In vivo disruption of tolerogenic cross-presentation mechanisms uncovers an effective T-cell activation by B-cell lymphomas leading to antitumor immunity

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Bone marrow–derived antigen-presenting cells (APCs) play a central role in the induction of tolerance to tumor antigens expressed by B-cell lymphomas. Here we show that in vivo disruption of this APC-mediated tolerogenic mechanism unveils an intrinsic ability of malignant B cells to efficiently present tumor antigens to antigen-specific CD4+ T cells, resulting in a strong antitumor effect. This intrinsic antigen-presenting ability of malignant B cells is, however, overridden by tolerogenic bone marrow–derived APCs, leading instead to T-cell unresponsiveness and lack of antitumor effect. These results highlight the concept that therapeutic strategies aimed at enhancing the antigen-presenting function of B-cell lymphomas might not succeed unless the tolerogenic mechanisms mediated by bone marrow–derived APCs are disrupted in the first place. (Blood. 2006;107:2871-2878)

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Introduction

B-cell malignancies, including indolent and aggressive non-Hodgkin lymphomas, are the transformed counterparts of cells that are capable of activating antigen-specific T cells. Similar to normal B cells, malignant B cells express major histocompatibility complex (MHC) class I and II molecules and low but inducible levels of adhesion and costimulatory molecules.1,2 Indeed, much of what we have learned about the basic cell biology of MHC class I and II antigen processing and presentation, normal antigen-presenting cell (APC) homing, adhesion, and T-cell costimulation has been gleaned from analysis of B-cell lines.3-5 B-cell malignancies seem, therefore, well equipped to provide the necessary signals for activation of tumor-antigen–specific T cells.

A number of studies have demonstrated that malignant B cells can process and present antigen to T cells in vitro, including the presentation of epitopes derived from their own unique immunoglobulin idiotype to CD4+ and CD8+ T cells.6-9 Furthermore, cross-linking of CD40 on lymphoma cells induces the up-regulation of adhesion and costimulatory molecules, resulting in a markedly enhanced T-cell response to B-cell tumors in vitro.10 It is paradoxical that in spite of their intrinsic antigen-presenting capabilities, B-cell tumors fail to be eliminated in the very same compartment—lymph nodes—in which tumor-antigen–specific T-cell responses are initiated.

Reminiscent of the previously published in vitro studies, we have found that a murine B-cell lymphoma cell line, A20, transfected to express the model antigen influenza hemagglutinin (HA), efficiently activates HA-specific CD4+ T cells from T-cell receptor (TCR) transgenic mice in vitro.11 However, when these same transgenic T cells are adaptively transferred to mice bearing A20HA lymphoma, the observed outcome is the induction of antigen-specific CD4+ T-cell anergy rather than T-cell activation.12 One potential explanation for these findings is that perhaps B-cell tumors are not efficient APCs in vivo and their direct encounter with T cells is responsible for the induction of T-cell anergy. More recent studies using parent-into-F1 bone marrow (BM) chimeras ruled out this possibility, pointing instead to cross-presentation by host APCs, not lymphoma cells themselves, as the dominant mechanism responsible for the induction of tumor-antigen–specific CD4+ T-cell tolerance.13 This involvement of a “third party” in vivo, represented by host APCs, provided an explanation for the divergent outcomes in T-cell responses to malignant B cells in the in vitro (T-cell activation) and in vivo (T-cell tolerance) settings.

Although our previous studies have indicated that the intrinsic APC capabilities of B-cell lymphomas have no influence on the induction of T-cell tolerance in vivo,14 questions that remain are whether malignant B cells are capable of directly presenting tumor antigens in vivo and whether this putative T-cell/B-cell interaction could lead to T-cell activation rather than to tolerance. To answer these questions, we evaluated lymphoma growth and antigen-specific T-cell function in experimental models in which cross-presentation by BM-derived APCs is not operative and malignant B cells are the only cells capable of presenting tumor antigen to CD4+ T cells. The results of this analysis demonstrate that direct presentation of tumor antigens by B-cell lymphoma in vivo leads to T-cell activation and to the development of effective antilymphoma immunity.

Materials and methods

Mice

Male BALB/c severe combined immunodeficiency (SCID) or C57BL/6 SCID mice, aged 6 to 8 weeks, were purchased from Jackson Laboratories.

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Reisolation of clonotypic T cells after in vivo transfer of activated T cells to allow for the establishment of memory anti-HA CD4+ T cells were injected into the tail veins of immune reconstituted chimeric SCID mice, and bone marrow was harvested by flushing the bones with RPMI at 4°C. Single-cell suspensions were obtained by passing bone marrow cells through nylon wool.

**Construction of bone marrow chimeric mice**

Femurs and tibiae were obtained from BALB/c SCID mice and C57BL/6 SCID mice, and bone marrow was harvested by flushing the bones with RPMI at 4°C. Single-cell suspensions were obtained by passing bone marrow cells through nylon wool. The cells were washed 3 times in sterile Hanks balanced salt solution (HBSS), and 10^6 BM cells from either BALB/c SCID mice (H-2^d) or C57BL/6 SCID mice (H-2^b) were injected into the tail veins of irradiated (10 Gy [1000 rad]) BALB/cxC57BL/6 F1 (H-2^d/b) recipients. A 3-month period was allowed for immune reconstitution before adoptive transfer of T cells.

**Adoptive transfer of antigen-specific T cells**

For the adoptive transfer of anti-HA–specific CD4+ T cells, we used BALB/c TCR transgenic mice to C57BL/6 mice to generate H-2^d/b F1 TCR transgenic offspring. It was necessary to use F1 TCR transgenic donors to ensure that any nontransgenic T cells transferred to the chimeras or SCID mice were not alloreactive to the recipient, thus triggering graft-versus-host disease (GVHD). The transgenic donor population was obtained from the thymi of H-2^d/b F1 TCR transgenic animals to avoid any contaminating MHC class II–bearing APCs. CD8+ T cells and double-negative thymocytes were depleted using the antibodies 3.155 and J.11.d.2, respectively. This was followed by FITC goat-anti–mouse IgG2a secondary antibody (BD Biosciences) and analysis on a FACScalibur (BD Biosciences) using FlowJo software (Tree Star, Ashland, OR).

**Flow cytometric analysis**

T cells were stained with FITC-conjugated goat anti–mouse CD4 (Caltag, Burlingame, CA) and biotinylated rat anti–clonotypic TCR antibody mAb 6.5, followed by PE-conjugated streptavidin (Caltag). Fifty thousand gated events were collected on a FACScalibur (BD Biosciences) and were analyzed using FlowJo software (Tree Star). Values represent the mean ± SE of the percentage of cells expressing the clonotypic TCR. Background staining of splenocytes from naive F1 mice is usually less than 0.1%.

**Tissue sections**

Tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Deparaffinized tissue sections were immersed in 1% H2O2 for 10 minutes to block endogenous peroxidase activity and were subjected to epitope retrieval treatment by heating of the slides in a commercial steamer with 0.1 M sodium citrate. Sections were stained with goat anti–mouse CD3-ε (Santa Cruz Biotechnology, Santa Cruz, CA) or rat anti–mouse B220 (Caltag) with the use of Vectastain ABC Kits (Vector Laboratories, Burlingame, CA). Double immunohistochemistry was performed first for B220 and then for CD3-ε; the epitope retrieval step was repeated after the first staining to quench any biotin or peroxidase activity. Stained sections were mounted in Vectamount (Vector Laboratories, Burlingame, CA). Double immunohistochemistry was performed first for B220 and then for CD3-ε; the epitope retrieval step was repeated after the first staining to quench any biotin or peroxidase activity. Stained sections were mounted in Vectamount (Vector Laboratories) and analyzed under a Leica DMLB 100S microscope (Leica Microsystems, Wetzlar, Germany) using either a 20×/0.50 HC PL Fluotar or a 40×/0.75 HCX PL Fluotar objective lens (Leica Microsystems). Photographs were taken with an RT Color Spot camera (Diagnostic Instruments, Sterling Heights, MI) and analyzed with Image-Pro Plus 5.0 software (Media Cybernetics, Silver Spring, MD).

**BrdU incorporation**

BrdU (BD Biosciences) was diluted to 0.8 mg/mL in drinking water and was given to the experimental animals starting 2 weeks before tumor challenge until the end of the experiment. Every other day, the drinking water was changed with freshly made BrdU solution. BrdU incorporation on clonotypic T cells was determined by staining purified T cells with PE-conjugated anti-CD4 (BD Biosciences) and biotinylated rat anti–clonotypic TCR antibody mAb 6.5, followed by APC-conjugated streptavidin (BD Biosciences). Cells were then fixed, permeabilized, and stained for BrdU with the BrdU Flow Kit (BD Biosciences), according to the manufacturer’s instructions. Fifty thousand gated events were collected on a FACScalibur (BD Biosciences) and were analyzed using FlowJo software (Tree Star) by first gating on the clonotypic T-cell population and then assessing the intensity of staining for BrdU.

**Functional assessment of dendritic cells**

CD11c+ DCs were purified from collagenase-digested spleens using CD11c microbeads and LS columns (both from Miltenyi Biotech, Auburn, CA) according to the manufacturer’s instructions. DCs (2 × 10^5) were then plated with HA peptide (12.5 μg/mL) and purified T cells (1 × 10^6) from the spleens of TCR-transgenic animals. Four hours later, GolgiStop (BD Biosciences) was added to the wells. Ten hours after plating, cells were...
stained with PerCP-conjugated anti–mouse CD4 (BD Biosciences) and biotinylated anti–clonotypic TCR antibody mAb 6.5, followed by PE-conjugated streptavidin (Caltag). After fixation and permeabilization with CytoFix/CytoPerm Plus Kit (BD Biosciences), the cells were stained with FITC-conjugated anti–mouse IL-2 (BD Biosciences) and APC-conjugated anti–mouse IFN-γ (BD Biosciences). Flow cytometric analysis was performed as described.

Statistical analysis
Two-way analysis of variance (ANOVA) was used to evaluate the magnitudes of tumor-induced effects on cytokine production by clonotypic T cells. Differences in survival were assessed with the log-rank test.

Results
Rejection of B-cell lymphoma by antigen-specific CD4+ T cells in the absence of tolerogenic mechanisms mediated by APCs

In previous studies, the use of parent SCID-into-F1 bone marrow chimeras allowed us to unambiguously demonstrate the dominant role of APCs in the induction of tumor-antigen–specific T-cell tolerance during the progression of a B-cell lymphoma expressing a model tumor antigen (A20HA). In this system, naive anti-HA CD4+ transgenic T cells adaptively transferred into H-2dSCID→H-2dxb tumor-bearing chimeras, in which host APCs expressed the restricted element I-E2 required for the presentation of the HA antigen, were rendered fully unresponsive. In sharp contrast, in H-2dSCID→H-2dxb tumor-bearing chimeras, in which only malignant B cells displayed the restricted element required for antigen presentation, CD4+ HA-specific T cells remained functional. However, no significant differences in tumor growth rates were observed in either set of tumor-bearing chimeras. This failure of naive antigen-specific CD4+ T cells to reject A20HA lymphoma led us to explore whether memory antigen-specific CD4+ T cells would be better in controlling lymphoma growth. Therefore, we adoptively transferred 1×106 in vitro activated anti-HA transgenic CD4+ T cells reisolated from H-2bSCID/H-2dxb tumor-bearing chimeras, and they were inspected twice weekly for the development of tumors. Six mice were included in each group (P < .01). Data are representative of 3 experiments with similar results.

Figure 1. Rejection of A20HA B-cell lymphoma in the absence of cross-presentation mediated by BM-derived APCs. H-2dSCID→H-2dxb F1 mice were lethally irradiated (10 Gy [1000 rad]), and each received a graft consisting of 4×106 bone marrow cells from either BALB/c SCID (H-2k) or C57BL/6 SCID (H-2b) donors. Three months after bone marrow reconstitution, either group of chimeras intravenously received 1×106 in vitro activated CD4+ H-2dSCID→H-2dxb TCR-transgenic T cells (H2dxb) specific for HA/IEG. Four weeks later, all the mice were challenged intravenously with 1×106 A20HA cells, and they were inspected twice weekly for the development of tumors.

To better understand the survival advantage observed in Figure 1, we determined next the fate and functional characteristics of naive and in vitro activated anti-HA transgenic CD4+ T cells transferred to H-2dSCID→H-2dxb or H-2bSCID→H-2dxb chimeras. We followed an experimental design similar to that in Figure 1. The only difference was that tumor-bearing chimeras and tumor-free controls were killed 3 weeks after challenge with A20HA. In addition, we assessed in vivo T-cell proliferation by feeding a cohort of mice BrdU from day 14 after tumor challenge until the end of the experiment (day 21).

Previously, we have shown that adoptive transfer of clonotype-positive T cells into A20HA-bearing mice resulted in initial clonal expansion, followed by contraction, of this T-cell population. Although the peak of this expansion occurred 6 days after T-cell transfer, by day 20 there was still a slightly higher number of clonotype-positive T cells in A20HA-bearing mice than in tumor-free animals. However, as early as 1 week after adoptive transfer, antigen-specific CD4+ T cells displayed impaired proliferative response (as determined by 3H-thymidine incorporation) and cytokine production in response to cognate antigen in vitro.22 Reminiscent of these findings in H-2d recipients, we found that by day 21 after T-cell transfer, the percentage of antigen-specific T cells was also above baseline in H-2dSCID→H-2dxb tumor-bearing chimeras compared with tumor-free animals (Figure 2A, left). As determined by in vivo BrdU incorporation, only a small percentage of clonotype-positive T cells were still dividing in H-2dSCID→H-2dxb tumor-bearing chimeras compared with tumor-free mice (Figure 2A, right). This finding suggested that T cells from tumor-bearing chimeras might have encountered antigen in vivo, most likely by interacting with malignant B cells. This putative B-cell/T-cell interaction is further supported by the strong in vivo proliferation of clonotype-positive T cells in tumor-bearing mice compared with
Antigen-specific T cells were still proliferating in vivo (Figure 3B, left). Furthermore, T cells displayed blunted IL-2 production in response to in vitro restimulation with cognate antigen (Figure 3C, left), indicative that memory T cells were also rendered tolerant in H-2<sup>b</sup>SCID→H-2<sup>ab</sup> tumor-bearing chimeras. In contrast, in the absence of cross-presentation, memory CD4<sup>+</sup> T cells displayed strong antigen-specific responses as determined by their clonal expansion (Figure 3A, right), sustained in vivo proliferation (80.6% vs 14.6 in tumor-free chimeras) (Figure 3B, right), and preserved the ability to produce IL-2 in response to cognate antigen (Figure 3C, right).

Naive and memory antigen-specific CD4<sup>+</sup> T cells were rendered fully unresponsive when cross-presentation mechanisms mediated by BM-derived APCs were operative in vivo. In the absence of such a mechanism, naive and memory antigen-specific T cells seemed to encounter tumor antigen directly on malignant B cells, resulting in antigen-specific T-cell proliferation and preservation, rather than impairment, of their ability to produce IL-2 in response to cognate antigen.
Disruption of tolerogenic cross-presentation unveils an intrinsic ability of B-cell lymphomas to directly prime antigen-specific CD4+ T cells in vivo

Our findings suggest that in the absence of cross-presentation mechanisms, B-cell lymphomas themselves could present tumor antigens to CD4+ T cells in vivo. However, it could be argued that in the process of generating the chimeras, some H-2dxb F1 APCs might have survived lethal irradiation and been present after immune reconstitution. This is especially relevant for the reconstituted H-2dSCID→H-2dxb F1 chimeras, in which antigen presentation by residual host APCs rather than by malignant B cells could be a confounding factor. To completely rule out this possibility and to prove that direct T-cell priming by malignant B cells occurs in vivo, we evaluated the fate and function of naive anti-HA transgenic T cells transferred directly to C57BL/6 SCID (H-2b) or BALB/c SCID (H-2d) mice. In this experimental system, BM-derived APCs from C57BL/6 SCID did not have the restricted ability to effectively activate naive antigen-specific T cells in vivo. The results clearly confirmed that B-cell lymphomas have an intrinsic ability to present the antigen to naive HA CD4+ T cells.

To further prove that a direct T cell/B-cell interaction actually occurs in vivo, we analyzed by immunohistochemistry (IHC) the spleen sections from C57BL/6 SCID tumor-bearing mice adoptively transferred with transgenic T cells. As shown in Figure 5A, T cells were in close contact with malignant B cells in the spleens of these animals (inset). Next, we assessed the fate of these T cells by evaluating whether they were capable of infiltrating lymphoma nodules that developed in the livers of C57BL/6 SCID tumor-bearing mice or BALB/c SCID tumor-bearing mice used as controls. Although an almost complete absence of T cells was found in the lymphoma nodules of BALB/c SCID (Figure 5B, left panel), adoptively transferred T cells were clearly infiltrating lymphoma nodules in C57BL/6 SCID mice (Figure 5B, right panel).

Rejection of A20 wild-type lymphoma in the absence of cross-presentation of tumor antigens

To extend our observations beyond the TCR transgenic experimental system, we determined next whether animals with normal T-cell repertoires but disrupted cross-presentation mechanisms were capable of rejecting wild-type B-cell tumors in vivo. With the use of bone marrow cells from BALB/c or C57BL/6 immunocompetent donors, we generated 2 sets of BM chimeras in which APCs expressed H-2d or H-2b. In this model, the bone marrow graft also provided the necessary precursors for T-cell generation in the thymus of the recipient animal. Given that H-2d and H-2b elements are present in the thymus of H-2dxb mice, the reconstituted T-cell repertoire would contain H-2d-restricted and H2d-restricted mature T cells, irrespective of the BM-graft haplotype. Because A20 B cells express H-2d but not H-2b, only the H-2d-restricted T cells would be able to recognize tumor antigens directly on the lymphoma cells and, therefore, could potentially elicit an antitumor immune response. As seen in Figure 6A, in the presence of APCs able to tolerate H-2d-restricted tumor-reactive T cells (H-2d-H2dxb chimeras), lymphoma developed in all the animals. In sharp contrast, a significant delay in the growth of A20 tumor was observed in H-2b-H2dxb chimeras. Long-term follow up (up to 150 days) revealed that in the absence of cross-presentation to H2d-restricted T cells, 40% of these mice rejected A20 lymphoma. This antitumor effect was not NK-mediated because depletion of this cell population in H-2d→H-2dxb chimeras did not change the observed survival outcome (data not shown). Furthermore, we ruled out the possibility that H-2d alloreactive T cells in reconstituted H-2b→H-2dxb chimeras could be responsible for A20WT rejection because T cells from these chimeras did not proliferate in mixed-leukocyte reaction (MLR) in the presence of irradiated H-2d, H-2b, or H-2dxb cells whereas they proliferated vigorously in the presence of third-party H-2b cells (data not shown). Instead, in vivo depletion studies using anti-CD4 or anti-CD8 antibodies confirmed that CD4+ T cells were mainly responsible for the antitumor effect.
Histologic sections were performed and stained as detailed. Cells led to significant antitumor immunity. C57BL/6 SCID mice were adoptively transferred intravenously with 1 × 10⁶ A20WT tumor cells. Ten mice were included in each group, and they were inspected twice weekly for the development of tumor (P < .01 for the comparison between both chimeras). (B) H-2b → H-2db chimeras were treated with anti-CD4+- or anti-CD8+-depleting antibodies (3 times per week or received no treatment [no depletion]) before challenge with A20WT tumors. Mice received depleting antibodies once a week thereafter until the end of the experiment. Ten mice were included in each group (P < .01 for CD4-depleted vs -nondepleted groups).

Discussion

These findings demonstrate that tumor-antigen–specific CD4⁺ T cells can be efficiently primed in vivo by malignant B cells, leading to antilymphoma immunity only when unopposed by the dominant-negative effect of cross-presentation mediated by BM-derived APCs.

In the immune response against tumors, it is likely that malignant cells themselves and BM-derived APCs present tumor antigens to antigen-specific T cells. This explanation is more plausible for tumors that were derived from cells with intrinsic antigen-presenting capabilities and that have developed in the same organs in which antigen-specific T-cell responses are initiated, as is the case for B-cell lymphomas. As shown here, the fate of tumor-antigen–specific CD4⁺ T cells is divergent when tumor antigens are presented by lymphoma cells or by BM-derived APCs. Although T-cell priming and generation of effective immunity are elicited by direct tumor-antigen presentation by B-cell lymphomas, cross-presentation by BM-derived APCs leads instead to antigen-specific T-cell tolerance. The latter mechanism is clearly dominant over direct presentation because induction of T-cell unresponsiveness and tumor growth represents the ultimate outcome for B-cell lymphomas in vivo.
Figure 7. Dendritic cells from A20 lymphoma-bearing mice have impaired antigen-presentation capabilities. BALB/c mice were injected intravenously with \(1 \times 10^6\) A20 WT lymphoma cells. Three weeks later, animals were killed and CD11c\(^{+}\) splenic DCs were isolated by magnetic sorting. Purified anti-HA T cells (2 \( \times \) 10\(^5\)) were then plated for 10 hours with 2 \( \times \) 10\(^5\) DCs from lymphoma-bearing (right) or control tumor-free (left) mice in the presence or absence of cognate peptide. Each dot plot is gated on the CD4\(^{+}\) /6.5\(^{+}\) clonotypic population and shows the percentage of anti-HA T cells positive for IL-2 or IFN-\(\gamma\), as determined by intracellular cytokine staining. DCs were obtained from 4 mice, and the experiment was repeated twice with similar results.

Several factors might account for the predominant effect of cross-presentation over direct tumor-antigen presentation. First, although B-cell lymphomas arise in the same organs in which T-cell responses are elicited, it is plausible that, at least early during lymphoma development, either cell population would be confined to its respective B-cell or T-cell zone within the secondary lymphoid organs, making direct interaction unlikely. Second, the known migratory capabilities of BM-derived APCs might favor the exposure of antigen-specific T cells to antigen cross-presented by APCs migrating from the tumor site (B-cell zone). Such an early encounter, which usually occurs in the absence of inflammation, has been shown to result in the induction of T-cell tolerance rather than T-cell activation.\(^{15}\) Noninflammatory BM-derived APCs, with their low levels of MHC, costimulatory molecules, and other adhesion molecules that participate in T-cell priming, can induce a “partial” T-cell activation state that, in the absence of additional signals capable of sustaining this initial response or in the presence of dominant-suppressive mechanisms, is rapidly followed by T-cell unresponsiveness.\(^{3,16}\) The potential role of such a suppressive mechanism(s) has been recently highlighted by our demonstration that antigen presentation by host APCs led to the induction of regulatory T cells (TRegs) that dominantly suppress activated Th1 effector cells primed by A20HA cells directly.\(^{17}\)

Our findings that memory CD4\(^{+}\) T cells are also rendered unresponsive in tumor-bearing mice in which both mechanisms of tumor-antigen presentation are operative (H-2\(^d\) into H-2\(^{ab}\) chimeras or H-2\(^d\)/SCID) further confirmed the dominant-negative effect of cross-presentation over direct presentation. Memory T cells, because of their migratory capabilities,\(^{18,19}\) are likely to encounter tumor antigens in malignant B cells residing in B-cell zones and in BM-derived APCs cross-presenting tumor antigens. Although it is plausible that some memory T cells could be directly activated by malignant B cells, the fact that the overall outcome is tolerance induction suggests that most T cells might have encountered tumor antigen on APCs or that they are actively suppressed by APC-induced TRegs.\(^{17}\) Needless to say, the demonstration that memory CD4\(^{+}\) T cells are also rendered unresponsive during the in vivo growth of B-cell tumors has sobering implications for immunotherapy of this disease and provides one important explanation for the observed limited efficacy of T-cell adoptive therapy against B-cell malignancies.

B cells, macrophages, and DCs are all BM-derived cells that express MHC class II molecules and, as such, are capable of presenting tumor antigen to antigen-specific CD4\(^{+}\) T cells. However, several lines of evidence have pointed to DCs as playing a central role in initiating T-cell responses in vivo given their superior efficiency in antigen uptake, processing, MHC-peptide complex display, and migration to the T-cell areas of secondary lymphoid organs.\(^{14}\) Such a DC–T-cell encounter in vivo has not always led to T-cell priming and could result instead in the induction of T-cell unresponsiveness.\(^{15,20}\) Presentation of tumor antigens by DCs seems, therefore, to be the most likely mechanism involved in lymphoma-induced CD4\(^{+}\) T-cell tolerance. Interestingly, we have found that CD11c\(^{+}\) DCs from lymphoma-bearing mice are impaired in their antigen-presenting capabilities (Figure 7), suggesting that lymphoma-induced T-cell tolerance could be attributed, at least in part, to suboptimal tumor-antigen presentation by CD11c\(^{+}\) DCs. We are assessing whether these cells are tolerogenic in vivo and whether the impairment in antigen presentation is restricted to a specific DC subpopulation.

The finding that malignant B cells can efficiently prime antigen-specific CD4\(^{+}\) T cells is not surprising, considering the resemblance to their normal counterparts. Normal B cells have long been known to interact with CD4\(^{+}\) T cells during physiologic immune responses in a process that involves the presentation of peptide–MHC class II complexes, along with costimulatory signals to antigen-specific T cells.\(^{21,22}\) Similarly, some studies have shown that malignant B cells display well-preserved antigen-presenting capabilities.\(^{3,11,12,23,24}\) In contrast to these findings, others have found that B cells not only fail to activate naive T cells but may also induce unresponsiveness of antigen-specific T cells.\(^{10,25,27}\) Therefore, significant controversy remains about the ability of B cells to stimulate T-cell responses in vivo. The results presented here provide support for the intrinsic priming ability of B cells and indicate that such a T-cell/B-cell interaction in vivo is associated with the elicitation of antilymphoma immunity (Figures 1, 6). Although we realize that not all B-cell lymphomas have preserved antigen-presenting capabilities, our results indicate that even when they do, as is the case for A20, host APC-dependent tolerance is dominant over B-cell–dependent T-cell activation.

For many years, a number of different approaches have been used to enhance the antigen-presenting capabilities of malignant B cells, mainly by genetically modifying B cells to enforce the expression of immunologically relevant molecules such as adhesion/costimulatory molecules or cytokines.\(^{11,28–30}\) Although these strategies can induce systemic immunity in vivo, the effect observed has been transient and insufficient to induce effective lymphoma eradication. One potential explanation for the limited success of these strategies is that they have focused on enhancing the APC function of the malignant B cells while ignoring the other important component represented by the dominant effect of cross-presentation of tumor antigens.

Therefore, from a therapeutic perspective, it is likely that elicitation of effective antilymphoma immunity will require a rational combination of therapeutic approaches aimed at enhancing the antigen-presenting function of B cells with strategies targeting the remarkable barrier imposed by tolerogenic cross-presentation mediated by BM-derived APCs.

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References


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