ADAPTIVE DIFFERENTIATION OF MURINE LYMPHOCYTES

I. Both T and B Lymphocytes Differentiating in
F1 → Parental Chimeras Manifest Preferential
Cooperative Activity for Partner Lymphocytes Derived
from the Same Parental Type Corresponding to the Chimeric Host*

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Approximately 5 yr ago the first observations that documented genetic restrictions imposed
by genes within the major histocompatibility complex (MHC)† upon cooperative interactions
between T lymphocytes and macrophages and between T lymphocytes and B lymphocytes
were described (1–3). Later, it was found that the most efficient lysis of target cells by specific
cytotoxic T lymphocytes (CTL) occurred when the CTL and target cell, respectively, shared
gene identities in the mouse H-2 complex (4–8). Genetic mapping studies documented that
gene(s) controlling T-B-cell interactions are located in the I-region of the mouse H-2 complex
(9), whereas those involved in CTL-target interactions are located in the K and D regions of H-
2 (10, 11).

The subject of MHC-linked genetic restrictions on cell-cell communication processes has
evoked controversy both in terms of the extent of such constraints on cell-cell interactions and
on the best possible interpretations of such restrictions (12–14). Essentially two major concepts
have evolved to explain these genetic restrictions on cell interactions. The first hypothesis,
which stemmed from analysis of such restrictions in T-B-cell interactions, considered that
interactions among various cell types in the immune system are mediated by cell interaction
(CI) molecules located on the cell surface, at least some of which are encoded by MHC genes
(i.e. I-region genes in this case), and which are quite distinct from the lymphocyte receptors
specific for conventional antigens (13–15). The CI molecule concept therefore emphasizes a
dual recognition mechanism which involves at least two distinct molecular interactions in
lymphocyte activation, one utilizing antigen-specific receptors and the second consisting of
reactions between the relevant CI structures and their corresponding receptors. The second
major concept, derived primarily from studies in the CTL systems, considered that T lympho-
cytes have receptors which recognize not antigen alone, but antigen in some form of association
with MHC gene products on cell surface membranes; this concept of “altered-self” (16)
recognition by T lymphocytes differs substantially from the CI molecule concept in predicting
the existence of a single receptor on T cells simultaneously recognizing modified determinants
on the cell surface. To date, no definitive proof has been obtained to establish which of these
two models is correct.

* Publication 59 from the Department of Cellular and Developmental Immunology and publication
1479 from the Immunology Departments, Scripps Clinic and Research Foundation, La Jolla, Calif.
Supported by U. S. Public Health Service grant AI-13781 and National Foundation grant 1-540.
† Supported by a Fellowship from The Arthritis Foundation.

† Abbreviations used in this paper: Alum, aluminum hydroxide gel; ASC, Ascaris suum extract; C, complement;
CI molecules, cell interaction molecules; CTL, cytotoxic T lymphocytes; DNP, 2,4-dinitrophenyl; K.I.H,
keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; SCRF,
Scripps Clinic and Research Foundation.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/78/0901-0727$1.00
The validity of the original interpretation of the basis for genetic restrictions dictating the most effective interactions between T and B lymphocytes in the development of antibody responses came under question amidst the reports of other investigators who failed to find similar restrictions in different systems in which T-B-cell cooperative responses were analyzed (17–19); more recent studies in these very same laboratories have clarified these matters somewhat (20–22). The most compelling of such experiments were those performed with cells obtained from tetraparental bone marrow chimeric mice (17). In such circumstances, T lymphocytes originally derived from donor bone marrow of different H-2 haplotypes (i.e. parent A and parent B) but which had differentiated together within a lethally irradiated (A × B)F1 host environment, were found to be independently capable of interacting effectively with B cells derived from conventional donors of the opposite parental type.

Since the parental A and B lymphoid populations of such chimeras were mutually tolerant of one another (i.e. unable to exert reciprocal alloreactivity), it was logical to question whether failure to observe effective cell interactions between the partner cells derived from nontolerant histoincompatible donors might reflect the existence of some inhibitory consequences of mixing such cells. However, this explanation seemed untenable for a number of reasons discussed more fully elsewhere (14, 15), not the least of which was our inability to detect suppression in appropriate cell mixture experiments (23, 24).

In view of the (a) striking degree of MHC-linked genetic restrictions imposed upon effective T-B-cell interactions, (b) absence of demonstrable suppressive influences to explain such genetic restrictions, and (c) seemingly contradictory data obtained with T and B lymphocyte populations derived from bone marrow chimeras, we proposed that this collection of observations could be logically explained by a concept of adaptive differentiation of lymphoid cell precursors (24–27). This concept, in brief, predicted that (a) lymphoid cell precursors differentiate in such a way as to learn the relevant compatibilities required of it for effective cell-cell interactions and, moreover, (b) the crucial lesson that must be learned is dictated by the MHC genotype of the environment in which such differentiation takes place.

In this manuscript we describe experiments documenting that differentiation of both T and B lymphocytes in appropriate bone marrow chimeric mice follows the rules of adaptive differentiation. This process is expressed phenotypically in the capacities of such lymphocytes to optimally interact with reciprocal (i.e. B or T) partner cells of the same H-2 haplotype as that of the chimeric host. The implications of these findings for understanding the mechanisms by which cells of the immune system communicate effectively and unmistakably with one another will be discussed.

Materials and Methods
The proteins, reagents, and preparation of hapten-protein conjugates were the same as those described in earlier reports (3, 28–30). 9 mol of 2,4-dinitrophenyl (DNP)/100,000 daltons of keyhole limpet hemocyanin (KLH) (DNPh-KLH) and 2.1 × 10⁻⁷ mol of DNP/mg of Ascaris suum (DNPs-ASC) were employed in these studies. The preparation of anti-θ serum, its characterization and method of anti-θ serum treatment of spleen cells, determination of serum anti-DNP antibody levels by radioimmunoassay, and the method for enumerating DNP-specific plaque-forming cells (PFC) of the IgG class are described elsewhere (29, 31).

Animals and Immunizations. Inbred BALB/c (H-2a) mice were obtained from Simonsen Laboratories, Gilroy, Calif. or from the Scripps Clinic and Research Foundation (SCRF) mouse breeding colony. Inbred A/J (H-2d) and (BALB/c × A/J)F1 hybrids (CAF1, H-2d/a) were obtained from The Jackson Laboratory, Bar Harbor, Maine or from the SCRF mouse breeding colony. Donors of hapten-primed B cells or carrier-primed T cells were immunized i.p. with, respectively, 10 μg of DNP-ASC precipitated with 4 mg of aluminum hydroxide gel (alum) or 20 μg of KLH emulsified in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.). Conventional (i.e. non-chimeras) donor mice were immunized generally at 8- to 12-wk of age; bone marrow chimeras were immunized as cell donors 3 mo after bone marrow reconstitution (see below). Typically, both hapten- and carrier-primed donor mice were boosted i.p. with 10 μg of the respective antigen in saline 3–4 wk after initial priming; spleen cells were
used 2–4 wk later for either adoptive transfer in vivo assays (3, and Results) or for microculture in vitro assays (29). All X-irradiation was done with a 137Cs cesium irradiator (Gammacell 40, Atomic Energy Limited of Canada).

Preparation of Bone Marrow Chimeras. Bone marrow chimeras were prepared by repopulating lethally X-irradiated (900 rads) recipient mice with donor bone marrow cells in a manner similar to that described by von Boehmer et al. (32) and Sprent et al. (33). Lethally irradiated 12- to 15-wk old CAF1, A/J, or BALB/c recipients were injected intravenously with 15 × 10^6 viable donor bone marrow cells which had been treated with anti-θ serum plus complement (C) to deplete any contaminating T lymphocytes. The mice were housed in cages covered with protective caps; oxytetracycline (Pura-Mycin,Ralston Purina Co., Checkerboard Square, St. Louis, Mo.) was added to the drinking water as a prophylactic measure against infection.

Chimeras were prepared in the following donor → recipient combinations: (a) CAF1 → CAF1, (b) A/J → CAF1, (c) BALB/c → CAF1, (d) CAF1 → A/J, (e) CAF1 → BALB/c, and (f) A/J → BALB/c → CAF1.

Determination of Lymphoid Cell Chimerism. All chimeras were rested after reconstitution for approximately 3 mo before analysis for chimerism. Chimerism was ascertained by analyzing peripheral blood lymphocytes for susceptibility to cytolysis by A/J anti-BALB/c and BALB/c anti-A/J antisera by using a microcytotoxicity assay described elsewhere (34); both antisera lysed > 90% of specific target cells at dilutions of 1:500. Details of the preparation of these antisera and the procedure of this assay, which is highly sensitive and permits analysis of small numbers of peripheral blood lymphocytes, will be reported elsewhere.3

In all, nearly 200 chimeras consisting of the various types indicated above were prepared and analyzed for chimerism by these techniques. Criteria for chimerism consisted of appropriate unilateral sensitivity to cytolysis in the cases of parental → F1 chimeras and bilateral sensitivity to both anti-BALB/c and anti-A/J antisera in the cases of F1 → parent chimeras; any inappropriate lysis differing more than 2% from medium + C controls were grounds for discarding the chimera from the study. Approximately 80% were found to be true chimeras by these criteria; the remaining 20% were either questionable or clearly nonchimeric and were removed from the study. Only after typing for chimerism were mice primed with either KLH or DNP-ASC for use as T- and B-cell donors, respectively.

Data Presentation and Statistical Analyses. Because of the nature and complexity of the type of cell mixture experiments to be described in this report, the results obtained in any given group reflect the interplay of at least three definable variables: (a) the inherent strength of each carrier-specific T-cell population as a result of antigen sensitization; (b) the inherent strength of each DNP-specific B-cell population as a result of priming with DNP-ASC; and (c) the composition of a given chimera serving as donor of helper T cells relative to the type of B cell (i.e. conventional, parent → F1; or F1 → parent chimera) used in a particular mixture. Since all three variables must be taken into account to make meaningful comparisons of the degree of helper T-cell activity of a given donor cell population for each of the different B-cell populations, we have presented the data from individual groups in two different ways, which are depicted in separate panels in each figure. The first way (panel A, Figs. 2–7) presents the degree of helper activity of a given T-cell type provided to each different B-cell type as a relative measurement (expressed as percent of control) based upon the magnitude of helper activity that such T cells provide to B cells derived from isologous donors. For example, the mean response of a group of recipients of isologous mixtures of CAF1 T cells and CAF1 B cells was taken as the 100% control value against which to compare the responses in all other recipient groups in which CAF1 T cells were used as helpers for each of the different B-cell types employed.

This comparison alone is insufficient, however, because it does not take into account differences in the inherent strengths of the various B cells in terms of their capacities for antibody production (variable 2). Therefore, the second method of presenting the data (panel B, Figs. 2–7) compares the magnitude of response developed with a given mixture of helper T cells with a particular B-cell type relative to the magnitude of response developed by that

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2 B. J. Skidmore and L. Miller. 1978. A new microcytotoxicity method for determining lymphoid chimerism by examination of peripheral blood lymphocytes of murine bone marrow chimeras. Manuscript submitted for publication.
particular B-cell type when mixed with helper T cells of isologous type. For example, the response developed by a mixture of CAF1 helper cells and CAF1 B cells is taken as a 100% control value against which to compare the magnitudes of responses developed by the same CAF1 B cells when helped by T cells of another type (e.g. A/J → CAF1 chimera T cells). Since neither conventional A/J nor BALB/c helper T cells were used in these experiments, the responses obtained in cooperative mixtures with CAF1 T cells were taken as the isologous control responses for A/J and BALB/c B cells, respectively. The absolute values for the PFC responses obtained in each control group which received isologous mixtures of T and B cells are depicted beside the corresponding bar on each individual graph.

The fact that all of the various cell mixtures employed in the in vivo assays were prepared from common pools of donor cells and transferred to adoptive recipients on the same day, makes this type of double comparison valid as well as necessary. Moreover, as can be seen from the results, double comparisons of this type make the data considerably more meaningful since criteria were established demanding concordance between both methods of data analysis before conclusions were drawn from any given group.

Statistical analyses were made with geometric means and standard errors calculated from individual DNP-specific PFC values in each respective group. P values from comparison of relevant experimental and control groups were ascertained by Student’s t test.

Results

Lymphoid Cells from Parent → F1 and F1 → Parent Chimeras Lack the Capacity to Exert Allogeneic Effects in Vivo. One of the most sensitive tests for allogeneic-type cell interactions is the measurement of the capacity of a given cell population to exert an allogeneic effect on antibody production in vivo (14 and 35). Thus, even in circumstances where no other manifestation of allogeneic interactions can be detected, such as in vitro mixed lymphocyte reactivity or in vivo graft rejection, subtle interactions of this type can be reflected by significant facilitation of antibody responses (36).

The protocol and results of studies to test chimeric donor lymphocytes as potential inducers of an allogeneic effect are summarized in Fig. 1. In these experiments, 10 × 10⁶ DNP-ASC-primed CAF1 spleen cells were transferred to irradiated CAF1 recipients either (a) alone, (b) in the presence of 8 or 10 × 10⁶ conventional CAF1 KLH-primed helper cells, or (c) comparable numbers of unprimed spleen cells from either conventional F1 or parental donors (Exp. I) or from the various chimeras indicated (Exp. II). In both experiments challenge with the homologous antigen, DNP-ASC, stimulated good secondary anti-DNP antibody responses (group I), whereas challenge with DNP-KLH, in the absence of any added helper cells, failed to elicit significant responses (group II). Addition of KLH-primed CAF1 helper T cells (group III), but not unprimed F1 cells (group IV), permitted the development of excellent secondary responses to DNP-KLH. Concomitant transfer of unprimed parental A/J or BALB/c spleen cells resulted in significant secondary anti-DNP antibody responses (Exp. I, groups V and VI), a manifestation of the capacity of such cells to exert a facilitating allogeneic effect as previously described (32). In sharp contrast, none of the chimeric donor spleen cells manifested any capacity to exert a similar type of allogeneic effect (Exp. II, groups V–IX), thereby providing strong evidence for the lack of any appreciable alloreactivity in these chimeras against either parental H-2 antigens.

Analysis of in Vivo Helper Activity of Chimeric T Cells for Parental, F1, and Chimeric B Cells from DNP-Primed Donors. The capacities of KLH-primed T lymphocytes from either conventional CAF1 donor mice or from the various bone marrow chimeras to provide helper activity for DNP-primed B lymphocytes of various donor origins were measured
SECONDARY CHALLENGE

\[ 10 \mu g \text{DNP-ASC (Alum)} \]

\[ 20 \mu g \text{DNP-KLH (Alum)} \]

**EXPERIMENT I**

| Group       | Unprimed or Primed Helper T Cells |
|-------------|-----------------------------------|
| I           | None                              |
| II          | None                              |
| III         | Unprimed A/J                       |
| IV          | Unprimed BALB/c                    |
| V           | Unprimed A/J                       |
| VI          | Unprimed BALB/c                    |

**EXPERIMENT II**

| Group       | Unprimed or Primed Helper T Cells |
|-------------|-----------------------------------|
| I           | None                              |
| II          | None                              |
| III         | KLH-/+ CAF1                       |
| IV          | Unprimed CAF1                     |
| V           | Unprimed A/J                       |
| VI          | Unprimed BALB/c                    |
| VII         | Unprimed A/J                       |
| VIII        | Unprimed BALB/c                    |

**Figure 1.** Spleen cells from parent \( \rightarrow \) F1 and F1 \( \rightarrow \) parent bone marrow chimeras fail to exert allogeneic effects on DNP-primed conventional F1 spleen cells adoptively transferred to CAF1 recipients. The protocol is summarized on the left. 7 days after cell transfer and secondary challenge, spleens were removed from individual recipient mice and analyzed for IgG DNP-specific PFC. Results are presented as the mean IgG PFC of each group of four mice.

in a standard adoptive transfer system in which both cell types were concomitantly transferred into 675 rads X-irradiated CAF1 recipients. In all instances, the B-cell populations were depleted of T lymphocytes by in vitro treatment with anti-T serum plus complement. Not depicted graphically are the responses of control groups of recipients of each different DNP-primed B-cell population transferred alone in the absence of helper T cells. The values of IgG PFC/10^6 cells for these groups are as follows for each different B-cell type: (a) CAF1 = 146; (b) A/J = 29; (c) BALB/c = 16; (d) CAF1 \( \rightarrow \) CAF1 = 75; (e) A/J \( \rightarrow \) CAF1 = 35; (f) BALB/c \( \rightarrow \) CAF1 = 22; (g) CAF1 \( \rightarrow \) A/J = 15; (h) CAF1 \( \rightarrow \) BALB/c = 13.

The cooperative activities of KLH-primed helper T cells from conventional CAF1 or from the various chimeric donors for DNP-primed B cells from three conventional and five chimeric donor types are summarized in Figs. 2–7. The essence of the results obtained with each different type of helper T-cell population is as follows.

**CAF1 Helper T Cells (Fig. 2).** Conventional CAF1 helper T cells provided helper activity for all of the various B cells employed, although differences in relative magnitudes of helper activity among the various groups were obvious. Fig. 2 emphasizes the importance of expressing the data from each group by both methods.
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Fig. 2. In vivo helper activity of KLH-primed conventional CAF1 T cells for parental, F1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

presented in panels A and B, respectively. Thus, differences between groups that are clearly significant when responses are expressed in relation to CAF1 helper T-cell activity provided to isologous CAF1 B cells (panel A) are not necessarily significant when expressed in relation to the amount of helper activity received by each different B-cell type from its own isologous T-cell type (panel B), and vice-versa. Only in the case of the helper activity for BALB/c → CAF1 B cells (group 6) was there concordance in significant differences from controls in both methods of data presentation.

CAF1 → CAF1 Chimeric Helper T Cells (Fig. 3). In all instances, these T cells provided very effective help for primed B cells whether derived from conventional or chimeric donors. The only significant differences from controls were related to responses of higher, rather than lower, magnitude and concordance in this respect was observed only in cooperative activity with conventional BALB/c B cells (group 11).

A/J → CAF1 Chimeric Helper T Cells (Fig. 4). In contrast to the preceding results, helper T cells from A/J → CAF1 chimeras showed clear disparities in their ability to provide helper activity for certain of the B-cell types. Thus, while effective help was provided to conventional CAF1 and A/J and to chimeric CAF1 → CAF1, A/J → CAF1 and CAF1 → A/J B cells, little or no demonstrable helper activity was provided for B cells derived from either conventional BALB/c (group 19) or BALB/c → CAF1 (group 22) chimera donors. These results indicate quite clearly that T cells in the A/J → CAF1 chimera retain the phenotype of the original parental strain in terms of genetic restriction in their cooperative activity (2, 3). The significance of the defect in
cooperative interactions between A/J → CAF1 chimeric T cells and CAF1 → BALB/c chimeric B cells (group 24) will be discussed below.

**BALB/c → CAF1 Chimeric Helper T Cells (Fig. 5).** Similar to the results obtained with the reciprocal parent → F1 chimera, BALB/c → CAF1 chimeric T cells effectively helped B cells from conventional CAF1 and BALB/c and chimeric CAF1 → CAF1, BALB/c → CAF1, and CAF1 → BALB/c donors. Such cells failed to provide helper activity for conventional A/J (group 26) or A/J → CAF1 (group 29) chimeric B cells.

**CAF1 → A/J Chimeric Helper T Cells (Fig. 6).** The notable results with these helper T cells were their failures to provide effective help for either conventional BALB/c (group 35) or chimeric BALB/c → CAF1 (group 38) donors. This contrasts sharply with the effective help such cells provided to B cells from conventional CAF1 and A/J and from chimeric CAF1 → CAF1, A/J → CAF1 and CAF1 → A/J donors.

**CAF1 → BALB/c Chimeric Helper T Cells (Fig. 7).** These results were almost precisely the reciprocal of the results obtained with CAF1 → A/J chimeric T cells (Fig. 6). Due to an unexplained high mortality incidence in recipients of isologous CAF1 → BALB/c T and B lymphocytes (group 48), the data from this group cannot be presented. The results obtained with the mixture of CAF1 → BALB/c T cells and CAF1 → CAF1 B cells (group 44) were therefore selected as the 100% control values. The notable results with these helper T cells were their failures to interact effectively with B cells from either conventional A/J (group 42) chimeric A/J → CAF1 (group 45) or chimeric CAF1 → A/J (group 47) donors; the significance of the latter finding will be discussed further below.

**Titration of Helper Activity of KLH-Primed Chimeric T Cells For DNP-Primed B Cells from Conventional Donor Mice in Secondary In Vitro Antibody Responses.** The deficiencies of F1 → parent chimeric T cells in providing helper activity for conventional parental B cells of the opposite haplotype were further analyzed in in vitro secondary anti-DNP antibody responses. KLH-primed CAF1 → CAF1, CAF1 → A/J, and CAF1 → BALB/c...
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| Group | KMH-Primed Helper T Cells | Anti-κ-Treated CNP-Primed B Cells |
|-------|--------------------------|----------------------------------|
| 17    | CAF_{i} → A/J            |                                  |
| 18    | CAF_{i} → BALB/c         |                                  |
| 19    | A/J → CAF_{i}            |                                  |
| 20    | A/J → CAF_{i}            |                                  |
| 21    | CAF_{i} → A/J            |                                  |
| 22    | CAF_{i} → BALB/c         |                                  |
| 23    | CAF_{i} → A/J            |                                  |
| 24    | CAF_{i} → BALB/c         |                                  |

**SECONDARY CHALLENGE**

20 μg DNP-KLH (Alum)

**A PROVIDED TO**

| Group |
|-------|
| 17    | P < 0.001   |
| 18    | P = 0.002  |
| 19    | P < 0.001  |

**B RECEIVED BY**

| Group |
|-------|
| 17    | P < 0.001   |
| 18    | P < 0.001  |
| 19    | P < 0.001  |

**ANTI-DNP RESPONSE**

(IgG PFC/10^6 Spleen Cells)

(% of control)

![Graph](image)

Fig. 4. In vivo helper activity of KMH-primed A/J → CAF_{i} chimeric T cells for parental, F_{i}, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

B Lymphocytes Undergo Adaptive Differentiation in F_{i} → Parent Chimeras To Cooperate Preferentially with Helper T Cells from Isologous Parental Donors. Two results in the preceding studies suggested that B lymphocytes might undergo adaptive differentiation in the bone marrow chimera environment. Thus, A/J → CAF_{i} chimeric T cells and CAF_{i} → BALB/c chimeric B cells did not interact very effectively as indicated by the concordance of significant differences in both panels A and B of Fig. 4. Similarly, CAF_{i} → BALB/c chimeric T cells interacted poorly with B cells from CAF_{i} → A/J chimeric donors resulting in concordantly significant differences in secondary responses compared to controls (Fig. 7). Since the helper T cells in both instances were quite effective in providing helper activity for conventional CAF_{i} B cells, these results indicate that a significant shift may have occurred with respect to the ability of F_{i} B cells to be effectively helped by the chimeric T cells, presumably because these B cells differentiated in the environment of a parental host. Due to the potential importance of this finding in terms of clarifying our understanding of the...
mechanism(s) by which lymphocytes effectively communicate with one another, further analysis, both in vivo and in vitro, of this possibility was carried out. Since the in vivo analysis required the use of conventional parental helper T lymphocytes which are fully competent to react against irradiated F1 recipients, the in vivo experiment summarized in Fig. 9 employed the standard 2-stage adoptive transfer technique originally devised in our laboratory for analysis of genetic restrictions in T-B-cell interactions in the mouse (3).

As illustrated in Fig. 9, B lymphocytes from CAF1 → CAF1, CAF1 → A/J, and CAF1 → BALB/c chimeric donors were tested for their cooperative activity with KLH-primed T cells from conventional CAF1 → A/J or BALB/c donors. The notable results were the relative deficiencies in cooperative activities between primed B cells from CAF1 → A/J and CAF1 → BALB/c chimeras with T cells derived from conventional BALB/c (group VI) and A/J (group VIII) donors, respectively. This contrasts directly with the indiscriminate cooperative interactions of CAF1 → CAF1 chimeric B cells with all three T-cell types and the ability of the F1 → parent chimeric B cells to interact with either conventional F1 or corresponding parental type T cells.

This important manifestation of haplotype preference in primed chimeric B lymphocytes was confirmed by a titration analysis carried out in vitro secondary antibody responses (Fig. 10). In contrast to the ability of CAF1 → CAF1 chimeric B cells to interact comparably well with either A/J or BALB/c T cells over the whole range of cell doses employed (top panel), were the results obtained with CAF1 → A/J chimeric B cells (bottom panel). These cells (a) displayed considerably higher responses when cocultured with A/J T cells over the entire cell dose range employed; and (b) the cooperative activity with helper T cells from BALB/c donors were substantially lower than the responses with A/J T cells. Although it may appear that some defect existed in the capacity of F1 T cells to help the CAF1 → A/J chimeric B cells, the magnitude of such responses most likely reflects a lower level of overall helper activity of this F1 T-cell population as indicated by the lower responses

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Fig. 5. In vivo helper activity of KLH-primed BALB/c → CAF1 chimeric T cells for parental, F1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.
obtained when such cells were cocultured with CAF1 → CAF1 chimeric B cells (top panel).

The normal cooperative activity between either of the F1 → parent chimeric B cells and CAF1 T cells argues against the possibility of a subtle suppressive mechanism explaining these results. Nevertheless, this possibility was directly tested by analyzing the effects chimeric B cells might have on normal cooperative T-B cell interactions in vitro. As summarized in Table I, IgG DNP-specific PFC responses in cultures containing cooperative mixtures of CAF1 → CAF1 B cells and either conventional CAF1, A/J, or BALB/c T cells were of substantial magnitude. Moreover, such responses were not appreciably affected by the concomitant presence of additional B cells obtained from either CAF1 → CAF1 or CAF1 → A/J chimeras.

Discussion

The results presented here demonstrate that in appropriate circumstances both T lymphocyte precursors of the regulatory helper cells for antibody production and B lymphocyte precursors of antibody-secreting cells differentiate along pathways dictated, to a considerable extent, by the environmental milieu in which such differentiation occurs. Phenotypic manifestations of this process are reflected in the relative haplotype preference displayed by a given population of (a) T lymphocytes for providing the most effective cooperative helper activity for primed B lymphocytes of various donor origins, and (b) B lymphocytes insofar as their cooperative capabilities of interacting effectively with helper T cells from various conventional and chimeric donors. These results provide, therefore, strong support for the concept of adaptive differentiation which we originally proposed several years ago (24–27) to account for several discrepancies among various studies analyzing the presence or absence of H-2-linked genetic restrictions in T-B-cell cooperative interactions. Moreover, the present
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Fig. 7. In vivo helper activity of KLH-primed CAF1 → BALB/c chimeric T cells from parental, F1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

studies complement recent observations of other investigators (22, 37–39) which similarly support the general model of adaptive differentiation of lymphocytes.

The most crucial information, upon which the aforementioned conclusion is based, can be summarized as follows: in general, parallel results were obtained with both lymphocyte types and demonstrated quite clearly that (a) F1 → F1 chimeric lymphocytes displayed no restriction in terms of cooperative activity with all of the various partner cell combinations, results which parallel precisely the cooperative capabilities of conventional F1 T cells; (b) parent A → (A × B)F1 and parent B → (A × B)F1 chimeric lymphocytes behaved phenotypically in a manner indistinguishable from conventional parental cells in cooperating effectively with partner cells only from F1 donors or from parental donors corresponding to the H-2 haplotype of the original bone marrow donor; and (c) (A × B)F1 → parent A and (A × B)F1 → parent B chimeric T and B cells displayed restricted haplotype preference in cooperating best with partner lymphocytes sharing the H-2 haplotype (either entirely or codominantly) of the parental chimeric host. In other words, cells originally of F1 donor origin no longer behaved as typical F1 cells, but rather displayed restricted cooperative activity similar to that which would be observed in interactions employing conventional parental cells. Similar findings have been made in the CTL-target cell systems (37–39), although these studies were restricted to T cells.

Two points are worth emphasizing about these observations. First, failure of parent → F1 or F1 → parent chimeric T or B cells to cooperate with partner lymphocytes of the opposite parental haplotype cannot be explained by the existence of some type of suppressive mechanism, whether subtle or otherwise. This possibility was argued against by the capability of such cells to cooperate effectively with partner lymphocytes from either conventional F1 or F1 → F1 chimeric donors. Furthermore, this possibility was tested directly by experiments in which chimeric cells of F1 → parent
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Fig. 8. Titration of in vitro helper activity of KLH-primed chimeric T cells for DNP-primed B cells from conventional parental or F1 donors. Microcultures containing the various cell mixtures indicated were stimulated with DNP-KLH (0.05 μg/well) and assayed after 4 days. All cultures were set up in triplicate. Control cultures of such B cells incubated in the absence of antigen resulted in PFC values in the range of 10–45.

origin were mixed with appropriate combinations of syngeneic and semiallogeneic T and B lymphocytes. In no case was there any detectable inhibitory effect of such chimeric cells on the normal cooperative responses generated in such mixtures. This is shown in the case of B lymphocytes in Table I; absence of suppressive effects due to chimeric T cells was confirmed in similar cotransfer experiments as well (our unpublished observations).

The second point worth emphasizing is that the finding that lymphocytes from semiallogeneic parent → F1 chimeras were unquestionably incapable of interacting with partner cells of the opposite parental haplotype is inconsistent with certain (40, 41), but not other (42), studies on cooperative T-B-cell interactions with chimeric lymphocytes. Moreover, the failure of T lymphocytes from single parent → F1 chimeras to interact effectively with B cells of the opposite parental type contrasts with the ability of T lymphocytes from double parent → F1 chimeras to reciprocally interact with B cells of opposite parental type (17). As discussed below, the basis for these differences appears to be an important clue to the mechanism(s) underlying adaptive differentiation.

The results of these studies appear to answer two of the essential questions that have been facing immunologists in recent years: (a) do lymphocytes of various classes and subclasses interact with one another via cell surface molecules, or CI structures that are entities quite distinct from conventional antigen-specific receptors? (b) Is the process of effective cell-cell communication one which can be learned during certain stages of differentiation such that the cells involved are selected appropriately to optimize the communications system? From the aforementioned observations it now seems possible to state that the answers to both of these questions are yes.

This conclusion is particularly strengthened by the findings made with B lymphocytes which had differentiated in F1 → parent chimeras. Quite unlike the situation
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**Fig. 9.** B lymphocytes adaptively differentiate in F₁ → parent chimeras to cooperate preferentially with helper T cells from isologous parental donors. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge. Control values for responses of the respective B-cell types in the absence of helper T cells were as follows: (a) CAF₁ → CAF₁ = 50; (b) CAF₁ → A/J = 42; (c) CAF₁ → BALB/c = 26. The positive control values for the three respective B-cell types were (a) CAF₁ → CAF₁ = 5,194; (b) CAF₁ → A/J = 1,655; and (c) CAF₁ → BALB/c = 1,829.

with T cells, where the argument can be made that H-2 restrictions manifested by T lymphocytes could reflect the specificity of their receptors for antigen-plus-"self" (16), this argument does not easily explain the findings concerning B cell adaptive differentiation. Such results in the case of the latter cell class are best explained by a process of selection of the relevant B cells during differentiation and priming. Remote possibilities for explaining the data otherwise, such as inappropriate macrophage-lymphocyte interactions or something from the chimeric host sticking on the surface of the primed B cells thereby interfering with cooperative activity can be dismissed because: (a) macrophages of the neutral F₁ adoptive recipient should provide indiscriminate macrophage-lymphocyte interaction capability in vivo, and macrophages from both chimeric and conventional donor origin were present in the in vitro culture; and (b) primed chimeric B cells (as well as helper T cells) that have been serially transferred through successive adoptive hosts retain the same cooperative phenotype manifested when first removed from chimeric donors (our unpublished observations).

The striking contrast between the remarkable ease with which adaptive differentiation of lymphocytes can be demonstrated in F₁ → parent chimeras versus the difficulty in demonstrating the same phenomenon in reciprocal parent → F₁ chimeras deserves additional comment because the message these differences convey to us is obviously very important. First, it is pertinent to stress that our demonstration of adaptive differentiation of murine lymphocytes represents quantitative differences in phenotypic expressions of preference for interacting partner cells. It would be indeed surprising if this were not the case inasmuch as the concept of adaptive differentiation assumes that each individual of the species possesses the genotypic potential for encoding the entire repertoire of CI molecules, and their corresponding receptors, of
the species. Adaptation reflects, therefore, the selection process that follows interactions of developing cells with the surrounding environment and amongst themselves. As a consequence of these early interactions, the relevant CI phenotype(s) that will be expressed on functionally mature cells is (are) selected. Hence, while we, and others, can experimentally program a cell population to interact preferentially with one of two possible choices of partner cells, the potential ability of cells within that population to interact with a second alternative choice has not by any means been irrevocably eliminated. That is precisely why lymphocytes derived from an F1 → A chimera, although clearly interacting best with partner cells of parent A type, can still display interacting capabilities (albeit of lower efficiency) with partner cells from parent B, a point illustrated perhaps most clearly with F1 → parent chimeric B cells.

Any hypothesis concerning the process of adaptive differentiation must take into consideration the following four points: (a) lymphocytes differentiating in F1 → parent chimeras express the cooperating phenotype of the parental host; (b) lymphocytes differentiating in double parent → F1 chimeras express reciprocal cooperating phenotypes for interacting with partner cells of opposite parental type; (c) lymphocytes differentiating in single parent → F1 chimeras retain the cooperating phenotype of the original parent donor; and (d) adaptive differentiation is a general process applicable to B lymphocytes as well as T lymphocytes.

The hypothesis that emerges in our minds to explain these findings can be briefly summarized as follows: in any individual, the stem cell population possesses the genotypic library for expressing and recognizing all possible CI phenotypes of the species. This library spans not only many different specificities, but a whole spectrum

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1 D. H. Katz 1978. Adaptive differentiation of lymphocytes: theoretical implications for mechanisms of cell-cell recognition and regulation of immune responses. Manuscript submitted for publication.
TABLE I
F1 → Parent Chimeric B Cells Fail to Exert Suppressive Effects on Cooperative T-B Cell Interactions in Secondary in Vitro Anti-DNP Responses*

| Chimeric B cells tested for suppression (× 10^-6) | Anti-θ-treated CAF1 → CAF1 B cells plus KLH-primed helper T cells from: |
|-----------------------------------------------|--------------------------|
| None                                         | CAF1        | A/J     | BALB/c  |
|                                              | 17,867      | 18,560  | 18,347  |
| CAF1 → CAF1                                 | 23,830      | 26,596  | 32,074  |
|                                              | 17,340      | 25,170  | 29,149  |
|                                              | 16,011      | 22,872  | 26,756  |
| CAF1 → A/J                                  | 21,277      | 26,170  | 28,511  |
|                                              | 16,702      | 22,979  | 27,340  |
|                                              | 16,469      | 21,915  | 32,660  |

*Microcultures containing (a) 0.5 × 10^6 CAF1 → CAF1 anti-θ-treated DNP-ASC-primed B cells; (b) 0.5 × 10^6 irradiated (770 rads) KLH-primed helper T cells from either CAF1, A/J, or BALB/c donors; and (c) varying numbers (or none) of anti-θ-treated chimeric B cells from DNP-ASC-primed CAF1 → CAF1 or CAF1 → A/J donors were stimulated with 0.05 µg DNP-KLH per well for 4 days.

†Results are expressed as IgG DNP-specific PFC/10^7 cultured cells (triplicate cultures).

of binding affinities between any two interacting CI molecules. In any set of two interacting CI molecules, one can be considered to be a target whereas the second molecule is most likely a specific receptor for that target; moreover, at least one of the two CI molecules is a product of MHC gene(s). Early in ontogeny, stem cell progeny express the entire range of CI molecule specificities and affinities characteristic of the species. However, as differentiation proceeds, those cells capable of recognizing the CI phenotype of the native environment undergo selection in which those with high affinity binding receptors for "self" are deleted (not necessarily eliminated, but rendered functionally sterile). The remaining self-recognizing cells are those with low-to-moderate affinity binding receptors and these cells mediate functional communication processes necessary for regulating immune responses.

Concomitantly, those cells recognizing CI phenotypes of other individual members of the species undergo a somewhat different type of selection. In the absence of environmental selection, cells with predominantly high, rather than low-to-moderate, affinity receptors for other CI molecules of the species emerge. The pressure for maintaining such cells may be the need for a suitable mechanism for limiting the numbers of low-to-moderate affinity cells of corresponding CI phenotype; the latter cells have no useful purpose in the inappropriate environment and without an effective surveillance mechanism to limit their growth, they might simply proliferate uncontrollably. The high affinity cells could perform this function; in addition, they most likely represent some, if not all, of the cells we call alloreactive.

When F1 lymphocytes differentiate in the environment of parent A, environmental selection would maintain predominantly low affinity cells recognizing parent A. Absence of environmental selection for the parent B specificity would result in diminution of the functional interacting cells of low-to-moderate affinity of this type. Those cells recognizing parent B would emerge as high as well as low-to-moderate
affinity cells. The net result is predominance of functional interacting cells with CI molecules of anti-A specificity and hence preferential interactions of lymphocytes in such a chimera for partner cells of parent A type; this is precisely what the present studies demonstrate. The fact that lymphocytes differentiating in single parent → F₁ chimeras retain the interacting phenotype of the donor parent reflects complex regulatory events that are discussed more fully elsewhere. The contrasting ability of lymphocytes from double parent → F₁ chimeras to reciprocally cooperate is explainable by this model by considering that in each respective parental population low-to-moderate affinity cells reactive with the opposite CI specificity emerge in this situation (with a concomitant disappearance of high affinity cells of the same specificity).

Considerable further investigation is needed to validate this model and to ascertain the cellular and molecular processes involved. Preliminary evidence suggests that a mechanism similar (or identical) to the allogeneic effect may play a critical role in determining at least certain of the events. Studies currently underway are designed to resolve these and related questions.

Summary

The concept of adaptive (selective) differentiation predicts that early differentiation of lymphocytes is conditioned by the environment in which such differentiation takes place. These processes appear to involve selection of lymphocytes according to their self-recognition capabilities for engaging in the most effective cell-cell interactions. Since self-recognition between interacting lymphocytes is, at least in part, controlled by major histocompatibility complex-linked genes, then adaptive differentiation is also controlled by these genes. In these studies, we have tested the capacities of helper T lymphocytes and hapten-specific B lymphocytes primed in the environments of various combinations of bone marrow chimeras prepared between two parental strains (i.e. A/J and BALB/c) and their corresponding F₁ hybrid (CAF₁) to interact with primed B and T lymphocytes derived from conventional parent and F₁ donors as well as all of the corresponding bone marrow chimera combinations. The results demonstrate clearly that (a) F₁ → F₁ chimeric lymphocytes display no restriction in terms of cooperative activity with all of the various partner cell combinations; (b) parent → F₁ chimeric lymphocytes manifest effective cooperative activity only for partner cells from F₁ or parental donors corresponding to the haplotype of the original bone marrow donor, thereby behaving phenotypically just like conventional parental lymphocytes; and (c) F₁ → parent chimeric lymphocytes display restricted haplotype preference in cooperating best with partner lymphocytes sharing the H-2 haplotype, either entirely or codominantly, of the parental chimeric host. The implications of these findings for understanding certain controlling mechanisms for lymphocyte differentiation are discussed.

We are very grateful to Andree Carter, Robert Gordon, and John Hokanson for outstanding technical assistance in the performance of these studies. We also thank Doctors. Larry Miller and Soldano Ferrone for their contributions in the successful development of the cytotoxicity assay used for typing the chimeras used herein, which is the subject of a separate report. Finally, we greatly appreciate the excellent secretarial assistance of Judy Henneke in the preparation of this manuscript.

Received for publication 28 February 1978.
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