The CD70/CD27 Pathway Is Critical for Stimulation of an Effective Cytotoxic T Cell Response against B Cell Precursor Acute Lymphoblastic Leukemia

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For effective immunotherapy, maintaining the frequency and cytotoxic potential of effector cells is critical. In this context costimulation via the CD70/CD27 pathway has been proven essential. CD70 has been reported to be expressed to varying degrees on malignant B cells. However, in B cell precursor acute lymphoblastic leukemia, the most common childhood malignancy, the role of CD70 in stimulation of antileukemic T cell responses has so far not been delineated. Herein we demonstrate that in B cell precursor acute lymphoblastic leukemia expression of CD70 is low but can be induced upon blast activation via CD40. Both CD70 and CD80/CD86 up-regulated on CD40-stimulated blasts contribute to primary stimulation of T cell proliferation and cytokine production in an additive manner. These two signals also cooperate in the prevention of T cell anergy. In contrast to blockade of CD70 during the effector phase, inhibition of CD70-mediated costimulation during generation of antileukemic T cells prevents effector cell proliferation and reduces their cytotoxic capacity. Modulation of the CD70/CD27 pathway may thus represent a novel therapeutic approach for augmenting magnitude and quality of the antileukemic response in B cell precursor acute lymphoblastic leukemia. The Journal of Immunology, 2009, 182: 718–725.

Adequate expansion of functionally competent cytotoxic effector cells is key to any effective immunotherapy approach. In this aspect CD70/CD27 interactions differ critically from the well-characterized classical costimulatory CD80/CD86 pathway. While during primary T cell activation there seems to be a certain redundancy in CD80/CD86 and CD70 costimulation, it is triggering of CD27 on T lymphocytes by its ligand CD70 that augments the magnitude of Ag-specific cytotoxic T cell responses both by enhancing initial expansion and survival of Ag-specific T cells as well as by improving their cytotoxic capacity (1–4).

In acute leukemia, deficient immunogenicity of immature leukemic blasts may in principle be due to defective Ag processing, Ag presentation, or lack of costimulatory molecules. As the majority of B cell leukemias express MHC molecules to high levels, their reduced T cell stimulatory capacity is largely attributed to deficient costimulation. However, similar to normal B cells, cross-linking of CD40, detected to varying degrees in most B cell malignancies, improves their Ag-presenting capacity due to significant up-regulation of the costimulatory molecules CD80 and CD86 (5–10). CD80 and CD86 belong to the Ig superfamily of costimulatory molecules, and their capacity to facilitate primary Ag-specific T cell activation has been well characterized (11, 12). It has recently been recognized, however, that in the generation of CTLs, the costimulatory molecule CD70 serves a role independent of the classical costimulatory signals mediated by CD80/CD86 (1, 4, 13, 14). As members of the TNF ligand/receptor superfamilies, CD70/CD27 interactions deliver critical costimulatory signals for the expansion and maturation of Ag-specific T effector cells, thus governing magnitude and maintenance of the cytotoxic response (2, 3, 15). CD70 is a type II transmembrane glycoprotein and is physiologically found on a small subset of activated memory B cells. It is also transiently up-regulated on activated T cells (16–18). Its receptor CD27, a type I glycoprotein, is expressed on most cytotoxic peripheral blood T cells, NK cells (19), and subpopulations of B memory cells (17, 20). In T cells, ligation of CD27 by CD70 results in T cell activation, cytokine production, and proliferation (21, 22). When effector cells differentiate, however, they down-regulate CD27 expression, loose their proliferative potential, and at the same time increase their cytotoxic activity. Because of its pivotal role in immune activation, CD70 expression is physiologically highly restricted to ensure the transient availability of this potent costimulatory signal (2, 20, 21).

The occasional presence of CD70 on germinal center lymphocytes also suggests a role of CD70/CD27 interactions in clonal B cell expansion. Indeed, CD70 has been reported to be expressed to varying degrees on malignant B cells, including chronic lymphocytic leukemia (CLL), various subtypes of non-Hodgkin lymphoma, and myeloma cells (23–27). In these mature B cell malignancies, deregulated and increased CD70 expression has been found on most CLL and

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4 Abbreviations used in this paper: CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; BCP-ALL, B cell precursor ALL; CD40-stim. ALL, CD40-activated ALL blasts; SI, stimulation index; unstim. ALL, unstimulated ALL blasts.
mature lymphoma subtypes. This is in contrast to acute lymphoblastic leukemia (ALL), the most common childhood malignancy, where only low-level expression of CD70 on B cell precursor blasts has been documented (28). Given the differential baseline expression pattern of CD70 in immature vs mature B cell malignancies, it is in fact critical to investigate under which conditions the expression level of CD70 becomes increased on immature ALL blasts in view of enhancing cytotoxic antileukemic T cell response. Indeed, although CD70 has been reported to enhance the magnitude and quality of the cytotoxic response, the role of CD70 in antileukemic T cell responses in B cell precursor ALL (BCP-ALL) has not been delineated. Thus, we explored the relevance of CD70 expression in BCP-ALL with regard to activation and expansion of functionally competent antileukemic T effector cells and provide first evidence for its contribution to induction and maintenance of the cytotoxic response in primary human ALL.

Materials and Methods

Patients

Leukemic blasts from 31 patients with newly diagnosed BCP-ALL (24 common ALL, 7 pre-B-ALL) treated according to the German Cooperative ALL Study Group (CoALL06-07) protocol were assessed for surface expression of costimulatory molecules. The scientific substudy was approved by the ethics committee of the CoALL central trial office in Hamburg and the local ethics committee in Duesseldorf. Informed consent was obtained from the parents or legal guardians before analysis. Fresh or cryopreserved bone marrow or peripheral blood samples contained 50% and 61% ALL blasts in two cases, while blast content in the remaining samples ranged from 77% to 98% (median of 90%).

Primary cell isolation

For functional assays, ALL blasts and PBMC were isolated by standard Ficoll gradient centrifugation. As effector cells, T cells were then isolated by addition of neuraminidase-treated sheep erythrocytes (Dade Behring). Erythrocyte-bound T cells were pelleted by a second Ficoll-density centrifugation. Erythrocytes were lysed by ammonium chloride and T cells were washed. Purity was documented by flow cytometry as >90% CD3-positive cells.

Flow cytometry analysis

For flow cytometry analysis a standard panel of murine fluorescein-conjugated mAb and respective isotype controls (BD Biosciences) was employed for diagnosis of BCP-ALL and costimulatory molecule detection. Analysis of CD40, CD80, CD86, and CD70 surface expression on ALL blasts was performed by double staining with anti-CD19 or anti-CD10 and respective mAbs against the TNF receptor and costimulatory molecules (BD Biosciences) on a FACScan (BD Biosciences) according to standard protocols utilizing the CellQuest software for analysis. Results were calculated as the percentage of positive blasts.

Stimulation of leukemia cells by CD40 cross-linking

For cross-linking of the CD40 receptor, genetically modified fibroblasts expressing the human CD40L in trans were used. Unmodified and CD40L-transduced fibroblasts were treated with mitomycin C (0.01 mg/ml) at 37°C for 2 h and washed twice before 10^6 ALL blasts were added. Blocking anti-CD80, -CD86, and -CD70 mAbs were added as described above. Eighteen hours before harvest, cells were pulsed with 1 μCi of [3H]thymidine (Amersham Biosciences) and [3H]thymidine incorporation (cpm) was measured by liquid scintillation spectrophotometry (Beckman Coulter). The stimulation index (SI) was calculated for each individual experiment as follows: SI = cpm (T cells + ALL cells)/cpm (T cells).

Results

Up-regulated CD70 improves the T cell stimulatory capacity of CD40-stimulated ALL blasts

First we assessed the effect of blast activation via the CD40 receptor on surface expression of CD70 as well as the critical costimulatory molecules CD80 and CD86. Overall CD40 expression on ALL samples ranged from 60% to 100% (median of 94%). Ligation of the CD40 receptor by coculture of blasts on feeder cells transgenically expressing CD40L resulted in up-regulation of CD70 on leukemic blasts from 17 ± 3% to 63 ± 5% (mean ± SE) (Fig. 1). Also, significant up-regulation of CD80 from 11 ± 3% to 41 ± 4% and CD86 from 36 ± 5% to 75 ± 4% was observed (Fig. 1).
Next, the T cell stimulatory capacity of CD40-activated ALL cells was determined with CD40-ALL blasts as stimulator and allogeneic T cells as responder cells in a MLR. CD40-activated blasts exhibited a 3-fold higher T cell stimulatory capacity (SI 22 ± 3.8; mean ± SEM) compared with unstimulated ALL cells (7 ± 1.5) (Fig. 2A). Additionally, coculture of T lymphocytes with

FIGURE 1. Up-regulation of CD70 and CD80/CD86 on ALL blasts after CD40 stimulation. After CD40 activation, surface expression of CD70, CD80, and CD86 on ALL blasts was analyzed by flow cytometry (n = 31). Results are shown as the mean percentage of positive blasts ± SE for unstimulated ALL blasts (unstim. ALL) cultured for 3–4 days on nonmodified feeder cells and CD40-activated blasts (CD40-stim. ALL) cultured on fibroblasts expressing CD40L in trans. There is a significant increase in surface expression of all costimulatory molecules after CD40 stimulation (p < 0.01).

FIGURE 2. Impact of costimulatory signals on proliferation and IFN-γ secretion in allogeneic T lymphocytes activated by CD40-stimulated ALL blasts. Unstimulated and CD40-stimulated ALL blasts were cultured with allogeneic T cells from healthy donors at a ratio of 1:2 for 5 days. Blocking Abs directed against CD70 and CD80/CD86 were added at initiation of culture. A, Proliferation of T cells was measured by [3H]thymidine incorporation for the last 18 h (n = 18). Results represent the means ± SEM of the SI. CD40-activated blasts exhibit significantly higher T cell stimulatory capacity than did unstimulated blasts (p < 0.01). After blockade of costimulatory molecules either alone or in combination, T cell proliferation is significantly reduced (p < 0.03). Combined blockade of CD70/CD80/CD86 results in significantly stronger T cell inhibition compared with blockade of either CD70 or CD80/CD86 alone (p < 0.01). B, IFN-γ secretion likewise assessed in supernatants after 5 days was reduced after blockade of costimulatory molecules (n = 4). Both blockade of CD70 alone (p = 0.04) and combined blockade of CD70/CD80/CD86 (p = 0.03) result in significant reduction of IFN-γ secretion.
CD40-ALL blasts resulted in markedly enhanced IFN-γ production (1764 ± 566 IU/ml; mean ± SE) compared with IFN-γ production in T cells cultured in the presence of unstimulated blasts (464 ± 127 IU/ml) (Fig. 2B). To determine the role of CD70 vs CD80/CD86 as costimulatory signals delivered by CD40-activated blasts, blocking mAbs directed against each of these costimulatory molecules were added to the MLR (Fig. 2A), resulting in comparable inhibition of both T cell proliferation (SI 18 ± 3.6 and 17 ± 3.3) and IFN-γ production (781 ± 309 and 836 ± 141 IU/ml) in the presence of anti-CD70 vs anti-CD80/CD86 mAbs, respectively. More importantly, simultaneous blockade of all costimulatory molecules was additive with inhibition of T cell proliferation (SI 10 ± 2.3) to levels as low as observed after coculture with nonactivated ALL blasts alone (SI 7 ± 1.5). Simultaneous blockade of costimulatory molecules CD70 and CD80/CD86 also resulted in pronounced decrease of IFN-γ secretion in effector T cells stimulated with CD40-activated blasts (502 ± 250 IU/ml) (Fig. 2B). Taken together, our results document that costimulation via CD70 delivers an equally strong stimulatory signal compared with CD80 and CD86. Moreover, costimulation mediated through both the CD28/CD80/CD86 and the CD27/CD70 pathways is additive.
CD70 and CD80/CD86 are critical costimulatory signals for T cell responsiveness to ALL blasts

To delineate the role of CD70 costimulation in antileukemic T cell responses further, allogeneic T cells initially primed by unstimulated or CD40-activated ALL blasts were assessed in a secondary MLR for induction of T cell proliferation (Fig. 3A). With the aim to differentiate the contribution of CD70 and CD80/CD86 expression during primary T cell stimulation, the individual costimulatory molecules were blocked by addition of the respective Abs during primary T cell contact. In contrast to T cells stimulated with CD40-activated blasts during both primary and secondary MLR (SI 6.47 ± 3.18), primary T cell contact with unstimulated ALL blasts substantially impaired T cell proliferation even upon secondary encounter with CD40-activated blasts (SI 2.12 ± 0.44), indicating induction of T cell anergy by primary contact with naïve blasts. Blockade of CD70 costimulation during first stimulation with CD40-activated blasts resulted in inhibition of secondary T cell proliferation (SI 3.14 ± 1.66; mean ± SEM) comparable to blockade of CD80/CD86, although CD40-activated ALL blasts were used as the secondary stimulus. Inhibition of T cell proliferation was even more pronounced following combined blockade of CD80/CD86 and CD70 costimulation (SI 2.09 ± 0.72). Interestingly, a similar pattern was observed when unstimulated ALL blasts were used as a primary stimulator, suggesting that even the low level of costimulatory molecules sufficed to allow for effective secondary stimulation by CD40-activated blasts. Thus, after blockade of CD70, T cell proliferation was consistently even lower than after primary stimulation with unstimulated blasts (SI 1.34 ± 0.44 with CD70 blockade vs 2.12 ± 0.44 without CD70 blockade). However, T cell hyporesponsiveness was again most profound following combined blockade of CD70 and CD80/CD86 even during primary T cell contact with unstimulated blasts, resulting in significant suppression of the secondary T cell response (SI 0.78 ± 0.2 vs 2.12 ± 0.44; p < 0.03) (Fig. 3A).

Next we assessed if addition of IL-2 during secondary T cell encounter with CD40-activated blasts can compensate for anergy induced in the absence of adequate costimulation during primary ALL/T cell encounter. Following primary stimulation with CD40-activated ALL blasts, addition of IL-2 during secondary stimulation resulted in significant increase of T cell proliferation compared with secondary stimulation with CD40-activated ALL blasts only (SI 10.1 ± 3.3 vs 6.5 ± 3.2; p = 0.056). T cell proliferation was still enhanced by addition of IL-2 during secondary stimulation even when CD80/CD86 was blocked during first T cell/ALL contact (SI 10.3 ± 3.5; p = 0.05). Whereas the inhibitory effect of CD80/CD86 blockade during primary stimulation was completely reversible by addition of IL-2 during secondary stimulation, following CD70 blockade during primary T cell contact with ALL cells addition of IL-2 during secondary stimulation only partially reversed T cell unresponsiveness (SI 3.1 ± 1.7 vs 5.9 ± 2.6; p = 0.2) (Fig. 3B).

CD70 costimulation is critical for the generation of functionally competent antileukemic CTL

Next we explored the contribution of up-regulated costimulatory molecules on CD40-activated blasts for the generation of ALL-specific CTL. ALL blasts from three patients were used to generate CTL from donors matched for HLA-A, HLA-B, and HLA-DR by three rounds of stimulation with either unstimulated or CD40-activated blasts at 10-day intervals followed by culture with IL-2 and IL-7. Phenotypic analysis of the generated lines revealed a mixed population of CD4+, CD8+, and CD56+ T cells. In a standard
51Cr-release assay T cell lines generated using CD40-activated ALL blasts demonstrated a high level of specific cytotoxicity against unstimulated ALL blasts (Fig. 4A) as well as against CD40-ALL blasts (Fig. 4B). While T cell lines repeatedly restimulated with unstimulated ALL blasts also recognized and lysed ALL blasts, cytotoxic activity was consistently lower than observed with CTL generated by repeated stimulation with CD40-activated ALL blasts at different E:T ratios. * indicates significant blockade of CTL generated in the presence of CD70 Abs in both unstimulated (p = 0.0004) and stimulated ALL blasts (p = 0.048).

To determine the role of signaling via the CD70/CD27 pathway in the generation of CTLs stimulated by CD40-activated ALL blasts, two of the three T cell lines were prepared in the presence or absence of blocking anti-CD70 Abs. CD40-ALL blasts induced strong proliferation of effector cells with 37- and 89-fold expansion after 3 wk of culture, which was reduced to 15- and 3-fold in the presence of anti-CD70 mAb, corresponding to a 60% and 97% reduction of expansion after CD70 blockage. Additionally, generation of cytotoxic effector cells in the presence of CD70 blockade significantly decreased the cytotoxic response against ALL blasts. Of note, a 30–60% reduction of cytotoxic T cell response relative to that without CD70 mAb was observed against unstimulated ALL blasts and a 20–25% reduction against CD40-stimulated ALL blasts (Fig. 5, A and B). The more pronounced decrease in cytotoxic activity of CTL generated in the presence of CD70 blockade when unstimulated ALL blasts were used for stimulation indicates that up-regulation of CD80/CD86 costimulatory signals following CD40 activation can compensate in part for the CD70 blockade. In a separate experiment, CD70-blocking Abs were added during the cytotoxicity assay only. However, addition of CD70-blocking Abs during the effector phase alone had no effect on target cell lysis (Fig. 5, C and D).

Discussion
The lack of costimulatory surface molecules is one of the postulated mechanisms by which leukemia cells are thought to escape immune surveillance. While the role of CD80/CD86-mediated as well as CD70-mediated costimulation has been extensively studied in different mature B cell malignancies (5–10, 29, 30), in BCP-ALL until to date little was known about CD70 as the second signal for effective antileukemic T cell activation. Herein we demonstrate for the first time that in primary BCP-ALL baseline expression of CD70 is low but can be induced upon blast activation via CD40. These CD40-stimulated ALL cells then induce proliferation and enhance the cytotoxic potential of antileukemic cytotoxic effector T cells in a CD70-dependent manner, and we further show that in contrast to the well-known costimulatory molecules CD80 and CD86, CD70 not only contributes to the activation of CTLs but is a critical signal during the expansion phase of the cytotoxic T cell response.

Physiologically CD70/CD27 interactions are thought to take place early in the immune response. Thus, shortly after recognition of Ag, CD27 is highly up-regulated on the T cell surface and provides signals to allow continued cell division initially regulated by CD28. CD70/CD27 interactions also seem to prevent T cell death several days into the immune response, determining the absolute number of effector T cells that are generated and subsequently the frequency of memory T cells (4, 14, 31). Thus, in view
of the fact that CD70 is critically involved in Ag-dependent T cell activation, constitutive low-level expression of CD70 may contribute to the poor Ag-presenting capacity in BCP-ALL. However, just as nonmalignant B cells are capable of up-regulating their T cell stimulatory potential upon CD40 stimulation, the Ag-presenting capacity of ALL cells can be enhanced by CD40 ligation. The importance of CD80/CD86/CD28 up-regulation for improved immunogenicity of CD40-activated ALL has long been recognized (8–10). Reaching beyond these observations, we now demonstrate increased expression of CD70 on CD40-activated ALL blasts. In more mature malignant B cells, such as follicular lymphoma cells and chronic lymphocytic leukemia cells, variable levels of CD70 baseline expression have been detected (23–27) and CD40-mediated up-regulation of CD70 is associated with enhanced immunogenicity in these mature B cell leukemias (25, 32, 33). However, while in mature B cell malignancies baseline CD70 expression is already adequately high to contribute to cellular T cell response even in the absence of additional CD40 ligation, acute lymphoblastic leukemia blasts are generally characterized by an immature phenotype with negligible expression levels of the costimulatory molecules CD80, CD86, and, as shown here, also of CD70.

We itemized for the first time the significance of the two costimulatory pathways in BCP-ALL and could show that both CD70 and CD80/CD86 become up-regulated on CD40-stimulated blasts and contribute to primary stimulation of T cell effector functions such as T cell proliferation and cytokine production in an additive manner. These two signals also cooperate in the prevention of T cell anergy. The importance of sufficient costimulation during primary contact is emphasized by the fact that blockade of CD70 during primary T cell contact reduces the secondary T cell response to CD40-activated blasts. The same holds true for blockade of CD80/CD86 during primary T cell activation. Moreover, T cell hyporesponsiveness is most profound in the absence of both costimulatory signals and is also no longer reversible during second challenge even with CD40-activated blasts. Interestingly, suppression of the secondary T cell response is also observed after blockade of low-level CD70 expression typically found on unstimulated blasts, underscoring the notion that CD70 is critical for preventing T cell anergy even at low levels. Thus, CD70 blockade also impairs IL-2–induced augmentation of the T cell proliferative response during secondary stimulation with CD40-activated BCP-ALL blasts, while CD80/CD86 blockade has no such effect. In contrast to our study with allogeneic peripheral blood T lymphocytes as effector cells in which CD40-activated ALL blasts prevent anergy, for T cells derived from cord blood it has been shown that CD40-activated ALL cells may induce anergy via enhanced expression of IL-10 (34), while in our system the stimulatory effects mediated by up-regulated CD70 and CD80/CD86 supervene. These differences may be attributed to the different responsiveness of mature peripheral blood vs naive cord blood T cells toward costimulatory signals delivered by CD40-activated blasts. Reversal of blast-specific anergy observed in peripheral blood but not in cord blood T cells may have implications with regard to the choice of the stem cell source when considering enhancement of the antileukemic response by application of a CD40-licensed vaccine in the setting of hematopoietic stem cell transplantation.

In addition to effective T cell priming, increasing and maintaining the frequency of CTLs is an essential prerequisite for any successful immunotherapy approach. In viral disease, vaccine efficacy was significantly improved by coadministration of the relevant viral Ag with soluble CD70, resulting in massive expansion of Ag-specific CD8+ T cells. In another in vivo model, CD70 was also shown to quantitatively and qualitatively enhance the cytotoxic response to influenza virus and an EBV-mediated B cell lymphoma. High numbers of Ag-specific T cells in the memory phase were maintained and effector cell function was significantly enhanced (35, 36). More importantly, it has recently been reported that even in the absence of additional T cell help, triggering of CD27 on T cells by CD70 expressed on CD40-licensed APCs augments the cytotoxic T memory pool (31). We have explored the potential of CD40-stimulated ALL blasts to generate HLA-matched allogeneic cytotoxic leukemia-specific T cells and for the first time delineated the role of CD70 to this effect. Indeed, T cell lines generated by stimulation with CD40-activated ALL cells proved highly capable of lysing both unstimulated as well as CD40-stimulated blasts. As a novel finding we document the requirement of CD70-mediated costimulation for expansion of antileukemic CTLs. Thus, proliferation of effector cells stimulated with CD40-stimulated blasts was profoundly inhibited by addition of CD70-blocking Abs at the initiation of culture. Of note, in contrast to CD80/CD86 blockade, inhibition of CTL expansion by CD70 blockade could not be compensated for by the addition of IL-2 or even IL-7 to cell culture, emphasizing that CD70-mediated costimulation acts independently of these cytokines known to provide T cell help.

As costimulation via CD70 has been shown to improve the cytotoxic potential of effector cells, we also investigated the role of CD70 costimulation on the cytolytic activity of CTLs and tested the ability of T cell lines generated in the presence of blocking anti-CD70 Abs to lyse the leukemic targets. Cytotoxicity of T cells generated with CD40-activated or even naive ALL blasts was significantly weaker when CD27/CD70 interaction was blocked during CTL generation. It has previously been shown that CD27/CD70 interaction plays a crucial role in ameliorating cytotoxic T cell and NK cell activity (37) in a perforin/granzyme-dependent manner (3). Thus, perforin expression by CTLs induced by EBV-transformed B cells was diminished in the presence of anti-CD70-blocking Abs during CTL generation (3). In addition to the perforin/granzyme pathway, however, CD95–CD95L interactions are involved in T cell-mediated cytotoxicity (38–40). In ALL, we have recently shown up-regulation of CD95 receptor expression following CD40 activation, which is in keeping with our observation made here that CTLs lysed CD40-stimulated blasts more efficiently than did unstimulated ALL cells (41). Of note, up-regulation of the costimulatory molecule CD70 on CD40-activated ALL cells does not contribute to the enhanced target susceptibility of CD40-stimulated blasts, as CD70 blockade did not affect CTL-mediated lysis of target cells. Thus, in the antileukemic response, CD70 delivers critical signals for effector T cell expansion and cytotoxic potential but not for target cell recognition.

Much of what we know about the relevance of CD70/CD27 interaction for reversal of T cell unresponsiveness and maintenance of a memory T cell pool stems from studies on viral immunity and transgenic murine models. It is the capacity to trigger expansion of effector T cells by which CD70 recommends itself as a highly relevant costimulatory signal, as documented by a number of murine models for cancer vaccines (42–44). Our own data showed that in leukemic mice, CD70 expressed by the vaccine cells does indeed induce a potent antileukemic response, which is predominantly mediated by CD8+ T cells. CD4+ T cells were not required to maintain long-term protection, again emphasizing that CD70-mediated costimulation obviates the need for additional T cell help (44). As CD70 interacts with its ligand CD27 expressed on T as well as NK cells, in vivo triggering of IFN-γ and perforin release from NK cells following CD27 ligation also serves to improve the tumor-specific cytotoxic T cell responses, providing a link between innate NK cell-dependent responses and adaptive T cell immunity (37). Taken together, we provide the first evidence that in CD40-activated primary BCP-ALL cells, CD70 and CD80/CD86 costimulation are additive in primary T cell activation. Furthermore,
CD70/CD27 interaction provides a critical stimulus for expanding effector T cells and enhancing their cytotoxic activity. Understanding the mechanisms that are responsible for enhancing the Ag-presenting capacity of leukemic blasts has significant implications for the development of novel immunotherapy approaches in BCP-ALL. Here, CD70-mediated costimulation provided by CD40-activated blasts is shown to improve the antileukemic effector response in a qualitative as well as quantitative manner. Characterization of the molecules involved in enhancing blast immunogenicity may form the basis not only for vaccine approaches but also for Ab/ligand-mediated therapy.

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Disclosures

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