Distinct pathways of B cell differentiation. I. Residual T cells in athymic mice support the development of splenic germinal centers and B cell memory without an induction of antibody.

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Distinct Pathways of B Cell Differentiation

1. Residual T Cells in Athymic Mice Support the Development of Splenic Germinal Centers and B Cell Memory without an Induction of Antibody

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B cell memory to T cell-dependent Ags develops in the germinal centers (GC). Here we report that thymus-deficient, nu/nu mice immunized with phosphorylcholine coupled to keyhole limpet hemocyanin (EPC-KLH) develop GC in the spleen in the absence of Ab-forming cell (AFC) response. However, the formation of GC on EPC-KLH immunization requires T cells, because 1) CB.1.7-scid mice reconstituted with B lymphocytes failed to develop GC without a supplement of CD4+ cells and 2) in vivo administration of an anti-CD4 mAb abolished the GC response in euthymic mice. Thus, it appears that the formation of GC in nu/nu mice was due to a low number of T cells that were detectable in situ within the splenic lymphoid follicles. The numbers of GC in individual Ag-stimulated nu/nu mice appeared to correlate with the density of T cells in the splenic sections. The B cells in these GC expressed T15, the dominant Id of anti-PC Ab, and became primed for an anamnestic response. Secondary challenge with EPC-KLH resulted in an increased number of GC without detectable AFC. However, when the Ag-primed nu/nu mice received CD4+ lymphocytes 1 day before the challenge, they demonstrated a vigorous AFC response that was predominantly IgM and significantly higher than the secondary response of nu/nu mice that had been reconstituted with CD4+ cells during both primary and secondary immunizations. Therefore, it appears that immunization of nu/nu mice may lead to an early step of B cell activation and memory development even though the T lymphocytes in these mice are incompetent to provide help for Ab formation. The memory and Ab pathways of B cell differentiation may involve different mechanisms of T cell help. Journal of Immunology, 1994, 152:1718.

In the course of immune response, the Ag-reactive B lymphocytes in the peripheral lymphatic tissues follow two distinct pathways of differentiation that are separated anatomically and functionally, i.e., formation of GC and development of foci of AFC. GC in B cell follicles are regions of intense cell proliferation (1, 2) and represent the site of the somatic mutations of rearranged IgV genes (3), the selection of cells on the basis of their receptor affinity for the Ag (4, 5), and the generation of a pool of memory B cells that produce Ab on secondary immunization (6). B cells in GC are readily identified by avid binding of a lectin, PNA (7). The formation of GC is T cell dependent (8-10). On the other hand, B cells in periarteriolar lymphatic sheath proliferate without undergoing somatic mutations (11), bind PNA poorly (7), and differentiate into AFC (up to 103 AFC/focus) (11) that release the bulk of primary Ab. The development of AFC in response to T-dependent Ags requires cooperation with CD4+ Th cells.

Although both GC and AFC foci require T cells, there may be significant differences in the mechanisms of T cell help for the respective pathways of B cell differentiation. The Ab foci develop in close contact with the surrounding masses of T cells (12) and the mechanisms of T-B cooperation in AFC formation, including the cognate cell interaction and release of well-characterized interleukins.
have been thoroughly studied (13, 14). Much less is known about the role of T cells in GC formation. Variable numbers of CD4+ cells were found interspersed with the GC B cells in mice (15), rats (16), and humans (17). However, the CD4+ lymphocytes isolated from GC appear to represent a subset of T cells (16, 18, 19) that express an NK cell marker, Leu-7, and that differ functionally from the classical CD4+ helper cells (18, 19). These results raise the intriguing possibility that the mechanisms of T-B cell interaction in the GC/memory pathway are different from those in differentiation of AFC.

Experiments of Vonderheide and Hunt (10) with adoptive transfer of B and T cells in rats implied that the GC formation by B cells may be reconstituted with relatively few CD4+ cells. Based on their results, we speculated that the GC response requires at least an order of magnitude fewer Th cells than the Ab formation does, and we tested this hypothesis using an adoptive transfer of varying numbers of normal CD4+ lymphocytes into athymic (nu/nu) mice immunized with a T-dependent Ag. To our surprise, nu/nu mice that did not receive any T cells developed large numbers of GC in the splenic lymphatic follicles, despite the fact that they did not respond by the production of Ab. Further investigation indicated that the small number of peripheral T lymphocytes in nu/nu mice appears to be sufficient for initiation of the GC pathway of B cell response but not for the AFC differentiation, and that these GC B cells develop a memory response to Ag challenge. We suggest that nu/nu mice provide an experimental system for delineation of a minimal T cell signal(s) that is required for the GC memory pathway of B cell differentiation.

Materials and Methods

Mice

BALB/cAnTac and C57BL/6NTac athymic (nu/nu) (2 mo of age) mice were purchased from Taconic Farms (Germantown, NY). BALB/c AnNCr athymic (nu/nu) and euthymic mice were obtained from Charles River Laboratories (Wilmington, MA). CB.17 SCID mice from a colony maintained at the animal facility of University of Maryland School of Medicine were generously provided by Drs. John Sacci and Abdu Azad. All mice were maintained in a restricted animal room in sterile microisolator cage system (Lab Products, Inc., Mywood, NJ) on a 12-h day/night cycle.

Ags and immunizations

KLH and conalbumin were purchased from Sigma (St. Louis, MO). EPC-KLH, (EPC) conjugated to KLH according to Spande (20) was kindly provided by Dr. James J. Kenny (National Cancer Institute, Frederick, MD). PC conjugates of BSA, CGG (both from Sigma), and BA standard tube Ag (National Veterinary Services Labs, Ames, IA) were prepared by reacting diazotized p-aminophenyl phosphonylclohexine (Sigma), respectively, with the proteins and with the BA suspension (21). A lyophilized vaccine from Ph strain R36a was prepared as previously described (22). All Ags were administered as a PBS solution i.p.

Lymphocyte preparations and cell transfer

Splenocyte suspensions were prepared by teasing spleens in RPMI 1640 medium supplemented with 25 mM HEPE (GIBCO, Grand Island, NY) and 0.5% BSA (Sigma). T lymphocytes were depleted by two treatments with a cocktail of mAb H013-4 (anti-Thy 1.2), GK 1.5 (anti-CD4), and 3-I55 (anti-CD8) from ascitic fluids (American Type Culture Collection, Rockville, MD) for 30 min at room temperature followed by a pretreated, normal rabbit serum as a source of complement for 40 min in a 37°C bath. The resulting B cell fraction contained <1% Thy 1.2-positive cells by FACS analysis.

T cell-enriched splenocyte populations were prepared by filtration through nylon wool columns (Wako BioProduct, Richmond, VA) using the manufacturer's protocol. Nonadherent cells were treated once with mAb 3.155 plus rabbit complement to eliminate CD8+ T cells. The resulting T cell fractions contained >80% of Thy 1.2+/CD4+ cells, <10% of spl+ cells, and <2% of CD8+ cells as determined by FACS analysis.

Cells for adoptive transfer were resuspended in 0.5 ml of PBS containing 1% (v/v) of normal mouse serum and injected in the tail vein 16 h before immunization.

FACS analysis

Cells were incubated with biotinylated goat anti-mouse Ig (Fisher Biotech, Pittsburgh, PA) or with biotinylated anti-Thy 1.2 (Becton Dickin-son, San Jose, CA) followed by staining with strepavidin-FITC (Fisher). The binding of mAb GK1.5 and 3.155 was visualized with goat anti-rat Ig-FITC conjugates (Fisher). Cells were analyzed with a flow cytometer (Becton Dickinson).

Serum Ab

Levels of PC Ab were determined by standard ELISA techniques using PC-BSA as Ag in solid phase and a goat anti-mouse Ig Ab labeled with β-galactosidase (Fisher) as a probe. The serum Ab concentration was determined by extrapolation from a standard curve.

Enumeration of AFCs

Splenocytes producing PC-specific Ab of different isotypes were enumerated by a modified ELISPOT assay. Spleen cells were suspended in RPMI 1640 medium with 5% FCS and distributed into 96-well, round-bottom culture plates (Costar, Cambridge, MA) in triplicate aliquots of 1 to 3 X 10^5 cells in 100 μl. Nitrocellulose filter sheets (0.45 μm pore size; Schleicher and Schuell, Keene, NH) were coated with a solution of PC-BSA (100 μg/ml in PBS) overnight at 4°C and then incubated in a blocking solution of 10% BSA in PBS for 2 h at 37°C. Filters coated with BSA were used as a control. The nitrocellulose filters were placed on the top of the culture plates and held firmly in place with the lid using binder clips. The cells were transferred onto the nitrocellulose by turning the plate upside down and placing it in a 37°C incubator with 5% CO2 for 4 h. The filters were washed under tap water, treated with PBS-EDTA (10 mM) for 10 min, and rinsed in PBS. Bound PC Ab "spots" were visualized by staining with biotin-labeled goat Abs to mouse IgM, IgG, and IgA followed by SA-ALPH conjugate (all from Fisher) and a substrate (ProBoLit, NBT/BCIP Color Development System, Promega Corp., Madison, WI). The spots were scored under X15 magnification and expressed as means from triplicates.

Immunohistochemistry

The preparation of anti-id mAbs AB1.2 and B36-82 and their specificity for the T 15 Id was previously described (23). Conjugation of proteins AB1.2, B36-82, and PC-BSA with biotin-N-hydroxy succinimide (Vec- tor Laboratories, Burlingame, CA) was conducted according to the proto-col supplied by the manufacturers. These conjugates were used for staining of PC-specific AFC in combination with SA-ALPH and a sub- strate, ProtobLit (Promega), for blue color stain. CD4+ cells in sections were visualized by staining with mAb GBK1.5 and goat anti-rat Ig/ALPH conjugate (Fisher). GCs were stained either with biotinylated PNA (Vec-tor Laboratories) followed by SA-ALPH, or with PNA conjugated to horseradish peroxidase (E.Y. Laboratories, Inc., San Mateo, CA) and a substrate, 3-amino-9-ethyl-carbazole (Sigma), for red color stain.
Blocks of frozen spleens were cut in a cryostat as 6-µm thick longitudinal sections, thaw mounted onto silanated glass slides (Digene Diagnostic, Inc., Beltsville, MD), air-dried for 20 min, fixed in acetone for 10 min, and stored at −70°C until used. For immunohistochemical staining, sections were thawed, washed in PBS, blocked by incubation with 10% BSA (1 h), and incubated with the appropriate probes.

**Enumeration of GC and Ab foci**

GC centers were counted as PNA-stained areas within lymphoid follicles, and the number was expressed as percentage of all follicles in the section (mean from two sections). Ab foci were scored as discrete clusters of AFC in perifollicular lymphatic sheath per spleen section (mean from two sections).

A correlation between the development of Ab foci and the single cell AFC response in the spleens of mice immunized with EPC-KLH was determined in preliminary studies. A larger portion of each spleen (approximately 1/3) was frozen and sectioned, and a smaller portion (1/3) was used for single cell ELISPOT assay. It was found that Ab foci became apparent when the numbers of single cells increased above 100 AFC/10⁶ splenocytes (data not shown), which is in agreement with our previously published results (24). The Ab responses in the results section were expressed either as Ab foci/section or as AFC/10⁶ splenocytes according to the experimental design.

**Statistical analysis**

Statistical analysis was performed using the Wilcoxon nonparametric test.

**Results**

Spleenic responses of BALB/c euthymic and athymic (nu/nu) mice to a single immunization with soluble EPC-KLH in a representative experiment are shown in Table I. The euthymic animals developed larger numbers of PNA⁺ GC and PC-specific Ab foci (day 7 after the immunization). Unexpectedly, the nu/nu mice also formed GC (Table I) that were well developed and strongly stained with PNA (Fig. 1A) even though there were no detectable Ab foci in the spleen. The development of GC in nu/nu mice appeared to be Ag dependent because unimmunized animals did not have any PNA⁺ follicles in the spleen. Furthermore, a large proportion of GC in nu/nu mice and those in euthymic mice showed a specific reaction to PC hapten as revealed by staining with mAb AB1.2 that recognizes the TI5 Id on PC-specific Ab (Fig. 1). Reconstitution of nu/nu mice with unprimed, syngeneic CD4⁺ lymphocytes 1 day before the immunization with EPC-KLH resulted in an increase in the number of GC, and Ab foci were now also induced in numbers comparable to those of euthymic mice (Table I). These results demonstrated that the hapten-specific Ab response to EPC-KLH is T dependent but they raised a question as to whether the GC response to this Ag requires T cells. To address this question, we performed an adoptive cell transfer experiment in SCID mice (Table II). CB17 SCID recipients that were reconstituted with splenic B cells, depleted of T lymphocytes by repeated cytotoxic treatment, failed to develop GC and did not mount any significant AFC response to PC (Table II, second line). However, the animals that received B cells together with a CD4⁺ fraction of T lymphocytes responded to EPC-KLH with high numbers of both GC and AFC (Table II, third line).
Table II. GC formation in B cell-reconstituted SCID mice immunized with EPC-KLH: requirement for CD4+ T cells

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<th>SCID Mice Treatment*</th>
<th>Splenic Responses (day 6 after Immunization)</th>
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*CB17-SCID mice were reconstituted (i.v.) with splenic lymphocyte fractions from unimmunized BALB/c and immunized with soluble EPC-KLH (i.p.), as indicated.

^b n = number of animals.

^c Proportion (%) of lymphatic follicles containing PNA centers (day 6).

^d Splenocytes producing PC-specific IgM were enumerated by ELISPOT assay in a cell suspension that was prepared from approximately one-third of the spleen (see the Materials and Methods).

Having found that the generation of splenic GC with EPC-KLH Ag requires T cells, we reasoned that the pattern of response in nu/nu mice (Table I) was due to a low number of resident T cells (24, 25) sufficient to sustain the development of GC but not Ab formation. To test this hypothesis, adjacent sections from nu/nu spleens were stained for Thy 1.2+ cells in situ and for PNA+ GC, respectively. The results of such examinations are illustrated in Figure 2. The density of Thy 1.2+ cells in sections was arbitrarily scored as high (+; Fig. 2F), intermediate (±; Fig. 2D), or negative when no Thy 1.2+ cells were found (−; Fig. 2B). A more precise enumeration of Thy 1.2+ cells was not attempted owing to their uneven distribution in the sections. It was noted that in euthymic mice, Thy 1.2+ cells were densely packed in the periarterial sheaths (Fig. 2H), whereas the resident Thy 1.2+ of nu/nu mice appeared to be dispersed throughout the follicles (Fig. 2F).

Co-staining of sections with mAb GK1.5 and PNA revealed the presence of CD4+ cells within the PNA+ CG in the spleen of nu/nu mice (Fig. 3). Next, we examined the

![FIGURE 2.](http://www.jimmunol.org/)

**FIGURE 2.** Appearance of GC and Thy 1.2+ cells in the spleens of selected individual mice immunized with EPC-KLH. Adjacent splenic sections from nu/nu mice (A to F) and an euthymic mouse (G and H) were stained with PNA (upper panel) and anti-Thy-1 (lower panel). Note the absence of GC (A) in a nu/nu spleen that stained negatively (−) for Thy 1.2 (B). An increasing development of PNA+ GC is seen in nu/nu mice that have a low number (±) (C and D) or a moderate number (+) (E and F) of Thy 1.2+ splenocytes associated with the lymphoid follicles.

![FIGURE 3.](http://www.jimmunol.org/)

**FIGURE 3.** Distribution of CD4+ cells in the spleen of nu/nu (A) and euthymic (B) mice immunized (− 6 days) with EPC-KLH. A: CD4+ cells (blue) are scattered through the splenic lymphoid follicles and within the PNA+ GC (red) in an athymic mouse. B: Note the masses of CD4+ cells (blue) in the periarteriolar lymphatic sheath adjacent to the follicles with relatively few cells in GC (red).
expression of Thy 1.2 and GC formation in splenic sections of 16 nude mice that were immunized either with EPC-KLH or another PC Ag, Pn vaccine, in independent experiments. It appeared that the proportion of follicles with GC correlated with the estimated density of follicular Thy 1.2+ cells in a plot of values from individual nude mice (Fig. 4). Three of 16 animals, which were scored as having no cells stained for Thy 1.2 in situ, failed to develop GC. These results support the notion that the formation of splenic GC in nude mice after an immunization is related to the T cell background.

A FACS analysis of splenocytes from nude mice purchased either from Taconic Farms (the source of mice for these experiments) or Charles River Laboratories showed comparable low numbers of Thy 1.2+ cells at the age of 2 mo (Table III). CD4+ cells became detectable at 3 mo of age in animals from both vendors. An administration of soluble EPC-KLH either at 2 or 3 mo of age did not influence the appearance of CD4+ cells (Table III).

An attempt was made to prevent GC formation by depleting CD4+ cells in vivo. Injection (i.v.) of mAb GK1.5 in euthymic BALB/c mice 2 days before immunization with EPC-KLH abrogated the PC Ab response and reduced the GC formation approximately 10-fold to below that level seen in nude mice (Table IV). However, a similar treatment failed to inhibit the GC development in 3-mo-old nude mice (Table IV), presumably because of the previously reported low density of CD4 expression on splenic lymphocytes in these animals (27–29).

Development of GC was noted after an immunization of nude mice with PC hapten presented on various carriers (Table V, group A). Interestingly, the lowest induction of GC occurred with PC-B. abortus, which was also the only Ag that produced PC Ab foci in nude mice. Two soluble proteins not coupled with PC, KLH, and conalbumin (Table V, group B) also induced a moderate number of splenic GC; administration of passive anti-KLH, Ab together with the KLH, significantly increased the GC response (Table V, group B), demonstrating that the well-known role of immune complex in the induction of GC (31) also occurs in athymic mice.

The potential of the GC response in nude mice for the generation of B cell memory was investigated using the experimental protocol shown in Figure 5. A cohort of nude mice was immunized with EPC-KLH (Fig. 5, group A). A group of four animals was assayed for primary anti-PC response on day 7 after the immunization (Fig. 5, group A-1) and the remaining animals were rested for 30 days. Before a 2nd immunization with EPC-KLH the mice were divided into two groups, one of which (Fig. 5, A-2) was challenged without any other treatment, whereas the other group (Fig. 5, A-3) was reconstituted with 2 × 10^7 CD4+ lymphocytes from normal unimmunized BALB/c donors and then challenged with the Ag. Another nude cohort (Fig. 5, group B) received normal CD4+ cells 1 day before the primary immunization and was likewise divided into three groups: B-1, for assay of the primary response; B-2, for the secondary response; and B-3, for the secondary response with a second inoculum of normal CD4+ cells. Serum anti-PC Abs were monitored during the primary response and it was ascertained that the Ab titers in the responder mice (cohort B) dropped to background levels before the boost immunization (data not shown). All animals were killed on day 7 after the secondary immunization and tested for PC-specific AFC of different isotypes and for GC (Figs. 6 and 7).

The nude mice that were not reconstituted with CD4+ cells failed to respond to both 1st immunization (Fig. 6, A-1) and 2nd challenge (Fig. 6, A-2) with EPC-KLH. The numbers of PC-specific IgM AFC detectable in these animals by ELISPOT were comparable to the background of AFC in unimmunized mice. In marked contrast, the Ag-primed nude mice that were supplemented with CD4+ lymphocytes at the time of 2nd challenge (Fig. 6, group A-3) developed a vigorous IgM AFC response that was
higher than the 2nd IgM responses in the control groups (Fig. 6, groups B-2 and B-3). The anamnestic response of nu/nu mice in Figure 6, group A-3 included significant numbers of IgG and IgA AFC; however, the bulk of the response (>80%) was IgM, whereas the responses of mice that received helper cells before priming (Fig. 6, group B) were shifted to ≈50% of (IgG+IgA) isotypes. It appears that the immunization of nu/nu mice with EPC-KLH, which induces GC formation, primed the hapten-specific B cells for a strong, anamnestic IgM response with a limited isotype switch relative to mice with functional Th cells.

The development of splenic GC in the above groups of mice is shown in Figure 7. Note that the secondary immunization of nu/nu mice in group A-2 (i.e., the animals that were not reconstituted with CD4+ lymphocytes) resulted in increased formation of PNA+ GC compared with the primary GC response in group A-1 (A-2 > A-1 at p = 0.004). Moreover, the scores of GC in group A-2 were not significantly different from the numbers of secondary GC in the mice that received CD4+ cells (A-2 vs B-2 and A-3 in Fig. 7, p = 0.95) and in euthy whole mice (Fig. 7, group E). Because the mice in group A-2 were 3 mo old at the time of secondary challenge, it is appropriate to compare their GC response with that of nu/nu mice that received primary immunization at 3 mo (Table IV). The difference between these groups (secondary response > primary response at 3 mo, p = 0.003) indicates that the increased formation of GC in group A-2 resulted from the priming with Ag rather than from the low numbers of CD4+ splenocytes that become detectable in 3-mo-old nu/nu mice (Table III). Collectively, these observations support the notion that Ag-specific B cells expand in the GC of thymus-deficient mice but that an addition of Th cells is required for the realization of the potential to produce Ab.

**Discussion**

The thymus rudiment in mice homozygous for the nu gene is incapable of supporting the normal lymphocyte development, resulting in severe depletion of peripheral T cells and virtual absence of T cell-dependent immune responses...
to produce lymphokines are impaired (32, 33). The T cell helper/inducer functions are completely lacking in \textit{nu/nu} mice (26); however, it has been possible to generate low, variable CTL responses (34, 35) and to derive alloreactive T cell clones (33, 37, 38) from these animals at older ages (>6 mo). Alloreactive T cells in \textit{nu/nu} mice have a restricted repertoire (34, 37) and a very limited use of V\(\beta\) chains (33, 37, 39) compared with the CTL from euthymic mice. These results and the findings of very low levels of \(\alpha\)- and \(\beta\)-gene expression (40) indicate a severe limitation of TCR repertoire diversity in athymic mice.

Our results suggest, however, that the functionally compromised T cells of \textit{nu/nu} mice are capable of providing "help" for the development of GC and B cell IgM memory to a T-dependent Ag. Antigen-primed \textit{nu/nu} mice had to be supplemented with normal helper cells to express the potential for Ab memory response; without such supplement, the boost immunization resulted in an increased number of GC (Fig. 7) without Ab formation (Fig. 6). It was also noted that the anamnestic AFC responses in the spleen of Ag-primed, athymic mice were higher but that the isotype switch was less pronounced compared with the secondary responses of mice with competent T cells. Preliminary results from our laboratory indicate that the apparent differences in magnitude of the splenic secondary responses may be attributed to the changes in distribution of AFC between the spleen and bone marrow. When priming with EPC-KLH occurred in animals with competent T cells (i.e., euthymic mice or T cell-reconstituted \textit{nu/nu}), the majority of secondary AFC were found in bone marrow, whereas the anamnestic response after a priming of

### FIGURE 5

Experimental design for testing a development of immunologic memory to EPC-KLH in \textit{nu/nu} mice (see the text).

(25, 26). Nonetheless, small and variable numbers of Thy.1+ cells (<5% to 20%) are detectable in the spleens of 8- to 10-wk-old \textit{nu/nu} mice and increase with age (26, 27). These T cells, which presumably differentiate in extrathyemic tissues such as the small intestine (28), have abnormal phenotypic and functional properties in comparison with the thymus-derived cells from euthymic mice. A fraction of Thy 1+ cells in \textit{nu/nu} is CD4−/CD8−, the ratio of CD4+:CD8+ cells is low (28, 30) and the CD4 molecule is expressed at low density (28, 32). The proliferative responses of \textit{nu/nu} T cells to various stimuli and their ability

### FIGURE 6

Memory AFC response in Ag-primed \textit{nu/nu} mice that were supplemented with unprimed CD4+ T cells before (~1 day) the secondary challenge (2−). Splenic AFC-producing PC-specific Ab IgM (black columns), IgG (hatched columns), and IgA (dotted columns) were enumerated by ELISPOT assay at 7 days after primary (1°) and secondary (2°) immunization with EPC-KLH. The proportions of different isotypes are expressed as percentage of the total AFC response (empty columns). Each column represents a mean ± SE from two experiments (6 to 8 animals/group).
A careful survey of the literature revealed that the present findings should not have been entirely unexpected. Earlier studies had noted a variable development of GC in the spleens of nu/nu mice after immunization with flagellin (43) and BA (8) but not with type III polysaccharide (44). This is consistent with our observation (Fig. 4, Table V) that the GC responses in nu/nu mice vary according to the immunogen, the individual animal, and the level of preexisting Ab. Additionally, Diamantstein and Blitstein-Willinger (45) reported a long time ago that splenocytes from SRBC-primed nu/nu mice were able to mount a secondary AFC response in vitro when supplemented with T cells from SRBC-primed donors. The authors proposed that unlike the Ab response, the development of B cell memory to T-D Ags is independent of T cell help. A similar conclusion was drawn by Roelants and Askonas (46) from their finding of B cell memory development in thymectomized, irradiated bone marrow-reconstituted mice immunized with hemocyanin. These authors were more cautious in their interpretation, however, realizing that the bone marrow-reconstituted mice did have a low number of T cells.

Our interpretation of the above data, together with these results, is that the GC/memory pathway of B cell differentiation is T cell dependent but that the requirements for help are different compared with the Ab-forming pathway. This hypothesis is consistent with the facts that 1) B cells that were rigorously depleted of T lymphocytes did not develop GC after immunization in vivo (10; and this paper), 2) small numbers of T cells in nu/nu mice that have abnormal phenotype and restricted repertoire (see Discussion) suffice to "help" the GC/memory differentiation, and 3) T cells isolated from human tonsillar GC have different properties from the helper cells that drive the Ab response (16–19).

Acknowledgments

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