ROLE OF THE MAJOR HISTOCOMPATIBILITY GENE PRODUCTS IN REGULATING THE ANTIBODY RESPONSE TO DINITROPHENYLATED POLY(L-Glu^{55},L-Ala^{35},L-Phe^{9})$_n$[*]

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The role of the major histocompatibility gene complex (MHC)[1] products in the regulation of humoral immune responses has been an area of intensive investigation in recent years. It now appears that the gene products of the MHC are important in regulating collaborative interactions between T and B lymphocytes and thus play a crucial role in the development of effective humoral immune responses (1, 2). However, the mechanism by which the genes of the MHC serve to control cell-cell interactions in antibody responses is still controversial. Initial studies using in vivo cell-transfer protocols to examine the role of the MHC gene products in antigen-specific T cell-dependent antibody responses demonstrated an absolute requirement for T and B lymphocytes to share genes of the MHC (2, 3). Subsequent studies with irradiation bone marrow chimeras suggested that the genetic identity between T and B cells per se was less crucial than the ability of T cells to recognize the MHC gene products of the collaborating cells, either alone or in conjunction with the immunizing antigen (4-10). However, there has been little agreement as to the nature and extent of this restriction (11-16), and the level in cell-cell interactions at which these restrictions are imposed (17-20). This is a result of, in part, the use of a variety of sophisticated manipulations of T and B cell populations that renders direct comparisons difficult. Further uncertainties are introduced by the inherent difficulties of carrying out studies involving MHC-nonidentical cells in the absence of potential allogeneic effects (16, 21).

Consequently, a number of crucial questions concerning the role of the MHC gene products and their mechanism of action remain unresolved. First, there is little

agreement as to whether T cells and nonimmune or primary B cells from H-2-nonidentical inbred mouse strains can collaborate in humoral responses. Second, although antigen-specific interactions may be permitted between allogeneic antigen primed T and primary B lymphocytes, this may not be the case for all B cell subpopulations; in particular, immune or secondary B cells. Third, it has been shown that MHC-linked restrictions in both T cell proliferative responses and B cell stimulation may operate at the level of the interaction of T cells and macrophages (5–8, 17–20). Such findings imply that all MHC-imposed restrictions may be accounted for at the level of antigen processing. However, such a determinative regulatory role for macrophages in antibody responses remains controversial. Finally, the actual composition of the T cell repertoire within an inbred strain with respect to its collaborative capacity with B cells of MHC-dissimilar strains is not fully understood. Recently, bone marrow irradiation chimeras have been used to probe the extent of the influence of the differentiating environment in restricting interactions of T cells with B cells of other MHC haplotypes. Although somewhat controversial, these studies suggested that the parental differentiating environment affected the collaborative phenotypic expression of a multipotential T cell repertoire (10, 22, 23).

We have addressed each of these questions in the experiments described in this report. We have employed the splenic fragment culture system and used as a carrier antigen, the synthetic random copolymer poly(L-Glu^{55},L-Ala^{35},L-Phe^9)$_n$ (GLϕ9), the immune response to which has been shown to be under H-2-linked Ir (immune response) gene control (24–26).

The conclusions drawn from these studies can be summarized as follows: (a) As suggested in earlier studies, primary B cells are able to interact with MHC-nonidentical T cells, whereas secondary B lymphocytes required some form of MHC recognition by the collaborating T cell. (b) F$_1$ T cells interacting with antigen in the milieu of syngeneic macrophages are able to discriminate between GLϕ9 responder and nonresponder B lymphocytes. Thus, the GLϕ9 Ir gene defect can apparently operate at the level of the T cell-B cell interaction, rendering a determinative role for macrophage antigen presentation unlikely. (c) The GLϕ9 nonresponder strain investigated has demonstrable GLϕ9-specific T cell help for the antigen 2,4-dinitrophenylated (DNP)-GLϕ9. However, a subpopulation of the GLϕ9-specific T cells, i.e., those which could have collaborated with their own B cells in a fashion characteristic of MHC-identical T and B lymphocytes, is not detectable in the nonresponder. (d) The GLϕ9-specific helper T cell population of the responder strain that is capable of collaborating with nonresponder B cells appears to have been deleted from the (responder × nonresponder)F$_1$ presumably as a result of the presence of the nonresponder MHC gene products in the F$_1$ animal. Thus, the responder T cell repertoires of the F$_1$ and parental strains appear to be distinct and to be determined, to some extent, by the genes of the MHC.

Materials and Methods

Antigens. *Limulus polyphemus* hemocyanin (Hy) was purchased from Worthington Biochemical Corp., Freehold, N. J. DNP-Hy, 10 mol DNP/100,000 mol wt Hy (DNP$_{10}$-Hy) was prepared as described elsewhere (27). GLϕ9 was synthesized as described previously (28). An aqueous solution of the polymer was dialyzed free of the hydrogen bromide for 1 wk against two daily changes of distilled water and lyophilized. The lyophilized polymer was dissolved in water for use at a concentration of 10 mg/ml, the pH adjusted to 7.9 with 1 N NaOH, and

stored frozen at $-70°$ C. DNP_{10}-$GL\phi9$ was synthesized and the coupling ratio determined by methods described for other protein antigens (27).

Animals and Immunizations.   6- to 8-wk old male BALB/c mice were obtained from Carworth Farms, Wilmington, Mass. C57BL/6J, (C57BL/6J × BALB/cJ) F_1, SJL/J, and A/J 6- to 8-wk-old mice were purchased from The Jackson Laboratory, Bar Harbor, Maine D2.GD, B10.A(4R), and B10.A(5R) recombinant inbred mice were obtained through the Scripps Clinic and Research Foundation animal breeding facility Mice to be used as Hy-immunized recipients received one intraperitoneal injection of 0.1 mg Hy in complete Freund's adjuvant (CFA) 8–12 wk before use Secondary B cell donors were immunized by injection of 0 1 mg DNP-Hy in CFA intraperitoneally 6–8 wk before use. To consistently provide maximum helper function, particularly in the nonresponsder strain, mice to be used as $GL\phi9$-primed recipients were given three intraperitoneal injections of 0.1 mg $GL\phi9$; the first in CFA 16 wk before use, followed by two injections of 0.1 mg $GL\phi9$ in saline 9 and 5 wk before use To verify that the polymer and its immunization properties were consistent with previous reports (24, 25), the sera obtained from individual bleedings of mice 2–3 wk after the final injection of $GL\phi9$ were tested for the presence of $GL\phi9$-specific antibodies by a radioimmunoassay employing anti-Fab antibody as a detecting reagent, which allowed for the detection of both IgM and IgG antibody (11) The results showed the responder strains, BALB/c and CBF_1, produced appreciable amounts of antibody specific for the $GL\phi9$ antigen, whereas there was no detectable level of $GL\phi9$-specific antibody in the serum of the nonresponder strain C57BL/6.

Cell Transfers and Fragment Cultures.   The methods for obtaining monoclonal antibody responses in splenic fragment cultures and for the detection of antibody in culture fluids by a radioimmunoassay have been previously described (11, 12, 27, 29) The methods for removing T cells from the donor cell inoculum by anti-θ and complement treatment have been previously described (30). The anti-θ antisera used in these studies was the kind gift of Dr. Walter Gerhard, Wistar Institute, Philadelphia, Pa. The antisera and complement was cytotoxic for >95% of thymocytes by the trypan blue exclusion test. Macrophages were removed from the donor cell inoculum by passing donor cells over a Sepharose G-10 column obtained from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J. (31) The recovery of spleen cells from the G-10 Sepharose column was ~60%.

Results

The primary DNP-specific B Cell Response of Responder, Nonresponder, and F_1 Strains to DNP_{10}-$GL\phi9$.   Preliminary experiments were carried out to determine the ability of the $GL\phi9$-responder strain, BALB/c, the nonresponder strain, C57BL/6, and the F_1 responder strain, (BALB/c × C57BL/6)F_1 to collaborate in antibody responses to the antigen DNP-HY in various donor and recipient combinations. A summary of the results obtained is shown in Table I. As can be seen, the DNP-specific primary donor B cells responded in approximately the same frequency when transferred to MHC-syngeneic, -allogeneic, or -hemisyngeneic Hy-primed irradiated recipient mice. Variations in the responses appeared to be strain dependent rather than dependent on the particular donor-recipient combination employed. Although quantitatively similar in terms of the frequency of the response, the donor B cell responses differed strikingly in the heavy-chain isotype of the antibody produced, depending on the MHC identity of the donor cells and Hy-primed recipients. Syngeneic donor-recipient strain combinations and hemisyngeneic combinations involving F_1 and parental strains yielded clones, the majority of which synthesized IgG_1 antibody; whereas allogeneic strain combinations yielded IgM antibody-producing clones exclusively, which is consistent with previously published results (11).

Similar experiments were carried out to determine the helper T cell potential of $GL\phi9$-immunized responder, nonresponder, and F_1 recipient mice in collaboration with primary DNP-specific B cells in response to DNP_{10}-$GL\phi9$. When $GL\phi9$-immu-

TABLE I

The Frequency and Heavy-Chain Isotype of DNP-specific Primary B Cell Responses in Hy- and GLφ9-immunized Recipient Mice

Donor*	Recipient	Recipient immunization‡	In vitro antigen	Total number of cells transferred	Number of positive foci/10^6 cells transferred	Percentage of foci IgG$_1$
				× 10^{-6}		
BALB/c (d)§	BALB/c (d)	Hy	DNP-Hy	56	0 89	80
		GLφ9	DNP-GLφ9	64	0.89	60
	C57BL/6 (b)	Hy	DNP-Hy	40	0.60	<4
		GLφ9	DNP-GLφ9	30	0 30	<1
	CBF$_1$ (d/b)	Hy	DNP-Hy	16	0 75	83
		GLφ9	DNP-GLφ9	24	0.75	44
C57BL/6 (b)	BALB/c (d)	Hy	DNP-Hy	20	0 80	<6
		GLφ9	DNP-GLφ9	56	0 61	<2
	C57BL/6 (b)	Hy	DNP-Hy	20	0 60	83
		GLφ9	DNP-GLφ9	30	0.30	<3
	CBF$_1$ (d/b)	Hy	DNP-Hy	20	0 75	87
		GLφ9	DNP-GLφ9	60	0.30	<1
CBF$_1$ (d/b)	BALB/c (d)	Hy	DNP-Hy	20	0 85	82
		GLφ9	DNP-GLφ9	54	0.46	32
	C57BL/6 (b)	Hy	DNP-Hy	20	0.75	67
		GLφ9	DNP-GLφ9	32	0.41	<8
	CBF$_1$ (d/b)	Hy	DNP-Hy	16	0.94	81
		GLφ9	DNP-GLφ9	48	0 67	62

* Between 4 × 10^6 and 8 × 10^6 donor spleen cells were transferred to each recipient
‡ There were no detectable antibody responses when donor cells were transferred to recipients that were not carrier immunized
§ The H-2 haplotype of the strain is shown in parentheses.

nized BALB/c responder mice were used as recipients of either C57BL/6 nonresponder, BALB/c responder, or CBF$_1$ responder, nonimmune spleen cells, the DNP-specific antibody response to DNP-GLφ9 paralleled those obtained using the hapten carrier system DNP-Hy. As shown in Table I, syngeneic and hemisyngeneic donor B cells were stimulated in GLφ9-primed BALB/c recipients to yield IgG$_1$-antibody-producing clones, whereas allogeneic C57BL/6 DNP-specific clones synthesized only IgM antibody.

In contrast, the response of DNP-specific primary donor B cells transferred to GLφ9-primed F$_1$ responder mice was strikingly different from that predicted by the results obtained employing DNP-Hy. In GLφ9-primed CBF$_1$ recipients, the hemisyngeneic BALB/c responder donor B cells responded with IgG$_1$-antibody responses, as did the syngeneic CBF$_1$ responder donor B cells; however, hemisyngeneic C57BL/6 nonresponder B cells were stimulated to synthesize only IgM antibody. Thus, although the T cells of the CBF$_1$ responder recipient were capable of recognizing and responding to the antigen GLφ9, only the collaborative interactions with the parental responder or F$_1$ B cells can be described as homologous, as they yielded IgG$_1$-antibody-producing clones. The F$_1$ T cell population appears to be deleted of T cells capable of interacting

in a homologous fashion with parental nonresponder B cells and, therefore, interactions result in the synthesis of only IgM antibody. This contrasts with results obtained in Hy-primed CBF$_1$ recipients, which appeared to have populations of T cells that were capable of homologous interactions with both parental type DNP-specific primary B cells.

As shown in Table I, the GLφ9-primed nonresponder C57BL/6 recipients of responder, nonresponder, and F$_1$ primary donor B cells were capable of promoting antigen-dependent antibody responses to DNP-GLφ9, but not in a homologous fashion, as indicated by the exclusive IgM antibody responses. Thus, the nonresponder strain, although maintaining a repertoire of GLφ9-specific T cells, is apparently lacking that population capable of interacting in a homologous fashion with the MHC-identical nonresponder DNP-specific B cells or hemisyngeneic CBF$_1$ B cells. In this respect, the GLφ9-primed CBF$_1$ responder recipients were similar to the C57BL/6 nonresponder recipients because they lacked T cells that were able to recognize and interact in a homologous fashion with the C57BL/6 nonresponder DNP-specific primary B cells.

The Secondary DNP-specific B Cell Response of GLφ9 Responder, Nonresponder, and F$_1$ Strains to DNP$_{10}$-GLφ9. To establish a baseline for the current study, donor cells from DNP-Hy-immunized BALB/c, C57BL/6, and CBF$_1$ mice were transferred to Hy-primed irradiated recipients and fragment cultures were stimulated in vitro with DNP-Hy. Table II shows a summary of the results obtained. Consistent with earlier results, the majority of DNP-specific secondary B cell responses in both syngeneic and allogeneic Hy-primed recipients resulted in the synthesis of IgG$_1$ antibody and, thus, appeared homologous in nature. For example, secondary C57BL/6 B cells were stimulated to produce IgG$_1$ antibody in Hy-primed BALB/c recipients.

Experiments were carried out to assess the ability of secondary DNP-specific responder, nonresponder, and responder F$_1$ B cells to be stimulated in GLφ9-primed irradiated recipients. In general, responses to DNP$_{10}$-GLφ9 were lower in frequency than those to DNP$_{10}$-Hy in appropriately primed recipients. This presumably reflects poor priming to GLφ9, especially in the nonresponder strain, as well as a possibly limited presentation of the DNP determinant by a defined copolymer as opposed to the homologous carrier used for donor priming. The GLφ9-primed BALB/c responder strain was able to promote IgG$_1$-antibody responses by B cells of the responder, nonresponder, and F$_1$ responder strains. By analogy to the secondary DNP-specific responses obtained in Hy-primed recipients, one could conclude that BALB/c responder mice express populations of GLφ9-specific T lymphocytes that are capable of interacting in a homologous fashion with both responder B cells and nonresponder B cells. This is in sharp contrast to the response of secondary donor B cells transferred to the GLφ9-primed C57BL/6 nonresponder recipients. As shown in Table II, the GLφ9-specific T cells of the nonresponder strains were capable of promoting only the responses of secondary B cells of the responder BALB/c and CBF$_1$ strains but not the response of its own syngeneic C57BL/6 DNP-specific secondary B cells. The response of the CBF$_1$ and BALB/c secondary B cells were of the IgG$_1$ isotype, suggesting that these B cell responses resulted from homologous collaborative interactions available in the GLφ9-primed C57BL/6 nonresponder recipient. The inability of C57BL/6 secondary B cells to be stimulated in the GLφ9-primed C57BL/6 nonresponder strain suggests that secondary B cells, unlike primary B cells, were not able to collaborate

<div align="center">TABLE II</div>

The Frequency and Heavy-Chain Isotype of Secondary B Cell Responses in Hy- or GLφ9-immunized Recipient Mice

DNP-Hy-immunized donor*	Recipient	Recipient immuniza-tion‡	In vitro antigen	Total number cells trans-ferred	Number of posi-tive foci/ 10^6 cells trans-ferred	Percent-age of foci IgG₁
				$\times 10^{-6}$		
BALB/c (d)§	BALB/c (d)	Hy	DNP-Hy	36	3 08	86
		GLφ9	DNP-GLφ9	36	1 03	62
	C57BL/6 (b)	Hy	DNP-Hy	32	0 87	57
		GLφ9	DNP-GLφ9	92	0 92	95
	CBF₁ (d/b)	Hy	DNP-Hy	32	2 47	90
		GLφ9	DNP-GLφ9	88	1 02	82
C57BL/6 (b)	BALB/c (d)	Hy	DNP-Hy	32	0.59	95
		GLφ9	DNP-GLφ9	108	0.65	74
	C57BL/6 (b)	Hy	DNP-Hy	48	1 04	80
		GLφ9	DNP-GLφ9	108	0.05	—‖
	CBF₁ (d/b)	Hy	DNP-Hy	34	1 38	89
		GLφ9	DNP-GLφ9	148	0.07	—
CBF₁ (d/b)	BALB/c (d)	Hy	DNP-Hy	28	2 79	86
		GLφ9	DNP-GLφ9	27	1 26	74
	C57BL/6 (b)	Hy	DNP-Hy	32	2.34	93
		GLφ9	DNP-GLφ9	16	1 12	56
	CBF₁ (d/b)	Hy	DNP-Hy	28	2 00	86
		GLφ9	DNP-GLφ9	28	0.96	93

* Donor animals were immunized 6–8 wk before cell transfer with 0.1 mg DNP-Hy in CFA
‡ Unimmunized animals were used as recipients of immunized donor cells for all strain combinations shown When a total of 72 × 10⁶ DNP-Hy-immunized donor cells were transferred to unimmunized recipients and the fragment cultures stimulated in vitro with DNP-GLφ9, the number of positive foci/10⁶ cells transferred was always <0.03
§ The H-2 haplotype of the strain is shown in parentheses.
‖ Heavy-chain isotype not determined.

with the available GLφ9-specific T cells in these recipients and, thus, required a homologous mode of stimulation. In the absence of such cells, the secondary C57BL/6 B cells fail to be stimulated.

The responses of the responder, nonresponder, and F₁ responder secondary B cells in GLφ9-primed CBF₁ responder recipients are also summarized in Table II. Responder BALB/c and CBF₁ secondary donor B cells responded in CBF₁ recipients yielding IgG₁-antibody-producing clones; however, consistent with earlier findings of Katz and Benacerraf (3), secondary nonresponder B cells were unable to be stimulated in the CBF₁ responder recipients. It would appear that the C57BL/6 nonresponder B cells were not able to collaborate with the available GLφ9-primed T cells in the CBF₁ recipient. As suggested above, this would imply that the hemisyngeneic CBF₁ responder, like the C57BL/6 nonresponder, had no GLφ9 T cells that were able to interact with B cells of the nonresponder parent in a homologous fashion. Thus, in these respects, the CBF₁ responder T cell repertoire resembles the C57BL/6 nonre-

TABLE III

The Frequency and Heavy-Chain Isotype of Responses of Secondary Donor B Cells Transferred to Syngeneic and Allogeneic Responder and Nonresponder Strains

DNP-Hy-immunized donor*	Strain GLφ9 respon- siveness‡	Recipient	Strain GLφ9 respon- siveness‡	H-2 identity KABJECSD	Total number donor cells trans- ferred	Number of positive foci/10^6 donor cells trans- ferred	Percent- age of foci IgG$_1$
					× 10^{-6}		
B10 A (5R)	+	BALB/c	+	CSD	24	0 66	60
		C57BL/6	−	KAB	24	0.08	—‖
B10 A (4R)	−	BALB/c	+	—	32	0 38	50
		C57BL/6	−	BJECSD	64	0 02	—
SJL	−	BALB/c	+	—	28	1 29	56
		C57BL/6	−	—	44	0 61	52
A/J	−	BALB/c	+	CSD	40	0 90	78
		C57BL/6	−	—	32	0 78	100
D2 GD	−	BALB/c	+	KAB	32	0 62	85
		C57BL/6	−	JECSD	24	0 04	—

* Between 4 × 10^6 and 6 × 10^6 donor cells were transferred to each recipient All cultures were stimulated with DNP-GLφ9
‡ According to Benacerraf and Germain (8) and P Maurer Unpublished observations
‖ Heavy-chain isotype not determined

sponder repertoire in the deletion of this population of T cells. This deletion is presumably a result of the presence of the C57BL/6 nonresponder MHC haplotype in the CBF$_1$ strain. Because this MHC haplotype is absent in the parental BALB/c responder mice, these mice can collaborate with C57BL/6 secondary B cells.

Similar results were obtained when the donor cells from DNP-Hy immunized animals were anti-θ and complement treated before cell transfer. A total of 6 × 10^6 anti-θ-and-complement-treated donor cells of the BALB/c responder and C57BL/6 nonresponder were transferred to GLφ9-immunized BALB/c, C57BL/6, and CBF$_1$ recipients, and the responses were indistinguishable from those obtained with untreated donor cell populations. Additionally, when either BALB/c or C57BL/6 secondary donor cell populations were depleted of macrophages, responses were similar to those obtained with nondepleted populations.

The Genetic Locus Responsible for GLφ9 Responsiveness.   Responsiveness to GLφ9 has been demonstrated to be under the control of two distinct complementing Ir genes: one located in the I-A subregion, termed β, the other located in the I-E/I-C subregion, termed α (8, 25, 26, 32). Having described an apparent functional deletion of the subpopulation of T cells in the C57BL/6 nonresponder and CBF$_1$ responder strains that is capable of interacting with the C57BL/6 nonresponder strain B cells in a homologous fashion, we wished to address the question of whether such a deletion required the presence of both complementing genes in the responding B cells. When donor and recipient strains are appropriately selected for such an analysis, they also offer the potential to determine the extent to which allogeneic effects resulting from

MHC incompatibilities may have influenced the above results. Table III summarizes the results obtained when spleen cells from DNP-Hy immunized donors were transferred to GLϕ9-primed irradiated recipients.

C57BL/6 nonresponder recipient T cells were not able to collaborate with donor secondary B cells of strains that shared genes with C57BL/6 in either the I-A (B10.A[5R]) or the I-C (D2.GD and B10.A[4R]) subregion. This was independent of the ability of the donor strain itself to respond to GLϕ9. Thus, B cells from both the responder strain, B10.A(5R), and nonresponder strains, B10.A(4R) and D.2GD, were incapable of responding in the C57BL/6 recipient. Further strain analyses showed that secondary B cells of the nonresponder strains SJL and A/J, which are not identical in either α or β to the C57BL/6 nonresponder recipient, were able to respond in the C57BL/6 recipients. It thus appears that T cells are present in the C57BL/6 GLϕ9 nonresponder that are able to collaborate with the B cells of the nonresponder s and a haplotypes.

In addition, several of these strain combinations offer the potential for an allogeneic effect, and yet, responses occurred as predicted by the previous experiments. Thus, the B10.A(4R), D2.GD, and B10.A(5R) donor cells do not respond, although incompatibilities exist in the K and I-A subregions; the K, I-A, and I-B subregions; and the I-J, I-E, I-C, S, and D subregions. At the same time, incompatibilities in the entire region did not prohibit responses of BALB/c, A/J, and SJL donor cells. It appears, therefore, that allogeneic effects, either positive or negative, were not responsible for the responses observed.

Discussion

In this report, we present the results of studies designed to determine the ability of T cells from GLϕ9-immunized responder and nonresponder strains to collaborate with DNP-specific B cells in antibody responses to DNP-GLϕ9. Because our studies used the splenic fragment culture system, they differ importantly from other studies. Most significant is that when recipients are adequately carrier primed, it is possible to obtain both secondary and primary B cell responses in collaboration with MHC-nonidentical carrier immunized T cells. Consequently, it is possible to discriminate between two modes of primary B cell responses by virtue of the heavy-chain isotype of the antibody produced; those resulting from interactions of MHC-identical T and B cells and those of nonidentical T and B cells. By extending this system to an MHC-linked responder-nonresponder antibody response, it has now become possible to define experimentally three modes of T cell-B cell interactions that appear to be the predominant, although not the exclusive, interactions when T cells and B cells are derived from various sources: (a) The first of these, which we will refer to as homologous collaborative interactions, occurs between T cells and primary or secondary B cells from MHC-identical responder mouse strains. These interactions result in predominantly IgG$_1$ antibody-producing clones. (b) The second are those interactions that occur between T cells and secondary B cells of MHC-nonidentical strains. These interactions, like homologous interactions, yield IgG$_1$ antibody-producing clones. However, there is no evidence that these T cells are, in fact, identical to the syngeneic T cell populations in their recognition of the antigen or B cells. One might predict that because these T cell populations have not previously encountered the MHC cell-

surface gene products of the allogeneic B cells, they may not have been selected to interact with these B cells. Consequently, they may represent a unique subpopulation not present in the syngeneic T cell repertoire. In addition, as noted, they are incapable of yielding IgG$_1$ antibody primary B cell responses. We, therefore, propose to term such interactions isologous interactions, to distinguish them from homologous interactions. (c) The third mode of T cell-B cell collaboration, which we will refer to as heterologous interactions, occurs between nonidentical T and primary B cell populations and does not appear to be adequate for secondary B cell stimulation. These interactions characteristically result in small IgM antibody-producing clones.

Previous studies have indicated that primary B cells could be triggered by antigen-specific allogeneic T cells in a frequency not significantly lower than the frequency of B cells stimulated with antigen-specific syngeneic T cells; however, the responses obtained in the allogeneic collaborative interactions were solely of the IgM-antibody class (11). The capacity of appropriately triggered allogeneic antigen-specific T cells to enable an IgM response of primary B cells has subsequently been confirmed by several laboratories (15, 18, 20, 33) and has been interpreted as reflecting an antigen-driven T cell-dependent stimulatory phenomenon that may be mediated via mitogen receptors of primary B cells for T cell products (34). At the time, it also appeared that such allogeneic recognition could enable some secondary B cells to be triggered to yield IgG$_1$-antibody-producing clones in a manner similar to syngeneic collaborative interactions. We have now demonstrated that an antigen-specific T cell population can be deleted of T cells that have the ability to collaborate with MHC-identical secondary B cells and, at the same time, retain the ability to enable IgM antibody responses of the MHC-bearing primary B cells. This finding clearly implies that two populations of GLϕ9-specific T lymphocytes with different collaborative potentials are present in the responder strain: one essential for the promotion of the IgG$_1$ antibody responses of primary and secondary B cells, and the second, a population with the potential to promote only IgM primary B cell responses. We suggest the latter population needs no MHC-recognition identity with the responding B cell. The GLϕ9-primed nonresponder T cell population appears to be deleted of the first category of T cells: those selected to interact with their own MHC-identical B cells. The remaining GLϕ9-specific T cell population in the nonresponder appears to be unbiased with respect to its capacity to collaborate in an isologous fashion with B cells of other MHC phenotypes

It would appear that individuals of all murine strains normally maintain a population of T cells that are capable of productive and specific homologous-like interactions with MHC-nonidentical B cell populations. This is most clearly evidenced by the demonstration that secondary B cells of a nonresponder strain fail to be stimulated in collaboration with syngeneic T cell populations, yet retain responsiveness in collaboration with MHC-allogeneic strains. The degree of restriction in the recognition of MHC determinants for B cell-T cell collaboration, T cell-macrophage interactions, and antigenic recognition by cytolytic T cells is a highly controversial subject at the present time. Results from this laboratory and others (10-12, 16, 20, 35) have implied that individuals of any strain inherit the capability for homolgous interaction with all other MHC haplotypes and, in fact, express this capability after maturation. The majority of published results, however, report that the recognition of self MHC, particularly in immunized individuals, is nearly absolute (2, 3, 5-9, 15,

36). Most of these latter findings have utilized chimeric animals constructed between F_1 and parental mouse strains or, alternatively, have attempted to eliminate alloaggressive interactions between allogeneic cell populations via filtration through hemisyngeneic individuals. It is possible that the use of such protocols to eliminate allogeneic effects has also deleted much of the antigen-specific collaborative potential of the resulting T cell population with respect to that particular allogeneic B cell population. These experimentally manipulated T cell populations, which lack the ability to collaborate with B cells from a given allogeneic strain, may be akin to the physiologically observed deletion of $GL\phi9$-specific T cells in the responder F_1 strain capable of interacting isologously with secondary B cells of the nonresponder haplotype. The parental responder T cells that have differentiated in the absence of the nonresponder MHC haplotype collaborate quite well with the same nonresponder B cells.

We can draw several important conclusions concerning the basis of I region-determined nonresponsiveness from the studies presented here. First, there appear to be T cells in the $GL\phi9$ nonresponder strain that have the potential to recognize the antigen $GL\phi9$, but do so only in the context of non-self MHC phenotypes. Such responses are observed in collaboration with either responder B cells or MHC-dissimilar nonresponder B cells. Thus, within the repertoire of a particular mouse strain, even within a nonresponder strain, the potential to collaborate in an isologous fashion with B cells of MHC-nonidentical mouse strains is maintained. Second, the inability of the F_1 T cell population to collaborate with the nonresponder secondary B cells appears to be a result of a specific deletion of T cells by the presence of the MHC-nonresponder phenotype. This is evidenced by the ability of T cells of the parental responder strain to collaborate with the nonresponder B cells. Third, these findings serve as strong confirmation of the previously reported complementation of Ir genes in the I-A and I-E/I-C subregion that control responsiveness to the antigen $GL\phi9$ (8, 25, 26, 32). The inability of the responder secondary B10.A(5R) B cells to respond in $GL\phi9$-immunized C57BL/6 recipients that share the nonresponder haplotype only in the IA subregion demonstrates that the sharing of one of the two complementary nonresponder genes is sufficient for nonresponsiveness. Thus, the nonresponder T cell population appears to have been deleted of cells capable of homologous interactions with B cells that share either of the two complementing nonresponder genes. The C57BL/6 nonresponder T cells are, however, capable of interacting in an isologous fashion with nonresponder B cells of MHC-dissimilar strains such as SJL and A/J. This clearly implies that the basis of $GL\phi9$ nonresponsiveness in the C57BL/6 and SJL are distinct, and that there is a polymorphism in the complementary genes controlling $GL\phi9$ responsiveness as was previously implied by the theory of coupled complementation (32). Our studies would, therefore, predict that isologous collaborative interactions may occur between any two nonresponder strains for which there is no cross-reaction at the level of the deletion of specific T cells.

Finally, our data argue against a determinative role of macrophages in the processing and presentation of antigenic determinants, as has been postulated to explain the linked recognition of MHC and antigenic determinants (5–8). Because responses in this system are independent of donor T cells or of macrophages, the collaborating T cells are presumably stimulated in the environment of their own

macrophages and in the environment in which they were initially primed. Therefore, there appear to be no limitations imposed on T cell-macrophage interactions, and yet, there are clear differences in the degree of responsiveness, depending on the MHC haplotype of the responding B cells. This can only be interpreted on the basis of T cell recognition of antigen presented in the context of B cells per se. Thus, the population of GLϕ9-specific T cells in a CBF$_1$ recipient in the presence of CBF$_1$ macrophages can clearly delineate between BALB/c and C57BL/6 primary and secondary B cells.

It is crucial to all of these speculations that the collaborative interactions observed in this study are not the result of any of a wide range of phenomena that have been termed allogeneic effects. We had previously concluded that allogeneic effects play little or no role in the stimulation in the fragment culture system because: (a) Attempts to induce responses in the fragment culture system via allogeneic effects have invariably failed. (b) All stimulatory interactions require that both the T cells and B cells recognize the stimulating antigen, and that both determinants must be on the same antigen molecule. (c) The extensive irradiation is likely to eliminate most allogeneic stimulatory interactions. In this paper, we present further convincing evidence that nonspecific allogeneic stimulatory effects play little or no role in this system. We found that secondary B10.A(4R) cells were not stimulated when transferred to GLϕ9-primed C57BL/6 recipient mice. Although these strains are allogeneic in the I-A subregion that is responsible for most nonspecific allogeneic effects, these differences do not appear adequate to circumvent the nonresponsiveness imposed by the shared genes of the I-E/I-C subregion.

In toto, the findings reported here present new and important evidence that first, individuals within given murine strains possess the capacity to recognize, in a homologous fashion, B cells of totally allogeneic individuals; and second, that the nonresponder status is the result of a deletion of T cells capable of recognizing antigen in the context of B cells of the nonresponder haplotype. In addition, it appears that this deletion is the result of the presence of the nonresponder haplotype within the individual during development or antigen priming. Thus, whatever the mechanism of T cell-B cell recognition, it is clear that the presence or absence of certain MHC determinants in the environment of the T cells is completely determinative for the recognition of presumably unrelated antigens in the context of B cells bearing given MHC. Thus, it would appear that the basis for haplotype differences in antigen recognition resides in the selective forces operative in establishing the T cell receptor repertoire per se. From the data presented here, it appears that two major roles of MHC could be viewed as that of an antigenic determinant whose presence during repertoire establishment is pivotol in dictating the deletion of certain receptor specificities and, thus, shaping the expressed repertoire and further by its need to be recognized on B cells during their antigen-mediated interactions with T cells. The mechanisms by which these influences exert themselves remain to be resolved by further investigation.

Summary

These studies were carried out to investigate the potential helper T cell repertoire specific for the random copolymer poly(L-Glu^{55},L-Ala^{35},L-Phe^9)$_n$ (GLϕ9) of responder, nonresponder, and (responder $\times$ nonresponder)F$_1$ murine strains. We tested the ability of these T cells to collaborate with dinitrophenyl (DNP)-specific primary and

secondary B lymphocytes of each strain in response to the antigen DNP-GLϕ9 in the splenic-fragment culture system. The results of these experiments show that there are GLϕ9-specific T lymphocytes in the responder, nonresponder, and F_1 strains; but that these three GLϕ9-specific T cell populations differ in their collaborative potential. Responder T cells are able to collaborate with their own syngeneic responder B cells as well as the allogeneic nonresponder B cells in a syngeneic fashion. The F_1 T cell population resembles that of the nonresponder in its ability to collaborate with only responder B cells in a syngeneic fashion. Analyses carried out using appropriately selected mouse strains indicate that these results are unlikely to be a result of positive or negative allogeneic effects. The results obtained suggest that individuals within a given murine strain do possess the capacity to collaborate in a syngeneic fashion with B cells of any other MHC-allogeneic strain as well as their own MHC-identical B cells. The nonresponder status in the response to GLϕ9 appears to be the result of a deletion of T cells capable of recognizing antigen in the context of B cells of the nonresponder haplotype. Thus, the MHC gene products appear to play a determinative role in shaping the expressed helper T cell specificity repertoire within an individual mouse strain.

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