ADAPTIVE DIFFERENTIATION OF MURINE LYMPHOCYTES

II. The Thymic Microenvironment Does Not Restrict the Cooperative Partner Cell Preference of Helper T Cells Differentiating in $F_1 \rightarrow F_1$ Thymic Chimeras*

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This study was conducted to analyze the extent to which the major histocompatibility complex (MHC)[1] genotype of the thymus restricts the cooperating phenotype of helper T cells with respect to their ultimate ability to interact effectively with partner B lymphocytes in the development of antibody responses. These studies, like others reported previously (1, 2), made use of artificially constructed bone marrow chimeras prepared by reconstituting adult-thymectomized, lethally irradiated F_1 mice with syngeneic F_1 bone marrow, together with transplanted thymuses from either F_1 or parental donors. Reconstituted mice of these types were then immunized with keyhole limpet hemocyanin (KLH) and their KLH-specific helper T cells so induced were tested for the cooperative helper activity they could provide to 2,4-dinitrophenyl (DNP)-primed B lymphocytes derived from conventional F_1 or parental donors in developing secondary anti-DNP antibody responses to DNP-KLH. The results clearly show that the thymus influences little, and certainly does not restrict, the partner cell preference displayed by helper T cells differentiating in such environments. Moreover, the extent of thymic influence differed depending on the class of antibody being produced with the help of such cells.

This investigation is an extension of earlier studies in this (3) and other laboratories (4–10) which have addressed the predictions of the concept of adaptive differentiation (3, 11–14). This notion ascribes the partner cell preferences of various cells of the lymphoid system, known to be genetically controlled by various regions of the MHC (15), to processes of selection that occur early during cell differentiation and which are determined by the MHC phenotype of the environment in which such differentiation occurs (3, 11–14). The first experiments supporting this concept were performed

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[1] Abbreviations used in this paper: ASC, *Ascaris summ* extract; CFA, complete Freund's adjuvant; CI molecules, cell interaction molecules; CTL, cytotoxic T lymphocyte(s); DNP, 2,4-dinitrophenyl hapten; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; SCRF, Scripps Clinic and Research Foundation.

by us using conventional (i.e. nonchimeric) mice several years ago (11). More recently, several investigators, including ourselves, have conducted such experiments using various types of bone marrow chimeras and analyzing the cells differentiating in such environments for either cytotoxic T-lymphocyte (CTL) activity (1, 2, 4–7) or for cooperative helper T-cell function (3, 8, 9). In addition, we have analyzed B lymphocytes from such chimeras for evidence of adaptive differentiation similar to that observed with T lymphocytes, and have demonstrated this to be true (3, 11). In general, the weight of evidence from all such studies makes it clear that lymphocytes do, indeed, differentiate adaptively in accordance with the MHC phenotype of the environment in which they live.

A significant point that has arisen from certain of these studies concerns the role played by the thymic microenvironment in directing the self-recognition capabilities of T lymphocytes. Thus, Zinkernagel et al. (1), and subsequently, Fink and Bevan (2), reported that ($A \times B$) F_1 precursors of CTL that have matured in an F_1 mouse whose thymus was replaced by a homozygous parent A thymus graft generate CTL (after immunization) which display a certain degree of restriction in their lytic activity for target cells possessing the same *H-2K* and/or *H-2D* genetic regions as parent A. When a similar analysis is made of helper T lymphocytes generated in such thymic chimeras, we find that the influence of the thymus in directing ultimate partner cell preference is partial at best. This suggests that either fundamental differences exist in the selection mechanisms for self-recognition among precursors of CTL and helper T lymphocytes, respectively, or that the bone marrow chimera systems may not be providing the clearest window through which to view these processes.

Materials and Methods

The proteins, reagents, and preparation of hapten-protein conjugates were the same as those described in earlier reports (3, 16). 9 mol of DNP/100,000 daltons of 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 (3, 16–18).

Animals and Immunizations. Inbred BALB/c ($H\text{-}2^d$) mice were obtained from Simonsen Laboratories, Gilroy, Calif., or from the Scripps Clinic and Research Foundation (SCRF) mouse breeding colony. Inbred A/J ($H\text{-}2^a$) and (BALB/c × A/J)F_1 hybrids (CAF_1, $H\text{-}2^{d/a}$) were obtained from The Jackson Laboratories, 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., nonchimeras) donor mice were immunized generally at 8–12 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 then removed 2–4 wk after boosting, and adoptively transferred to irradiated recipient mice for in vivo assay as previously described (3, Results). All irradiation was done with a ^{137}cesium irradiator (Gammacell 40, Atomic Energy Limited of Canada); adoptive recipients were administered 675 R several hours before cell transfer. Secondary challenge consisted of 20 μg of DNP-KLH precipitated with 2 mg of alum administered i.p.; all mice were bled for antibody titration and individual splenic DNP-specific PFC were enumerated 7 d after secondary challenge.

Preparation of Bone Marrow Chimeras. Bone marrow chimeras were prepared by repopulating either adult thymectomized or nonthymectomized, lethally irradiated (900 R) CAF_1 recipient

mice with 15 × 10^6 anti-θ serum + complement-treated CAF_1 bone marrow cells in the manner described previously (3, 19, 20). Thymectomy was performed on 12–15-wk-old CAF_1 mice, 2 wk before irradiation and bone marrow transplantation. 4–5 d after bone marrow transplantation, thymuses were transplanted to thymectomized recipient mice by insertion of four thymus lobes from donor mice, through a small surgical incision, subcutaneously in the mid-thoracic area of the back. Thymuses were obtained from 4–5-wk-old CAF_1, A/J, or BALB/c donors and prepared for transplantation in one of two ways: (*a*) Some donors were pretreated with 2.5 mg of cortisone acetate i.p. and 100 μl of anti-lymphocyte serum (ALS) i.v.; the thymuses from such mice were removed 48 h later and exposed to 1,500 R irradiation in vitro. (*b*) In other cases, thymuses were removed from donor mice which had not received prior treatment and then subjected to 875 R irradiation in vitro immediately before transplantation.

All chimeric mice were housed following the precautions described previously (3). 3–4 mo after reconstitution, the peripheral blood lymphocytes of all chimeras were analyzed by microcytotoxicity assay, as previously described (3, 21), to confirm that all cells were indeed of F_1 type. The chimeras were then primed with KLH (see above) for induction of helper T cells; each mouse was bled at various intervals thereafter and their serum analyzed for anti-KLH antibody levels; ≅85–90% displayed anti-KLH antibody responses comparable to those of conventional CAF_1 control mice, thereby indicating intact thymic function. Chimeras which failed to produce anti-KLH antibodies, or produced such antibodies in low quantities, were considered failures of thymus reconstitution and were discarded. Success of thymic reconstitution was also verified by direct inspection, grossly and microscopically, of the transplanted thymus lobes at the time such mice were killed.

Statistical Analysis of the Data. Statistical analyses noted in the text and in figures were made with geometric means and standard errors calculated from individual values of either serum anti-DNP antibody levels or splenic DNP-specific PFC in each group. *P* values from comparison of relevant experimental and control groups were ascertained by Student's *t* test.

Results

In Vivo Helper Activity of CAF_1 → CAF_1 Thymic Chimera T Cells for F_1 and Parental B Cells. CAF_1 → CAF_1 adult thymectomized bone marrow chimeras which had been transplanted with either CAF_1, A/J, or BALB/c thymuses were primed with KLH to generate KLH-specific T cells. 10 × 10^6 spleen cells from such thymic chimeras or from nonchimeric, conventional CAF_1 mice primed in the same way, were transferred together with 10 × 10^6 DNP-ASC-primed B cell from anti-θ serum-treated spleen taken from CAF_1, A/J, or BALB/c donor mice. Conventional CAF_1 mice served as irradiated recipients in all cases. On d 7, after secondary challenge with 20 μg of DNP-KLH, all mice were bled and their serum was analyzed for both IgG and IgE anti-DNP antibodies.

As shown in Fig. 1, control mice which received B cells alone, failed to develop detectable IgG responses and only barely detectable levels of IgE anti-DNP antibodies (groups I–III). Excellent cooperative responses were obtained between mixtures of conventional CAF_1 helper cells and all three B-cell sources; these responses were rather similar in magnitude in the IgG class, whereas A/J B cells displayed somewhat lower IgE secondary responses than those produced by CAF_1 or BALB/c B cells (groups IV–VI). The results obtained with helper T cells derived from F_1 chimeras reconstituted with either F_1, parental A/J, or parental BALB/c thymuses are illustrated by groups VII–XV. The data presented are those obtained with T cells from chimeras transplanted with donor thymuses which had been exposed to 875 R irradiation in vitro before transplantation, but not otherwise manipulated (i.e., no cortisone or ALS); comparable results were, however, obtained with thymic chimera T cells prepared by transplantation of thymuses from ALS and cortisone-pretreated donors followed by 1,500 R in vitro irradiation (data not shown).

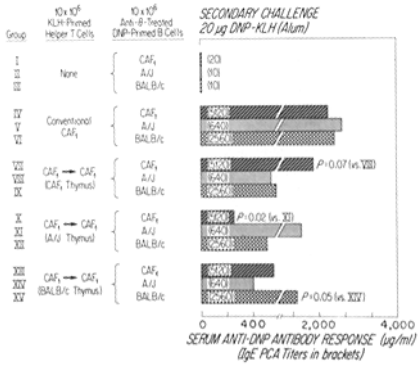

FIG. 1. In vivo helper activity of $CAF_1 \rightarrow CAF_1$ thymic chimera T cells for F_1 and parental B cells. Irradiated (650 R) CAF_1 recipients were injected intravenously with 10×10^6 helper T cells from KLH-primed conventional CAF_1 or CAF_1 thymic chimera donor mice together with 10×10^6 anti-θ serum-treated spleen cells from DNP-ASC-primed CAF_1, A/J, or BALB/c donor mice as indicated on the left of the figure. All recipients were secondarily challenged with 20 μg of DNP-KLH in alum shortly after cell transfer on day 0. 7 d later, all mice were bled before analysis of serum antibody levels and also killed for analysis of quantities of splenic DNP-specific PFC. On the right of the figure, the horizontal bars represent the geometric mean levels of serum anti-DNP antibodies in groups of four mice each. IgE anti-DNP antibody responses, as detected by passive cutaneous anaphylaxis analysis, are indicated within brackets enclosed in each corresponding horizontal data bar. With one exception, only those P values representing significant differences between relevant groups are indicated; although we do not consider a P value of 0.07 within significant limits, one such P value is indicated merely to illustrate the differences between groups 7 and 8.

Examination of the secondary anti-DNP antibody responses obtained with such thymic chimera helper T cells makes certain obvious points: First, in no instance was there any drastic restriction in helper cell activity for B cells of one or the other parent type. Second, F_1 T cells from chimeras reconstituted with A/J or BALB/c thymuses clearly displayed higher levels of helper activity for partner B cells derived from A/J and BALB/c donors, respectively; the level of help was significantly different from that provided to the other parent, however, only in the case of BALB/c thymus-reconstituted chimeras (group XV). Moreover, this pattern of preferential help manifested by parental thymus-reconstituted chimeras as contrasted to F_1 thymus-reconstituted chimeras, was only true for responses of the IgG class. It is noteworthy that the magnitudes of IgE anti-DNP antibody responses obtained with each different B-cell type were the same irrespective of whether conventional F_1 or thymic chimera F_1 helper T cells were used.

Titration of In Vivo Helper Activity of Thymic Chimera T Cells for F_1 and Parental B Cells. Fig. 2 summarizes the results of an experiment designed to test different doses of helper T cells obtained from either conventional F_1 or F_1 thymic chimera donors in terms of their ability to help F_1 or parental DNP-primed B cells. Thymic chimeras reconstituted with parental A/J thymuses by each of the two different methods employed for preparing donor thymuses before transplantation were tested in this study.

As shown in A, conventional F_1 helper T cells provided indiscriminant helper activity, at both doses tested, for both A/J and BALB/c B cells; the somewhat higher responses obtained with F_1 B cells reflects differences in the inherent strengths of the respective B-cell populations employed. In B, it can be seen that F_1 chimera T cells

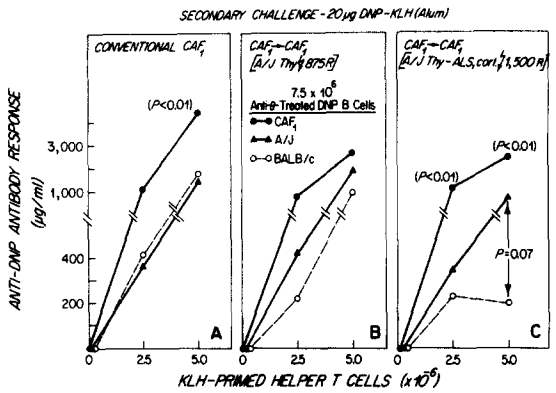

FIG. 2. Titration of in vivo helper activity of thymic chimera T cells for F_1 and parental B cells. Irradiated (650 R) CAF_1 recipients were injected with either 2.5 or 5.0 × 10^6 KLH-primed helper T cells from either conventional CAF_1 or $CAF_1 \rightarrow CAF_1$ thymic chimeras (reconstituted with A/J thymus) donors. Note that two different methods for preparation of donor thymuses for reconstitution were employed. Recipient mice were also injected with 7.5 × 10^6 DNP-ASC-primed anti-θ serum-treated B cells from either CAF_1, A/J, or BALB/c donor mice. All recipients were secondarily challenged with 20 μg of DNP-KLH in alum and bled 7 d later for titration of IgG anti-DNP antibody levels. The data presented are mean levels of serum anti-DNP antibodies in groups of four mice each. Pertinent *P* values are illustrated; note that although the thymic chimera T cells provided greater help for A/J, than for BALB/c, B cells at the highest dose of helper T cells employed in the far right panel, this difference was not statistically significant.

from donors reconstituted with A/J thymuses exposed only to 875 R in vitro irradiation before transplant, provided effective help for all three B-cell population, the highest responses being obtained with F_1 B cells. As in the preceding experiment, there is some indication of preference for helping A/J rather than BALB/c B cells, particularly at the lowest helper cell dose employed, but the differences between the helper activity provided to each respective parental B cell were not significantly different at either cell dose. On the other hand, F_1 chimeras reconstituted with parental A/J thymus taken from donors pretreated with cortisone and ALS and then subjected to 1,500 R in vitro irradiation, displayed a more exaggerated preference in helper activity provided to A/J B cells as compared to BALB/c B cells; these differences still were not significant, although clearly approaching significance at the highest helper T-cell dose employed.

Spleen Cells from F_1 Thymic Chimeras Fail to Exert Suppressive Effects on Cooperative T-B-Cell Interactions in Adoptive Secondary Anti-DNP Responses. To determine whether the slight cooperative preference observed with parental thymus-reconstituted F_1 thymic chimera T cells reflected the existence of any suppressive mechanisms, the following experiment was performed: 5 × 10^6 KLH-primed spleen cells from conventional CAF_1 donors were transferred together with 5 × 10^6 anti-θ serum-treated DNP-primed B cells from either F_1, A/J, or BALB/c donors into irradiated CAF_1 recipients. In addition, certain recipients received 5 × 10^6 cells from KLH-primed conventional CAF_1 donors or F_1 thymic chimeras. All recipients were challenged with 20 μg of DNP-KLH. 7 d later, these mice were bled, killed, and their spleens removed for determinations of DNP-specific PFC. As shown in Table I, the cooperative responses developed between conventional F_1 helper cells, and each of the three partner B-cell types were in no way affected by the additional transfer of either conventional F_1 or

TABLE I

Spleen Cells from $CAF_1 \rightarrow CAF_1$ Thymic Chimeras Fail to Exert Suppressive Effects on Cooperative T-B-Cell Interactions in Adoptive Secondary Anti-DNP Responses *

5×10^6 KLH-primed cells tested for suppression	5×10^6 Conventional CAF_1 KLH-primed helper T cells plus 5×10^6 anti-θ-treated DNP-primed B cells from:‡		
	CAF_1	A/J	BALB/c
None	22,986	31,197	20,961
Conventional CAF_1	34,655	29,156	18,475
$CAF_1 \rightarrow CAF_1$ [F_1 thymus]	29,959	25,036	23,025
$CAF_1 \rightarrow CAF_1$ [A/J thymus]	19,732	21,760	15,001
$CAF_1 \rightarrow CAF_1$ [BALB/c thymus]	20,298	25,228	22,879

* 5×10^6 spleen cells from KLH-primed conventional CAF_1 mice were transferred together with 5×10^6 anti-θ-treated DNP-ASC-primed spleen cells from CAF_1, A/J or BALB/c mice into 675 R irradiated CAF_1 recipients. In addition, certain groups received 5×10^6 spleen cells from KLH-primed conventional CAF_1 or F_1 thymic chimeras to test for possible suppressive effects of the latter cells. All recipients were challenged with 20 μg of DNP-KLH in alum.

‡ IgG DNP-specific PFC/10^6 spleen cells determined on day 7 after cell transfer and challenge. No statistically significant differences existed among the various groups.

F_1 thymic chimera spleen cells, thus arguing against the likelihood of suppressive mechanisms contributing to the results described above.

Discussion

This study was designed to determine whether the cooperating phenotype of helper T cells, which are *H-2I*-restricted, is influenced in the same way and to the same extent by the MHC phenotype of the thymus as reported previously to be the case for CTL, which are *H-2K* and *H-2D*-restricted (1, 2). This was tested by constructing $F_1 \rightarrow F_1$ irradiation bone marrow chimeras using adult thymectomized F_1 recipients which were subsequently reconstituted with either F_1 or parental thymus grafts. KLH-primed helper T cells obtained from these various thymic chimeras provided effective help for DNP-primed B cells obtained from either F_1 or parental donors. When parental thymus grafts were used to reconstitute $F_1 \rightarrow F_1$ chimeras, the resulting helper T cells provided somewhat more efficient help for B cells of the corresponding parental type, but were nevertheless capable of providing substantial (albeit less) helper activity for partner B cells derived from the opposite parent.

This moderate partner cell preference of helper T cells from parental thymus-grafted F_1 chimeras represents a significant difference from the results reported by Zinkernagel et al. (1) and Fink and Bevan (2) who observed a somewhat greater restriction in self-specificity of CTL imposed by the thymic microenvironment in which precursors of such cells had differentiated. It is unlikely that any obvious technical points, such as contaminating parental T cells leaking out of the parental thymus graft or the existence of some type of suppressive mechanism, could explain these differences in outcome because these possibilities were explored, both in their studies (1, 2) and our own, and were found to be absent. Indeed, our own concern about the technique of thymus grafting prompted us to use two different methods of preparing donor thymuses before transplantation. The results obtained were comparable in either case and, if anything, we found a tendency toward a somewhat greater

parental cell preference when more rigorous procedures were followed for the preparation of the donor parental thymus grafts (Fig. 2).

Frankly, we do not have an adequate explanation for these apparent differences in the strength of thymic influence on self-specificity of CTL and helper T cells, respectively. It is clear that the thymus exerts some influence in this regard on CTL precursors (1, 2) and also on precursors of helper T lymphocytes although, as shown here, the influence exerted on the latter seems to be considerably less than on the former. These differences could reflect the different genetic regions of the MHC involved, a point about which one may speculate but unfortunately cannot prove at the moment. If this is, in fact, true, then it becomes a significant point to consider when developing approaches to be employed for immunologic reconstitution in certain clinical circumstances.

It is perhaps equally important that these differences may be signaling to us a need to examine more critically, and to interpret more cautiously, the data obtained with the increasingly popular bone marrow chimera systems. Recently, bone marrow chimeras of various types have been employed in immunological studies conducted in a number of different laboratories. When all of the different findings are carefully examined, one is struck by the obvious diversity in experimental results obtained, not only from one laboratory to another but also within the same laboratory from one year to the next (compare references 22, 23). This is not altogether surprising when one considers that many variables, some readily apparent and some not, determine the success and extent of reconstitution, the health of the chimeras and, hence, the ultimate immunologic function manifested by chimeric cells. Moreover, differences in genetic restriction patterns of chimeric cell function have been observed depending on whether such functions were analyzed using in vivo or in vitro systems, a point emphasized very nicely by the recent studies of Erb et al. (24).

It seems, therefore, that bone marrow chimeras may simply pose too many variables that we cannot adequately control, and which make such experimental animals different enough from a conventional intact mouse, that we should perhaps reconsider the extent to which the chimera models are employed as the sole approach to answer many of the questions for which they are now used. We know, for example, that a sublethally irradiated parental mouse exerts a very definite allogeneic effect on transferred antigen-primed F_1 donor cells (25). We were surprised to find in recent studies that exposure of parental mice to lethal doses of x-irradiation (900 R and greater) resulted in allogeneic effects exerted on transferred F_1 donor cells that were as strong or stronger than those observed at lower sublethal doses (Fig. 3). This raises an obvious question as to what extent, if any, the parental environment may exert and/or influence selection processes by virtue of such allogeneic effects. Indeed, this may be a reasonable explanation for the more striking cooperative restrictions manifested by lymphocytes obtained from $F_1 \rightarrow$ parent chimeras that we observed in our earlier studies (3), as contrasted with the considerably less restriction observed in the parental thymus-grafted $F_1 \rightarrow F_1$ chimeras employed in the present studies. It should be emphasized that these discrepancies do not argue against the ability of lymphocytes to learn their preferential cooperating phenotype, but rather, it emphasizes the caution we must use in ascribing the major source of the environmental influence imposed. Simply on the basis of the differences obtained with $F_1 \rightarrow$ parent chimeras described earlier (3), and those reported here with F_1 thymic chimeras, it

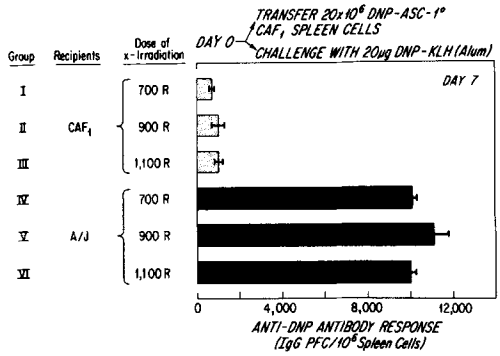

FIG. 3. Radiation resistance of the positive allogeneic effect exerted by irradiated parental recipients on adoptively transferred F_1 spleen cells. At 20 × 10^6 DNP-ASC-primed spleen cells from conventional CAF_1 donor mice were injected intravenously into either CAF_1 recipients (groups I–III) or A/J parental recipients (groups IV–VI) which had been irradiated with either 700, 900, or 1,100 R. All mice were challenged with 20 μg of DNP-KLH in alum shortly after cell transfer on day 0. On day 7, all mice were killed and their spleens analyzed for levels of DNP-specific PFC of the IgG class. The data are presented as geometric mean levels of individual splenic PFC in groups of four mice each. Statistical comparisons yielded P values of < 0.001 between responses obtained in all groups of A/J parental recipients versus the groups of CAF_1 recipients. These results therefore illustrate the capacity of irradiated parental recipients to exert positive allogeneic effects on F_1 DNP-ASC-primed spleen cells at all doses of irradiation employed.

seems most likely that a significant extrathymic influence, in addition to any exerted by the thymus itself, must play a critical role in determining the ultimate cooperating phenotype of, at least, helper T cells which have differentiated under these circumstances. Consistent with this interpretation are the studies of Kindred (26, 27) who has reproducibly failed to find evidence of intrathymic learning in nude mice grafted with allogeneic thymuses.

As this manuscript was being completed, the observations of Waldmann et al. (28) and Bevan and Fink (29), which both concern the effects of thymus grafts on development of *H-2* restrictions in helper T-cell functions, appeared in print. Both of these studies were interpreted as indicating a more definite thymic influence on helper T-cell differentiation than reported in the present paper. Careful scrutiny of the data, however, clearly reveals variable leakiness in the responses that should have displayed greater restriction if the thymus were solely responsible for directing adaptive differentiation of helper T cells, and in this sense are more consistent than conflicting with the present observations. We do not believe that it is any longer tenable to dismiss the observed leakiness as unexplained (and therefore unimportant), whereas deciding instead, to place the greatest emphasis on those data which are more compatible with the original aims of the experiments being performed.

Finally, it is worth emphasizing two additional points that are pertinent to these and similar studies with respect to the types of results obtained.

First, it should be noted that *H-2* restriction in T-B-cell cooperation as we originally defined it in conventional mouse systems is a true restriction, i.e., incompatible cell mixtures fail to respond altogether (15). The results described here and elsewhere (28, 29) with thymic chimeras represent examples of preference in the sense that higher responses are obtained with certain cell mixtures than with others. The significant qualitative difference between true restriction, on the one hand, and preference, as

seen with thymic chimeras, on the other underscores the importance of extrathymic influences on helper T-cell adaptive differentiation.

Secondly, the fact that we find only marginal or sometimes no thymic influence on preference of helper T-cell activity whereas others seem to observe more influence of the thymus (28, 29) could reflect the choice of thymic chimeras used for such analyses. Thus, as pointed out in Materials and Methods, we carefully screened all thymic chimeras for functional reconstitution according to their abilities to develop essentially normal anti-KLH antibody responses, an excellent criterion, we believe, for establishing whether KLH-specific helper T-cell activity has been restored to normal levels. Because we discarded those chimeras which developed lower-than-normal anti-KLH responses, we may have selected for those helper T cells which had passed through all stages of the normal T-cell differentiation pathway and hence, been influenced by both intra- and extrathymic events. In the other studies in these systems (1, 2, 5, 7, 28, 29), comparable functional criteria were not employed as guidelines for selecting the thymic chimeras actually employed and this could very well account for some of the differences in results obtained.

Summary

The cooperating preference of helper T cells originating from F_1 bone marrow, but differentiating in adult thymectomized, lethally irradiated F_1 recipients reconstituted with either F_1 or homozygous parental thymus grafts was investigated. Cooperating preference was assayed by determining the levels of helper activity provided by antigen-primed T cells derived from such thymic chimeras for hapten-primed B lymphocytes obtained from conventional F_1 or parental donors in adoptive secondary antibody responses in vivo. The results of these analyses revealed a tendency of helper T cells derived from parental thymic chimeras to provide better help for B cells of the same parental type corresponding to the origin of the thymus graft than for the opposite parent. Such preference was, however, only marginal and rarely were differences in levels of helper activity provided to the respective parental types statistically significant. Moreover, this marginal preference, when observed, pertained only to responses of the IgG class; no concordant preference in providing helper activity for IgE antibody responses was observed even with the same populations of thymic chimera helper T cells. Finally, in no instance was there any evidence of restriction in the classical sense of presence versus absence of help as we have routinely observed in all of our previous studies concerning genetic restrictions of T-B-cell cooperative interactions.

Although the basis for differences in the studies reported here when compared to observations made in cytotoxic T-lymphocyte systems is unclear, and could reflect genuine mechanistic requirements concerning what directs *H-2* restrictions in helper T cells and cytotoxic T lymphocytes, respectively, it is also possible that we are placing too much faith in our interpretations of data obtained in bone marrow chimera systems than is perhaps justified by the potentially great fragility of such systems.

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