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The Role of B Cells for in Vivo T Cell Responses to a Friend Virus-Induced Leukemia

KIRK R. SCHULTZ,* JAY P. KLARNET, RANDALL S. GIENI, KENT T. HAYGLASS, PHILIP D. GREENBERG

B cells can function as antigen-presenting cells and accessory cells for T cell responses. This study evaluated the role of B cells in the induction of protective T cell immunity to a Friend murine leukemia virus (F-MuLV)–induced leukemia (FBL). B cell–deficient mice exhibited significantly reduced tumor-specific CD4$^+$ helper and CD8$^+$ cytotoxic T cell responses after priming with FBL or a recombinant vaccinia virus containing F-MuLV antigens. Moreover, these mice had diminished T cell responses to the vaccinia viral antigens. Tumor-primed T cells transferred into B cell–deficient mice effectively eradicated disseminated FBL. Thus, B cells appear necessary for efficient priming but not expression of tumor and viral T cell immunity.

B CELLS ARE PART OF A HETEROGENEOUS population of antigen-presenting cells (APCs) that can activate CD4$^+$ helper T cells (*1*). The role of B cells in the induction and expression of in vivo T cell responses has been previously evaluated by rendering mice deficient in B cells by treatment from birth with high doses of rabbit antibody to mouse immunoglobulin M (IgM) (anti-μ) (*2*). These B-cell–deficient mice do not generate antigen-specific T cell responses after in vivo priming with hapten, a defect reflecting the requirement for B cells to function in vivo as APCs for T cells (*3*). The role of B cells in the in vivo presentation of larger, more complex antigens and the induction of CD4$^+$ helper and CD8$^+$ cytotoxic T cells is less clear. However, in vitro analysis of the response to large proteins such as thyroglobulin and vesicular stomatitis virus has demonstrated efficient in vitro activation of primed T cells by B cells, suggesting that B cell–APCs can potentially contribute to priming to large protein and viral antigens in vivo (*4*).

B cells could be particularly important for the induction of T cell responses to tumor cells, since limitations in antigen presentation could result from deficiencies in APC number and function that occur secondarily to progressive tumor growth or therapy (*5*). We have previously evaluated the T cell responses of C57BL/6 (B6) mice to FBL, a Friend murine leukemia virus (F-MuLV)–induced leukemia that expresses retrovirally encoded antigens and only class I major histocompatibility complex (MHC) antigens, but induces both CD4$^+$ and CD8$^+$ T cell responses (*6, 7*). The CD4$^+$ T cell response requires APC-expressing class II MHC antigens to process and present FBL, includes T cells that produce both interleukin-2 and interleukin-4 (IL-2 and IL-4), and is primarily directed at F-MuLV envelope epitopes. The cytolytic CD8$^+$ T cell response includes IL-2–dependent and IL-2–producing T cells that predominantly recognize F-MuLV Gag epitopes (*6, 7*). Adoptive transfer of FBL-specific T cells can completely eradicate disseminated FBL (*6, 8–10*). This FBL tumor model, with well-characterized T cell responses to tumor-associated retroviral antigens, was used to evaluate the role of B cells in the induction and expression of T cell responses to a tumor. The results demonstrate that B cells can play an essential role in the induction of in vivo T cell responses to retrovirally induced tumor cells.

Anti-μ–treated B6 mice were injected with FBL tumor cells, and the participation of B cells during in vivo priming was evaluated by measuring in vitro secondary T cell responses to FBL (*7*). B cell depletion was complete and selective; no IgM$^+$ cells or serum IgM could be detected, the B cell mitogen lipopolysaccharide (LPS) elicited no proliferative response, whereas the T cell mitogen conconavalin A (Con A) elicited a normal response (*11*). After immunization with FBL, the proliferative response to FBL of splenic T cells from anti-μ–treated mice was 50% that of control mice (Fig. 1), and essentially no response was detected in lymph node cells. These data, consistent

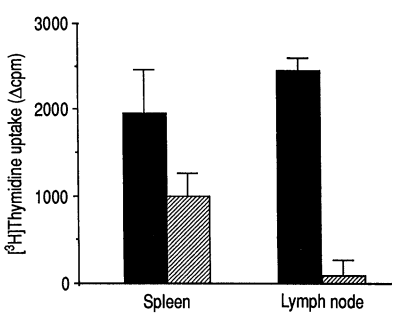

Fig. 1. Contribution of B cells to primary FBL-specific proliferative T cell responses. Spleen or lymph node cells were obtained from B6 mice (*H-2^b*) (Jackson Laboratory, Bar Harbor, Maine) treated from birth with rabbit IgG (solid bars) or anti-μ (hatched bars) and primed intraperitoneally at 6 to 9 weeks of age in vivo with 10^7 irradiated FBL. Responder cells (5×10^5), obtained 6 weeks after priming, and irradiated stimulator cells were cultured in 96-well, flat-bottom plates in RPMI 1640 containing 10% fetal bovine serum (FBS), 2.5×10^5 M 2-mercaptoethanol, penicillin (100 U/ml), streptomycin (100 mg/ml), and L-glutamine. Cells were stimulated in vitro for 72 hours at a responder to stimulator ratio of 100:1 and assayed for incorporation of [^{3}H]thymidine. Data are presented as the mean difference with standard error bars in [^{3}H]thymidine uptake in FBL-stimulated and unstimulated cultures and represent one of three experiments.

K. R. Schulz, Department of Pediatrics, University of Washington, Seattle, WA 98195.
J. P. Klarnet and P. D. Greenberg, Departments of Medicine and Immunology, University of Washington, and Fred Hutchinson Cancer Research Center, Seattle, WA 98195.
R. S. Gieni and K. T. HayGlass, Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

*To whom correspondence should be sent at Department of Pediatrics, Wayne State University, Detroit, MI 48201.

with results obtained with other less complex immunogens, show that B cells make a more significant contribution to the priming of lymph node T cells than splenic T cells. This could result from splenic B cells having functional qualities distinct from lymph node B cells such as class II–antigen density or from the fact that alternative APC are more available for priming in the spleen (*12, 13*). The effector T cell response to FBL includes, in addition to the proliferative helper and inflammatory $CD4^+$ T cells, cytolytic $CD8^+$ T cells (*6–8*), and a weak $CD8^+$ FBL-specific T cell response was also detected in B cell–depleted mice (Fig. 2). Thus, a deficiency of B cells resulted in inefficient in vivo priming of proliferative $CD4^+$ and cytotoxic $CD8^+$ T cell responses to immunogenic tumor cells.

The weak FBL-specific cytotoxic T cell responses detected in the B cell–depleted mice may result from the lack of sufficient FBL-specific $CD4^+$ T cell help required to generate the cytotoxic T cell response, or, alternatively, efficient priming of the $CD8^+$ T cell population may require a direct effect of accessory B cells on this subset. Although $CD8^+$ FBL-specific T cells that produce IL-2 are present after priming (*9*), the frequency of these helper-independent $CD8^+$ T cells reactive with FBL was too low to detect efficient priming of the $CD8^+$ subset in mice depleted of $CD4^+$ T cells (*14*). In contrast, vaccinia-specific $CD8^+$ T cells can be primed to the more strongly immunogenic vaccinia virus in $CD4^+$-depleted mice (*15, 16*). Therefore, the role of B cells during in vivo priming of both $CD8^+$ and $CD4^+$ T cell subsets was assessed by examining, in anti-μ–treated mice, both the FBL-specific proliferative response after priming with a recombinant vaccinia virus containing the F-MuLV envelope gene (vac-env), an immunogen capable of inducing $CD4^+$ FBL-specific T cell responses, and the vaccinia-specific proliferative response of $CD4^+$ and $CD8^+$ T cells (*7, 15–17*). Vac-env immunization, in IgG-treated control mice, primed mice for a strong proliferative T cell response to FBL (Fig. 3A), as well as to vaccinia antigens (Fig. 3B). In contrast, B cell–depleted mice had no significant proliferative T cell response to FBL or vaccinia (Fig. 3, A and B). Thus, B cell depletion interfered with priming to FBL tumor antigens, even if presented in the context of a highly immunogenic recombinant vaccinia virus. Moreover, the lack of a detectable significant T cell response to vaccinia in B cell–depleted mice implied that B cells may be important for priming both T cell subsets. This was further examined by evaluating the response of each T cell subset from intact mice primed to vaccinia antigens. Purified $CD8^+$ and $CD4^+$ T cells proliferated in response to vaccinia (Fig. 3C), comparable to the responses detected in the unfractionated T cell population. This is consistent with studies demonstrating vaccinia can prime $CD8^+$ cytotoxic T cells by way of a $CD4^+$ helper T cell–independent pathway (*15, 16*). Thus, B cells may be required to make a direct contribution for efficient in

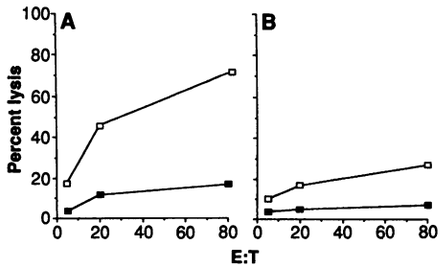

Fig. 2. Contribution of B cells to priming for cytolytic responses to FBL in (**A**) spleen and (**B**) lymph nodes. B6 responder splenocytes, primed as in Fig. 1, from control rabbit IgG–treated mice (□) or anti-μ–treated mice (■) (6×10^6 cells per well), were cultured with 3×10^5 irradiated FBL (10,000 R) in 24-well plates (Costar, Cambridge, Massachusetts) for 5 days with 10% FBS. Cytotoxicity of the effector cells was evaluated in a standard 4-hour ^{51}Cr-release assay with labeled FBL targets at the indicated effector to target ratios (E:T). Percent-specific lysis was evaluated by measuring the experimental minus spontaneous ^{51}Cr released divided by total minus spontaneous ^{51}Cr released. Specificity was demonstrated by the ability of the FBL-reactive effectors present in rabbit IgG–treated control mice to lyse FBL, but neither chemically induced syngeneic EL-4 tumor cells nor autologous Con A blasts. Similar results were obtained in two independent experiments.

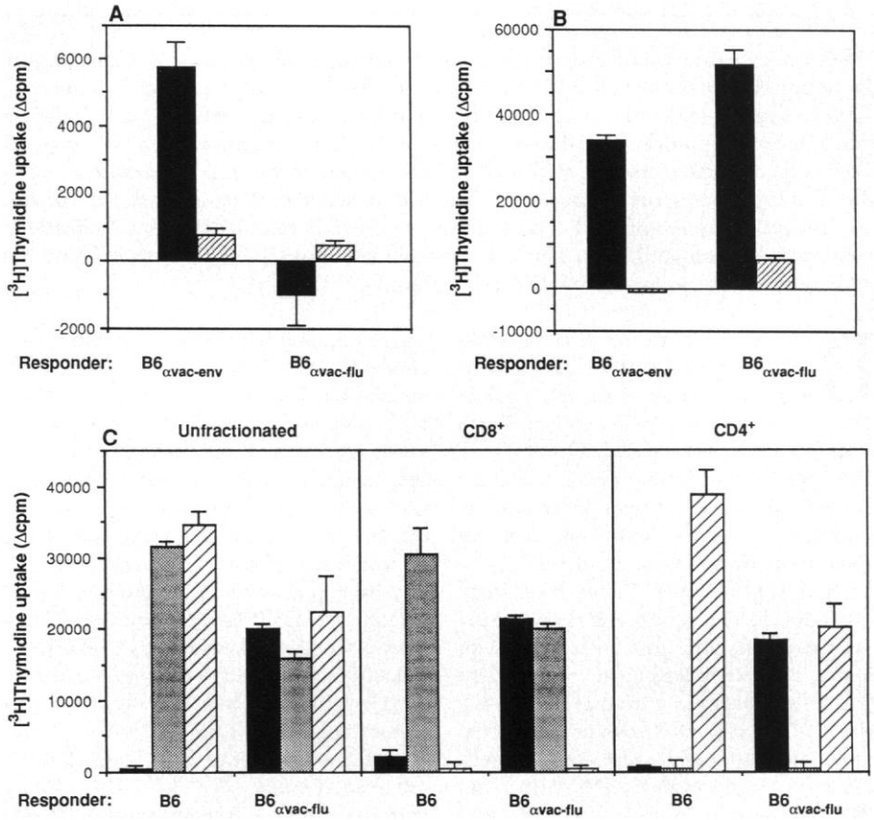

Fig. 3. Contribution of B cells to priming with a recombinant vaccinia virus. Spleen cells were obtained from control rabbit IgG–treated mice or anti-μ–treated mice immunized by tail scarification with 10 μl (10^8 plaque-forming units per milliliter) of either vac-env (recombinant vaccinia virus expressing the F-MuLV envelope), $B6_{\alpha vac\text{-}env}$, or vac-flu (recombinant vaccinia virus expressing the influenza hemagglutinin gene), $B6_{\alpha vac\text{-}flu}$ (provided by P. Earl and B. Moss) (7) and evaluated 4 weeks later. (**A**) Responder splenocytes from either control (solid bars) or B cell–depleted (hatched bars) mice were stimulated in vitro with irradiated FBL for 3 days, and proliferation was measured as in Fig. 1. (**B**) Responder splenocytes as defined above were stimulated with macrophages infected with vac-flu at a multiplicity of infection of 1:1 and proliferation measured as in Fig. 1. (**C**) Spleen cells from unprimed B6 mice or vac-flu–primed mice were used either unfractionated or after purification into $CD8^+$ and $CD4^+$ T cell subsets as previously described (7). Subset purity was documented phenotypically with fluorescent antibodies and functionally by the ability of only $CD8^+$ T cells to proliferate in response to the unique class I allo-stimulator $B6^{bm1}$ or $CD4^+$ T cells to the unique class II allo-stimulator $B6^{bm12}$ (7). Responder cells (8×10^5 per well) were cultured with either 2×10^5 irradiated spleen cells infected with vaccinia virus (solid bars) at a multiplicity of infection of 1:1 or 4×10^5 irradiated $B6^{bm1}$ (shaded bars) or $B6^{bm12}$ (hatched bars), as indicated, and proliferation measured as described in the legend to Fig. 1. The data represent one of three experiments.

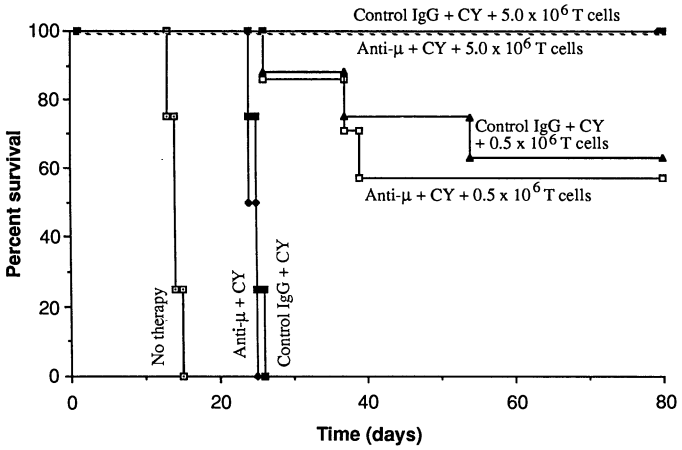

Fig. 4. Effect of B cell depletion on adoptive T cell therapy of disseminated FBL. B6 mice treated from birth with either rabbit (control) IgG or anti-μ antibody were inoculated with 5×10^6 FBL tumor cells on day 0 and received either no therapy, cyclophosphamide (CY) (180 mg/kg) on day 5, or CY plus a high dose (5×10^6) or low dose (0.5×10^6) of purified B6 anti-FBL immune T cells (>95% Thy1.2$^+$) with four to eight mice per group. Immune cells were generated by immunization of B6 mice with 10^7 FBL intraperitoneally 2 weeks apart (*8, 10*). Mice were monitored for tumor growth and survival, and all deaths resulted from progressive tumor growth.

vivo priming of both the CD8$^+$ and CD4$^+$ T cell subsets.

B cells may be important for induction of both CD4$^+$ and CD8$^+$ T cell responses to tumor and viral antigens through several mechanisms. B cells can act as APCs for CD4$^+$ T cell responses. The efficiency of B cells functioning as APCs is enhanced approximately 1000-fold if the antigen is directly bound by the Ig expressed on the B cell membrane (*12, 18*). This results both in internalization of antigen in endocytic vesicles, with processing into peptide fragments and presentation with class II MHC molecules and B cell activation, which enhances APC function (*18, 19, 20*). Consequently, B cells can serve a distinct APC function by taking up small amounts of tumor antigen present in the circulation or sequestered in lymph node follicular dendritic cells, for presentation to distant and nearby CD4$^+$ T cells (*21, 22*). These B cell–dependent presentation mechanisms may be particularly important at times when limited amounts of antigen are present (*23*). Thus, even in hosts with no viral B cell function, tumor antigens potentially recognizable by CD4$^+$ T cells but lacking B cell epitopes may not benefit from antigen presentation by B cells and may not elicit efficient CD4$^+$ T cell responses. Finally, although there is no evidence that B cells can take up cellular tumor antigens and introduce them into the endogenous pathway for presentation in the context of class I MHC molecules, B cells may function as accessory cells for CD8$^+$ T cell responses by secretion of IL-1 or IL-6 (*24, 25*). Tumor-specific IL-2–producing CD8$^+$ cytolytic T cells, which may play a critical role in antitumor immunity because of their independence from CD4$^+$ helper T cells (*26*), express IL-1 receptors, and require IL-1 to initiate IL-2 secretion and proliferation (*9*). Thus, accessory cells such as B cells may be particularly important for inducing CD8$^+$ cytotoxic T cell responses to tumors in settings in which tumor-specific CD4$^+$ T cells are deficient or absent.

B6 mice with disseminated FBL leukemia can be cured by adoptive chemoimmunotherapy with cyclophosphamide and FBL-primed T cells (*6, 8, 9*). Since therapeutic efficacy is dependent on antigen-induced in vivo proliferation of transferred cells (*27*), we examined whether B cells were important for the expression of adoptively transferred T cell tumor immunity. Therapy of B6 mice with adoptively transferred FBL-primed, purified T cells (>95% Thy 1.2$^+$) resulted in a dose-dependent therapeutic effect, with a high cell dose (5×10^6) curing 100% of mice and a lower cell dose (0.5×10^6) curing 63% of mice (Fig. 4). Treatment of B cell–deficient mice with these FBL-immune T cells resulted in an equivalent therapeutic effect at both cell doses. Thus, host B cells do not appear to make a requisite contribution to the therapeutic response of adoptively transferred tumor-primed T cells. These results suggest that B cells may be most important during the induction rather than expression of T cell responses to tumor. This may reflect the less stringent stimulatory requirements for secondary T cell responses than primary responses (*28*). The demonstration of the importance of B cell participation in the induction of anti-tumor and anti-viral T cell immunity suggests that efforts to promote reconstitution of B cell function in immunocompromised patients in which B cells do not directly contribute to expression of immunopathology (*29*) may not only enhance antibody responses but may also result in improved T cell immunity.

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