Brief Definitive Report

Strong Priming of T Cells Adoptively Transferred into *scid* Mice

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Summary

We have examined the requirements for activating unprimed T cells in vivo by transferring T cells into *scid* mice, which lack mature B and T cells. Purified adult thymocytes and a protein antigen, keyhole limpet hemocyanin (KLH), were injected into *scid* mice. *scid* mice injected with T cells and KLH developed cellular lymph nodes containing $CD4^+$ and $CD8^+$ T cells. Cells recovered from the lymph nodes of injected *scid* mice proliferated and secreted interleukin 2 in response to KLH in vitro. The results indicate that T cells can be primed to KLH in the *scid* mouse in the absence of B cells.

The question of which APC induces primary T cell responses in vivo is not currently resolved. Several studies, in chickens and humans, indicate that B cells are not necessary for certain T cell responses. Thus, congenitally B cell–deficient humans mount normal delayed type hypersensitivity (DTH) responses (1, 2). Furthermore, bursectomized chickens mount normal allograft and DTH responses (3), and can prime T cells specific for SRBC (4). Moreover, antigen-pulsed dendritic cells, but not B cells, prime mice to protein antigens (5). These findings, however, do not rule out a role for B cells in events subsequent to the initial priming events. The involvement of B cells in events distal to the initial priming has been suggested by Ron and Sprent (6), who observed some priming of helper cells in mice chronically treated with anti-μ antibodies. These authors proposed that B cells amplify T cell responses initiated by dendritic cells. In contrast, other studies have indicated an apparently essential role for B cells in T cell priming. Lymph node cells from mice depleted of B lymphocytes by treatment with anti-μ antibodies or by x-irradiation failed to make secondary responses to protein antigens (7–10). Similarly, cells from a set of transgenic mice, expressing an MHC class II E molecule normally in APC but in only 2% of the animals' B cells, did not mount a proliferative response to an E-restricted antigen (11). None of these studies, however, looked directly at the status of dendritic cells in these mice.

The *scid* mouse offers a distinct experimental approach to study APC function for T cell priming since these mice are known to have APC function in vitro (11), but lack mature B and T cells. By adoptively transferring unprimed T cells, in the presence or absence of injected B cells, into *scid* mice and subsequently priming the mice with KLH, we show that endogenous APC are sufficient to prime the T cells to antigen in vivo, judged by several criteria. Our results indicate that T cell priming in *scid* mice does not require mature B cells.

Materials and Methods

Mice. C.B-17 *scid* mice, originally the generous gift of Dr. Mel Bosma, (Fox Chase Cancer Center, Philadelphia, PA), were bred and maintained under sterile conditions in the Animal Colony of the Tufts University Medical School. *scid* mice determined to have circulating levels of IgM or IgG >5 μg/ml were considered leaky and were not used in the study. 4–5-wk old female BALB/c mice were obtained from Jackson Laboratory, (Bar Harbor, ME) and Taconic Farms, Inc. (Germantown, NY) and maintained in the same animal colony under specific pathogen free conditions.

Antigens. KLH, Con A, LPS from *Escherichia coli* 0111:B4, and OVA were purchased from Sigma Chemical Co., St. Louis, MO.

Reconstitution and Priming. Thymocyte suspensions were prepared from 6–8-wk-old normal BALB/c mice. Thymocytes were passed twice over nylon wool columns. 4–7-wk-old *scid* mice were injected with 15–40 $\times$ 10^6 thymocytes ($scid_{THY}$) per site in the footpad, base of the tail, and intraperitoneally. In some experiments, mice were injected with BALB/c spleen cells, treated with anti-Thy1.2 and rabbit c, and thymocytes. These T cell–depleted spleen cell populations failed to respond to Con A and showed an enhanced response to LPS compared with untreated spleen cells. Such cells are subsequently referred to as B cells. 1 d later all reconstituted *scid* mice, uninjected *scid* mice, and a BALB/c control were primed in the same sites with 10 μg KLH in CFA.

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Cell Culture. 9 d after antigen priming, spleens and draining lymph nodes were removed and single-cell suspensions were prepared in RPMI 1640, supplemented as previously described (13), but containing 1% heat-inactivated autologous serum rather then FCS. 3×10^5 lymph node cells were cultured in 3–6-well replicates with 50 μg/ml KLH, 50μg/ml OVA, or 2.5 μg/ml Con A in 200 μl. Supernatant was removed after 18–24 h for cytokine bioassay (13). For the cytokine bioassay, 8×10^3 CTLL cells were incubated with two to three dilutions of supernatants for 36 h. 1 μCi [^{3}H]TdR was added for the final 18 h. Standard curves were set up with recombinant IL-2 and IL4. The presence of IL-2 and IL-4 in the supernatants of antigen-activated cells was assessed as follows: antibodies S4B6 and 11B11, specific for IL-2 and IL-4, respectively, were added to supernatants and assayed on the indicator CTLL population, as described above. The specificity of the antibodies was confirmed in our studies by using recombinant mouse interleukins.

FACS® Analysis. Conjugated antibodies were diluted to optimal concentrations and centrifuged for 15 min at 10,000 *g*. 2–10 $\times 10^5$ cells were incubated for 30 min at 4°C with the following FITC-conjugated antibodies: F(ab')$_2$ goat anti-mouse IgG (H + L) (Cappel Laboratories, Cochranville, PA), hamster anti-mouse CD3 ε (Boehringer Mannheim Diagnostics, Inc., Houston TX), and anti-mouse Lyt2 from Becton Dickinson and Co., Mountain View, CA. Phycoerythrin-conjugated anti-mouse L3T4 was also used (Becton Dickinson and Co.). The cells were washed in PBS containing 1% normal rabbit serum and then analyzed on a FACScan® (Becton Dickinson and Co.).

Serum Antibody Levels. Immulon 2 96-well plates (Dynatech Corp., Chantilly, VA) were coated overnight at 4°C with 1 μg/well of either goat anti-mouse IgG, Fc-specific or goat anti-mouse IgM, μ chain specific (Sigma Chemical Co., St. Louis, MO), at pH 8.6. After extensive washing with PBS, pH 7.4, plus 1% Tween 20, the wells were incubated for 8 h at room temperature with 3% BSA, pH 7.4. Duplicate wells were then incubated overnight at 4°C with doubling dilutions of mouse IgG 2a (κ) or mouse IgM (κ), both from Sigma Chemical Co., or sera from injected mice. Wells were then incubated for 6 h at room temperature with 1:1,000 dilutions of Sigma alkaline phosphatase–conjugated anti-mouse IgG (whole molecule) or anti-mouse IgM (μ chain specific). Solution containing Sigma 104 substrate tablets (5 mg/5 ml sodium carbonate buffer, pH 9.5) and 2 mM magnesium chloride was added to all wells. Plates were read on a Dynatech MR 650, at 410 nm, after 60-min incubation at room temperature.

Results and Discussion

We first established the phenotype and function of the cells that we were to inject into the *scid* mice. FACS® analysis indicated that thymocytes, treated as described in Materials and Methods, showed an insignificant level of staining with all B cell–specific reagents; T cell staining with anti-CD3 was >98% (data not shown). The proliferative response of these purified thymocytes to both KLH and the B cell mitogen LPS was extremely low, even in the presence of 2,000 rad irradiated BALB/c spleen cells. Supernatants from wells containing KLH and purified thymocytes, in the presence or absence of irradiated spleen cells, did not activate CTLL (data not shown). Thus, all these data indicate that (*a*) the level of B cell contamination in the injected T cell population was very low, and (*b*) the cells were not previously primed to KLH.

Table 1. *Supernatants of Lymph Node Cells from scid Mice Reconstituted with Thymocytes Alone or Thymocytes Plus B Cells, and Injected with KLH, Contain IL-2*

	IL-2			
Exp. 1	–	OVA	Con A	KLH
			U/ml	
BALB/c	<0.01	<0.01	>1.0	0.490 ± 0.070
$scid_{THY}$	<0.01	<0.01	>1.0	0.193 ± 0.041
$scid_{THY+50B}$	<0.01	ND	>1.0	0.138 ± 0.014
$scid_{THY+15B}$	<0.01	ND	>1.0	0.153 ± 0.016
$scid_{THY+5B}$	<0.01	<0.01	>1.0	0.136 ± 0.011
scid	<0.01	<0.01	0.025 ± 0.018	<0.01

	KLH-restimulated supernatants		
Exp. 2	–	+ Anti-IL-2	+ Anti-IL-4
		U/ml	
BALB/c	0.634 ± 0.089	<0.01	0.606 ± 0.058
$scid_{THY}$	0.236 ± 0.034	<0.01	0.252 ± 0.024
$scid_{THY+10B}$	0.205 ± 0.022	<0.01	0.218 ± 0.013

In Exp. 1, *scid* mice were injected with 100×10^6 thymocytes and in Exp. 2, 70×10^6 thymocytes plus the indicated numbers of B cells in millions. The *scid* control in Exp. 1 is the supernatant of spleen cells from *scid* mice injected with KLH alone. The anti-IL-4 used in Exp. 2 inhibited the activity of 12.5 U/ml recombinant IL-4.

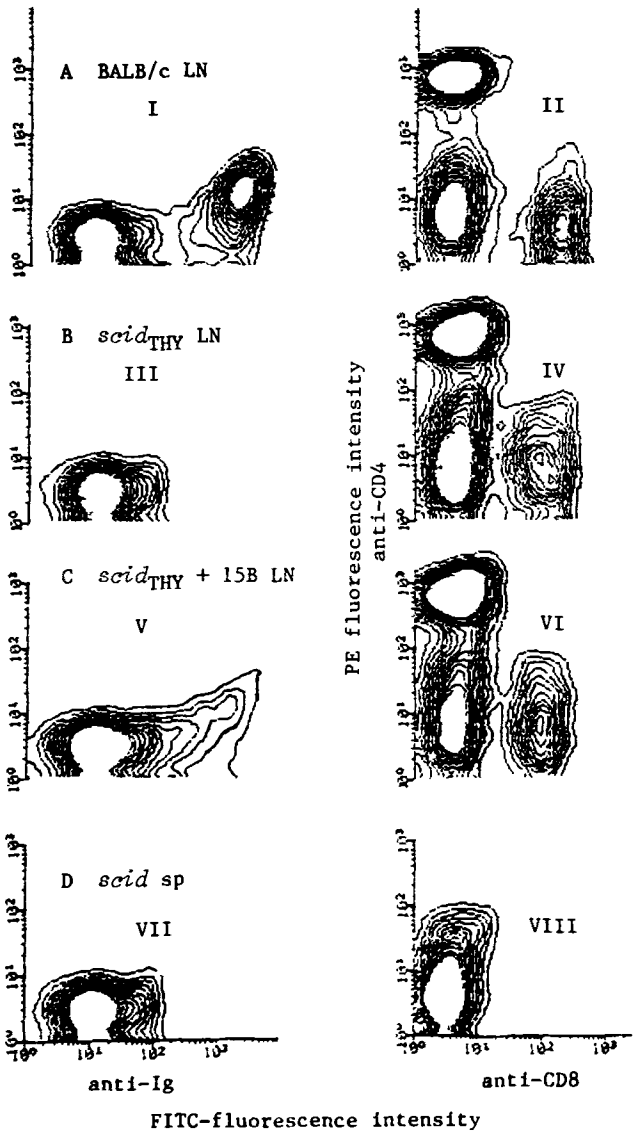

Figure 1. FACS® profiles of lymph node cells from (*A*) a KLH-primed BALB/c mouse, (*B*) *scid* mice injected with KLH and 100 × 10^6 treated thymocytes and (*C*) *scid* mice injected with KLH, 100 × 10^6 thymocytes, and 15 × 10^6 B cells. (*D*) The staining of spleen cells from a *scid* mouse injected with KLH alone. Cells in *I*, *III*, *V*, and *VII* were stained for B cells with FITC-conjugated F(ab')$_2$ goat anti-mouse IgG (H + L). Cells in *II*, *IV*, *VI*, and *VIII* were stained for CD4$^+$ and CD8$^+$ cells with both phycoerythrin-conjugated anti-L3T4 and FITC-anti-mouse Lyt 2.

9 d after injecting antigen - (10 d after injecting cells) draining lymph nodes and spleens were removed. *scid* mice injected with thymocytes plus antigen developed draining lymph nodes containing lymphocytes (1–2% of injected T cells). *scid* mice injected with cells alone or antigen alone, however, failed to develop cellular lymph nodes. Thus, the development of lymph nodes was an indicator of the induction of an immune response. 0.7–2% of injected B cells were recovered from the nodes of *scid* mice given B cells and thymocytes. The number of injected B cells did not appear to enhance the recovery of T cells in the nodes of reconstituted mice: T cell recovery was between 1 and 2% in all sets of mice.

Our initial experiments indicated that lymph node cells from T cell–reconstituted *scid* mice proliferated in vitro to KLH, suggesting that T cells injected into these mice had been primed in vivo (data not shown). To further test for priming of T cells in vivo, supernatants of KLH-restimulated lymph node cells derived from reconstituted *scid* mice were examined for the presence of T cell–derived cytokines. Table 1, representative of four experiments, indicates that lymph node cells derived from *scid* mice injected with either T cells alone or injected with T and B cells secreted comparable levels of IL-2, after restimulation by KLH in vitro. IL-4 was not found in the supernatants of any KLH-stimulated lymph node population.

To compare the composition of the lymph nodes from reconstituted *scid* mice and normal BALB/c mice, FACS® analysis was performed. In a series of experiments, the percentage of CD3$^+$ T cells recovered from the nodes of mice injected with 70–100 × 10^6 thymocytes and varying numbers of B cells (up to 50 × 10^6), and the nodes of primed BALB/c mice, were comparable at 60–65%. Similarly, the percentages of CD4$^+$ and CD8$^+$ cells were comparable in the nodes of *scid* mice reconstituted with T cells in the presence or absence of B cells, and normal BALB/c mice. The results of one experiment, representative of four similar experiments, are shown in Fig. 1. *scid* mice were injected with 100 × 10^6 thymocytes in the presence (*V* and *VI*) or absence (*III* and *IV*) of 15 × 10^6 B cells. Panel *III* indicates that fewer than 1% B cells could be detected in mice injected with purified T cells. Panel *IV* illustrates that the lymph nodes of such T cell–reconstituted *scid* mice contain both CD4$^+$ and CD8$^+$ cells. Panels *V* and *VI* indicate that B cells, as well as CD4$^+$ and CD8$^+$ T cells, were detected in the lymph nodes of mice injected with both thymocytes and B cells.

The absence of B cells in *scid* mice injected with thymocytes alone was confirmed by other criteria. The response to

Table 2. *Low levels of Circulating Ig in scid Mice Injected with T Cells and KLH*

	IgM	IgG
	μg/ml	
BALB/c	1212, 924, 448, 1562	3704, 4198, 704, 2010
$scid_{THY}$	0.5, 0.6, 1.3, 1.5	0.1, 0.1, 4.8, 3.6
$scid_{THY+10B}$	197, 97, 164, 137	31, 12, 420, 625
$scid_{THY+2B}$	150, 126	252, 233
scid	4.8, 0.1, 0.1, 1.3	0.2, 1.3, 0.5, <0.1

scid mice were injected with 75 × 10^6 thymocytes alone (*line 2*), thymocytes + 10 × 10^6 B cells (*line 3*), thymocytes + 2 × 10^6 B cells (*line 4*), or no cells (*line 5*). All mice were killed 9 d after injecting KLH. Figures shown are serum levels of individual mice at time of death. In BALB/c mice, preinjection levels of IgM were 2–3 times lower and IgG 4–7 times lower than the numbers shown.

LPS of spleen cells from these mice was consistently very low (data not shown). Moreover, Table 2 indicates that the circulating levels of IgG and IgM in *scid* mice that received antigen and T cells were as low as background, whereas mice injected with both B and T cells had detectable levels of both IgM and IgG.

Taken together, these data indicate that unprimed T cells can be primed to KLH in the *scid* mouse in the absence of B cells. As a consequence of priming, the T cells proliferate and secrete IL-2 in response to KLH in vitro. We therefore conclude that in mice, as in humans and chickens, mature B cells are not necessary for the induction of primary immune responses *in vivo*. These data suggest that in previous studies, in which normal mice were depleted of B cells, the antigen-presenting function of cells other than B cells might have been affected (6–10). In the set of MHC class II E transgenic mice lacking E expression on B cells, it is also possible that the proliferative response was not a sensitive assay of T cell priming, as suggested by the authors, since cells from only 50% of the positive control mice responded to the E-restricted antigen (11).

Our data also confirm the recent finding that mature T cells injected into *scid* mice do not induce B cell differentiation (14). Although our studies cannot establish which cell or cells may be responsible for the induction of primary immune responses to protein antigens, the previously described potency of dendritic cells in inducing T cell responses makes them strong candidates (5, 13, 15).

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