ROLE OF THE MAJOR HISTOCOMPATIBILITY GENE
PRODUCTS IN REGULATING THE ANTIBODY
RESPONSE TO DINITROPHENYLATED
POLY(l-Glu\textsuperscript{55}, l-Ala\textsuperscript{35}, l-Phe\textsuperscript{9})\textsubscript{n}*

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The role of the major histocompatibility gene complex (MHC)\textsuperscript{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

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Abbreviations used in this paper: CFA, complete Freund's adjuvant, DNP, 2,4-dinitrophenylated, GL\textsubscript{69}, poly(l-Glu\textsuperscript{55}, l-Ala\textsuperscript{35}, l-Phe\textsuperscript{9})\textsubscript{n}, Hy, L\textsubscript{ymnulus polyphemus} hemocyanin, MHC, major histocompatibility complex

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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, L-Ala, L-Phe)n (GL<9), 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-nonident-
tical T cells, whereas secondary B lymphocytes required some form of MHC recognition
by the collaborating T cell. (b) F1 T cells interacting with antigen in the milieu of
syngeneic macrophages are able to discriminate between GL<9 responder and non-
responder B lymphocytes. Thus, the GL<9 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 GL<9 nonresponder strain investigated has
demonstrable GL<9-specific T cell help for the antigen 2,4-dinitrophenylated (DNP)-
GL<9. However, a subpopulation of the GL<9-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 GL<9-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 × nonre-
sponder)F1 presumably as a result of the presence of the nonresponder MHC gene
products in the F1 animal. Thus, the responder T cell repertoires of the F1 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 Biochem-
ical Corp., Freehold, N. J. DNP-Hy, 10 mol DNP/100,000 mol wt Hy (DNP<9-Hy) was
prepared as described elsewhere (27). GL<9 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<sub>10</sub>-GL<sub>4</sub>9 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<sub>1</sub>, 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 nonresponder strain, mice to be used as GL<sub>4</sub>9-primed recipients were given three intraperitoneal injections of 0.1 mg GL<sub>4</sub>9; the first in CFA 16 wk before use, followed by two injections of 0.1 mg GL<sub>4</sub>9 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<sub>4</sub>9 were tested for the presence of GL<sub>4</sub>9-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 C57BL/6, produced appreciable amounts of antibody specific for the GL<sub>4</sub>9 antigen, whereas there was no detectable level of GL<sub>4</sub>9-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<sub>1</sub> Strains to DNP<sub>10</sub>-GL<sub>4</sub>9.

Preliminary experiments were carried out to determine the ability of the GL<sub>4</sub>9-responder strain, BALB/c, the nonresponder strain, C57BL/6, and the F<sub>1</sub> responder strain, (BALB/c × C57BL/6)F<sub>1</sub> 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 H-primed recipients. Syngeneic donor-recipient strain combinations and hemisyngeneic combinations involving F<sub>1</sub> and parental strains yielded clones, the majority of which synthesized IgG<sub>1</sub> 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<sub>4</sub>9-immunized responder, nonresponder, and F<sub>1</sub> recipient mice in collaboration with primary DNP-specific B cells in response to DNP<sub>10</sub>-GL<sub>4</sub>9. When GL<sub>4</sub>9-immu-
TABLE I
The Frequency and Heavy-Chain Isotype of DNP-specific Primary B Cell Responses in Hy- and GL\textsubscript{49}-
immunized Recipient Mice

| Donor\* | Recipient | Recipient immunization\(†\) | In vitro antigen | Total number of cells transferred | Number of positive foci/10\(^6\) cells transf. | Percentage of foci IgG\(_\text{a}\) |
|-------|----------|-----------------------------|----------------|----------------------------------|---------------------------------------------|---------------------------------|
| BALB/c (d)\| BALB/c (d) | Hy | DNP-Hy | 56 | 0.89 | 80 |
| GL\textsubscript{49} | DNP-GL\textsubscript{49} | 64 | 0.89 | 60 |
| C57BL/6 (b) | Hy | DNP-Hy | 43 | 0.60 | 43 |
| GL\textsubscript{49} | DNP-GL\textsubscript{49} | 30 | 0.30 | 1 |
| CBF\(_1\) (d/b) | Hy | DNP-Hy | 18 | 0.75 | 83 |
| GL\textsubscript{49} | DNP-GL\textsubscript{49} | 24 | 0.75 | 44 |
| C57BL/6 (b) | BALB/c (d) | Hy | DNP-Hy | 20 | 0.80 | 6 |
| GL\textsubscript{49} | DNP-GL\textsubscript{49} | 56 | 0.61 | 2 |
| C57BL/6 (b) | Hy | DNP-Hy | 20 | 0.60 | 83 |
| CBF\(_1\) (d/b) | Hy | DNP-Hy | 30 | 0.30 | 3 |
| GL\textsubscript{49} | DNP-GL\textsubscript{49} | 60 | 0.30 | 1 |

* 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-GL\textsubscript{49} paralleled those obtained using the hapten carrier system DNP-Hy. As shown in Table I, syngeneic and hemisyngeneic donor B cells were stimulated in GL\textsubscript{49}-primed BALB/c recipients to yield IgG\(_\text{a}\)-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 GL\textsubscript{49}-primed F\(_1\) responder mice was strikingly different from that predicted by the results obtained employing DNP-Hy. In GL\textsubscript{49}-primed CBF\(_1\) recipients, the hemisyngeneic BALB/c responder donor B cells responded with IgG\(_\text{a}\)-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 GL\textsubscript{49}, only the collaborative interactions with the parental responder or F\(_1\) B cells can be described as homologous, as they yielded IgG\(_\text{a}\)-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 CBF1 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 GL49-primed nonresponder C57BL/6 recipients of responder, nonresponder, and F1 primary donor B cells were capable of promoting antigen-dependent antibody responses to DNP-GL49, but not in a homologous fashion, as indicated by the exclusive IgM antibody responses. Thus, the nonresponder strain, although maintaining a repertoire of GL49-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 hemisynthetic CBF1 B cells. In this respect, the GL49-primed CBF1 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 Seconda O, DNP-specific B Cell Response of GL49 Responder, Nonresponder, and F1 Strains to DNP10-GL49. To establish a baseline for the current study, donor cells from DNP-Hy-immunized BALB/c, C57BL/6, and CBF1 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 IgG1 antibody and, thus, appeared homologous in nature. For example, secondary C57BL/6 B cells were stimulated to produce IgG1 antibody in Hy-primed BALB/c recipients.

Experiments were carried out to assess the ability of secondary DNP-specific responder, nonresponder, and F1 B cells to be stimulated in GL49-primed irradiated recipients. In general, responses to DNP10-GL49 were lower in frequency than those to DNP10-Hy in appropriately primed recipients. This presumably reflects poor priming to GL49, 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 GL49-primed BALB/c responder strain was able to promote IgG1-antibody responses by B cells of the responder, nonresponder, and F1 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 GL49-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 GL49-primed C57BL/6 nonresponder recipients. As shown in Table II, the GL49-specific T cells of the nonresponder strains were capable of promoting only the responses of secondary B cells of the responder BALB/c and CBF1 strains but not the response of its own syngeneic C57BL/6 DNP-specific secondary B cells. The response of the CBF1 and BALB/c secondary B cells were of the IgG1 isotype, suggesting that these B cell responses resulted from homologous collaborative interactions available in the GL49-primed C57BL/6 nonresponder recipient. The inability of C57BL/6 secondary B cells to be stimulated in the GL49-primed C57BL/6 nonresponder strain suggests that secondary B cells, unlike primary B cells, were not able to collaborate
The Frequency and Heavy-Chain Isotype of Secondary B Cell Responses in Hy- or GL49-immunized Recipient Mice

| DNP-Hy-immunized donor* | Recipient | Recipient immunization† | In vitro antigen | Total number of positive foci/10^6 cells transferred | Percentage of foci IgG1 |
|-------------------------|-----------|-------------------------|-----------------|-----------------------------------------------------|------------------------|
| BALB/c (d)§             | BALB/c (d) | Hy                      | DNP-Hy          | 36 × 10^6                                           | 3.08 86                |
| C57BL/6 (b)             | GL49      | Hy                      | DNP-Hy          | 36 × 10^6                                           | 1.03 62                |
| CBFl (d/b)              | GL49      | Hy                      | DNP-Hy          | 32 × 10^6                                           | 0.92 95                |
| BALB/c (d)†             | GL49      | Hy                      | DNP-Hy          | 32 × 10^6                                           | 2.47 90                |
| C57BL/6 (b)             | GL49      | Hy                      | DNP-Hy          | 32 × 10^6                                           | 8.80 82                |
| CBFl (d/b)              | GL49      | Hy                      | DNP-Hy          | 88 × 10^6                                           | 1.02 82                |

* 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 × 10^6 DNP-Hy-immunized donor cells were transferred to unimmunized recipients and the fragment cultures stimulated in vitro with DNP-GL49, the number of positive foci/10^6 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 GL49-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 GL49-primed CBFl responder recipients are also summarized in Table II. Responder BALB/c and CBFl secondary donor B cells responded in CBFl 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 CBFl responder recipients. It would appear that the C57BL/6 nonresponder B cells were not able to collaborate with the available GL49-primed T cells in the CBFl recipient. As suggested above, this would imply that the hemisyngeneic CBFl responder, like the C57BL/6 nonresponder, had no GL49 T cells that were able to interact with B cells of the nonresponder parent in a homologous fashion. Thus, in these respects, the CBFl responder T cell repertoire resembles the C57BL/6 nonre-
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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- | Strain GL49 | Strain GL49 | H-2 Identity | Total number of positive foci/10^6 donor cells transferred | Number of positive foci/10^6 donor cells transferred | Percentage of foci IgG |
| immunized | GL49 responsiveness* | GL49 responsiveness† | K J E C S D | donor cells transferred | donor cells transferred | |
| donor* | | | | | | |
| B10 A (5R) + BALB/c + | C57BL/6 | CSD | 24 | 0.66 | 60 |
| B10 A (4R) - BALB/c + | C57BL/6 - J E C S D | 64 | 0.02 | - |
| SJL - BALB/c + | C57BL/6 - | 28 | 0.29 | 56 |
| A/J - BALB/c + | C57BL/6 - | 44 | 0.61 | 52 |
| D2 GD - BALB/c + | C57BL/6 - | 32 | 0.78 | 100 |

* Between 4 × 10^6 and 6 × 10^6 donor cells were transferred to each recipient. All cultures were stimulated with DNP-GL49.
† According to Benacerraf and Germann (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 CBF1 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 GL49-immunized BALB/c, C57BL/6, and CBF1 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 GL49 Responsiveness. Responsiveness to GL49 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 CBF1 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 D2GD, 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 IgG1 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 IgG1 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 IgG1 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 IgM-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 GL49-specific T lymphocytes with different collaborative potentials are present in the responder strain: one essential for the promotion of the IgG1 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 GL49-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 GL49-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 homologous 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,
Most of these latter findings have utilized chimeric animals constructed between F1 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 GL49-specific T cells in the responder F1 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 GL49 nonresponder strain that have the potential to recognize the antigen GL49, 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 F1 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 GL49 (8, 25, 26, 32). The inability of the responder secondary B10.A(5R) B cells to respond in GL49-immunized C57BL/6 recipients that share the nonresponder haplotype only in the IA subregion demonstrates that the sharing of one of the two complementing 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 GL49 nonresponsiveness in the C57BL/6 and SJL are distinct, and that there is a polymorphism in the complementary genes controlling GL49 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 GL49-specific T cells in a CBF1 recipient in the presence of CBF1 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 GL49-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 pivotal 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(1-Glu₃₅,1-Ala₃₅,1-Phe₃₅)ₖ (GL49) of responder, nonresponder, and (responder × nonresponder)F₁ 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-GL49 in the splenic-fragment culture system. The results of these experiments show that there are GL49-specific T lymphocytes in the responder, nonresponder, and F1 strains; but that these three GL49-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 F1 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 GL49 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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