Anti-CD70 antibodies: a potential treatment for EBV$^+$ CD70-expressing lymphomas

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Abstract

A monoclonal antibody (Rituximab) directed against the B-cell surface antigen, CD20, is increasingly used as a therapy for B-cell lymphomas. However, CD20 is expressed on all normal mature B cells and hence is not a specific tumor target. In contrast, CD70 is expressed on highly activated lymphocytes as well as on many B-cell and T-cell lymphomas but is not expressed on the great majority of B cells and T cells. In this report, we have explored the potential utility of anti-CD70 monoclonal antibodies for treatment of CD70$^+$ EBV$^+$ B-cell lymphomas. Using two Burkitt's lymphoma lines (Raji and Jijoye) that express surface CD70 and a CD70$^-$ Burkitt's lymphoma line (Akata), we show that two different monoclonal antibodies directed against human CD70 allow rabbit and human complement to kill EBV$^+$ B cells in a CD70-dependent manner *in vitro*. In the absence of complement, neither anti-CD70 antibody induced *in vitro* killing of CD70$^+$ cell lines. Importantly, i.p. injection of anti-CD70 antibodies also inhibited the growth of CD70$^+$ Burkitt's lymphoma cells in severe combined immunodeficient mice but did not inhibit the growth of CD70$^-$ Burkitt's lymphoma cells. These results suggest that anti-CD70 antibodies may be useful for the treatment of CD70$^+$ B-cell lymphomas. [Mol Cancer Ther 2005; 4(12):2037–44]

Introduction

The presence of specific surface markers on tumor cells has allowed the effective development of monoclonal antibodies for use as targeted therapeutic agents. The antigen-binding regions of these antibodies provide specificity to the tumor-killing effects. Whereas some antibodies kill tumor cells by blocking cell surface receptors that promote tumor growth, such as epidermal growth factor receptor and HER-2 (1, 2), other antibodies may induce cell killing by activating signal transduction cascades downstream of the receptor that result in apoptosis (3, 4). In addition, antibodies can kill cells via complement-mediated cell lysis (5). The term ''complement'' refers to a large category of circulatory proteins that act, on triggering, to induce a cascade of enzymatic events resulting in injury to cells (6). Antibodies binding to an antigen are cross-linked by the first component (C1q) of the classic complement pathway, which is sufficient to initiate the full cascade. The anti-CD20 antibody, Rituximab, kills B cells through complement-mediated lysis (7–12) and, in addition, induces a signal transduction cascade that induces apoptosis (13–15).

In this study, we have investigated the potential therapeutic effect of two different anti-CD70 monoclonal antibodies in regard to their ability to specifically kill CD70$^+$ tumor cells. Although CD70 expression on nonmalignant B cells and T cells is restricted to a small, highly activated subset, a variety of lymphoid malignancies constitutively express CD70. CD70 expression has been reported on 50% of B-cell chronic lymphocytic leukemia cases, 33% of follicular lymphomas, 25% of mantle cell lymphomas, and 71% of diffuse large cell lymphomas (16–19) as well as some T-cell malignancies (20, 21). In addition, certain forms of latent EBV infection have also been shown to induce expression of CD70 on host cells, and this effect is probably mediated in part by the viral latent membrane protein 1 (22). Burkitt's lymphoma lines containing the most stringent form of EBV latency (type I), in which EBNA-1 is the only viral protein produced, do not express CD70. However, Burkitt's lymphoma lines with type III latency (in which up to nine viral proteins are made, including latent membrane protein 1) express CD70 (23). Similarly, EBV-immortalized lymphoblastoid cell lines, which have type III latency, also universally express CD70 (24–26). Furthermore, tumors with type II EBV latency (expressing EBNA-1 and latent membrane protein 1 but not EBNA-2, EBNA-3, EBNA-4 EBNA-5, or EBNA-6), such as nasopharyngeal carcinomas and Hodgkin's disease, also express CD70, although CD70 is not expressed on normal epithelial cells (27–29). Latent membrane protein 1 expression alone in epithelial cells is sufficient to activate CD70 (22). Thus, CD70 activation is a marker for certain types of EBV infection as well as lymphoid malignancies.

CD70, also known as CD27 ligand, is a member of the tumor necrosis factor gene family and is normally expressed only on the surface of highly activated B cells, T cells, and dendritic cells (30–32). CD27 (a tumor necrosis factor receptor homologue; ref. 33) is expressed in hematopoietic stem cells

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(34), T cells (35), and memory B cells (36, 37). CD70 ligation to CD27 receptor on the surface of memory B cells induces activation and differentiation into plasma cells (38–40). CD70 ligation of CD27 on the surface of T cells promotes differentiation of CTLs in the absence of $CD4^+$ T-cell help (41–44). In addition, several reports have shown that ligation of CD70 also induces a signal transduction pathway that results in activation and proliferation of B cells and T cells (16, 45–48).

Here, we have examined the effects of anti-CD70 antibodies in regard to their ability to kill $CD70^+$ Burkitt's lymphoma cells *in vitro* as well as in a severe combined immunodeficient (SCID) mouse model of lymphoma. Although neither antibody caused killing of $CD70^+$ cells in the absence of complement *in vitro*, both antibodies mediated complement-dependent killing of Burkitt's lymphoma cells *in vitro* in a CD70-specific manner. Furthermore, anti-CD70 antibody also significantly inhibited the growth of $CD70^+$, but not $CD70^-$, Burkitt's lymphomas in SCID mice. These results suggest that anti-CD70 antibodies may potentially be useful for treating $CD70^+$ malignancies.

Materials and Methods

Cell Lines

EBV^+ Burkitt's lymphoma cell lines that express CD70 (Raji and Jijoye) or do not express CD70 (Akata) were grown in RPMI supplemented with 5% heat-inactivated fetal bovine serum. Each of these lines also expresses CD20 (49, 50).

Antibodies

Supernatant was harvested from hybridomas secreting murine monoclonal anti-CD70 antibodies LD6 (IgG2b) and Ki-24 (IgG3). The LD6 and Ki-24 (a gift from Harald Stein, Institut für Pathologie, Charité-Campus Benjamin Franklin, Medical University Berlin, Berlin, Germany) antibodies have been described previously (30, 51). Control antibodies used were purified murine IgG1 for LD6 experiments and purified murine IgG3 for Ki-23 experiments (both purchased from Sigma, St. Louis, MO). The LD6 and Ki-24 antibodies were purified over a protein G or A column, respectively. Azide-free control antibodies were filter sterilized through low protein binding 0.2-μm filters. All concentrations of antibodies were quantitated by both spectrophotometry using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, Inc., Hercules, CA) and by direct visualization of silver-stained aliquots run on SDS-PAGE (data not shown). Functional binding of antibody was verified by fluorescence-activated cell sorting (FACScan, Becton Dickinson, Franklin Lakes, NJ) analysis of known $CD70^-$ and $CD70^+$ control cell lines (data not shown).

Complement

Aliquots of commercially available rabbit complement (Dynal Co., Brown Deer, WI) were kept at −20°C. To obtain human complement, blood was collected from healthy volunteers following an institutional review board–approved protocol. The blood was allowed to clot at room temperature for 30 minutes and spun down, and the serum was then aliquoted and frozen at −20°C.

Complement-Dependent Cytotoxicity Assays

Aliquots of rabbit (Dynal) or human serum were thawed on ice. Heat-inactivated complement controls were generated by heating an aliquot to 55°C for 30 minutes and then returning the sample to the ice. Cells (1×10^4/well) were plated in 96-well plate with antibody (control or anti-CD70, at a final concentration of 10 μg/mL) and serum (heat-inactivated or active complement at a final concentration of 5% rabbit serum or 20% human serum) in a final volume of 100 μL. After 3 hours, an aliquot from the well was counted on a hemocytometer using trypan blue and the viable cells were counted. Each condition was done at least thrice, and the results were normalized to the negative control group (no antibody, no complement).

Western Blot

EBV^+ B cells (1×10^6) were treated with either active or inactivated complement in the presence of antibody (control, anti-CD70, or anti-CD20) at a concentration of 1 μg/mL. After 2 days, the samples were washed thrice and whole-cell extracts were made from the cell pellets with 100 μL NP40 lysis buffer. Protein concentrations of extracts were determined using the Bio-Rad reagent, and material (30 μg) was loaded according to SDS-PAGE protocol. Blots were probed with a primary antibody directed against the EBV early lytic protein, BMRF1 (Vector Laboratories, Burlingame, CA), and secondary anti-mouse antibody, and the blots were treated with enhanced chemiluminescence kit according to the manufacturer's instructions.

Tumor Response to Anti-CD70

Following a protocol approved by the University of North Carolina Animal Facility, SCID mice 4 to 6 weeks old were inoculated s.c. into both flanks with 5×10^6 Jijoye Burkitt's lymphoma cells. Tumors were allowed to grow until first palpable (12 days), and the animals then received i.p. either 500 μg LD6 or 500 μg control antibody. The tumors were then measured regularly by calipers in three dimensions to calculate the absolute volume of the tumors. At least six tumors were treated and monitored for each group. A similar experiment was done with Akata Burkitt's lymphoma cells, except that mice were injected i.p. with 500 μg LD6 or control antibody on day 9 (when tumors were first palpable) and day 11 after injection of tumor cells. The timing for the first dose of antibody was chosen based on the time required for each of the Burkitt's lymphoma lines to form a palpable tumor in SCID mice. The $CD70^-$ tumors (Akata) were treated with two doses (rather than one dose) of CD70 antibody to confirm that the antibody had no nonspecific antitumor effect even when given at higher doses.

Results

Anti-CD70 Antibody Directs Complement-Mediated Lysis of Burkitt's Lymphoma Cells in a CD70-Dependent Manner

To determine if anti-CD70 antibodies induce killing of CD70-expressing cells in the presence or absence of complement, EBV^+ Burkitt's lymphoma cells that express

CD70 (Raji) were treated for 3 hours with either a control monoclonal antibody or an anti-CD70 antibody (LD6) in the presence of either no complement, inactivated (heated) rabbit complement, or activated rabbit complement. Cells from each condition were then counted by trypan blue exclusion to determine effects on cell viability. Anti-CD70 antibody alone did not induce killing of CD70$^+$ Raji cells after 3 hours (Fig. 1A) or after 48 hours (data not shown). However, in the presence of active (but not inactive) rabbit complement, the anti-CD70 antibody induced efficient killing of Raji cells, whereas the control antibody had little effect. This anti-CD70-mediated complement-dependent killing was comparable with that achieved using the anti-CD20 antibody, Rituximab. Similar effects were obtained with another CD70-expressing Burkitt's lymphoma line (Jijoye; Fig. 1B). These results indicate that the LD6 anti-CD70 antibody can mediate effective complement-dependent killing of CD70$^+$ Burkitt's lymphoma cells.

Anti-CD70-Directed Lysis of Cells Is CD70 Dependent

To confirm that anti-CD70 antibody killing requires CD70 expression on cells, CD70$^-$, CD20$^+$ Burkitt's lymphoma cells (Akata) were treated with control antibody, anti-CD70 antibody, or anti-CD20 antibody (Rituximab) in the presence or absence of activated rabbit complement. Although some nonspecific killing of these cells was observed in the presence of inactivated serum (although this trend did not reach statistical significance as determined by overlapping confidence intervals), the anti-CD70 antibody did not enhance cellular toxicity in comparison with the control antibody. In contrast, the anti-CD20 antibody resulted in a significant complement-dependent decrease in viability (Fig. 2). These results indicate that anti-CD70-mediated complement-dependent killing requires CD70 expression on the tumor target.

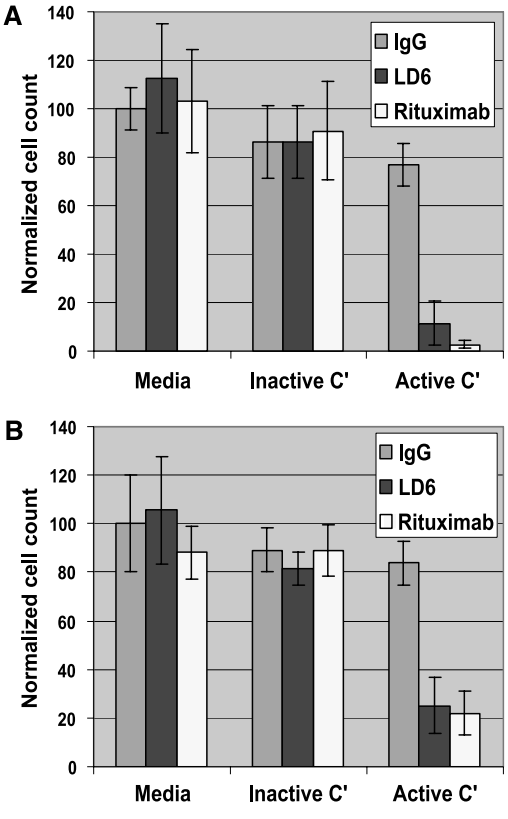

Figure 1. CD70$^+$ Burkitt's lymphoma cell lines are lysed by the combination of anti-CD70 and active rabbit complement. **A**, CD70$^+$ Raji cells incubated in medium, inactivated rabbit complement (*C'*), or activated complement were treated with control antibody, anti-CD70 antibody (LD6), or Rituximab. **B**, CD70$^+$ Jijoye cells were treated as in **A**. The number of viable cells in each treatment group is indicated (normalized to viability of cells treated with control IgG and medium).

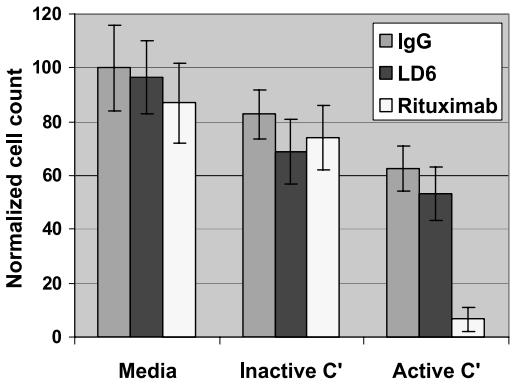

Figure 2. CD70$^-$ Burkitt's lymphoma cell lines are not lysed by the combination of anti-CD70 and active rabbit complement. CD70$^-$ Akata cells incubated in medium, inactivated rabbit complement, or activated complement were treated with control antibody, anti-CD70 antibody (LD6), or Rituximab. The number of viable cells in each treatment group is indicated.

The Ki-24 Anti-CD70 Antibody Also Mediates Killing of CD70$^+$ Cells via Complement

Whereas some anti-CD70 antibodies, including LD6, are thought to induce a signal transduction cascade in CD70$^+$ cells by cross-linking the CD70 receptor (51), other anti-CD70 antibodies, such as Ki-23, are thought to inhibit CD70 activation by its natural ligand, CD27 (31). To determine if the effects of blocking versus stimulating anti-CD70 antibodies were different in regard to their ability to mediate complement-dependent killing, we examined CD70-dependent cell killing by the IgG3 anti-CD70 monoclonal antibody, Ki-24, in the presence and absence of activated rabbit complement (Fig. 3). The Ki-24 antibody also killed CD70$^+$, but not CD70$^-$, Burkitt's cells in a complement-dependent manner. Longer treatments of CD70$^+$ cells with the Ki-24 antibody in the absence of complement still did not induce cell killing (data not shown). These results indicate that both CD70-stimulating and CD70-blocking antibodies can be used to kill CD70$^+$ cells in the presence of complement but that neither type of antibody induces killing of CD70$^+$ Burkitt's lymphoma cells in the absence of complement.

Human Complement Can Be Directed by Anti-CD70 Antibody to Kill CD70$^+$ Lymphoma Cells

Human cells express surface inhibitors of complement (CD55, CD46, and CD59), which preferentially recognize human complement versus complement from other species

(52). Hence, complement from nonhuman species is known to induce more complement-mediated killing of human cells than does human complement. To determine if anti-CD70 antibody can also direct complement-mediated killing in the more physiologic setting of human complement, CD70-expressing Raji Burkitt's lymphoma cells were incubated with medium, inactivated human complement, or active human complement and then treated with control monoclonal immunoglobulin, anti-CD70 (LD6), or anti-CD20 (Rituximab). As shown in Fig. 4A, significant lysis of $CD70^+/CD20^+$ Raji cells was mediated by both anti-CD70 and anti-CD20 antibodies in the presence of active human complement. In contrast, $CD70^-/CD20^+$ Akata cells were lysed by active complement in the presence of anti-CD20 antibody but not in the presence of anti-CD70 antibody (Fig. 4B), confirming that the effect of the anti-CD70 antibody was dependent on expression of CD70 on the cells.

Neither Anti-CD70 nor Complement Results in Activation of Lytic EBV Gene Expression

Several stressful stimuli (including chemotherapy and irradiation) have been shown previously to induce the lytic form of EBV infection through mitogen-activated protein kinase pathways (53, 54), and partial lysis of cells by complement is capable of activating these pathways (55). Thus, complement-dependent killing of EBV^+ B cells could potentially be amplified through the induction of the lytic EBV infection. To determine if this is the case, latently infected Raji and Jijoye cells were treated with either anti-CD70 or control antibodies (10 μg antibody/mL) in the presence of either active or inactivated rabbit complement (5%). After 2 days, cells were harvested and expression of the early lytic EBV protein, BMRF1, was quantitated by immunoblot. The anti-CD70 antibody (LD6) did not induce the expression of the early lytic EBV protein in the presence or absence of complement (Fig. 5). Thus, stimulation of the CD70 receptor with LD6 does not activate lytic EBV infection, and the anti-CD70 antibody/complement-mediated lysis of EBV infected cells does not require the lytic form of viral infection.

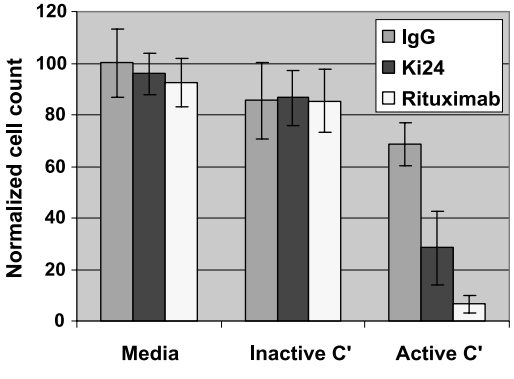

Figure 3. The anti-CD70 antibody, Ki-24, also directs complement-mediated lysis of $CD70^+$ Burkitt's lymphoma cells. $CD70^+$ Raji cells incubated in medium, inactivated rabbit complement, or activated complement were treated with control antibody, anti-CD70 antibody (Ki-24), or Rituximab.

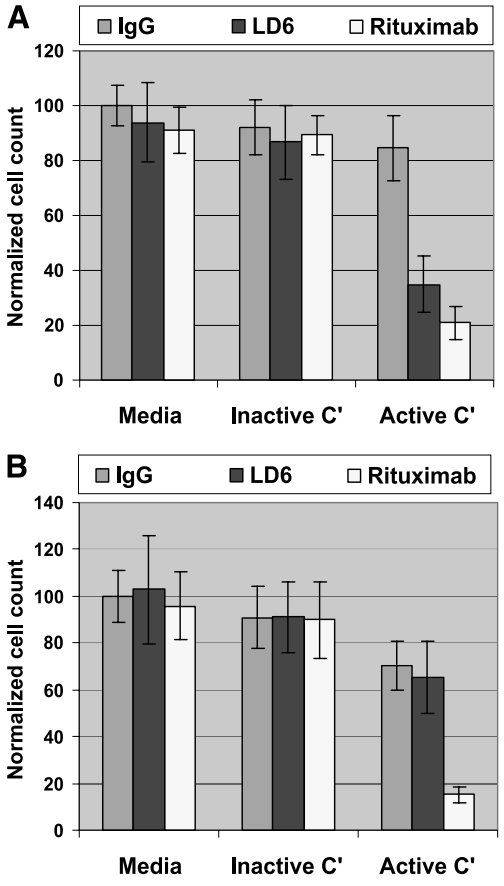

Figure 4. $CD70^+$ Burkitt's lymphoma cell lines are specifically lysed by the combination of anti-CD70 and active human complement. **A,** $CD70^+$ Raji cells incubated in medium, inactivated human complement, or activated complement were treated with control antibody (IgG), anti-CD70 antibody (LD6), or Rituximab. **B,** $CD70^-$ Akata cells were treated as in **A**.

Anti-CD70 Antibody Inhibits the Growth of EBV^+ Burkitt's Lymphomas in Mice

The previous *in vitro* results suggested that the anti-CD70 antibodies could potentially inhibit the growth of $CD70^+$ lymphomas *in vivo*, because complement is present in serum. To determine if this is the case, $CD70^+$ Burkitt's lymphoma cells (Jijoye) were injected into the flanks of SCID mice. When palpable tumors had formed (12 days after inoculation), mice were treated i.p. with 500 μg of either control murine immunoglobulin or anti-CD70 (LD6) antibody. Tumors were measured in three dimensions by calipers every 2 to 3 days thereafter. At 2 weeks following the development of palpable tumors, the animals treated with the anti-CD70 antibody had significantly smaller tumors than the mice treated with the control antibody (Fig. 6).

To characterize the *in vivo* effects of anti-CD70 antibody on EBV^+ Burkitt's lymphoma tumors, Jijoye tumors were also examined histologically. No significant cellular infiltrate was apparent in tumors from mice treated with either the anti-CD70 antibody or the control antibody (data not

shown). In addition, no expression of the lytic EBV protein BMRF1 was observed in tumors from mice treated with either anti-CD70 antibody or the control antibody (data not shown). These results suggest that the LD6 anti-CD70 antibody can inhibit the growth of CD70$^+$ Burkitt's lymphomas in SCID mice in the absence of a significant inflammatory response or the induction of lytic EBV infection.

To determine if the antitumor effect of the LD6 antibody required tumor cell expression of CD70, we inoculated mice with CD70$^-$ Burkitt's lymphoma cells (Akata) and treated the mice with LD6 versus control antibody (1 mg i.p. every other day for 3 days). In contrast to the therapeutic effect observed with the CD70$^+$ tumor, the LD6 anti-CD70 antibody did not inhibit the growth of the CD70$^-$ Burkitt's lymphoma tumor (Fig. 7) even when given at a higher dose. These results indicate that anti-CD70 antibodies may be useful for treatment of CD70$^+$ lymphomas in patients.

Discussion

Therapies for cancer are evaluated according to their effectiveness compared with their toxicity, and increasing the specificity of molecular targets is a rational approach to improving treatment. The development of therapeutic monoclonal antibodies, such as Rituximab, has been an important milestone in the treatment of cancer partly because such antibodies are at least somewhat specific in their toxicity. Although therapeutic monoclonal antibodies are generally not adequate single agents for treatment of most malignancies, mechanisms for increasing their activity, including linking radioactive tags and improving epitope-antibody surface kinetics, are being refined (56). Here, we show that monoclonal antibodies directed against the CD70 protein could potentially be useful for treatment of CD70$^+$ lymphomas. Because many B-cell and T-cell lymphomas express CD70, whereas only rare, highly activated, normal B cells and T cells express CD70, the CD70 receptor could potentially serve as a relatively specific therapeutic target for the treatment of both B-cell and T-cell lymphomas.

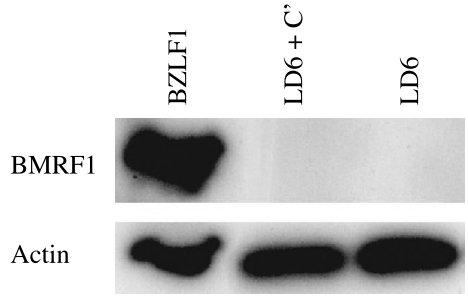

Figure 5. Anti-CD70 antibody (with or without activated complement) does not activate lytic EBV infection. Jijoye cells were incubated with LD6 (anti-CD70 antibody) with or without activated rabbit complement. Cell extracts were harvested 48 h later and immunoblot analysis was done to quantitate expression of an early lytic EBV protein (BMRF1) as well as the cellular actin protein. Jijoye cells transfected with the EBV immediate-early gene BZLF1 are included as a positive control for BMRF1 expression.

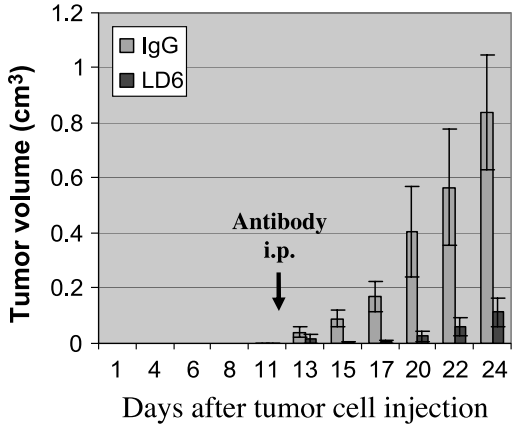

Figure 6. Anti-CD70 antibody inhibits the growth of CD70$^+$ Burkitt's lymphoma tumors in SCID mice. Jijoye cells were introduced s.c. into the flanks of SCID mice. At the earliest time that tumors were palpable, either control or anti-CD70 antibody (LD6) was injected i.p. Tumor size was evaluated thrice weekly. The size of tumors in each treatment group (average with 95% confidence intervals) is indicated at different time points.

Rituximab is a recombinant murine anti-human CD20 antibody genetically engineered to have the human Fc fragment for the immunoglobulin heavy chain (57). It is an effective agent when used for a variety of B-cell malignancies. Rituximab has been proposed to kill CD20$^+$ cells by several different mechanisms (56, 58, 59), including antibody-directed complement-dependent cytotoxicity with resultant chemotactic attraction of cellular immune cells (8–10), antibody-directed cellular cytotoxicity (9, 60, 61), and direct signaling via CD20 resulting in apoptosis (3, 13). Although short courses of Rituximab are generally well tolerated, this antibody eliminates normal mature B cells in addition to lymphoma cells. Consistent with this, Rituximab treatment has been associated with persistent panhypogammaglobulinemia (62). In addition, Rituximab may cause increased granulocytopenia (63).

In the current study, we have focused on the CD70 surface marker as a potential target for therapeutic monoclonal antibodies, because its expression on nonmalignant cells is relatively limited in comparison with the CD20 surface marker. Although highly activated T cells and B cells express CD70, there is no expression by B cells or T cells in the memory or naive compartments, unlike CD20, which is expressed in all but the most immature B cells. In addition, unlike Rituximab, anti-CD70 antibodies could potentially be used to treat T-cell as well as B-cell lymphomas. Furthermore, many EBV-associated malignancies, including nasopharyngeal carcinoma, also have CD70 expression (23, 27, 28). Our results indicate that an anti-CD70 antibody mediates complement-dependent killing of CD70$^+$ Burkitt's lymphoma cells *in vitro* and inhibits the growth of CD70$^+$ lymphoma cells in mice. This report is the first attempt to use CD70 as a therapeutic target in a mouse model of cancer.

There are several potential mechanisms by which abnormally activated CD70 expression could contribute to

cancer pathogenesis. Interestingly, some glioblastomas also express CD70 (64), and in these tumors, CD70 expression protects the tumor from lysis by CD27-expressing cytotoxic T cells (65). Whereas CD70 serves as the ligand for the CD27 receptor, there is also evidence that CD27 serves as a ligand for the CD70 receptor and induces a signal transduction cascade in $CD70^+$ lymphocytes that results in mitogenic and activating stimulation (45, 48, 51). Because many $CD70^+$ lymphomas also express CD27, interactions between CD27-expressing and CD70-expressing lymphoma cells could potentially support the growth of these lymphomas.

Rituximab has been reported to induce killing of $CD20^+$ B cells *in vitro* even in the absence of complement, and the mechanism for this effect is thought to be due to the ability of this antibody to stimulate a CD20-mediated signal transduction cascade that results in apoptosis. In contrast, in this study, we found that neither CD70-stimulating nor CD70-blocking antibodies significantly affected the viability of CD70-expressing Burkitt's lymphoma cells *in vitro* in the absence of complement. Nevertheless, in the presence of complement, the killing effect of the anti-CD70 antibodies was similar to that of Rituximab. Thus, the CD70 receptor is expressed at sufficient level on $CD70^+$ tumor cells to efficiently bind complement in the presence of anti-CD70 antibody and mediate cell killing. Furthermore, the LD6 anti-CD70 antibody strikingly inhibited the growth of $CD70^+$ Burkitt's lymphoma cells in SCID mice while not affecting the growth of $CD70^-$ Burkitt's lymphoma cells. Based on our *in vitro* results and the lack of an inflammatory infiltrate in the tumors (as might occur with antibody-dependent cellular cytotoxicity), this inhibitory effect of the anti-CD70 antibody on $CD70^+$ tumor growth in SCID mice is presumably due primarily to complement-mediated killing.

Complement is an important effector system in both nonadaptive and antibody-dependent adaptive immune responses. The complement cascade can be initiated by different mechanisms, including cross-linking of antibodies by C1q complement proteins and spontaneous deposition in lipid membranes of C3. Either event results in sequential activation of regulatory complement proteins that activate a final common pathway to produce the membrane attack complex, a collection of complement proteins (C5-C9) that insert into lipid bilayer membranes and form pores. Sufficient membrane injury results in lysis of the target cell. Cleavage byproducts of the early phases of the complement cascade, C3a and C5a, serve as chemotactic agents that attract cellular components of the immune system.

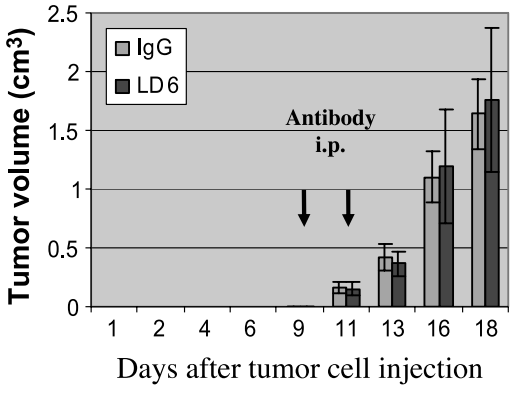

Figure 7. Anti-CD70 antibody does not inhibit the growth of $CD70^-$ Burkitt's lymphoma tumors. Akata cells were introduced s.c. into the flanks of SCID mice. At the earliest time that tumors were palpable, either control or anti-CD70 antibody (LD6) was injected i.p. Tumor size was evaluated thrice weekly. The size of tumors in each treatment group (average with 95% confidence intervals) is indicated at different time points.

The effect of complement-mediated tumor killing may be underestimated in tumor studies done in SCID mice, in which T and B lymphocytes cannot contribute or amplify the antitumor response mediated by complement, although neutrophils do participate in SCID mouse antitumor response with Rituximab (56, 66). On the other hand, animal cells are protected from complement-mediated killing by inhibitory proteins on their surface membranes, and these inhibitory proteins are less active in protecting cells against complement from other species (52). Hence, the tumor-killing activity of the SCID mouse complement for human $CD70^+$ Burkitt's lymphoma cells might actually be enhanced in comparison with what would occur in patients.

As CD70 stimulation of the CD27 receptor expressed on B cells and T cells is required for efficient differentiation of memory B cells into plasma cells as well as efficient generation of cytotoxic T cells, anti-CD70 antibodies may also be useful for inhibiting various types of autoimmune diseases. Consistent with this, previous studies showed that antibodies directed against the mouse CD70 protein inhibited the development of experimental autoimmune encephalitis in mice (67) and promote graft survival of heart transplants in a mouse model (44). Furthermore, in these previous studies, treatment of mice with an antibody directed against mouse CD70 (rather than human CD70, as used here) did not result in obvious toxicity. Nevertheless, as our results here suggest that anti-CD70 antibodies would not only inhibit the interactions between CD70 and CD27 but also promote complement-mediated killing of highly activated B cells and T cells, long-term treatment with anti-CD70 antibodies could potentially result in immunosuppression. Chronic CD27 stimulation in CD70 transgenic mice results in severe immunodeficiency (68).

This report is the first to show that anti-CD70 antibodies may be useful for inhibiting the growth of $CD70^+$ tumors. Most studies suggest that the major mechanism of cell killing by Rituximab in patients is through complement-dependent lysis. Our *in vitro* studies showed that anti-CD70 antibody and Rituximab produced similar killing $CD70^+$/$CD20^+$ tumor cells in the presence of human complement. Anti-CD70 antibody also inhibited the growth of $CD70^+$ tumors in SCID mice. Thus, anti-CD70 antibodies should be further explored as a potentially effective therapeutic modality for treating CD70-expressing tumors in patients.

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