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Cell transfer studies in a genetically controlled immune response+

Fetal liver cells from 14–17-day old mouse embryos of inbred strains which are genetic responders (C57BL/10) or nonresponders (CBA) to a branched synthetic polypeptide [(T, G)-A--L] were injected into reciprocal (nonresponder or responder) strain, lethally irradiated adult mice. These radiation chimeras were then tested for: (a) their ability to respond to (T, G)-A--L; (b) the allotype for specific anti-(T, G)-A--L antibody produced; and (c) the degree of tolerance for CBA (nonresponder) histocompatibility antigens of C57BL/10 (responder) cells taken from high responder chimeras.

Eleven high responder radiation chimeras had specific anti-(T, G)-A--L antibody, which appeared to be exclusively responder (C57BL/10) allotype. However, since all the high responder chimeras were predominantly C57BL/10 in total serum immunoglobulin allotype and lymphoid cell type, this result is not conclusive.

Six high responder chimeras had C57BL/10 spleen cells which were tolerant of CBA (nonresponder) histocompatibility antigens in the Simonsen discriminant spleen assay.

1. Introduction

The immune response in mice to a series of multichain synthetic polypeptide antigens, (T, G)-A--L, (H, G)-A--L, and (Phe, G)-A--L, is largely controlled by a single, autosomal, dominant and determinant-specific gene, termed "Ir-1" (immune response-1), which is closely linked to the major histocompatibility (H-2) locus in the IXth mouse linkage group [1]. The mechanism of action of this gene has not yet been elucidated. One possibility is that the inability to produce high antibody response in the low responder is due to a defect at the level of the antibody-producing cell. Two indirect pieces of evidence suggest that this is not the case. When low responders to (T, G)-A--L are immunized with this antigen coupled electrostatically to a highly charged immunogenic molecule (*i.e.*, methylated bovine serum albumin (MBSA)), the low responders produce a high response to (T, G)-A--L [2]. This finding, that the low responders are actually capable of producing antibodies to (T, G)-A--L if it is presented in the correct manner, suggests that the basic defect causing some strains to be low responders is probably not at the level of the antibody-producing cell. Electrostatic coupling of (T, G)-A--L to MBSA may enable the low responder to circumvent a defect and properly "process" (or recognize) (T, G)-A--L to induce high amounts of antibody production. An additional point supporting this concept is the fact that many low responders to (T, G)-A--L are high responders to immunization with (H, G)-A--L [1]. However, the anti-(H, G)-A--L antibodies produced by these mice also react strongly with (T, G)-A--L. The antigens do not, however, cross-immunize [2], but antibodies to either antigen cross-react well. Thus, immunization with (H, G)-A--L is another situation in which low responders to (T, G)-A--L can be shown to produce high titers of antibody capable of binding (T, G)-A--L.

Based on this information, the major defect in low responders may occur in some step in antigen processing or recognition prior to the involvement of the antibody-producing cell. Thymus-derived, antigen-reactive cells would be a conceivable site for such a defect [3].

If one assumes that the above hypothesis is correct, and that low-responder cells are indeed capable of producing high titered anti-(T, G)-A--L responses when the antigen is properly "processed" and "presented", then, in chimeric mice containing both low- and high-responder strain lymphoid cells, one might expect that only the high-responder, thymus-derived cells could interact effectively with antigen. However, if cell cooperation between cells from the two strains can occur [3], then the anti-(T, G)-A--L antibodies produced could be of both low- and high-responder strain immunoglobulin allotypes. If cell cooperation between cells of different strains could not occur, or if low-responder cells are unable to produce anti-(T, G)-A--L antibodies, then the antibodies observed would be of high-responder allotype only. In order to test the above hypothesis, irradiation chimeras were used in combination with immunoglobulin allotype markers to attempt to determine which of the cell types in the chimeras were producing the anti-(T, G)-A--L antibody.

Since this immune response, as well as the immune responses to a variety of other antigens, has been found to be closely linked to the major histocompatibility (H-2) locus [4], the possibility must be considered that the reason for the existence

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Abbreviations: **(T,G)-A--L:** Poly-L(tyrosine, glutamic acid)-poly-DL-alanine--poly-L-lysine **(Phe,G)-A--L:** Poly-L(phenylalanine, glutamic acid)-poly-DL-alanine--poly-L-lysine **(H,G)-A--L:** Poly-L(histidine, glutamic acid)-poly-DL-alanine--poly-lysine **MBSA:** Methylated bovine serum albumin **MEM:** Minimal essential medium **GVH:** Graft-versus-host **ABC:** Antigen-binding capacity

of low-responder strain types is due to some sort of cross-reaction between self-histocompatibility antigens in the low responders and the immunizing antigens in question. No cross-reaction has been demonstrable to date. Anti-(T, G)-A–L antibodies do not specifically bind to low-responder strain lymphoid cells or erythrocytes*. Antibodies to low responder H-2 specificities do not bind to (T, G)-A–L*. Stable irradiation chimeras containing low- and high-responder cell types apparently tolerant to one another's H-2 antigens have been shown to be capable of a high anti-(T, G)-A–L response [5]. However, the nature of such a cross-reaction could be quite complex, perhaps involving recessive histocompatibility, or related, linked antigens restricted in expression to certain cell types (similar to the thymus leukemia [TL] antigens) [6].

The possibility has been raised that "stable" responder irradiation chimeras could nevertheless be undergoing a chronic graft-versus-host (GVH) reaction** so that the responding cells might *not* be tolerant to the histocompatibility antigens of the low-responder strain. Therefore, in the present investigation, the lymphoid cells of the irradiation chimeras were also studied using the Simonsen discriminant spleen assay to determine the state of tolerance of these cells with respect to one another.

In a recent extension of this work, allophenic (tetraparental) chimeric mice were also studied in an attempt to determine not only which strain's cells can be antibody producers, but also the possible role of histocompatibility cross-tolerance as the cause of genetic nonresponsiveness.

2. Materials and methods

2.1. Animals

CBA, C57BL/10, and C57BL/10 Sn mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, USA.

2.2. Antigen, immunization and antibody assay

The antigens used, immunization procedures and antibody assay have been described previously [7]. The assay was modified only in that 0.0005 μg (T, G)-A–L-509-^{125}I per serum aliquot (specific activity approximately 10 μCi/μg) was used instead of 0.0025 μg. Antigen-binding capacity (ABC) titers were expressed as the reciprocal of that dilution giving 50 % antigen bound. Titers of less than 250 were considered non-responders. (CBA mice have an average ABC = 60, range $<$ 10–200; C57BL/10 mice have an average ABC = 1200, range 300–15 000.)

2.3. Determination of allotype levels

Serum immunoglobulin allotype levels for the γG_{2a} (Ig-1 allotype) and γG_1 (Ig-4 allotype) classes were determined by the method of Herzenberg et al. [8] using antisera, diluents and appropriate ^{125}I myeloma proteins prepared as previously described [8]. The allotype levels found in the chimeras were compared to those observed in pooled serum from mice of each strain following the usual (T, G)-A–L immunization regimen.

2.4. Determination of specific anti-(T, G)-A–L allotype

The allotype of the anti-(T, G)-A–L antibodies in the chimeras was determined by a technique developed for this study. Pooled anti-allotype sera specific for each allotype group, *a* or *b*, but capable of precipitating several classes (γG_{2a}, γG_1, γG_{2b}) of immunoglobulins were allowed to react with chimeric anti-(T, G)-A–L as well as known positive antisera of each allotype under conditions selected for maximum precipitation (see below). The allotype-anti-allotype complexes were then sedimented by centrifugation and the supernatant was assayed for remaining anti-(T, G)-A–L activity in the usual manner. The anti-allotype sera, as well as normal mouse serum controls, were diluted in 3 % BSA in 0.04 M Tris-HCl buffer, pH 7.3, containing 10 % of a redissolved 33 % $(NH_4)_2SO_4$ precipitate from normal rabbit serum. This material was found to enhance specific precipitation to an even greater extent than 10 % normal rabbit serum alone, by virtue of its content of C1q and its relatively low molarity [9,***]. The anti-(T, G)-A–L sera were diluted in 3 % BSA in 0.04 M Tris-HCl, pH 7.3.

In a typical experiment, 50 μl aliquots of a 1 : 25 dilution of each anti-allotype serum were mixed with 50 μl aliquots of anti-(T, G)-A–L at dilutions ranging from 1 : 500 to 1 : 4000 and incubated 3 h at 37 °C, followed by incubation overnight at 4 °C. The mixtures were then centrifuged at 10 000 x g for 15 min at 4 °C in a Sorval GSA rotor, and two 25 μl aliquots of the supernatant were removed for titration in the usual assay for anti-(T, G)-A–L. The amount of polyvalent rabbit anti-mouse γ-globulin serum used to give maximum percent antigen bound was determined empirically and depended upon the amount of mouse γ-globulin in the original mixture, most of which came from the anti-allotype sera.

The percent of anti-(T, G)-A–L antibody precipitated by each anti-allotype serum was determined by comparing the 50 percent antigen-binding titers with controls in which the anti-(T, G)-A–L serum was mixed under identical conditions with normal mouse serum instead of anti-allotype serum. The anti-allotype-*a* serum used could precipitate 75–80 % of the anti-(T, G)-A–L antibody in an allotype-*a* responder serum [CBA (H-$2^{k/k}$, nonresponder) immunized with (T, G)-A–L-MBSA, or C3H.SW (H-$2^{b/b}$, responder) immunized with (T, G)-A–L].

The anti-allotype-*b* serum used could precipitate a similar percentage of the anti-(T, G)-A–L antibody from an allotype-*b* responder (C57BL/10) serum. Each of the anti-allotype sera was specific and precipitated only the antibodies of the allotype to which they were directed. In preliminary experiments, done using mixtures of known titer allotype *a* and *b* sera, it was determined that the minimum detectable percentage of anti-(T, G)-A–L of either allotype in such mixtures was 25 %.

2.5. Irradiation chimeras

Two-to-three-month old male and female CBA (low responder, H-2^k, allotype *a*) or C57BL/10 (high responder, H-2^b, allotype *b*) mice were given 850 r whole body irradiation (250 kV, 15 mA, 80 cm, filter $\frac{Al}{Cu}$). These mice were then given an intravenous injection containing 20 x 10^6 – 100 x 10^6 fetal liver cells of the opposite strain obtained from 14- to 17-day old fetuses by gentle dissociation into a single cell suspension

* Edidin, M. and McDevitt, H.O., unpublished data

** Simonsen, M., personal communication

*** Herzenberg, Lenore A., personal communication

at 4 °C in minimal essential medium (MEM). Control mice were given no fetal liver cells and always died 7 to 12 days post-irradiation. The mortality of the chimeras produced was approximately 50 % over the first 6 to 8 weeks with only rare deaths occurring after the eighth week. Surviving chimeras were immunized in the usual manner 3 to 5 months following irradiation and cell transfer.

2.6. Simonsen discriminant spleen assay [10]

Following immunization, bleeding and titering, animals were sacrificed and spleen and lymph nodes were dissociated in cold MEM. Twenty to 30 x 10^6 cells were then injected intraperitoneally into newborn CBA or C57BL/10 mice as well as into 6- to 8-day old (CBA x BALB/c)F_1 and (C57BL/10 x BALB/c)F_1 mice. The recipient mice were killed 10 days after cell transfer, and the degree of GVH reaction was determined by calculating the spleen index (spleen weight (mg) per 10 gm body weight) and expressed relative to uninjected litter mate controls (*i.e.*, = index 1.0). The F_1 mice were used to detect the presence of either CBA or C57BL/10 cells capable of giving a GVH reaction against a third strain type (BALB/c). For instance, 6-day old (CBA x BALB/c)F_1 mice received large doses (30 x 10^6) of C57BL/10 cells with no resulting GVH reaction; however, even low doses (1 x 10^6 – 5 x 10^6) of CBA cells were not rejected, and these cells caused a vigorous GVH reaction against the recipient due to its BALB/c histocompatibility specificities. A similar situation occurred in reverse in the (C57BL/10 x BALB/c)F_1. Newborn CBA and C57BL/10 recipients were used to determine whether or not the cells shown to be present by GVH in the F_1's were tolerant to CBA and C57BL/10 tissues respectively. Normal C57BL/10 and CBA spleen and lymph node cells were injected into all recipient types as positive and negative controls each time this experiment was performed.

2.7. Allophenic mice

Two allophenic mice (L288-1 and L288-2) composed of C57BL/10 and C3H tissues were studied [11]. L288-1 was mostly agouti (C3H) in coat color, and the degree of chimerism determined by hemoglobin typing was 50 % C3H, 50 % C57BL/10. L288-2 was mostly black (C57) in coat color, and the hemoglobin types were 10 % C3H, 90 % C57BL/10.

3. Results

3.1. Composition of radiation chimeras and allotype of anti-(T, G)-A--L produced by responders

Nineteen of the chimeras were nonresponders (ABC < 250), and twenty were responders. In Table 1 it can be seen that the nonresponders have a relatively low level of C57BL/10 (responder) immunoglobulins (Ig-1^b and Ig-4^b), an average of 26 % of normal in the case of CBA fetal liver into irradiated C57BL/10 chimeras and 51 % in the case of C57BL/10 fetal liver into irradiated CBA chimeras. However, in both groups there were several animals with near normal levels of C57BL/10 immunoglobulins, which were nevertheless nonresponders. Most of the nonresponder chimeras had C57BL/10 cells detectable in the discriminant spleen assay (Table 1).

The results for responder chimeras are given in Table 2. Both groups of responder chimeras have very low levels of CBA immunoglobulins and near normal levels of C57BL/10 immunoglobulins. The analysis of cell types by discriminant spleen assay showed that in most animals both cell types were present. Eleven of the responder chimeras had anti-(T, G)-A--L titers high enough for efficient determination of specific anti-(T, G)-A--L antibody allotype (Tables 2 and 3). In all eleven animals only anti-(T, G)-A--L antibody of C57 (high-responder) allotype was detectable. Included in Table 3 are the results of allotyping of anti-(T, G)-A--L antibodies from 10 (CBA x C57BL/10)F_1 animals. It is clear that in the case of the F_1 mice the anti-(T, G)-A--L detected may be of either or both allotypes. It should also be noted that in several of the chimeras and F_1 animals, in spite of relatively high titers of anti-(T, G)-A--L, neither type of anti-allotype serum precipitated a detectable amount of anti-(T, G)-A--L. This could have been due to the presence of anti-(T, G)-

Table 1. Non-responder chimeras (ABC < 250)

Fetal liver donor	Recipient	Ig-1a % Normal	Ig-4a % Normal	CBA cells present	Ig-1b % Normal	Ig-4b % Normal	C57 cells present
C57BL/10J	CBA	4	< 40	n.t.[a]	46	92	yes
		7	< 40	n.t.	38	105	no
		11	< 40	n.t.	< 2	< 0.4	no
		1	< 40	n.t.	36	32	yes
		1	< 40	n.t.	58	83	yes
		7	< 1	n.t.	58	19	yes
		26		n.t.	2	≃ 0.5	no
C57BL/10Sn	CBA	1	2	yes	86	83	yes
		2	< 40	no	24	24	yes
		2	< 40	inc.	13	27	yes
		2	< 40	yes	31	30	yes
		2	2	no	165	105	yes
CBA	C57BL/10J	< 1	3	no	103	156	yes
		100	330	no	31	32	n.t.
		100	130	n.t.	5	6	yes
		100	125	n.t.	5	3	yes
		100	370	n.t.	5	< 2	yes
CBA	C57BL/10Sn	63	230	n.t.	5	< 2	n.t.
		35	310	n.t.	4	3	n.t.

a) n.t. = not tested.

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Table 2. Responder chimeras (ABC >250)

	#	Anti-(T, G)-A–L titer	Ig-1[a] level [a]	Ig-4[a] level [a]	GVH assay in: 6–8-day old CBA x BALB/c [b] Index [c]	GVH [d]	Newborn C57BL/10 [b] Index [c]	GVH [d]	Ig-1[b] level [a]	Ig-4[b] level [a]	GVH assay in: 6–8-day old C57BL/10 x BALB/c [b] Index [c]	GVH [d]	Newborn CBA [b] Index [c]	GVH [d]
#1–15	1	9 000	7	2	1.84	+	0.80 0.85	–	29	62	1.77 1.95	+	1.44 1.02	±
Fetal liver donor: C57BL/10	2	1 250	8	3	1.38 1.88	+	0.82	–	82	57	2.25 2.40	+	inc.	
Irradiated recipient: CBA	3	3 000	<5	2	1.37	±	0.89 0.98	–	48	119	2.16 2.82	+	1.21 0.77	–
	4	2 000	26	33	1.64 2.44	+	1.00	–	25	57	1.97	+	inc.	
	5	2 500	<5	2	1.00	–	1.02	–	87	34	n.t. [e]		inc.	
	6	7 200	8	3	1.45	±	inc.		85	109	n.t.		1.73	+
	7	10 300	<5	2	3.06 2.62	+	1.36 1.04	±	81	216	2.60 2.55	+	inc.	
	8	2 200	1	3	inc.		n.t.		103	130	3.30 3.20	+	1.18	–
	9	860	<5	3	1.06	–	1.03	–	110	104	n.t.		1.28	–
	10	320	<5	2	1.59 1.55	±	1.00	–	70	32	1.89 2.00	+	0.54 1.02 1.32	–
	11	520	<5	2	1.05 0.96	–	1.00	–	96	90	1.67 1.42	+	1.82 2.30	+
	12	450	1	3	1.84 2.15	+	n.t.		114	83	3.45 3.50	+	1.30	±
	13	920	1	2	1.95	+	n.t.		83	50	3.20 3.30	+	1.08	–
	14	840	2	2	0.83 0.92	–	n.t.		97	70	2.10 2.60	+	0.92	–
	15	2 000	1	2	inc.		n.t.		76	140	3.80 4.40	+	inc.	
#16–20	16	1 400	22	38	inc.		n.t.		55	204	1.42	±	1.14	–
Fetal liver donor: CBA	17	9 000	<5	2	1.31 1.42	–	1.08 0.83	–	100	125	1.98 2.04	+	1.71 1.63	–
Irradiated recipient: C57BL/10	18	3800	<5	2	1.18 1.22	–	1.44 1.15	±	67	125	1.91 2.38	+	2.04	+
	19	4 000	<5	2	1.56 1.09	±	1.06 1.23	–	104	90	1.02 1.57	±	inc.	
	20	1 400	<5	2	0.90	–	1.00	–	87	42	1.33 0.82	–	2.90	+
#21 & 22 C57BL/10×C3H tetrapatental	21	2 200	20	5	n.t.		n.t.		93	92	n.t.		n.t.	
	22	8 800	2	1	n.t.		n.t.		74	195	n.t.		n.t.	
Control mice for the discriminant spleen assay	C57BL /10	300–1 900	0	0	avg. 1.16 range: 0.67–1.66		avg. 1.04 range: 0.73–1.28		100	100	avg. 2.78 range: 2.00–4.30		avg. 2.34 range: 1.32–3.55	
	CBA	<10–200	100	100	avg. 2.54 range: 1.43–4.80		avg. 2.06 range: 1.44–3.05		0	0	avg. 1.05 range: 0.74–1.42		avg. 1.02 range: 0.85–1.32	

a) Ig allotype levels are given as percent of average levels found in immunized control mice of each strain.
b) Strain type of animal used as recipient for testing chimera lymphoid cells in discriminant spleen assay.
c) Relative spleen index. "inc." = inconclusive results due to technical difficulties, *i.e.* test animal died or in some groups the (CBA x BALB/c)F_1 mice were too young to reject negative control (C57BL/10) cells and these cells gave rise to a positive graft-vs-host reaction.
d) GVH = graft-vs.-host reaction.
+ if spleen index within range given by positive control or allogeneic cells.
– if spleen index within range given by negative control or syngeneic cells.
± if spleen index within range of overlap between values occasionally given by positive and negative control cells.
e) n.t.: not tested.

A--L antibodies of classes not detected (IgA, IgM) or weakly precipitated (IgG_{2b}) by the antiallotype serum used; however, no further proof that this was the case is available at this time.

In summary, responder chimeras of both types had predominantly C57BL/10 lymphoid cells and serum immunoglobulins. Only C57BL/10 type anti-(T, G)-A--L was detected; however, the levels of total CBA immunoglobulins were so low in responder chimeras that this cannot be considered evidence that CBA cells are defective and cannot synthesize anti-(T, G)-A--L antibodies.

3.2. State of tolerance of lymphoid cells in radiation chimeras

Table 2 gives the overall results for the discriminant spleen assay of chimeric lymphoid cells in the four types of recipient used. In 16 of 20 high-responder chimeras, C57BL/10 cells were detectable by the presence of a GVH reaction in (C57BL/10 x BALB/c)F_1 recipients. Three of these mice were not tested in (C57BL/10 x BALB/c)F_1 recipients, and one gave no GVH reaction in the F_1 recipients, but a positive GVH reaction in CBA recipients. The C57BL/10 cells of these 20 high responders, as judged by the presence or absence of a GVH reaction in newborn CBA recipients, were tolerant to CBA histocompatibili-

Table 3. Allotype of anti-(T, G)-A--L antibody

#	Donor	Recipient	Anti-(T, G)-A--L titer	Per cent decrease in titer after precipitation with anti-allotype serum		
				anti-*a*[a]	anti-*b*[b]	solvent[c] alone
1	C57BL/10	CBA	9 000	<25	63	34
2	C57BL/10	CBA	1 250	<25	82	<25
3	C57BL/10	CBA	3 000	<25	82	<25
4	C57BL/10	CBA	2 000	<25	79	26
5	C57BL/10	CBA	2 500	<25	69	75 c)
6	C57BL/10	CBA	7 200	<25	84	<25
7	C57BL/10	CBA	10 300	<25	79	<25
8	C57BL/10	CBA	2 200	<25	67	<25
16	CBA	C57BL/10	1 400	<25	68	<25
17	CBA	C57BL/10	9 000	<25	62	<25
18	CBA	C57BL/10	3 800	<25	60	<25
10	C57BL/10	CBA	320	<25	<25	<25
12	C57BL/10	CBA	450	<25	<25	<25
14	C57BL/10	CBA	840	<25	30	25
15	C57BL/10	CBA	2 000	<25	<25	<25
19	CBA	C57BL/10	4 000	<25	<25	<25
20	CBA	C57BL/10	1 400	<25	<25	73 c)
21	C3H↔C57BL/10 tetraparental		22 000	25	63	25
22	C3H↔C57BL/10 tetraparental		8 800	<25	<25	<25
23	(CBA x C57BL/10)F_1		4 400	<25	82	<25
24	(CBA x C57BL/10)F_1		9 500	56	28	<25
25	(CBA x C57BL/10)F_1		2 300	<25	25	<25
26	(CBA x C57BL/10)F_1		2 600	53	25	41 c)
27	(CBA x C57BL/10)F_1		3 600	47	<25	<25
28	(CBA x C57BL/10)F_1		9 000	53	<25	<25
29	(CBA x C57BL/10)F_1		5 600	<25	<25	<25
30	(CBA x C57BL/10)F_1		4 000	43	55	<25
31	(CBA x C57BL/10)F_1		4 000	43	38	40 c)
32	(CBA x C57BL/10)F_1		3 000	52	<25	63 c)

a) The anti-allotype *a* serum used precipitated 62 – 87 % of the detectable anti-(T, G)-A--L from C3H.SW or CBA mice.
b) The anti-allotype *b* serum used precipitated 73 – 91 % of the detectable anti-(T, G)-A--L from C57BL/10 mice.
c) As shown in this column, there were rare occasions when incubation of anti-(T, G)-A--L antisera with solvent (3 % BSA in 0.04 M Tris-HCl buffer, pH 7.3, with 10 % $(NH_4)_2SO_4$ precipitated normal rabbit globulins) alone resulted in a drop in anti-(T, G)-A--L titer. These decreases were unusual, but reproducible. No explanation has been found for these results.

ty antigens in nine cases, non-tolerant in five cases, and were of uncertain state due to recipient deaths in six cases.

Figure 1 presents the relative spleen indices in newborn CBA recipients injected with lymphoid cells from CBA mice, C57BL/10 mice, high-responder chimeras, and low-responder chimeras. The positive and negative controls are clearly distinct populations; however, the high-responder chimeras gave a continous spectrum of indices overlapping both the positive and negative control groups. In order to see if there was any correlation between anti-(T, G)-A--L ABC titer and degree of GVH reaction of C57 cells against CBA histocompatibility antigens, these two values were plotted together in Figure 2. The results show that no correlation is observed.

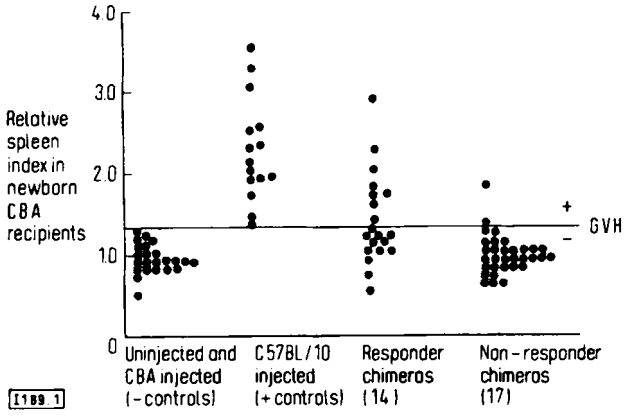

Figure 1. Relative spleen index in newborn CBA recipients injected with spleen cells from responder chimeras, non-responder chimeras, C57BL/10 positive controls and CBA negative controls

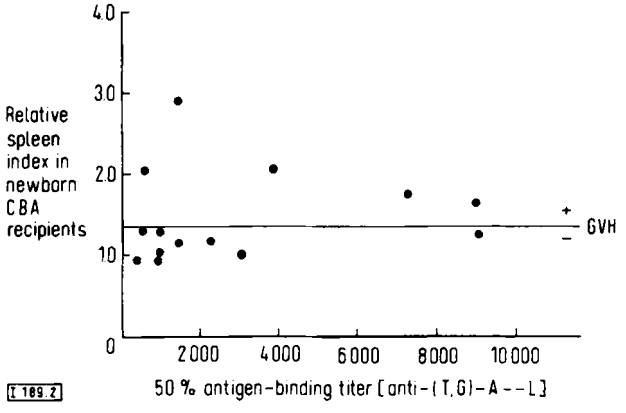

Figure 2. Fifty percent antigen-binding anti-(T, G)-A--L titer in responder chimeras, versus relative spleen index in newborn CBA recipients injected with 20 x 10^6 spleen cells from these chimeras

In summary, within the limits of detectability of the discriminant spleen assay in six animals, tolerance of high-responder (C57BL/10) lymphoid cells from radiation chimeras to low-responder (CBA) histocompatibility antigens did not result in a low-responder phenotype chimera. Therefore, tolerance to low-responder H-2 antigens would appear not to be responsible for the low-responder status of many strains of mice.

3.3. Allotype of anti-(T, G)-A--L antibodies in allophenic mice

Two allophenic mice, composed of C57BL/10 (H-2^b, allotype *b*) and C3H (H-2^k, allotype *a*) cells, have been tested to date. Both animals are high responders to (T, G)-A--L (Table 2). The allotype of the anti-(T, G)-A--L antibodies produced was all C57BL/10 type in the first allophenic; however, the level of total C3H immunoglobulins was so low that this result cannot be considered an adequate test of the ability of allophenic mice to make anti-(T, G)-A--L antibody of low-responder allotype.

It is clear that different degrees of chimersim occur in different tissues of the same animal. For example, the first animal was mostly agouti (C3H) in its coat color, had approximately

50 % C3H hemoglobin, but had only 20 % and 5 %, respectively, of normal C3H immunoglobulin levels of the γG_{2a} (Ig-1) and γG_1 (Ig-4) classes. However, since both mice are mosaics to at least some degree and since the lymphoid cells of an allophenic mouse are presumably mutally tolerant, the fact that both these mice are high responders to (T, G)-A–L further substantiates the conclusion of the preceding section that tolerance to low-responder histocompatibility antigens is not responsible for the low-responder phenotype.

4. Discussion

Analysis of the allotype of anti-(T, G)-A–L antibodies from 11 responder irradiation chimeras and two allophenic mice in the present study revealed that only responder type (C57BL/10) anti-(T, G)-A–L was produced in detectable quantities. However, the fact that significant degrees of chimerism of *lymphoid* tissues was not achieved prevents this from being an adequate test of the introductory hypothesis concerning whether low-responder, antibody-producing cells can cooperate with high-responder, thymus-derived antigen-reactive cells to produce low-responder allotype anti-(T, G)-A–L antibody. It is clear from the data presented here (and from other unpublished observations from this laboratory on animals given allogeneic bone marrow cells at birth, both with and without previous low-dose irradiation at 100 or 200 r) that animals which are 50 % mosaics of *lymphoid* cells of differing H-2 types are extremely difficult to produce and can probably best be obtained by creating chimerism very early in embryogenesis, such as the 6 – 8 cell stage, as has been done with allophenic (tetraparental) mice. Further experiments using this approach are currently under way in this laboratory.

Close linkage of several genetically controlled immune responses to major histocompatibility antigen loci is a finding of current interest [4]. The significance of this linkage with respect to the mechanism of the genetic control of specific immune responses is as yet unknown. The data in the present report further supports the concept that, at least in the Ir-1 system, tolerance to self (H-2) antigens is *not* the reason for inability of some strains of animals to mount an immune response to (T, G)-A–L.

With this possibility apparently ruled out, one can only speculate as to other mechanisms enabling H-2 antigens, or substances coded for by genes very closely linked to these antigens, to control the immune response of an animal to a variety of antigens. One possibility is that certain H-2 antigen specificities on the cell membrane might exert a positive non-specific effect which could facilitate interaction of cell receptors with particular antigenic determinants. In a similar manner, the Ir-1 gene could code for a separate non-H-2 membrane antigen with similar effects, but expressed only on certain cell types, such as thymocytes (analogous to the thymus leukemia, or TL, antigen locus which is also closely linked to H-2 [6]). It is conceivable that the Ir-1 effect is due to a separate class of antigen receptor found only on certain cell types and responsible for antigenic recognition. The present data do not exclude any of these possibilities, nor do they lend them any particular positive support.

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5. References

1 McDevitt, H.O. and Benacerraf, B., *Advan. Immunol.* 1969. *11*: 31.

2 McDevitt, H.O., *J. Immunol.* 1968. *100*: 485.

3 Miller, J.F.A.P. and Mitchell, G.F., *Transplant. Rev.* 1969. *1*: 3.

4 Benacerraf, B. and McDevitt, H.O., *Science* 1972. *175*: 273.

5 Tyan, M.L., McDevitt, H.O. and Herzenberg, L.A., *Transplant. Proc.* 1969. *1*: 548.

6 Boyse, E.A., Old, L.J. and Stockert, E., in Grabar, P., and Miescher, P.A. (Eds.), *Immunopathology, 4th Int. Symp.* Schwabe, Basel 1965, p. 23.

7 McDevitt, H.O. and Tyan, M.L., *J. Exp. Med.* 1968. *128*: 1.

8 Herzenberg, L.A., Warner, N.L. and Herzenberg, L.A., *J. Exp. Med.* 1965. *121*: 415,

9 Agnello, V., Winchester, R.J. and Kunkel, H.G., *Immunology* 1970. *19*: 909.

10 Simonsen, M., *Progr. Allergy* 1962. *6*: 349.

11 Wegmann, T.G. and Gilman, J.G., *Develop. Biol.* 1970. *21*: 281.