal to that obtained using SJL donors and recipients exclusively (14). Optimal cell-to-cell interaction between donors of the SJL and DBA/1 strains has also been demonstrated for hen egg white lysozyme (Mozes and Shearer, unpublished data). Thus, results obtained using marrow-thymus cell transfers from SJL low responder and DBA/1 high responder donors in irradiated DBA/1 recipients should generate response patterns reflecting the genetically determined immune response potential of the cells.

The results shown in Table VIII, using cell combinations containing an excess of DBA/1 thymocytes and limiting numbers of either DBA/1 or SJL marrow cells, indicate that the genetic defect is reflected in the marrow cell numbers, since 4×10^7 and 1×10^7 SJL marrow cells generated lower response frequencies than an equal number of marrow cells from DBA/1 donors (compare with upper portion of Table VI). Furthermore, the fact that these limiting inocula of SJL marrow cells in the presence of excess high responder DBA/1 thymocytes generated response frequencies not higher than those obtained using the

same marrow dilutions in the presence of 10^8 low responder SJL thymocytes indicates that the lower frequency of SJL marrow cells detected is not an artifact due to the reduced frequency of helper cell activity from SJL donors (compare with lower portion of Table VI).

The dilution data of Table VIII using a limiting inoculum of 10^7 DBA/1 or SJL thymocytes with a nonlimiting number of high responder DBA/1 marrow cells verifies the syngeneic results that this genetic defect is also reflected in the frequency of relevant thymocytes.

DISCUSSION

The conversion of low responder mice and guinea pigs to high responders by the transfer of lymphoid cells from high responder donors has demonstrated that genetic control of immune response is expressed by at least one population of immunocompetent cells (15–17). A number of experiments have been performed with the objective of establishing whether the functional cell type(s) responsible for the low- or nonresponder state belong to a population of immunocytes possessing helper function (i.e. relevant thymus-derived cells in the mouse) and/or to a separate category of immunocytes commonly referred to as marrow-derived cells, which are the ancestors of antibody-producing cells (3, 6, 18–20). Before questioning which cell type is responsible for low responsiveness, however, it would be helpful to characterize the cellular lesion(s) by asking: "How does the immune system of the low responder animal differ from that of the high responder at the cellular level in tissues which normally contain all the functional cells necessary for immunocompetence?" At the precursor cell level, the differences between high and low responders could reside in: (*a*) the relative numbers of immunogen-activated antigen-sensitive units; (*b*) the proliferative capacity of a given cell population after immunogenic stimulation; (*c*) the number of receptors per cell; (*d*) the quality of cell receptors (e.g. affinity); and/or (*e*) some aspects of cell-to-cell interaction. Results of this and other studies have demonstrated that the genetically controlled immune response potentials of SJL and DBA/1 mice to (T, G)-Pro--L (1), to the Pro--L– and (Phe, G)–specific responses of (Phe, G)-Pro--L (2), and to (Phe, G)-A--L (see Tables I and II; Figs. 1 and 2) are all reflected in the numbers of relevant immunocompetent precursors stimulated in the spleens of either immunized or unimmunized donors. These detected differences in precursor frequency could represent the real defect(s), and thereby indicate actual total cell numbers, or they could be the result of any of the other above mentioned possibilities, with the exception of proliferation of thymocytes responsive to (Phe, G)-Pro--L, which was shown not to be defective in the low responders (3). The possibility of a defect existing in the proliferative capacity of thymus-derived cells after immunization with (Phe, G)-A--L has not yet been examined. However, it is unlikely to be the only defect, since the low responsiveness of SJL mice to (Phe, G)-A--L can be attributed to both thymus and marrow cell

populations (Tables VI–VIII; Fig. 4). Irrespective of whether the precursor cell frequency differences observed between high and low responder animals represent the sole phenotypic abberation or whether they are only the manifestation of a more complex phenomenon, the observation that the spleens of the high responder strains exhibit from 8- to 24-fold greater immunocompetent activity has provided a useful approach for comparing these genetically determined lesions in thymus and marrow cell populations (3, 14).

The results obtained using cell dilutions for the (Phe, G)-A--L immunogen, which is known to lead only to an anti-(Phe, G) response, indicated differences of seven- and seventeenfold, respectively, for spleens of unimmunized and immunized high and low responder donors, (Tables I and II; Figs. 1 and 2). It is noteworthy that a significant increase in the frequency of precursors was obtained as a result of preimmunizing the DBA/1 high responders, whereas no change was detected in precursor frequency by preimmunizing the SJL low responder strain. Similarly, preimmunization had no effect on the DBA/1 low responders to (T, G)-Pro--L (1) which leads only to anti–Pro--L response (4), and on the SJL low responders to the (Phe, G) immunopotent region of (Phe, G)-Pro--L (2). The only case in which an increase in relevant splenic precursor cell frequency was obtained in a low responsive situation was for the DBA/1 response to the Pro--L portion of (Phe, G)-Pro--L. In this experiment the enhancement of the low anti–Pro--L response appeared to be due to the presence of a second (Phe, G) immunopotent region, to which DBA/1 mice are high responders. The presence of a second immunopotent area, however, does not necessarily ensure an increase in low responder spleen cell frequency as a result of immunization, since the low (Phe, G) secondary response potential of SJL mice was not enhanced by primary immunization with (Phe, G)-Pro--L (2). Possibly, a helper role may be envisaged for the memory of low responders controlled by *Ir-3* gene but not by *Ir-1* gene.

Similar to the results reported for (Phe, G)-Pro--L (3), as seen in Table III, cell-to-cell interaction between thymocytes and marrow cells is required for the generation of immune responses specific for (T, G)-Pro--L and for (Phe, G)-A--L in irradiated recipients immunized with these immunogens in complete Freund's adjuvant. Although immune responses were detected in a small percentage of recipients injected with thymocytes or marrow cells alone, these percentages were much lower than those observed using the cell mixtures, and may indicate contamination of the bone marrow and thymus, respectively, with an occasional helper cell or antibody-forming cell precursor.

The results obtained for (T, G)-Pro--L using limiting numbers of either marrow cells or thymocytes in the presence of a nonlimiting number of the complementary cell type are similar to the results reported for (Phe, G)-Pro--L (3). An eightfold lower frequency of relevant precursors was observed in the marrow of SJL low responders than in the marrow of high responder DBA/1 donors (Table IV, Fig. 3 A). In contrast, no differences were detected in the

frequencies of thymic helper cells required to generate (T, G)-Pro--L–specific responses in the high and low responder strains (Table V, Fig. 3 B). These cell frequency differences detected only in spleen (1) and bone marrow cell suspensions might reflect actual differences in antibody-forming cell precursors, but in view of recent results obtained with poly-alanine–poly-uridine (polyA-polyU) and peritoneal exudate cells (14, 21), they probably reflect an *Ir-3* genetic defect involving cell-to-cell interaction.

The results obtained with the bone marrow– and thymus cell–limiting dilutions for the (Phe, G)-A--L immunogen (controlled by *Ir-1* gene) are different from those seen for (T, G)-Pro--L (controlled by *Ir-3* gene), or for the Pro--L–specific (*Ir-3*) and (Phe, G)–specific (*Ir-1*) responses of (Phe, G) Pro--L (3). In contrast to the results obtained with these immunopotent specificities characteristic for the polyproline-based immunogens, we have used in these studies, dilution experiments for the low (Phe, G)–specific response on (Phe, G)-A--L in SJL mice indicated a clear defect in the population of thymic helper cells (Table VII, Fig. 4 B), as well as in the marrow population (Table VI, Fig. 4 A). Dilutions of SJL thymocytes conformed to the predictions of the Poisson model, whereas those from DBA/1 donors did not (see Fig. 4 B). This non-Poisson curve suggests not only that there are more thymus cells relevant for generating (Phe, G)-A--L responses in the DBA/1 than in the SJL strain (compare the 5×10^6 to 6×10^7 inocula of Table VII), but that the helper cell activity of DBA/1 thymocytes is more efficient than that of the SJL low responder strain. Strain-dependent variations involving lack of or adherence to a Poisson pattern generated by dilutions of marrow cells reactive with sheep erythrocytes have been reported (22, 23). The meaning of such differences is not fully understood, although for erythrocyte immunogens they appear to be associated with steps in cell differentiation which can be induced by immunization (24). In the present study the frequency of detected immune responses was not a function of DBA/1 thymocyte number and this might suggest some degree of thymus independence, or involvement of a humoral factor which might not be related directly to thymocyte number.

The finding that thymus cell suspensions from SJL donors contain only one detectable helper cell per 6×10^7 thymocytes could indicate that the data obtained for low responder marrow cell dilutions represent an artifact, since 10^8 syngeneic thymus cells would not contain a real excess of helper cell activity. However, the observation that the same frequency of responses was obtained with limiting dilutions of SJL marrow in the presence of 10^8 SJL, 2×10^8 SJL, or 10^8 DBA/1 thymocytes verifies that the lower precursor cell frequency detected in SJL bone marrow is not the result of an insufficient number of thymocytes (compare Tables VI and VII with Table VIII for equivalent inocula of limiting marrow cells mixed with 10^8 SJL or DBA/1 thymocytes).

The use of limiting dilutions for studying the cellular aspects of genetic control of immune responsiveness offers the advantage that transfers of syn-

geneic cell mixtures can be used, and thus avoids possible cell-to-cell or donor-host histocompatibility problems which could complicate interpretation of the results (18). In other words, when transfers of cell mixtures from donor mice differing in their *H-2* type as well as their immune response potential are used, one has to be certain that any low response observed is due to the genetic lesion, and is not the result of suboptimal cell-to-cell interaction due to *H-2* differences.

Thymus-marrow cell cooperation across an *H-2* barrier and the ability of these cell mixtures to generate immune responses in irradiated allogeneic or F_1 hosts appears to be a function of the particular mouse strains used (25). Published (14) and unpublished results from this laboratory, using (Phe, G)-Pro--L and lysozyme as immunogens, have indicated that combinations of thymocytes and marrow cells from SJL and DBA/1 donors in irradiated recipients of either parental strain generated responses identical to those from syngeneic combinations of equal cell numbers. The results summarized in Table VIII, using allogeneic cell mixtures, in which the donor of only one cell type was a low responder, are similar to the data obtained using syngeneic cell mixtures. This indicates that the low response potential of SJL mice to (Phe, G)-A--L is reflected in the frequencies of both thymus and marrow cell populations relevant for responsiveness to this synthetic polypeptide, and verifies the results obtained using cell combinations from syngeneic donors.

From these investigations a series of patterns are beginning to emerge concerning the interrelationships among the molecular, cellular, and genetic aspects of immunity (Table IX). The *Ir-1* genetic lesion, which is linked to *H-2*, cannot be corrected by polyA-polyU treatment or by syngeneic peritoneal exudate cells, although the low responses controlled by both genes are enhanced by complexing these immunogens electrostatically to methylated bovine serum albumin (14). The *Ir-1* defect appears to involve immunological memory and may be related to carrier function, since it is not subject to amplification in the low responder strain by the presence of a second determinant on the immunogen to which that strain responds normally. The non-*H-2*-linked *Ir-3* genetic defect appears to be a less severe lesion than *Ir-1* in the sense that the nonimmunogen-specific treatments of polyA-polyU and peritoneal cells corrected the response of the low responder DBA/1 mice to a specific immunogen (14) and that the ability to elicit an enhanced secondary response is present, provided that the immunogen contains a second immunopotent determinant to which this strain responds normally (2). The enhancement of the Pro--L-specific response in DBA/1 mice to the high responder level by polyA-polyU or by increasing the number of syngeneic peritoneal cells suggests that the *Ir-3* lesion may not involve a defect in the actual number of Pro--L-specific immunocompetent precursors in spleen and bone marrow, since it is unlikely that these treatments would be immunogen-specific. It is possible that the *Ir-3* defect involves a step in cell-to-cell interaction which would be seen in the relative numbers of

splenic antigen-sensitive units and of marrow precursors of antibody-forming cells stimulated (14).

The *Ir-1* defect is reflected in the limiting cell populations present in spleen and bone marrow for both the Pro--L and A--L series of branched chain polypeptides, but is detected in relevant thymocyte numbers only when the (Phe, G) immunopotent determinant is carried on a poly-DL-alanine side chain (Table IX). This latter observation suggests that different populations of thymocytes

TABLE IX

Emerging Patterns of Genetic Control of Immune Responsiveness in SJL and DBA/1 Mice to Synthetic Polypeptides Built on Multichain Poly-L-Proline or on Multichain Poly-DL-Alanine

Immunogen studied: Response specific for:	(T, G)-Pro--L Pro--L	(Phe, G)-Pro--L Pro--L	(Phe, G)-Pro--L (Phe, G)	(Phe, G)-A--L (Phe, G)
	Genetic characterization of response*			
Low responder strain	DBA/1	DBA/1	SJL	SJL
High responder strain	SJL	SJL	DBA/1	DBA/1
Linked to *H-2*	No	No	Yes	Yes
Designation of gene	*Ir-3*	*Ir-3*	*Ir-1*	*Ir-1*
	Low responders enhanced by‡			
PolyA-polyU treatment	Yes	Yes	No	No
Syngeneic peritoneal cells	Yes	Yes	No	No
Methylated bovine serum albumin	Yes	Yes	Yes	Yes
	Ratio of precursor cells in high to low responder donors§			
Spleen, secondary response	24:1	7:1	12:1	18:1
Spleen, primary response	4:1	11:1	3:1	7:1
Marrow, primary response (excess thymus)	8:1	5:1	5:1	5:1
Thymus, primary response (excess marrow)	1:1	1:1	1:1	(12–40):1

* See references 5, 6, and 9.

‡ See references 14 and 21.

§ See references 1–3 and this study.

respond to the Pro--L and A--L polypeptides, and that only the population stimulated by A--L may be defective in SJL mice. The observation that the genetic defect to (Phe, G) in this strain is reflected only in the number of marrow precursors when these peptides are attached to poly-L-proline whereas the defect is reflected in the number of both marrow and thymus immunocompetent cells when (Phe, G) is attached to poly-DL-alanine, stresses the importance of the chemical structure of the entire immunogenic macromolecule in the expression of the genetic defects for a given specificity at the cellular level.

SUMMARY

Genetic regulation of immunological responsiveness was studied at the cellular level by comparing the limiting dilutions of immunocompetent cells from spleen, thymus, and bone marrow of high and low responders as a function of the poly-L-prolyl and poly-DL-alanyl side chains of two synthetic polypeptide immunogens. The spleens of immunized and unimmunized high responder DBA/1 mice were found to contain respectively, 18- and 7-fold more limiting precursor cells specific for (Phe, G)-A--L than the spleens of SJL low responder donors. These results, using a synthetic polypeptide built on multichain poly-DL-alanine, confirm the findings reported for polypeptides built on multichain poly-L-proline (1, 2), that there is a direct correlation between immune response potential and the relative number of immunocompetent precursors stimulated.

Cell cooperation between thymocytes and bone marrow cells was demonstrated for both (T, G)-Pro--L and (Phe, G)-A--L. Limiting dilutions of thymus and bone marrow cells in the presence of an excess amount of the complementary cell type indicated an eightfold lower number of detected (T, G)-Pro--L-specific precursors in DBA/1 (low responder) marrow when compared with SJL (high responder) marrow. No differences were observed in the frequency of relevant high and low responder thymocytes for the (T, G)-Pro--L immunogen. These results are similar to those reported for the (Phe, G)-Pro--L (3). In contrast to the cellular studies reported for the Pro--L series of immunogens, the marrow and thymus cell dilution experiments for (Phe, G)-A--L revealed genetically associated differences in both the marrow and thymus populations of immunocytes from high (DBA/1) and low (SJL) responders. In addition to a fivefold difference in limiting marrow cell precursors (similar to that seen in the Pro--L studies), a striking difference was observed between the helper cell activity of high responder DBA/1 and low responder SJL thymocytes. This difference was indicated by the observation that low responder thymocyte dilutions followed the predictions of the Poisson model, whereas dilutions of high responder thymocytes did not conform to Poisson statistics. Transfers of allogeneic thymus and marrow cell mixtures from DBA/1 and SJL donors confirmed the syngeneic dilution studies showing that the genetic defect of immune responsiveness to (Phe, G)-A--L is expressed at both the thymus and marrow immunocompetent cell level.

The parameters presently known for genetic control of immune responses specific for (Phe, G) (*Ir-1* gene) and for Pro--L (*Ir-3* gene) have been compared. The *Ir-1* and *Ir-3* genes are not only distinct by genetic linkage tests (to *H-2*) (5, 6, 9), but they are also seen to be different by cellular studies. Furthermore, expression of low responsiveness within a given cell population was shown to depend on the chemical structure of the whole immunogenic macromolecule.

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