CELL-INTERACTION MOLECULES ON IMMUNOCOMPETENT LYMPHOCYTES

Development of Anti-Parent Cell-Interaction-Molecule-Receptor Reactions in F_1 Hybrid Mice and Evidence for a Unique F_1 Hybrid Subset of Interacting Cells*

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This report concerns some unique aspects of cooperative interactions between carrier-specific helper T lymphocytes of F_1 hybrid origin and hapten-specific B lymphocytes of parental origin in the development of adoptive, secondary hapten-specific antibody responses in mice. Specifically, our experiments illustrate that effective cooperation between $(A \times B)F_1$ helper T cells and B lymphocytes of parent A origin are significantly inhibited by the addition to such mixtures of unprimed lymphoid cells derived from donors of the opposite parent B type. Although on the surface this may seem simply an example of a straightforward allosuppression phenomenon, the evidence gathered in this study suggests that the mechanism underlying such inhibitory interactions may be of more fundamental importance to overall regulation of the immune system.

The rationale for initiating these studies stems from our belief that adaptive differentiation of lymphocytes, *i.e.*, learning the appropriate self-recognition repertoire, is a dynamic, rather than a static, process by which immunocompetent cells perceive their environmental milieu and develop, accordingly, the cooperative phenotype dictated by that environment (1–5; reviewed in 6). Evidence in support of this notion previously reported from this laboratory includes: (*a*) the demonstration that the restricted phenotypes of helper T cells primed *in situ* in $F_1 \rightarrow$ parent bone marrow chimeras or in neonatally tolerant parent environments are actually pseudo-restrictions resulting from some form of environmental restraint (7); and (*b*) the finding that it is possible to orchestrate the partner cell preferences of F_1 lymphocytes primed to antigen under the influence of a parental cell-induced allogeneic effect such that the ultimate cooperating phenotypes displayed by such cells deviate in their preference for partner cells originating from one or the other parental type (8, 9). Although direct evidence was lacking, we speculated that both environmental restraint and the ability to orchestrate the cooperating preferences of F_1 hybrid lymphocytes were manifesta-

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tions of the development of responses against receptors for self cell-interaction (CI)[1] molecules that are requisite participants in cell-cell interactions (6–9). Such haplotype-specific anti-CI receptor responses would readily explain the permissiveness of the development of one subpopulation of self-recognizing cells (corresponding to one of the parental haplotypes) in the face of nonpermissiveness of the development of the self-recognizing cell subpopulation corresponding to the second haplotype involved. These studies demonstrate that the cooperating phenotypes of cells previously primed in a conventional F_1 hybrid environment can be orchestrated by incorporating lymphoid cells of opposite parental type into the environment in which F_1-parent partner cell cooperation is taking place. For reasons discussed herein, these findings are likewise best explained by a mechanism of haplotype-specific anti-CI receptor responses.

Materials and Methods

The proteins, reagents, and preparation of hapten-protein conjugates were the same as those described in earlier reports (5, 10). 9 mol of dinitrophenyl (DNP)/100,000 dalton of keyhole limpet hemocyanin (KLH) (DNP_9-KLH) and 2.1×10^{-7} M of DNP/mg of *Ascaris suum* extract (ASC) ($DNP_{2.1}$-ASC) were employed in these studies. The preparation of anti-θ serum, its characterization and method of anti-θ serum treatment of spleen cells, the method for enumerating DNP-specific plaque-forming cells (PFC) of the IgG class, and the method for determining serum anti-DNP antibody levels by radioimmunoassay are described elsewhere (5, 10–12).

Animals and Immunizations. Inbred BALB/c ($H\text{-}2^d$) mice were obtained from the Scripps Clinic and Research Foundation (SCRF) mouse breeding colony, La Jolla, Calif. or from Simonsen Laboratories, Gilroy, Calif. Inbred A/J ($H\text{-}2^a$) and (BALB/c × A/J)F_1 hybrids (CAF_1, $H\text{-}2^{d/a}$) were obtained from the SCRF mouse breeding colony. Donors of DNP-specific B cells or KLH-specific T cells were immunized intraperitoneally with 10 μg of DNP-ASC adsorbed on 4 mg of aluminum hydroxide gel (alum) or 20 μg of KLH emulsified in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, Mich.), respectively generally at 8–12 wk of age. Such hapten- and carrier-primed donor mice were typically boosted intraperitoneally with 10 μg of the respective antigen in saline 3–4 wk after initial priming and again at monthly intervals thereafter; spleen cells were then removed 2–3 wk after the last booster immunization and adoptively transferred to irradiated recipient mice for in vivo assay according to experimental design as outlined in Results. In those experiments in which carrier-primed, irradiated recipients served as the source of helper T cells, such mice were primed with 20 μg of KLH in CFA 8 d before irradiation.

With one exception (see Results), CAF_1 mice served as recipients in all of the experiments presented. All recipients were exposed to 650 rad of irradiation delivered by a ^{137}Cesium irradiator (Gamma Cell 40; Atomic Energy Limited of Canada, Ottawa). All cell transfers were performed by the intravenous route and were carried out in two successive stages (see Results). Secondary challenge consisted of 10 or 20 μg of DNP-KLH adsorbed on 2 mg of alum administered intraperitoneally immediately after transfer of DNP-primed B cells. Magnitudes of DNP-specific antibody responses in recipient mice were ascertained 7 d after B cell transfer and secondary challenge, either by enumeration of IgG PFC in recipient spleens or by quantitation of DNP-specific serum antibodies. Statistical analyses were made with geometric means and SE calculated from individual PFC or serum antibody values in groups of 4–5 mice each. P values were ascertained by the Student's t-test.

[1] *Abbreviations used in this paper:* ASC, *Ascaris suum* extract; C, complement; CAF_1, (BALB/c × A/J)F_1; CFA, complete Freund's adjuvant; CI, cell interaction; DNP, dinitrophenyl; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cell(s); SCRF, Scripps Clinic and Research Foundation.

Results

Experimental Design. The general features of the experimental design in this study followed the original protocol for demonstrating genetic restrictions in T-B cell interactions that we developed and described some years ago (13, 14). To reiterate briefly, in this system cell transfers are performed in two stages on consecutive days in the following way: On day −1, unirradiated recipient CAF_1 mice are injected intravenously with 40×10^6–50×10^6 spleen cells from KLH-primed donor mice; in this particular study, KLH-primed CAF_1 mice served as donors in all experiments using this regimen. 24 h later, on day 0, after the injected carrier-primed helper cells have suitably migrated to the host lymphoid organs, all recipients are irradiated with 650 rad; this maneuver thus provides a suitable neutral F_1 environment that contains radioresistant KLH-specific helper T cells. At this time (day 0) a second cell transfer is performed using DNP-primed, T cell-depleted (by in vitro treatment with anti-θ serum plus complement [C]) spleen cells of parental A/J or BALB/c or homologous CAF_1 origin. Immediately thereafter, all recipients are secondarily challenged with DNP-KLH and the responses they generate determined 7 d later.

The significant alteration in this basic protocol that has been utilized in these experiments is the following: On day −1, unirradiated recipient mice were injected intravenously with 40×10^6–50×10^6 unprimed spleen cells of either parental A/J or BALB/c or homologous CAF_1 donor origin. In those experiments shown in which carrier-primed helper cells were injected into naive F_1 recipients, this meant that on day −1, two cell populations were transferred to appropriate recipient groups: one comprising the helper cell population, the other the unprimed parental or F_1 spleen cell population. In those experiments in which helper T cells were primed in the native environment of the recipient to be employed (i.e., carrier-primed recipients), on day −1 (before irradiation) such recipients were injected with only the one population of unprimed parental or F_1 spleen cells. In either case, irradiation was carried out on day 0; this means that both the carrier-primed helper cell population and the unprimed parental or F_1 spleen cell population were irradiated before the introduction of DNP-primed B cells into the system.

In another modification of this system, recipients were injected with two cell populations on day 0; thus, in addition to the population of DNP-primed B cells, a second cell population, consisting of either unprimed or KLH-primed spleen cells, was transferred. It is to be noted that these latter sources of helper T cell activities were not exposed to ionizing irradiation because they were injected together with B cells into the previously irradiated recipient environment.

In each experiment performed, appropriate control groups, consisting of irradiated recipients of DNP-primed, T cell-depleted B cell populations (of the three types employed), which were challenged with DNP-KLH in the absence of any available KLH-primed helper T cells, were included. To make graphic presentation of the data less cumbersome, these control groups have been omitted from the individual figures. In all experiments, however, responses manifested by such control groups were always $<1\%$ of the uninhibited positive control responses of the corresponding B cell population.

Inhibition of F_1 Hybrid T Cell Help for Parental, but Not F_1, B Cells by Concomitant Transfer of Unprimed Spleen Cells of Opposite Parental Type. Unirradiated CAF_1 recipients were injected on day −1 with 50×10^6 KLH-primed CAF_1 helper T cells and either no

additional cells or 50 × 10^6 unprimed spleen cells from either CAF_1, BALB/c, or A/J donors. On day 0, all recipients were irradiated and then injected with 15 × 10^6 DNP-primed, T cell-depleted B cells of either BALB/c, A/J, or CAF_1 origin; secondary challenge with DNP-KLH was performed immediately thereafter. The DNP-specific splenic PFC responses of the various groups of this experiment are summarized in Fig. 1.

Good cooperative T-B cell responses were obtained between F_1 helper cells and either parental BALB/c or A/J or homologous F_1 B cells in all groups which either received no additional unprimed spleen cells or when such cells were obtained from CAF_1 donors (groups I, II, V, VI, IX, X). In the case of cooperative responses with parental BALB/c and A/J cells, transfer of unprimed spleen cells of the homologous parental type exerted no significant effect on the cooperative responses obtained (groups III and VIII). In contrast, transfer of unprimed spleen cells of opposite parental type significantly diminished the magnitudes of cooperative responses obtained (groups IV and VII). Although these unprimed (and irradiated) parental spleen cells exerted significant inhibitory effects on cooperative F_1-(opposite) parent T-B cell interactions, these same parental cells failed to appreciably affect F_1 helper activity for homologous F_1 B cells (groups XI and XII).

Parental Cell Inhibition of F_1-Parent T-B Cell Cooperation: Homologous Parental Helper T Cells Fail to Rescue the Inhibited Response. One possible explanation for the preceding

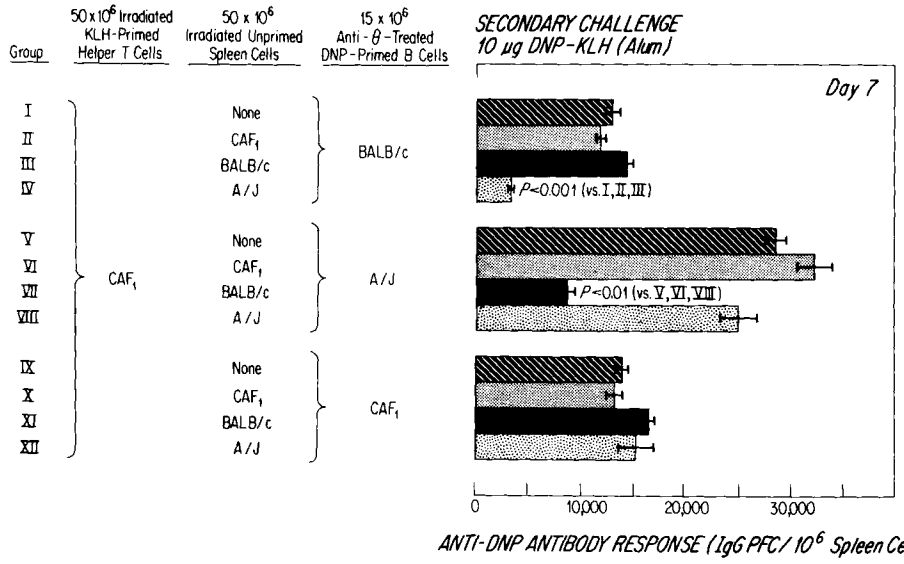

FIG. 1. Inhibition of F_1 hybrid T cell help for parental, but not F_1, B cells by concomitant transfer of unprimed spleen cells of opposite parental type. Unirradiated CAF_1 recipient mice were injected intravenously with 50 × 10^6 syngeneic KLH-primed spleen cells and 50 × 10^6 spleen cells from unprimed CAF_1, BALB/c, or A/J donor mice; control groups were not injected with unprimed spleen cells. 24 h later, all such recipient mice were irradiated (650 rad) and then injected intravenously with 15 × 10^6 DNP-primed B cells from either BALB/c, A/J, or CAF_1 donors. All recipients were challenged with 10 μg of DNP-KLH in alum administered intraperitoneally shortly after cell transfer. The data are presented as geometric mean levels of individual IgG DNP-specific PFC/10^6 spleen cells of groups of five mice each assayed on day 7 after the final cell transfer and secondary challenge. Horizontal lines represent SE and relevant *P* values depicting statistically significant differences between experimental and control groups are indicated beside the corresponding bars.

result is that the unprimed parental *A* spleen cells may induce an allogeneic effect on the B cells of parent *B* type, inducing the latter cell population to produce anti-parent *A* alloantibody. The anti-parent *A* alloantibody, in turn, could inhibit the cooperative activity of the CAF_1 KLH-primed T cells. If this explanation is correct, addition of KLH-primed helper T cells homologous to the relevant parental B cell population should restore the adoptive secondary anti-DNP response; conversely, failure to restore the response with homologous parental helper T cells would rule out anti-parental alloantibody-mediated inhibition of F_1-parent cooperation as the explanation.

The experiment summarized in Figs. 2 and 3 followed the design of the preceding experiment with the addition that either unprimed or KLH-primed spleen cells of homologous type to the parental B cells being assayed were cotransferred with those

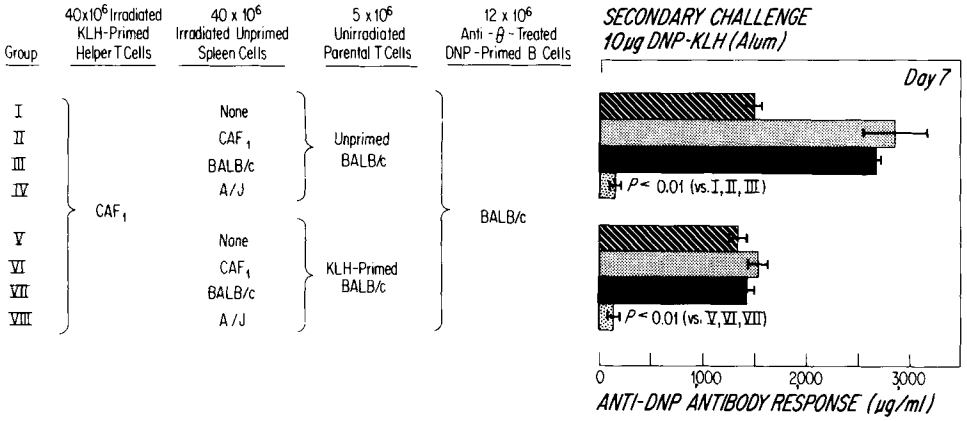

FIG. 2. Parental cell inhibition of F_1-parent T-B cell cooperation: homologous parental helper T cells fail to rescue the inhibited response. Same protocol in legend to as Fig. 1 except for the addition of homologous unprimed or KLH-primed parental spleen cells as indicated at the second stage cell transfer and the numbers of DNP-primed B cells employed. The data are presented as geometric mean levels of individual serum anti-DNP antibodies of groups of four mice each assayed on day 7 after final cell transfer and secondary challenge. Horizontal lines represent SE and relevant *P* values depicting statistically significant differences between experimental and control groups are indicated beside the corresponding bars.

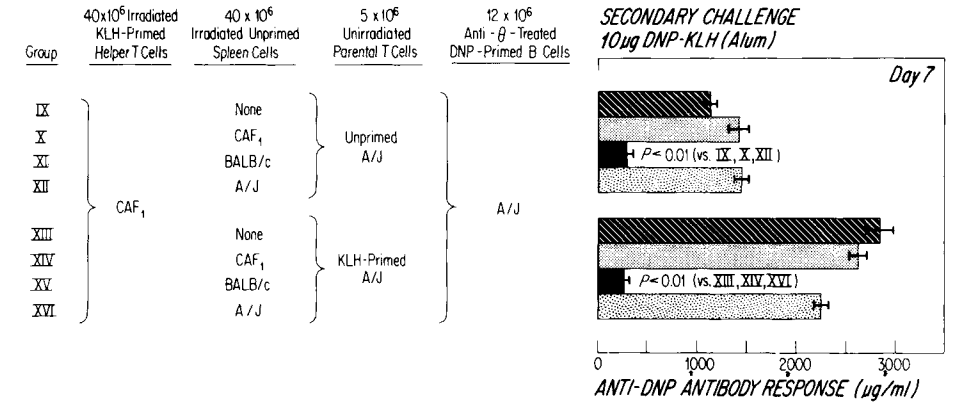

FIG. 3. Parental cell inhibition of F_1-parent T-B cell cooperation: homologous parental helper T cells failed to rescue the inhibited response. Same protocol as legend to Fig. 2.

B cells. It is quite clear that cooperative responses between CAF_1 helper T cells and BALB/c B cells (Fig. 2) are markedly inhibited by the presence of unprimed A/J spleen cells (cf. group IV versus groups I–III), and that such inhibited responses are not rescued by the additional transfer of unirradiated KLH-primed BALB/c helper T cells (cf. group VIII versus groups V–VII). The reciprocal phenomenon with respect to CAF_1 T cells cooperating with parental A/J B cells (Fig. 3) is likewise insensitive to restoration by the addition of KLH-primed A/J helper T cells. Although not shown in either of these Figures, control groups analyzing the helper T cell capacities of each of these respective parental T cell populations demonstrated excellent cooperative capabilities of both the BALB/c and A/J KLH-primed helper cell populations in straightforward adoptive secondary responses with the two respective homologous parental B cell populations.

Parental Spleen Cells Inhibit Cooperative Interactions between Opposite Parental B Cells and F_1 Hybrid, but Not Opposite Parental, Helper T Cells. In some of the earliest studies reported from our laboratory on the mechanisms of genetic restrictions in T-B cell cooperative interactions, we addressed the question of suppression as a possible explanation for the genetic restrictions observed (3, 15). The results of such studies argued rather definitively against this explanation, because cotransfer of KLH-primed cells of parental *B* origin never inhibited cooperative responses of homologous T and B cells of opposite parental *A* type, even over a substantial dose range (3, 15). It is possible, however, that the use of unprimed cells in the present system, as contrasted to those earlier studies in which the parental *B*-type cells had been KLH-primed, may make a difference in this respect, although this seems unlikely.

The experiment presented in Figs. 4 and 5 was designed to address this possibility. In this experiment, carrier-specific helper T cells were provided by the entire recipient environment inasmuch as irradiated, carrier-primed recipients were employed. Only in this way is it possible to address the aforementioned question, where necessary, in a manner relatively or completely devoid of any participation by F_1 lymphoid cells. As summarized in Fig. 4, it is clear that the presence of unprimed, irradiated lymphoid cells of opposite parental type significantly diminishes F_1-parent T-B cooperative cell interactions even when carrier-specific helper T cells are present in tremendous excess (*cf.* group IV versus I–III and group VII versus V, VI, and VIII). Once again, in contrast to the parental cell inhibition of F_1-parent cooperative interactions, no similar inhibition of homologous F_1-F_1 T-B cell interactions was observed (groups IX–XII).

As shown in Fig. 5, when carrier-primed parental, rather than F_1, recipients were employed as sources of helper T cells for DNP-primed B cells of homologous parental type, the presence of unprimed, irradiated lymphoid cells of opposite parental type failed to exert any detectable effect, either negative or positive, on the successful development of such responses (groups XIII–XX). These results are perfectly consistent with our previously reported observations in which cells of opposite parental type, but carrier-primed rather than unprimed, failed to suppress homologous T-B cell cooperative interactions (3, 15). Moreover, these data clearly indicate that whatever suppressive mechanism exists to explain these parental cell inhibition effects, they are not directly mediated by the parental cells themselves and, more importantly, require the presence of F_1 lymphoid cells in the system for such suppressive effects to be observed.

Parental Cell Inhibition of F_1-Parent T-B Cell Cooperation: T Cells Are Not Required in the

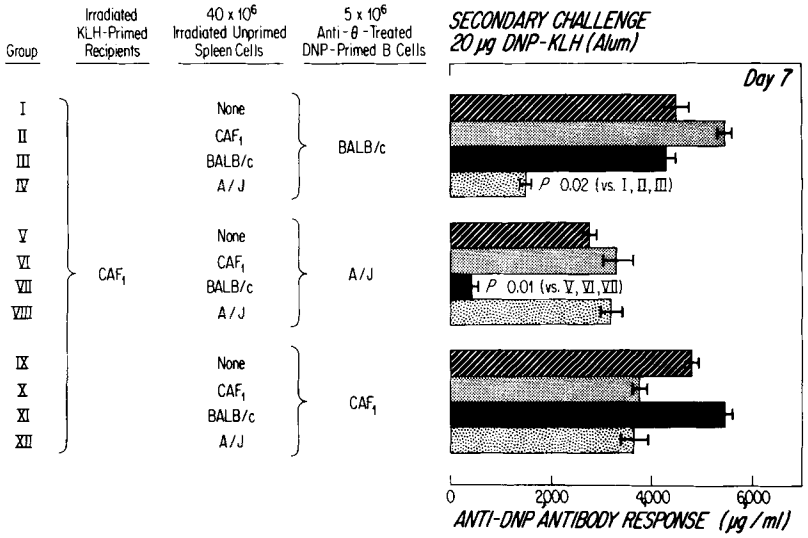

FIG. 4. Parental spleen cells inhibit cooperative interactions between opposite parental B cells and F_1 hybrid, but not opposite parental, helper T cells. Same protocol as Fig. 1 except for the use of KLH-primed CAF_1 recipients as source of helper T cells. All recipients were challenged with 20 μg of DNP-KLH in alum administered intraperitoneally shortly after cell transfer. The data are presented as geometric mean levels of individual serum anti-DNP antibodies of groups of four mice each assayed on day 7 after final cell transfer and secondary challenge.

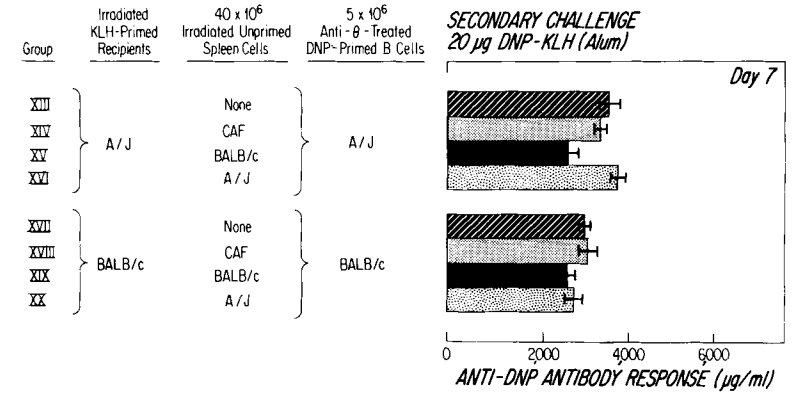

FIG. 5. Parental spleen cells inhibit cooperative interactions between opposite parental B cells and F_1 hybrid, but not opposite parental, helper T cells. Same protocol as in legend to Fig. 4, except for the types of KLH-primed recipients employed.

Opposite Parental Lymphoid Cell Population. An even stronger piece of evidence in support of the conclusion that the suppressive mechanism underlying the phenomenon described here is not directly mediated by parental cells derives from the experiment presented in Fig. 6. In this experiment, we investigated whether the presence of T lymphocytes in the irradiated, unprimed opposite parental spleen cell population was required for the development of inhibitory effects of F_1-parent cooperative cell interactions. This was accomplished by comparing the effects of opposite parental lymphoid cell populations transferred either as intact spleen cell populations or as T cell-depleted (by treatment in vitro with anti-θ serum plus C) spleen cells. As shown

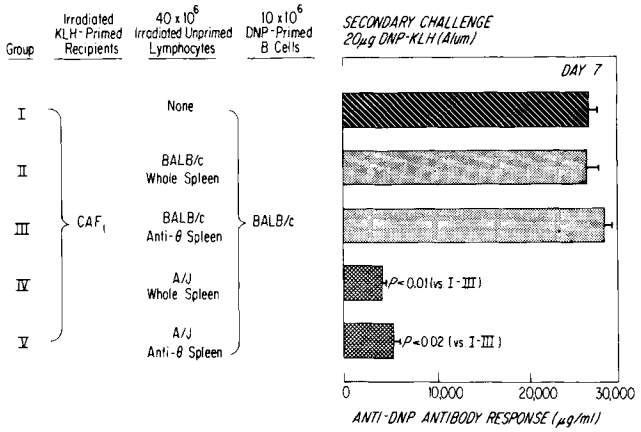

FIG. 6. Parental cell inhibition of F_1-parent T-B cell cooperation: T cells are not required in the opposite parental lymphoid cell population. Same protocol as in legend to Fig. 4, except for the use of either intact spleen cell populations or T cell-depleted spleen cells from each respective parental type.

in Fig. 6, CAF_1 helper T cells cooperate very well with DNP-primed BALB/c B cells in the absence of any additional unprimed parental cells (group I) or in the presence of either intact or T cell-depleted spleen cells from homologous BALB/c parental donors (groups II and III). In contrast, the additional transfer of lymphoid cells from opposite parental A/J donors significantly inhibits the F_1-BALB/c cooperative interactions irrespective of whether intact (group IV) or T cell-depleted cell populations are transferred.

Parental Cell Inhibition of F_1-Parent T-B Cell Cooperation Occurs Even When Parental B Cells Have Been Adoptively Primed in F_1 Recipients. The collective results of the preceding experiments direct us toward the conclusion that the presence of unprimed parental spleen cells results in the induction of anti-opposite parent-specific suppressive influences among the CAF_1 lymphoid cell population. Moreover, the induction of such suppressive influences does not require T lymphocytes in the parental lymphoid population. The next question we addressed pertained to the target(s) of such a suppressive mechanism. Specifically, we wished to address the possibility that F_1 lymphoid cells (carrier-primed or otherwise) are seeing something on conventional parental B cells that is different from what they see on F_1 partner B cells; this would explain the absence of any inhibitory effects of parental lymphoid cells on homologous F_1-F_1 cooperative interactions. If this were not the case, then perhaps priming parental B cells in an F_1 environment might select for cells possessing the same determinant structures present on F_1 cells and hence diminish the susceptibility of such adoptively primed parental B cells to the parental cell-induced inhibition phenomenon.

BALB/c B cells were adoptively primed to DNP-ASC in either homologous BALB/c or semisyngeneic CAF_1 recipients in the following manner: BALB/c and CAF_1 mice were primed with 20 μg of ASC in CFA to generate a substantial source of helper T cells. 8 d later, these carrier-primed mice were irradiated with 650 rad and then injected intravenously with 35×10^6 unprimed BALB/c spleen cells depleted of T cells by in vitro treatment with anti-θ serum plus C; immediately after cell transfer, all recipients were immunized with 20 μg of DNP-ASC adsorbed on 4 mg of

alum. 5 d later, all recipients were boosted with 10 μg of DNP-ASC in saline. 6 d after this secondary boost, spleen cells were removed from the first-stage transfer recipients, treatment with anti-θ serum plus C to eliminate any residual T cells derived from the first transfer hosts or otherwise, and then transferred into fresh irradiated, carrier-primed CAF_1 or BALB/c recipients (prepared in an identical manner to the first-stage recipients). These second-stage recipients were similarly immunized with 20 μg of DNP-ASC in alum and boosted with the same antigen and dose in saline 5 d later. 6 d after the booster immunization of these second-stage recipients, spleens were removed and treated with anti-θ serum plus C to provide the source of adoptively primed DNP-specific B cells for the experiment summarized in Fig. 7.

The experiment illustrated in Fig. 7 followed the same experimental protocol employed in Fig. 4 with the exception of the source of the DNP-primed B cells. As shown by groups I–IV, cooperative responses between CAF_1 helper T cells and BALB/c cells adoptively primed in irradiated BALB/c recipients were markedly diminished in the presence of unprimed, irradiated A/J (but not CAF_1 or BALB/c) lymphoid cells. Likewise, parental A/J lymphoid cells substantially inhibited collaborative interactions between F_1 helper cells and BALB/c cells adoptively primed in irradiated CAF_1 recipients (*cf.* group VIII versus V–VII). It is clear, therefore, that adoptive priming of the parental B cell partner in F_1 recipients does not alter the susceptibility of the system to parental cell-induced inhibition.

Parental Cell Inhibition of F_1-Parent T-B Cell Cooperation: Reversal of Inhibition after Cotransfer of Unprimed Spleen Cells of Both Parental Types. The presence of independent subsets of cooperating lymphocytes in F_1 hybrid individuals, one each corresponding to the haplotype specificity of the two respective parental haplotypes was first suggested by us several years ago as the best interpretation for experiments demonstrating restricted cooperative helper activity of (responder × nonresponder)F_1 hybrid T cells for B cells of the responder, but not of the nonresponder, parental type (16). This has since been verified by several investigators in a variety of different systems (17–22). Indeed, in the context of the present experiment, one might explain the

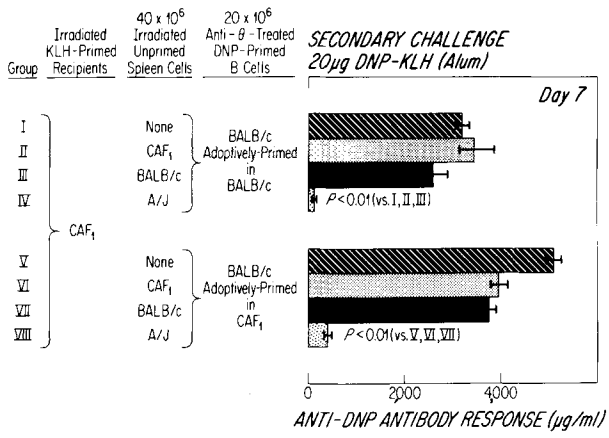

FIG. 7. Parental cell inhibition of F_1-parent T-B cell cooperation occurs even when parental B cells have been adoptively primed in F_1 recipients. Same protocol as in legend to Fig. 4 except for the use of 20 × 10^6 DNP-primed B cells of BALB/c origin, but which had been adoptively-primed either in BALB/c or CAF_1 recipients as described in Results.

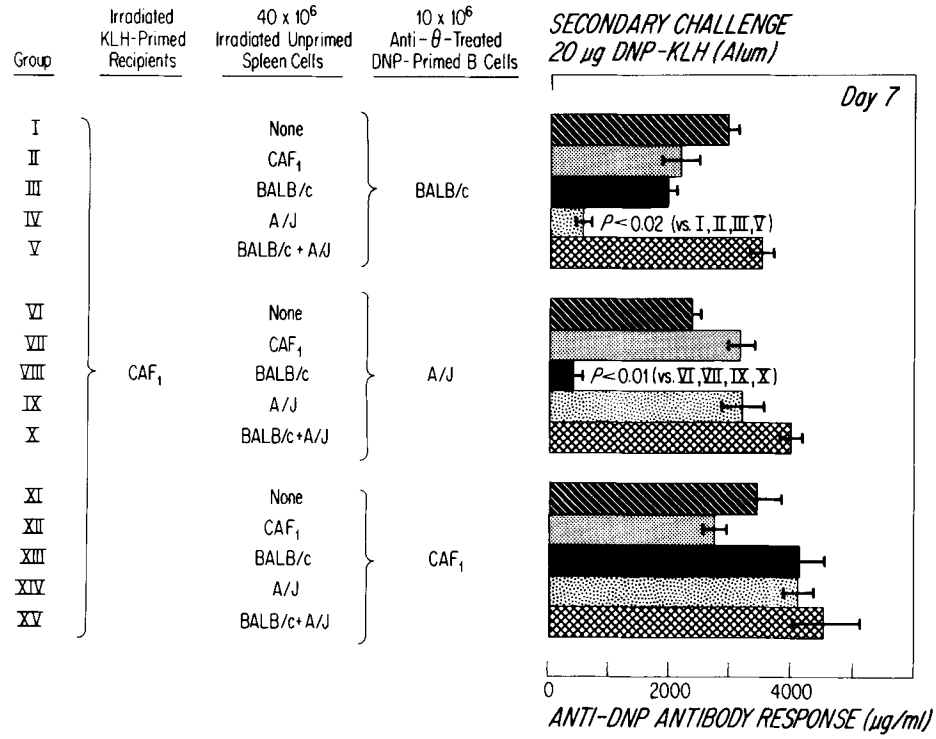

FIG. 8. Parental cell inhibition of F_1-parent T-B cell cooperation: reversal of inhibition after cotransfer of unprimed spleen cells of both parental types. Same protocol as in legend to Fig. 4 with the addition of groups receiving a mixture (40×10^6 of each) of unprimed spleen cells from BALB/c and A/J donors.

absence of parental cell-induced inhibitory effects on F_1-F_1 cooperative interactions on the basis that although the presence of unprimed parental cells might inhibit the cooperative capacities of the F_1 subset corresponding to the opposite parental specificity, the F_1 subset of helper cells corresponding to the second parental specificity (i.e., that identical with the unprimed parental cell type) would nevertheless still exist; consequently, cells with cooperative potential of at least one subset of F_1 cells would be present in either case after transfer of one type of unprimed parental lymphoid cells. Alternatively, we entertained the possibility that there may exist, in fact, 3 subsets of functional interacting F_1 cells—one each corresponding to the respective parental haplotypes and the third showing relatively unique F_1 specificity.

To address these two possibilities, an experiment was designed to determine whether the presence of unprimed lymphoid cells of both parental types would manifest some degree of inhibition of homologous F_1-F_1 cooperative interaction. We reasoned that if only two F_1 subsets existed, one each corresponding to their respective parental types, then the presence of a mixture of unprimed parental cells should effectively inhibit both F_1 subsets. On the other hand, the absence of any inhibitory effects of such a mixture of unprimed parental spleen cells might circumstantially indicate the existence of a third subset unique to F_1 specificities. The results of such an experiment are summarized in Fig. 8. Once again, carrier-primed helper T cells were provided by irradiated, carrier-primed CAF_1 recipients. Helper activity provided by such recipients

for BALB/c B cells (groups I–V) was significantly diminished by the presence of unprimed, irradiated A/J lymphoid cells (group IV), as expected. Likewise, helper activity for A/J B cells (groups VI–X) was significantly inhibited by the presence of BALB/c lymphoid cells (group VIII). In contrast, as shown previously, neither of these parental lymphoid cell populations exerted any inhibitory influence on the helper activity of F_1 cells for homologous F_1 B cells (groups XIII and XIV).

The unexpected finding in this experiment was that cotransfer of unprimed, irradiated lymphoid cells of both parental types not only failed to inhibit cooperative interactions between homologous F_1 T and B cells (group XV), but actually restored the F_1-parent cooperative responses in the case of both BALB/c (group V) and A/J (group X) DNP-primed B cells.

Discussion

These experiments demonstrate that F_1-parent T-B cell cooperation is significantly diminished by the addition of lymphoid cells of opposite parental type. The unprimed parental cells responsible for inducing such inhibition need not consist of T lymphocytes and do not need to continuously divide because, as the experimental protocol is designed, they are subjected to sublethal irradiation within 24 h after cell transfer. Thus, whatever degree of proliferation is important on the part of the parental lymphoid cell population, it is limited to the immediate 24 h after cell transfer.

The parental cell-induced inhibition of F_1-parent cooperative cell interactions cannot be reversed by addition of parental helper cells homologous to the parental B cells being assayed. Moreover, although the addition of irradiated unprimed lymphoid cells of parent *B* type significantly inhibits F_1-parent *A* T-B cooperation (and vice versa), the presence of parental lymphoid cells has no inhibitory effect on F_1-F_1 cooperative cell interactions. More important, the inhibition phenomenon absolutely requires the presence of F_1 cells, because the presence of parental lymphoid cells does not inhibit homologous T-B cell interactions of opposite parental type. Finally, although the presence of one parental cell population inhibits F_1-parent cooperative responses, the simultaneous presence of both parental cells restores such responses.

In considering the possible interpretations of these findings, several explanations have been addressed, either directly or indirectly, by the experiments presented here. First, it is quite clear that the inhibition phenomenon is not explained by production of conventional alloantibodies that reacted with, and inhibited the function of, the F_1 helper T cells. Thus, the experiment presented in Figs. 2 and 3 demonstrates that the inhibited F_1-parent cooperative interactions could not be restored by addition of a second population of carrier-primed helper cells homologous to the DNP-primed B cells employed. This inability of homologous parental T cells to rescue such responses also implies, albeit indirectly, that the DNP-primed B cell population involved is most likely the target of this inhibitory mechanism.

Second, the diminished F_1-parent cooperation is not a reflection of some form of macrophage/presenting cell imbalance resulting from the presence of opposite parental lymphoid cells in the system. This conclusion can be reached on the following grounds: (*a*) in quantitative terms, the numbers of antigen-presenting cells of F_1 type (which are unrestricted in their antigen-presenting capacities to partner cells of either parental type) was clearly in excess in all of the experiments performed; (*b*) even if one made the argument that irradiated antigen-presenting cells were less efficient in

their antigen-presenting capabilities than unirradiated antigen-presenting cells, it must be remembered that the opposite parental lymphoid cell population inducing diminished F_1-parent cooperation were likewise irradiated; (*c*) any postulated imbalance in antigen-presenting cells caused by the transfer of opposite parental lymphoid cells would have been overcome by the transfer of additional unirradiated parental lymphoid cells (either carrier-primed or not) homologous to the DNP-primed B cells employed, yet this was clearly not the case (Figs. 2 and 3); and (*d*) we have been able to inhibit F_1-parent cooperation with opposite parental lymphoid cells depleted of most macrophages by passage over Sephadex G-10 (data not shown). Collectively, these points argue forcefully against the possibility that a macrophage/antigen-presenting cell imbalance explains the observations presented.

After alloantibody or macrophage imbalance have been eliminated as likely mechanisms, one must consider what other type of suppressive mechanism might be generated in this cotransfer model to cause the observed inhibition. At first glance, the transferred parental lymphoid cell population itself seems the most likely suppressive element. However, there are several lines of evidence that argue against the parental population as the direct mediator of this suppression: First, one might expect that at least some inhibition of homologous F_1-F_1 T-B cell cooperation would have occurred if parental cells directly mediated the suppressive influence; it is clear, however, that the presence of unprimed parental cells does not appreciably disturb F_1-F_1 cooperative interactions. Second, one would have expected some inhibitory effects on homologous T-B cell interactions of opposite parental type; as shown in Fig. 5, and as reported elsewhere (3, 15), such is not the case. Finally, if parental cells do indeed directly mediate this suppressive mechanism, then the responsible cells belong to a non-T cell component of such populations, because parental lymphoid cells depleted of T cells are as effective in inducing the inhibitory phenomenon as T cell-containing parental cells (Fig. 6).

A more likely possibility is that the suppressive mechanism is actually effected by F_1 cells displaying anti-parent specificity, most likely directed against the parent type donating the primed, DNP-specific B cells. This conclusion is supported by the fact that: (*a*) F_1 cells must be present for the phenomenon to be observed; (*b*) F_1-F_1 cell interactions are not inhibited by the presence of parental lymphoid cells of either type; and (*c*) the inhibitory effect generated by the presence of one parental-type lymphoid cell population can be counter-balanced by incorporating lymphoid cells of the second parental type into the same system. This latter observation is most readily explained by considering that the numbers of parental cells transferred of each type were far in excess of the numbers of cooperating T and B lymphocytes in the system, and hence could serve as more accessible targets for the F_1 anti-parent reactions that are postulated to explain these observations.

Although direct proof is lacking at the moment, these inhibitory phenomena can best be explained by the development of self-specific responses against cell interaction structures. As discussed in detail elsewhere (6), such responses could be directed against either target CI molecules or corresponding receptors for such molecules (or both). In the context of the present experiments, we postulate that when unprimed lymphoid cells of parental *A* type are introduced into a cooperating mixture of $(A \times B)F_1$ and parental *B*-type partner cells, the presence of the parental *A* lymphoid cells induces a response within the F_1 population against CI structures specifically displayed

by the parental *B*-type partner cells. The consequence of such a response is a diminution in the capacity of the parental *B*-type partner cells to receive cooperative signals from potential helper cells either of F_1-type or even from homologous helpers of parental *B*-type.

This explanation raises an immediate question—why do lymphoid cells of parental *A* type induce responses in the F_1 against CI molecules and/or their receptors displayed by the opposite parental population, and not against themselves? In fact, there is no reason to conclude that F_1 responses against both parent CI phenotypes do not occur after all. That the presence of unprimed homologous parental *A*-type lymphoid cells fails to inhibit F_1-parent cooperative interactions when the parental B cells are of homologous parent *A*-type could be misleading. For, as argued above with respect to the counterbalancing effects of transferring both unprimed parental lymphoid cells, it might be that any anti-parent response directed against the inducing parental population might be masked or adsorbed by the presence of large numbers of the very cells inducing such responses.

If indeed the development of anti-CI receptor responses explains this phenomenon, then the data presented also provide evidence for the existence of one or more unique F_1 hybrid subsets of interacting cells; because F_1-F_1 cooperative interactions are not inhibited by the presence of either type of parental lymphoid cells. Indeed, that such anti-parent reactions can be generated, presumably for a worthwhile purpose, implies that there must be CI molecules and self-recognition capabilities that are uniquely F_1-type. Data from other systems clearly have documented unique F_1 specificities by biochemical (23) and antigenic (24, 25) criteria, and so it is not surprising that there should be unique F_1 specificities incorporated into the self-recognition repertoire.[2]

Recently, experiments performed by Miller and Derry (27) and Muraoka and Miller (28) have identified the existence of lymphoid cells capable of suppressing the development of in vitro cytotoxic reactions. Such cells are found in the spleens of athymic nude mice (27) and in the bone marrow and thymus (but not spleen) of normal mice (28) and display their suppressive activities on precursors of cytotoxic cells in a self-specific fashion. Although the experimental system differs considerably from the experiments reported here, it is our impression that the anti-self suppressor cells observed in their experiments may be related to the mechanism of anti-self CI receptor responses postulated as the explanation for parental cell-induced inhibition of F_1-parent T-B cell cooperation.

Finally, if experiments currently underway are successful in directly demonstrating the development of anti-self CI receptor responses in this experimental model, it is not difficult to envisage that such responses may occur as a normal component of immune regulation and probably will clarify our understanding of mechanisms underlying environmental restraint and adaptive differentiation.

Summary

The experiments presented herein demonstrate that F_1-parent T-B cell cooperation in vivo is significantly diminished by the addition of lymphoid cells of opposite parental type. This inhibition phenomenon is not a straightforward allosuppression mechanism as (*a*) it can be induced by parental lymphoid cells depleted of T cells, (*b*)

[2] Since submission of this manuscript, we have learned of experiments that directly demonstrate the existence of F_1-specific subsets of cooperating helper T cells (26).

it does not operate on cooperative interactions between homologous T and B cells of opposite parental type, and (*c*) absolutely requires the presence of F_1 cells as participants in the reactions generated. The possible involvement of alloantibodies produced aberrantly under the experimental conditions employed has been ruled out by direct experimentation, and the possibility that such inhibition reflects an imbalance in macrophage/antigen-presenting cell components of the reactions has been excluded. Because the presence of parental lymphoid cells only affects cooperative interactions between F_1 T cells and B lymphocytes of opposite parental type but has no inhibitory effect on cooperative interactions between homologous F_1, T, and B cells, this (and other points discussed herein) strongly argues for the existence of one or more subsets of F_1 interacting partner cells that are uniquely specific for F_1, as distinct from either parental type cell interaction determinants. For reasons discussed, it appears that the most likely mechanism underlying such parental cell-induced inhibitory effects on F_1-parent partner cell interactions is the development of anti-self cell interaction structure responses by F_1 cells against the relevant self-specific cell-interaction structures of the parental partner cells involved.

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