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)[1] 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 lymphocytes 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.

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 $(A \times B)F_1$ 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) (DNP$_9$-KLH) and 2.1 × 10^{-7} mol of DNP/mg of *Ascaris suum* (DNP$_{2.1}$-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 $(H$-$2^d)$ mice were obtained from Simonsen Laboratories, Gilroy, Calif. or from the Scripps Clinic and Research Foundation (SCRF) mouse breeding colony. Inbred A/J $(H$-$2^a)$ and (BALB/c × A/J)F$_1$ hybrids (CAF$_1$, H-$2^{d/a}$) 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 ^{137}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 CAF_1, 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) CAF_1 → CAF_1, (b) A/J → CAF_1, (c) BALB/c → CAF_1, (d) CAF_1 → A/J (e) CAF_1 → BALB/c, and (f) A/J + BALB/c → CAF_1.

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.[2]

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 → F_1 chimeras and bilateral sensitivity to both anti-BALB/c and anti-A/J antisera in the cases of F_1 → 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 → F_1 or F_1 → 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 CAF_1 T cells and CAF_1 B cells was taken as the 100% control value against which to compare the responses in all other recipient groups in which CAF_1 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

[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 CAF_1 helper cells and CAF_1 B cells is taken as a 100% control value against which to compare the magnitudes of responses developed by the same CAF_1 B cells when helped by T cells of another type (e.g. $A/J \rightarrow CAF_1$ 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 CAF_1 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 → F_1 and F_1 → 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 × 10^6 DNP-ASC-primed CAF_1 spleen cells were transferred to irradiated CAF_1 recipients either (*a*) alone, (*b*) in the presence of 8 or 10 × 10^6 conventional CAF_1 KLH-primed helper cells, or (*c*) comparable numbers of unprimed spleen cells from either conventional F_1 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 CAF_1 helper T cells (group III), but not unprimed F_1 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, F_1, and Chimeric B Cells from DNP-Primed Donors. The capacities of KLH-primed T lymphocytes from either conventional CAF_1 donor mice or from the various bone marrow chimeras to provide helper activity for DNP-primed B lymphocytes of various donor origins were measured
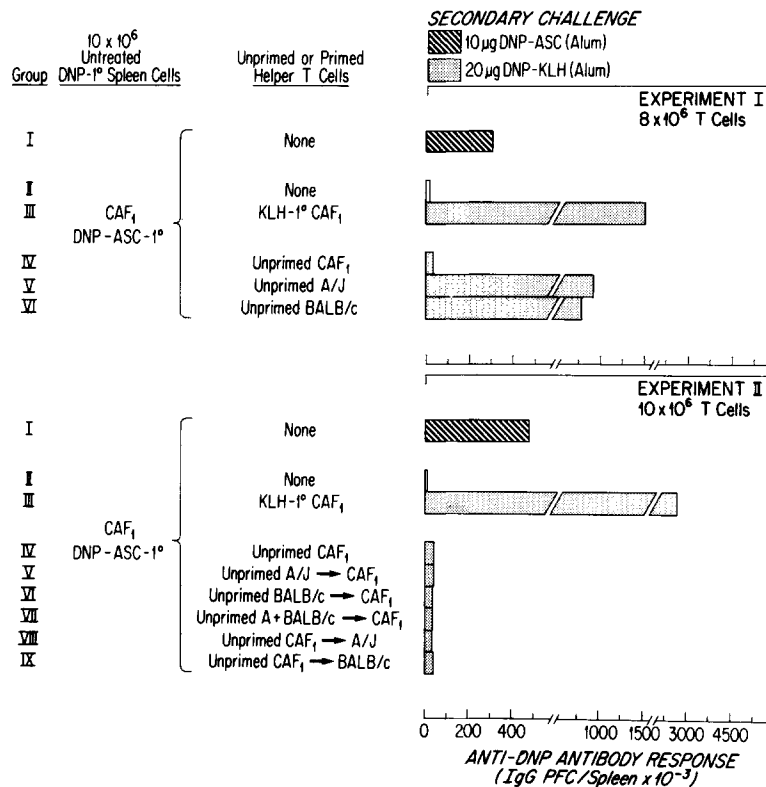
SECONDARY CHALLENGE
10 μg DNP-ASC (Alum)
20 μg DNP-KLH (Alum)

Group	10 x 10^6 Untreated DNP-1° Spleen Cells	Unprimed or Primed Helper T Cells	

EXPERIMENT I
8 x 10^6 T Cells

I		None	
II		None	
III	CAF₁ DNP-ASC-1°	KLH-1° CAF₁	
IV		Unprimed CAF₁	
V		Unprimed A/J	
VI		Unprimed BALB/c	

EXPERIMENT II
10 x 10^6 T Cells

I		None	
II		None	
III		KLH-1° CAF₁	
IV	CAF₁ DNP-ASC-1°	Unprimed CAF₁	
V		Unprimed A/J → CAF₁	
VI		Unprimed BALB/c → CAF₁	
VII		Unprimed A+BALB/c → CAF₁	
VIII		Unprimed CAF₁ → A/J	
IX		Unprimed CAF₁ → BALB/c	

0   200   400   1000   1500   3000   4500

ANTI-DNP ANTIBODY RESPONSE
(IgG PFC/Spleen x 10⁻³)

FIG. 1.   Spleen cells from parent → F_1 and F_1 → parent bone marrow chimeras fail to exert allogeneic effects on DNP-primed conventional F_1 spleen cells adoptively transferred to CAF_1 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 CAF_1 recipients. In all instances, the B-cell populations were depleted of T lymphocytes by in vitro treatment with anti-θ 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) CAF_1 = 146; (b) A/J = 29; (c) BALB/c = 16; (d) $CAF_1 \rightarrow CAF_1$ = 75; (e) A/J → CAF_1 = 35; (f) BALB/c → CAF_1 = 22; (g) $CAF_1 \rightarrow$ A/J = 15; (h) $CAF_1 \rightarrow$ BALB/c = 13.

The cooperative activities of KLH-primed helper T cells from conventional CAF_1 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 CAF_1 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
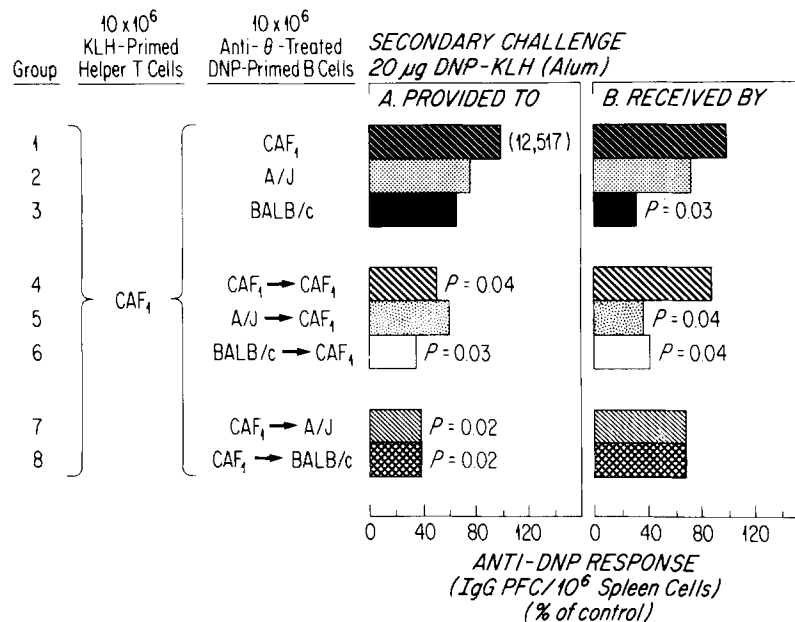
Fig. 2. In vivo helper activity of KLH-primed conventional CAF_1 T cells for parental, F_1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis 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 CAF_1 helper T-cell activity provided to isologous CAF_1 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 → CAF_1 B cells (group 6) was there concordance in significant differences from controls in both methods of data presentation.

CAF_1 → CAF_1 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 → CAF_1 Chimeric Helper T Cells (Fig. 4). In contrast to the preceding results, helper T cells from A/J → CAF_1 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 CAF_1 and A/J and to chimeric CAF_1 → CAF_1, A/J → CAF_1 and CAF_1 → 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 → CAF_1 (group 22) chimera donors. These results indicate quite clearly that T cells in the A/J → CAF_1 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
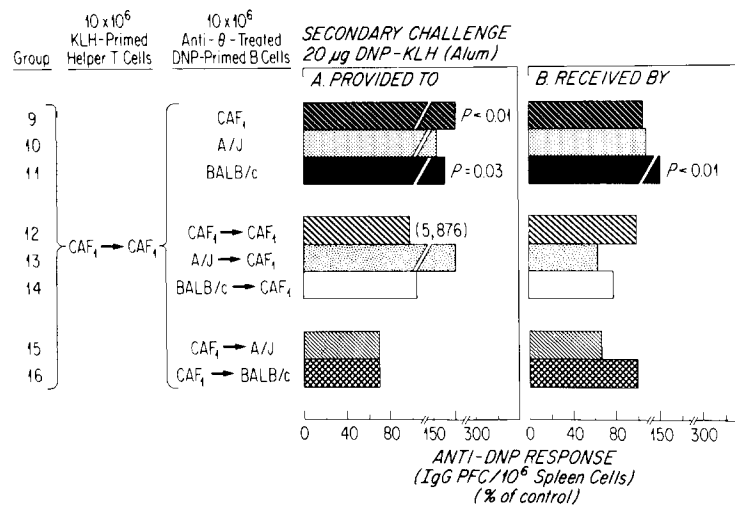
Fig. 3. In vivo helper activity of KLH-primed $CAF_1 \rightarrow CAF_1$ chimeric T cells for parental, F_1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

cooperative interactions between $A/J \rightarrow CAF_1$ chimeric T cells and $CAF_1 \rightarrow BALB/$ c chimeric B cells (group 24) will be discussed below.

$BALB/c \rightarrow CAF_1$ *Chimeric Helper T Cells (Fig. 5).*   Similar to the results obtained with the reciprocal parent $\rightarrow F_1$ chimera, $BALB/c \rightarrow CAF_1$ chimeric T cells effectively helped B cells from conventional CAF_1 and $BALB/c$ and chimeric $CAF_1 \rightarrow CAF_1$, $BALB/c \rightarrow CAF_1$, and $CAF_1 \rightarrow BALB/c$ donors. Such cells failed to provide helper activity for conventional A/J (group 26) or $A/J \rightarrow CAF_1$ (group 29) chimeric B cells.

$CAF_1 \rightarrow A/J$ *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 $BALB/c \rightarrow CAF_1$ (group 38) donors. This contrasts sharply with the effective help such cells provided to B cells from conventional CAF_1 and $A/$ J and from chimeric $CAF_1 \rightarrow CAF_1$, $A/J \rightarrow CAF_1$ and $CAF_1 \rightarrow A/J$ donors.

$CAF_1 \rightarrow BALB/c$ *Chimeric Helper T Cells (Fig. 7).* These results were almost precisely the reciprocal of the results obtained with $CAF_1 \rightarrow A/J$ chimeric T cells (Fig. 6). Due to an unexplained high mortality incidence in recipients of isologous $CAF_1 \rightarrow BALB/$ c T and B lymphocytes (group 48), the data from this group cannot be presented. The results obtained with the mixture of $CAF_1 \rightarrow BALB/c$ T cells and $CAF_1 \rightarrow$ CAF_1 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 $A/J \rightarrow CAF_1$ (group 45) or chimeric $CAF_1 \rightarrow A/J$ (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 F_1 $\rightarrow$ 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 $CAF_1 \rightarrow CAF_1$, $CAF_1 \rightarrow A/J$, and $CAF_1 \rightarrow BALB/$
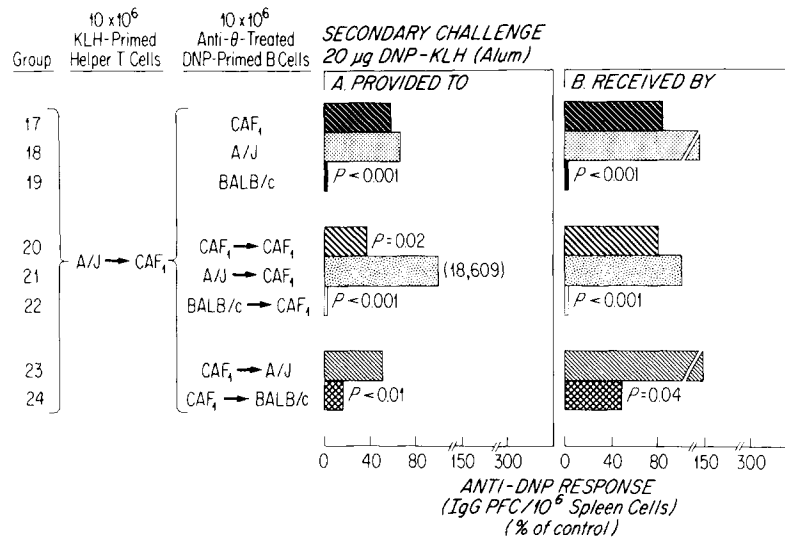
Fig. 4. In vivo helper activity of KLH-primed A/J → CAF₁ chimeric T cells for parental, F₁, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

c chimeric T cells, irradiated in situ before culture, were cocultured in varying quantities with a constant number (0.5×10^6) of anti-θ serum-treated DNP-ASC-primed B cells from either conventional A/J, BALB/c, or CAF₁ donor mice. The titration curves of cooperative anti-DNP responses to DNP-KLH (Fig. 8) are quite clear in illustrating (a) little difference in CAF₁ → CAF₁ chimeric T-cell helper activity provided to all three B-cell types (left panel); (b) striking disparities between the levels of effective helper activity, over the entire T-cell dose range provided by CAF₁ → A/J chimeric T cells to both A/J and CAF₁ B cells as contrasted to the hardly detectable helper activity provided to parental BALB/c B cells (middle panel); and, conversely, (c) the reciprocal situation with CAF₁ → BALB/c chimeric T cells which displayed little or no helper activity for parental A/J cells at all T-cell doses employed (right panel).

B Lymphocytes Undergo Adaptive Differentiation in F₁ → 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 → CAF₁ chimeric T cells and CAF₁ → 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, CAF₁ → BALB/c chimeric T cells interacted poorly with B cells from CAF₁ → 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 CAF₁ B cells, these results indicate that a significant shift may have occurred with respect to the ability of F₁ 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
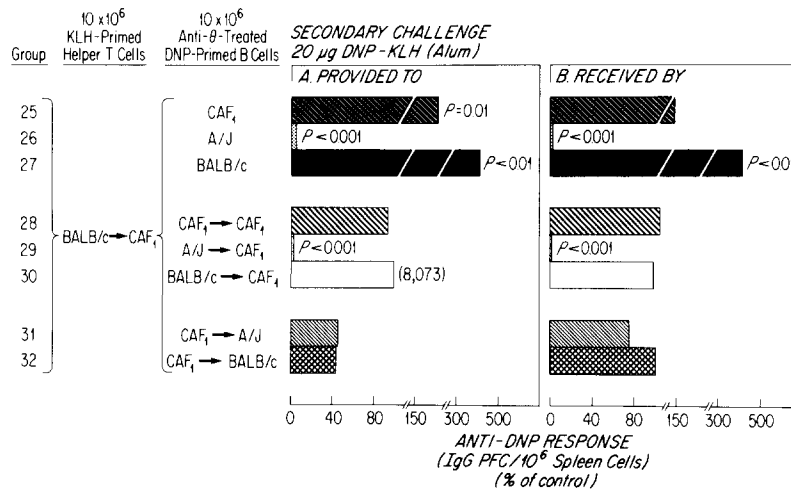
Fig. 5.   In vivo helper activity of KLH-primed BALB/c → CAF₁ chimeric T cells for parental, F₁, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

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 F_1 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 CAF_1 → CAF_1, CAF_1 → A/J, and CAF_1 → BALB/c chimeric donors were tested for their cooperative activity with KLH-primed T cells from conventional CAF_1 → A/J or BALB/c donors. The notable results were the relative deficiencies in cooperative activities between primed B cells from CAF_1 → A/J and CAF_1 → 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 CAF_1 → CAF_1 chimeric B cells with all three T-cell types and the ability of the F_1 → parent chimeric B cells to interact with either conventional F_1 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 in vitro secondary antibody responses (Fig. 10). In contrast to the ability of CAF_1 → CAF_1 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 CAF_1 → 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 F_1 T cells to help the CAF_1 → A/J chimeric B cells, the magnitude of such responses most likely reflects a lower level of overall helper activity of this F_1 T-cell population as indicated by the lower responses
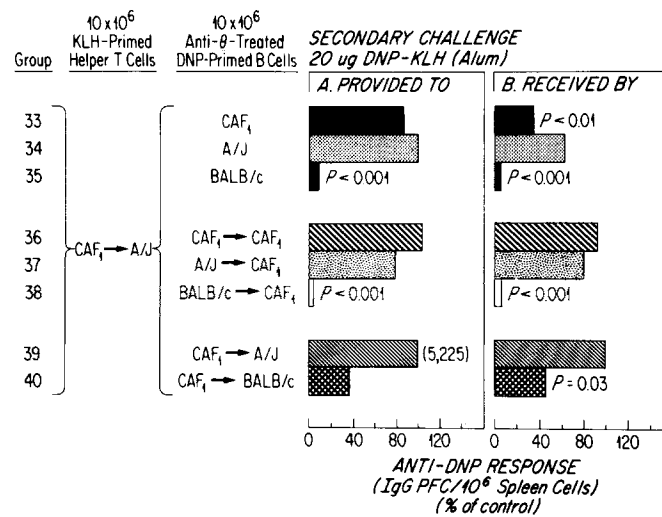
Fig. 6. In vivo helper activity of KLH-primed $CAF_1 \rightarrow A/J$ chimeric T cells for parental, F_1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge.

obtained when such cells were cocultured with $CAF_1 \rightarrow CAF_1$ chimeric B cells (top panel).

The normal cooperative activity between either of the $F_1 \rightarrow$ parent chimeric B cells and CAF_1 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 $CAF_1 \rightarrow CAF_1$ B cells and either conventional CAF_1, 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 $CAF_1 \rightarrow CAF_1$ or $CAF_1 \rightarrow A/J$ 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
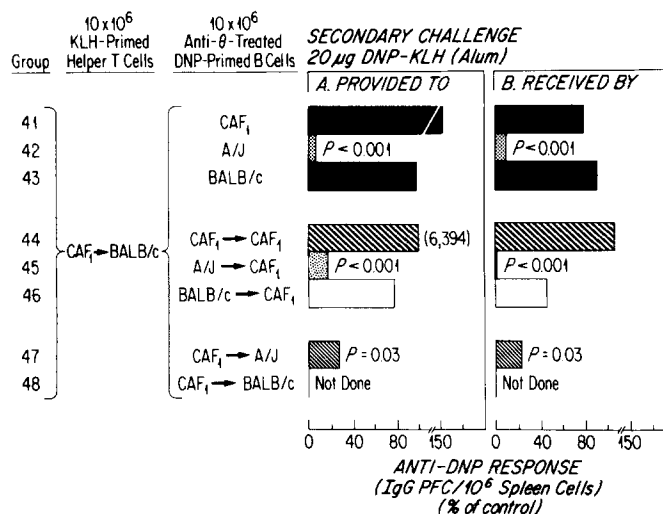
Fig. 7. In vivo helper activity of KLH-primed $CAF_1 \rightarrow$ BALB/c chimeric T cells from parental, F_1, and chimeric B cells relative to helper activity: A) provided to isologous B cells, or B) received by each B-cell type from isologous T cells. Spleens of all recipients (four per group) were 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) $F_1 \rightarrow F_1$ 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 F_1 T cells; (b) parent $A \rightarrow (A \times B)F_1$ and parent $B \rightarrow (A \times B)F_1$ chimeric lymphocytes behaved phenotypically in a manner indistinguishable from conventional parental cells in cooperating effectively with partner cells only from F_1 donors or from parental donors corresponding to the H-2 haplotype of the original bone marrow donor; and (c) $(A \times B)F_1 \rightarrow$ parent A and $(A \times B)F_1 \rightarrow$ 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 F_1 donor origin no longer behaved as typical F_1 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 $\rightarrow F_1$ or $F_1 \rightarrow$ 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 F_1 or $F_1 \rightarrow F_1$ chimeric donors. Furthermore, this possibility was tested directly by experiments in which chimeric cells of $F_1 \rightarrow$ parent
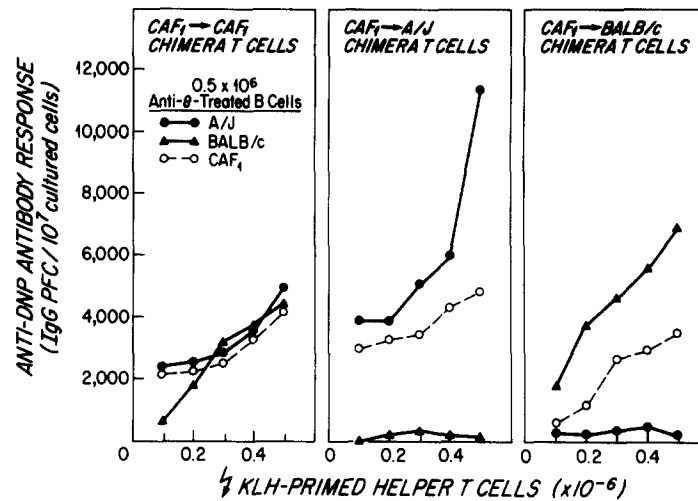
FIG. 8. Titration of in vitro helper activity of KLH-primed chimeric T cells for DNP-primed B cells from conventional parental or F_1 donors. Microcultures containing the various cell mixtures indicated were stimulated with DNP-KLH (0.05 μg/well) and assayed after 4 days. All cultures were set up in 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 → F_1 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 → F_1 chimeras to interact effectively with B cells of the opposite parental type contrasts with the ability of T lymphocytes from double parent → F_1 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 F_1 → parent chimeras. Quite unlike the situation
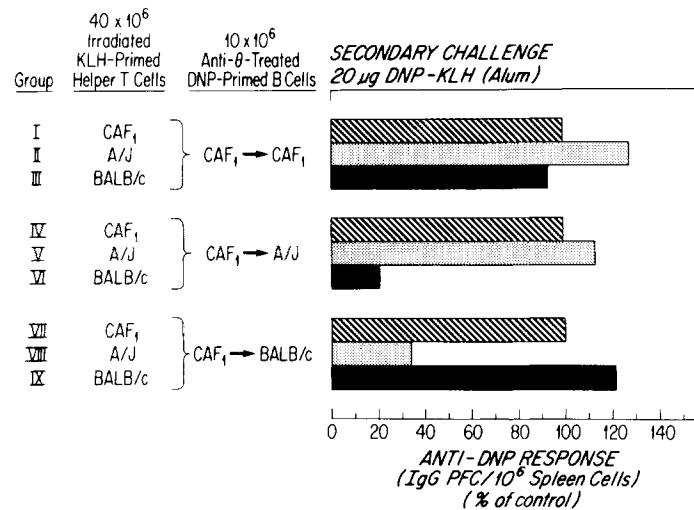
FIG. 9. B lymphocytes adaptively differentiate in $F_1 \rightarrow$ parent chimeras to cooperate preferentially with helper T cells from isologous parental donors. Spleens of all recipients (four per group) were removed for analysis of IgG DNP-specific PFC 7 days after transfer and challenge. Control values for responses of the respective B-cell types in the absence of helper T cells were as follows: (a) $CAF_1 \rightarrow CAF_1$ = 50; (b) $CAF_1 \rightarrow A/J$ = 42; (c) $CAF_1 \rightarrow BALB/c$ = 26. The positive control values for the three respective B-cell types were (a) $CAF_1 \rightarrow CAF_1$ = 5,194; (b) $CAF_1 \rightarrow A/J$ = 1,655; and (c) $CAF_1 \rightarrow BALB/c$ = 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 F_1 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 $F_1 \rightarrow$ parent chimeras versus the difficulty in demonstrating the same phenomenon in reciprocal parent $\rightarrow F_1$ 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
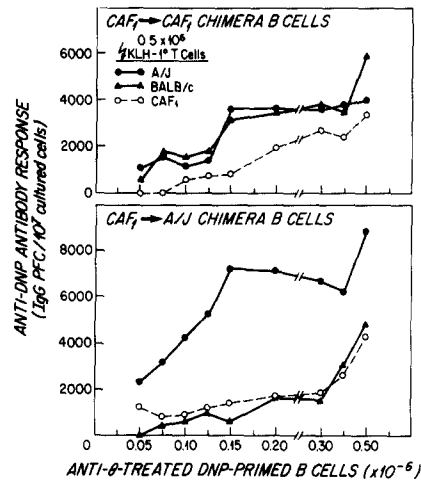
Fig. 10. Titration of in vitro cooperative activity of DNP-primed chimeric B cells with KLH-primed helper T cells from conventional parental or F_1 donors. Microcultures containing the various cell mixtures indicated were incubated for 4 days in the presence of DNP-KLH (0.05 μg/well). All cultures were set up in triplicate. Control values of such B-cell cultures in the absence of antigen were in the range of 0-30 PFC.

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 $F_1 \rightarrow A$ 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 $F_1 \rightarrow$ parent chimeric B cells.

Any hypothesis concerning the process of adaptive differentiation must take into consideration the following four points: (a) lymphocytes differentiating in $F_1 \rightarrow$ parent chimeras express the cooperating phenotype of the parental host; (b) lymphocytes differentiating in double parent $\rightarrow F_1$ chimeras express reciprocal cooperating phenotypes for interacting with partner cells of opposite parental type; (c) lymphocytes differentiating in single parent $\rightarrow F_1$ 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:[3] 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

[3] 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

$F_1 \rightarrow$ *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 ($\times 10^{-6}$)		Anti-θ-treated $CAF_1 \rightarrow CAF_1$ B cells plus KLH-primed helper T cells from‡		
		CAF_1	A/J	BALB/c
None	− 0.10	17.867	18,560	18,347
$CAF_1 \rightarrow CAF_1$	− 0.20	23,830	26,596	32,074
	− 0.30	17,340	25,170	29,149
		16,011	22,872	26,756
$CAF_1 \rightarrow$ A/J	− 0.10	21,277	26,170	28,511
	− 0.20	16,702	22,979	27,340
	− 0.30	16,489	21,915	32,660

* Microcultures containing (a) 0.5 × 10^6 $CAF_1 \rightarrow CAF_1$ anti-θ-treated DNP-ASC-primed B cells; (b) 0.5 × 10^6 irradiated (770 rads) KLH-primed helper T cells from either CAF_1, A/J, or BALB/c donors; and (c) varying numbers (or none) of anti-θ-treated chimeric B cells from DNP-ASC-primed $CAF_1 \rightarrow CAF_1$ or $CAF_1 \rightarrow$ 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 C‡ 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 F_1 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 → F_1 chimeras retain the interacting phenotype of the donor parent reflects complex regulatory events that are discussed more fully elsewhere.[3] The contrasting ability of lymphocytes from double parent → F_1 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).[3]

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 F_1 hybrid (CAF$_1$) to interact with primed B and T lymphocytes derived from conventional parent and F_1 donors as well as all of the corresponding bone marrow chimera combinations. The results demonstrate clearly that (a) F_1 → F_1 chimeric lymphocytes display no restriction in terms of cooperative activity with all of the various partner cell combinations; (b) parent → F_1 chimeric lymphocytes manifest effective cooperative activity only for partner cells from F_1 or parental donors corresponding to the haplotype of the original bone marrow donor, thereby behaving phenotypically just like conventional parental lymphocytes; and (c) F_1 → 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.

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