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*J Immunol* 2009; 183:3770-3777; Prepublished online 26 August 2009; doi: 10.4049/jimmunol.0901637

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Blocking of CD27-CD70 Pathway by Anti-CD70 Antibody Ameliorates Joint Disease in Murine Collagen-Induced Arthritis

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Rheumatoid arthritis (RA) is characterized by inflammation and cellular proliferation in the synovial lining of joints that result in cartilage and bone destruction. Although the etiology of RA is unclear, activated lymphocytes and proinflammatory molecules, in particular TNF superfamily members, have been implicated in the disease pathology. A TNF superfamily member, CD70, is found on activated lymphocytes and shown to be important in memory and effector responses of lymphocytes. CD70 is expressed at high levels on chronically activated T cells in patients with autoimmune disorders, including RA. The involvement of CD70 in the progression of RA, however, remains unknown. In this study, we report effects of targeting CD70 on disease pathogenesis by using an anti-mouse CD70 Ab in a murine model of collagen-induced arthritis (CIA). In addition to blocking CD70 binding to its receptor CD27, the anti-CD70 Ab used also engages Fc-dependent effector functions including Ab-dependent cellular cytotoxicity, phagocytosis, and complement fixation. Treatment of mice with anti-CD70 Ab both before the onset or after the established disease in CIA model resulted in marked improvements in disease severity and significant reduction in the production of autoantibodies. Histopathological analyses of the joints of mice revealed a substantial reduction of inflammation, and bone and cartilage destruction in response to the anti-CD70 Ab treatment. These results uncover a novel role for CD27-CD70 interactions in the regulation of in vivo inflammatory response leading to arthritis, and provide a molecular basis to support the rationale for anti-CD70 therapy for autoimmune and inflammatory diseases. The Journal of Immunology, 2009, 183: 3770–3777.

A member of the TNF superfamily, CD70 is expressed on activated B and T lymphocytes and dendritic cells (DCs) and plays an important role in lymphocyte effector functions (reviewed in Ref. 1–4). Expression of CD70 on lymphocytes is carefully controlled by a multitude of signals including cellular activation, stimulation by Ags, costimulatory signals, and cytokines (4, 5). Once induced, expression of CD70 on activated T and B cells persists for several days, particularly on primed effector cells, such as IFN-γ-producing T cells, and B cells involved in T-dependent antigenic responses (5–7). This highly restricted and temporally regulated expression of CD70 ensures only a transient opportunity for CD70 to exert its biological effects in a highly specific manner (5, 6). Expression of CD70 is also induced on mature DCs by triggering of CD40 or Toll-like receptors on these cells (8–10). CD70 binds to a unique receptor, CD27, which is expressed on T, B, and NK cells (11–13). CD27 is constitutively expressed on resting T cells, which can be further up-regulated upon T cell activation, particularly in CD45RA+ T cells (14–17). Activation of T cells can also lead to shedding of CD27 from the cell surface resulting in a soluble form (sCD27) (18). Expression of CD27 on T cells is tightly correlated with the effector functions of these cells (1), CD27 is also uniformly found on memory B cells (19). Resting and naive B cells usually do not express CD27; however, CD27 expression can be induced by activation of B cells, resulting in sustained expression over long periods of time (20, 21). Some low levels of sCD27 are detectable in the serum of healthy individuals, but sCD27 is highly up-regulated in many disease situations including human autoimmune diseases (18).

Signals through CD27 lead to activation of NF-κB and the c-Jun kinase pathways that regulate many cellular processes including cell proliferation, differentiation, and survival (22, 23). Thus, a key biological role for CD27-CD70 interactions in T cell priming, and in subsequent promotion of their survival resulting in the formation of effector and memory T cells, is well documented (24, 25). A role for CD70 has also been established in the induction of proliferation and cytokine secretion by both CD4 and CD8 T cells and in the development of CTL responses by CD8 T cells (5, 26).

In the B cell compartment, CD27-CD70 interactions are important in T-dependent Ab production by promoting B cell activation, germinal center formation, expansion of B cells and differentiation into plasma cells, and by enhancing Ig production (20, 21, 27, 28).

The in vivo role of CD70 in cellular immune responses has also been documented by demonstrating an accumulation of T cells with an effector phenotype, increased CD8 T cell response to influenza virus, and enhanced tumor clearance in CD70-transgenic mice (29, 30). In contrast, CD27-deficient mice have reduced...
expansion of T cells following primary or secondary infection with influenza virus, and T cells lacking CD27 are defective in mediating secondary and memory T cell responses (24, 31). In vivo studies in which CD27−CD70 interaction was blocked with Abs demonstrated significant efficacy in models of experimental autoimmune encephalomyelitis (32) and cardiac allograft rejection (33). An additional in vivo role for CD70 is also suggested by the discovery of a novel population of CD70+ APCs residing exclusively in the gut lamina propria of the mouse (34). These cells constitutively express CD70 and mediate the expansion and differentiation of Ag-specific T cells in the gut mucosa via CD70-dependent mechanisms. Discovery of this novel subset of APCs in humans has not been reported yet.

In addition to the normal role of CD70 in mounting an immune response, a role for CD70 in autoimmunity and chronic inflammatory conditions has also been proposed by several reports (35–38). For example, CD4 T cells in the synovium of rheumatoid arthritis (RA) and psoriatic arthritis patients have been shown to express high levels of CD70. Likewise, increased numbers of circulating CD70 expressing CD4 T cells in RA and systemic lupus erythematos (SLE) patients have been also documented (36–38). Not only is there an increased number of CD4 T cells expressing CD70 in SLE patients, but their frequency also correlates with disease severity (37, 38). The fact that T cells from SLE patients elicit enhanced Ig secretion by B cells, which can be abrogated with a blocking anti-CD70 Ab, further implicates a role for CD70 in the pathogenesis of this disease (35). CD70 overexpression on T cells in SLE patients is shown to be due to hypomethylation of DNA sequences that flank the CD70 promoter, which results in an inability to down-regulate CD70 expression once it is induced by the activation of T cells (36, 37, 39). Similarly, an increase in splenic CD70 expressing CD4 T cells has also been documented in the lupus-prone MRL/lpr strain of mice, which is also caused by defective DNA methylation of the CD70 gene (40).

Based on the involvement of CD70 in the immune response, particularly in the effector phase, and its potential role in autoimmune and inflammatory disease, CD70 makes an attractive target for Ab-based immunotherapy. Because of the restricted expression pattern of CD70, targeted therapy against CD70 by depletion or by blocking of CD70−CD70 interactions offers a means to selectively target activated cells of the immune system. Thus, to generate further proof-of-concept for using CD70−directed immunotherapy for human autoimmune disease, we examined the effects of targeting CD70 by anti-CD70 Ab on disease pathogenesis in a mouse model of collagen-induced arthritis (CIA). Treatment of mice with anti-CD70 Ab in this model resulted in marked improvement in the clinical symptoms of disease, as well as significant reduction in anti-CD70 Ab in the presence of 6% rabbit complement (Cedarlane Laboratories) for 2 h at 37°C. Five μg/ml propidium iodide was added to detect apoptotic cells. The percent nonviable cells were identified by flow cytometry. To address Ab-dependent cellular phagocytosis (ADCP), BCL-1 target cells were labeled using the PKH-26 Fluorescent Cell Linker Mini Kit (Sigma-Aldrich) according to the manufacturer’s instructions, and preincubated with varying concentrations of anti-CD70 Ab for 30 min on ice. Cells were washed and combined with monocyte-derived macrophages at a 4:1 target:macrophage ratio for 1–2 h at 37°C. Macrophages were then labeled with Alexa Fluor 488-conjugated anti-CD11b (BD Pharringen). The cell mixture was fixed with 1% paraformaldehyde, and analyzed by flow cytometry to detect CD11b+PKH26+ double-labeled fluorescent cells. Engulfed target cells are those that are red/green positive. Phagocytic activity was calculated as (percent double-labeled CD11b+/percent CD11b− cells) × 100.

Ab-dependent cellular cytotoxicity (ADCC) was measured using a standard 51Cr release assay. BCL-1 target cells were labeled with 51Cr (PerkinElmer) for 1 h and washed extensively. Effector cells were human NK cells enriched as previously described (42). Labeled targets were plated at standard 5000/well and incubated with varying concentrations of anti-CD70 Ab before being combined with effector cells at a 50:1 E:T ratio, for 4 h at 37°C. Supernatants were measured for the presence of 51Cr released by lysed target cells. Percent specific cell lysis was calculated as (test sample cpm − spontaneous cpm)/(total cpm − spontaneous cpm) × 100. Spontaneous and total cpm values were determined from the supernatants of target cells incubated in medium alone and from target cells lysed with 1% Triton X-100, respectively.

**Induction and evaluation of collagen II-induced arthritis**

DBA/1 mice were injected s.c. at the base of the tail with 100 μg of bovine type II collagen (CII) (Chondrex) emulsified in CFA (Chondrex) on day 0. At day 21 after primary immunization, a booster injection of 100 μg of CII in Freund’s incomplete adjuvant (Chondrex) was given s.c. in the tail. Arthritis development was assessed in the wrist and ankle joints every other day and scored as follows: 0, normal; 1, mild redness and swelling of ankle or wrist; 2, moderate to severe redness and swelling of ankle or wrist; 3, redness and swelling of entire paw, including digits; or 4, maximum inflammation including multiple joints. The maximal arthritic score per paw was 4, and the maximal disease score per animal was 16. Scoring was conducted in a blinded fashion.

**Determination of serum levels of Abs against mouse CII**

Serum concentrations of anti-mouse CII were measured using a Luminex 2-plex assay. In brief, prediluted mouse serum samples were incubated with Luminex carboxyl beads (MiraBio) coupled to mouse CII (Sigma-Aldrich) in Multiscreen HTS filter plates (Millipore) for 1 h at room temperature. Plates were washed and incubated with goat anti-mouse IgG (Sigma-Aldrich) conjugated to Alexa Fluor 532 (Molecular Probes) for 1 h at room temperature. Plates were washed and read on the BioPlex Luminex 200 instrument. Samples were diluted in a Tris-saline buffer.

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**Materials and Methods**

**Animals**

Male 4- to 6-wk-old DBA/1 mice were purchased from Harlan Laboratories. Mice were maintained in a pathogen-free animal facility throughout the experiments. All animal experiments were conducted in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited facility and conducted under Seattle Genetics’ Institutional Animal Care and Use Committee guidelines and approval.

**Generation of anti-CD70 Ab**

A previously described hamster Ab against mouse CD70, 3B9, was obtained from Dr. van Lier (University of Amsterdam, Amsterdam, The Netherlands) (9). This Ab has been documented to block the binding of CD70 to its receptor, CD27. To reduce immunogenicity of this hamster Ab in mice, we genetically constructed a chimeric Ab by replacing the Fc portion of this hamster Ab with a mouse Fc of IgG2a isotype. In short, we isolated the cDNAs encoding the heavy and a light chain fragment of 3B9 by RT-PCR using primer sets previously reported (41). These variable gene fragments were then spliced in-frame 5’ to gene fragments encoding mouse IgG2a (CH1-CH3, Igk-1a) and mouse Cε, respectively. The resulting Ab has the same binding properties as its parent molecule but has improved interactions with mouse FcRs and contains significant amino acid sequences of mouse origin. The engineered Ab was prepared by transfecting genes for chimeric H and L chains into CHO DG44 cells. The Ab was purified from the culture supernatants of these recombinant CHO cells using Protein-A columns.

**In vitro assays for characterization of anti-CD70 Ab**

To determine whether anti-CD70 Ab blocks the interaction of CD70 and CD27, ELISA plates were coated with 0.5 μg/ml recombinant murine CD70 (R&D Systems), followed by varying amounts of anti-CD70 Ab. Recombinant murine CD27-Ig (R&D Systems) was then added at 0.2 μg/ml and detected with HRP mouse anti-human Fc secondary reagent (Jackson ImmunoResearch Laboratories), followed by tetramethylbenzidine substrate (Sigma-Aldrich).

To assess complement-dependent cytotoxicity (CDC) activity, BCL-1 (a murine B cell lymphoma line expressing CD70) target cells were incubated with varying concentrations of anti-CD70 Ab in the presence of 6% rabbit complement (Cedarlane Laboratories) for 2 h at 37°C. Five μg/ml propidium iodide was added to detect apoptotic cells. The percent nonviable cells were identified by flow cytometry.

To address Ab-dependent cellular phagocytosis (ADCP), BCL-1 target cells were labeled using the PKH26 Fluorescent Cell Linker Mini Kit (Sigma-Aldrich) according to the manufacturer’s instructions, and preincubated with varying concentrations of anti-CD70 Ab for 30 min on ice. Cells were washed and combined with monocyte-derived macrophages at a 4:1 target:macrophage ratio for 1–2 h at 37°C. Macrophages were then labeled with Alexa Fluor 488-conjugated anti-CD11b (BD Pharmingen). The cell mixture was fixed with 1% paraformaldehyde, and analyzed by flow cytometry to detect CD11b+PKH26+ double-labeled fluorescent cells. Engulfed target cells are those that are red/green positive. Phagocytic activity was calculated as (percent double-labeled CD11b+/percent CD11b− cells) × 100.

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Histological examination of arthritic joints

Mouse fore- and hind paws were fixed in 10% buffered formalin, decalcified, and embedded in paraffin at IDEXX. Joint sections were prepared and stained with H&E (IDEXX). Microscopic evaluation of arthritic paws was performed by a veterinary pathologist in a blinded fashion. The joint(s) for each limb was scored histopathologically using a qualitative scale from 0 to 4 based on the degree of inflammation and erosion associated with arthritis. The scoring scale was defined as follows: 0, no lesion; 1, minimal; 2, mild; 3, moderate; 4, severe. Histopathologic images of arthritic limbs were taken using a Zeiss light microscope.

Statistics

Statistical analyses of the data were performed with GraphPad Prism 5.01 for Windows (GraphPad Software). The significance of data sets was determined using the Mann-Whitney U test. Values of $p < 0.05$ were considered significant. Error bars represent the SEM.

Results

Characteristics of anti-CD70 Ab

To effectively use anti-CD70 Ab in vivo, we prepared a recombinant chimeric Ab derived from a hamster anti-mouse CD70 mAb, as described in the Materials and Methods. The recombinant Ab was constructed by replacing the constant regions of the parent hamster Ab with a mouse Fc and IgG2a. The resulting chimeric Ab, hereon referred to as anti-CD70 Ab, was tested for its ability to bind mouse CD70, interfere with CD70/CD27 interactions, and for its ability to carry out effector functions associated with its Fc portion. In an ELISA designed to test both binding of Ab to CD70 as well as blocking of CD70-CD27 interactions, the anti-CD70 Ab was found to retain binding characteristics equivalent to those of the parent hamster Ab. In addition, the anti-CD70 Ab blocked binding of sCD27 to plate-bound recombinant CD70 in a dose-dependent manner (Fig. 1A). When tested for ADCC activity in a standard $^{51}$Cr release assay using NK cells as effector cells, the anti-CD70 Ab induced lysis of mouse CD70 expressing target cells in a dose-dependent fashion, while no lysis was achieved with a nonbinding control IgG2a Ab. Maximal lysis was achieved at Ab concentrations of 10 to 100 ng/ml (Fig. 1B), which are significantly lower amounts than that required to saturate target cells in a binding assay.

FIGURE 1. Characterization of anti-CD70 Ab. A, Anti-CD70 Ab blocks the interaction of CD70 and CD27. ELISA plates were coated with recombinant murine CD70, followed by varying amounts of anti-CD70 Ab. Recombinant murine CD27-Ig was then added and detected with HRP anti-human Ig secondary reagent, followed by tetramethylbenzidine substrate. Data are presented for the parent hamster Ab (○) and genetically engineered anti-CD70 Ab (●). B, Anti-CD70 Ab mediates ADCC activity. $^{51}$Cr labeled BCL-1 target cells were combined with effector human NK cells at a 50:1 E:T ratio for 4 h at 37°C. Supernatants were measured for the presence of $^{51}$Cr released by lysed target cells. Data are presented as percent specific lysis of target cells. C, Anti-CD70 Ab mediates ADCP. Fluorescent-labeled BCL-1 target cells were preincubated with varying concentrations of anti-CD70 Ab (●) or 2 µg/ml control IgG2a (□) and then cultured with monocyte derived macrophages in a 4:1 target:macrophage ratio for 1 h at 37°C. Macrophages were then labeled with a different fluorochrome and engulfed target cells that were double positive were identified by flow cytometry. Percent phagocytic cells is shown. D, Anti-CD70 Ab mediates CDC activity. BCL-1 (a murine B cell lymphoma line expressing CD70) target cells were incubated with varying concentrations of anti-CD70 Ab (●) or 10 µg/ml control IgG2a (□) in the presence of 6% baby rabbit complement for 1–2 h at 37°C. Apoptotic cells were detected by staining with propidium iodide. Data are presented as percent specific lysis of the target cells.
To determine whether anti-CD70 Ab facilitates phagocytic uptake of target cells, target cells were labeled with a lipophilic dye, coated with Ab, and then mixed with monocyte-derived macrophages. The macrophages were subsequently stained with a fluorochrome labeled anti-CD11b Ab, and phagocytosis was measured by the appearance of double-labeled coincident events by flow cytometry. Whereas phagocytosis in the presence of nonbinding, control IgG2a was less than 25% for the target cells tested, macrophages readily phagocytosed tumor cells coated with anti-CD70 in an Ab dose-dependent manner (Fig. 1C). Engulfment of target cells by macrophages was confirmed by fluorescence microscopy (data not shown). Similar to ADC, maximal phagocytosis, which ranged from ~45 to ~60%, was achieved at subsaturating concentrations of anti-CD70 Ab. The anti-CD70 Ab was further tested for its ability to induce murine tumor cell lysis by complement fixation (CDC). As shown in Fig. 1D, BCL-1 cells were lysed in an Ab-specific, dose-dependent manner upon addition of anti-CD70 Ab to target cells in the presence of rabbit complement. These data suggest that our chimeric anti-CD70 Ab has the potential to exert its effect by blocking CD27-CD70 interaction, and/or by depleting CD70+ target cells using Fc-mediated effector functions such as ADCC, ADCP, and CDC.

Treatment of mice with anti-CD70 Ab before onset of disease inhibits development of collagen-induced arthritis

As the role of CD27-CD70 interactions is well established in the induction and maintenance of the immune response, we wished to test the in vivo importance of this pathway in pathogenesis of disease using the CIA mouse model for arthritis. This was accomplished by immunizing mice with bovine CII to induce CIA and treating these mice with anti-CD70 Ab to determine its effect on the development of arthritis. In the first set of experiments, we investigated the effect of anti-CD70 on the preclinical phase of the disease. In this case, treatment with anti-CD70 Ab was started on day 21 after the initiation of the experiment. At this time, no clinical symptoms of arthritis were yet visible as judged by the clinical scoring method described in the Materials and Methods. The first signs of arthritis, erythema and swelling of the feet and ankles, are usually observed a few days after a second immunization with bovine CII. Mice were injected with either 5 mg/kg of anti-CD70 Ab or control Ab for a total of 6 doses given every fourth day starting on day 21 (Fig. 2A), and then monitored for clinical signs of arthritis. During the follow-up period, control Ab treated mice quickly developed signs of arthritis and reached peak disease development in the next 2–3 wk. Conversely, little to no disease development was observed in anti-CD70 Ab treated mice in the next 2 wk, and only mild forms of disease symptoms were observed in the remaining period of the experiment, indicating a profound delay in the onset of disease in anti-CD70 treated mice. The majority of paws from mice treated with control Ab manifested symptoms of severe arthritis, whereas only mild symptoms of arthritis were seen in the majority of paws from the anti-CD70 Ab treatment group (Fig. 2B). These data reveal that anti-CD70 Ab administered in this fashion delays the onset of disease, as well as significantly reduces the severity of arthritis development in treated mice. Thus, these results indicate an important in vivo role for the CD27-CD70 pathway in the inflammatory response leading to the development of CIA in this mouse model.

Effects of anti-CD70 Ab on established arthritis

The above studies establish the efficacy of anti-CD70 Ab treatment in the preclinical phase of CIA in mice. The late, chronic phase of CIA exhibits pathology more relevant to human RA, with apparent disease and dominating inflammatory mechanisms. Thus, to determine the effects of anti-CD70 treatment in this phase of CIA, a group of animals was also treated with anti-CD70 Ab after the onset of the arthritis. In this group, treatment with anti-CD70 Ab began after the onset of arthritis in the late chronic phase, where clinical symptoms of arthritis were highly visible. Starting on day 25, mice were treated with 5 mg/kg of anti-CD70 Ab for a total of 6 doses given every fourth day of the experiment and then monitored for clinical signs of arthritis. In this late phase, during the follow-up period, arthritis development progressed to a very severe state in the mice treated with control Ab, as shown in Fig. 2B. However, further progression of arthritis beyond the initial disease seen at the start of treatment was inhibited in the group treated with anti-CD70 Ab (Fig. 3). Data presented in Fig. 3 show that anti-CD70 Ab treatment in this late phase inhibits further disease progression as well as reduces the overall severity of arthritis development in the treated mice compared with the control group, shown again for comparison. Thus, these results demonstrate that anti-CD70 Ab treatment not only delays the onset of arthritis development when given in the preclinical phase but also significantly suppresses the progression of established arthritis when given after the onset of disease in the CIA model.
Erosive joint destruction is inhibited in anti-CD70 Ab-treated mice

To investigate the effects of anti-CD70 Ab on joint histopathology associated with arthritis development, paws were dissected from anti-CD70 Ab and control Ab-treated mice, and stained with H&E. Sections were then evaluated in a blinded fashion for signs of arthritis. In agreement with the clinical signs of arthritis, the histological analysis also showed more severe histopathological features of arthritis development in the group treated with control Ab as compared with the anti-CD70 Ab-treated group. Representative histological images of joint pathology are shown in Fig. 4. The control Ab-treated animals had significant numbers of proliferating synoviocytes and infiltrating inflammatory cells in their affected joints. Pannus development was pronounced, and erosions of bone and cartilage were highly evident. In contrast, anti-CD70-treated animals had mild to no signs of inflammation in the joints. Pronounced differences were found in proliferative changes, in the development of joint ankylosis, joint erosion, and joint space narrowing. All of these changes were markedly inhibited in the anti-CD70 Ab-treated mice both before (Fig. 4) and after (data not shown) the onset of the arthritis, suggesting that anti-CD70 Ab inhibits both the clinical symptoms and underlying pathology of collagen-induced arthritis in this model.

In addition to the histological analysis of joint pathology, histology scores using a qualitative scale from 0 to 4 were assigned to individual limbs based on the degree of severity of joint destruction. Histology scores for each individual mouse (n = 10 for each group) are shown in Fig. 5A, and represent the mean score for the four limbs of a given animal (except in one case where it was the mean score of three limbs due to one limb being unprocessable) when treatment was started on day 21. Individual histopathology scores assigned to each separate limb of control Ab (n = 40) and anti-CD70 Ab treated mice (n = 39; one limb unprocessable) are shown in Fig. 5B. A significant reduction was seen in the mean of individual histopathology limb scores in the group that was treated with anti-CD70 Ab. A large number of limbs from mice in the anti-CD70-treated group were assigned 0 or 1 using the scoring system defined in the Materials and Methods indicating no lesions or minimal inflammation in this group. In contrast, the majority of limbs from mice in the control Ab-treated group were assigned a score of 3 or 4, indicating moderate to severe inflammation and erosion. Mean histology scores were also calculated from each treatment group and are presented along with individual scores in Fig. 5B. Again, a marked reduction of joint and bone destruction was observed in the paws of anti-CD70 Ab treated mice compared with the control Ab-treated group. This histological benefit was also seen when histology scores from mice treated with anti-CD70 Ab with established arthritis were examined (data not shown).

Effect of anti-CD70 treatment on the Ab response to collagen

To investigate the influence of anti-CD70 treatment on the humoral immune response to collagen, sera obtained from mice at day 46 after initiation of bovine CII immunization were analyzed for mouse CII-specific IgG Abs by a Luminex-based assay. Mice treated with anti-CD70 in the preclinical phase of the disease had significantly lower concentrations of anti-mouse CII IgG Abs compared with mice treated with control Ab (Fig. 6). Similar results were obtained when sera from mice that received anti-CD70 after the onset of CIA were tested (data not shown). These results suggest that anti-CD70 treatment of mice attenuated the development of CIA, at least in part, by inhibiting B cell functions and production of autoantibodies specific to collagen.

Discussion

A large body of research over the past three decades has provided many insights into the molecular and cellular mechanisms of pathogenesis and the inflammatory processes involved in the development of human RA (43–47). A number of cell types as well as inflammatory mediators, including tissue-damaging enzymes
and proinflammatory cytokines, have been implicated in orchestrating the inflammatory response leading to the pathology of human RA. In particular, activated lymphocytes are considered to be the central players in coordinating the inflammatory reaction of RA. Activated T cells are shown to provide an activating stimulus to B cells and macrophages, both by secretion of cytokines and by cell-to-cell contact (45). T cells are particularly important for induction of TNF-α251 in macrophages, an effector cytokine important in human RA. In the synovium, DCs, B cells, and other APCs act to maintain continued T cell activation (46, 47). These intriguing insights have lead to a number of immunotherapeutic approaches to target key players involved in the inflammatory response in human RA, resulting in the approval of some biologics for human RA, with continued development of other novel therapies (46, 48–50).

These approaches have provided new promise and opportunity to treat human RA. They also make us acutely aware, however, of some of the unanticipated aspects and limitations of these therapies. Despite the expected specificity of present biologic therapies, significant issues with toxicity, clinical efficacy, and pharmacokinetic effects still exist (46, 51). As the pathology of human RA is complex and many cellular and molecular players orchestrate the inflammatory response in the synovium, there is a need for additional therapies (46). In this respect, a novel approach targeting CD70 offers a unique opportunity for Ab-based immunotherapy. Targeting CD70 is particularly interesting because of its restricted expression pattern in normal cells and over expression in rheumatoid and psoriatic arthritis and lupus patients (1, 2, 35, 38). Under physiological conditions, expression of CD70 is mostly restricted to activated lymphocytes and DCs, and is transient in nature, waning with the removal of the antigenic stimulus. Thus, in contrast to many immunosuppressive agents and currently used immunotherapies to treat autoimmune diseases which exhibit considerable toxicity (46, 48), targeted therapy against CD70 offers a potential mechanism to selectively target only the activated cells of the immune system, and potentially avoid generalized immunosuppression and overt toxicity. Based on the biology of the CD70 pathway, Ab-based immunotherapy for depletion of CD70-expressing cells or blocking of CD27-CD70 interactions offers a viable approach for human RA, and perhaps other autoimmune and inflammatory indications.

The results presented in this study provide evidence of a novel role for CD70 in the development of CIA. Treatment of mice with anti-CD70 Ab in a mouse model of CIA resulted in marked improvements in clinical symptoms of disease, inhibition of autoantibody production, and reduction of inflammation and subsequent bone and cartilage destruction. From these findings, it is evident that CD27-CD70 interactions play a prominent role in the development of CIA. Thus, our studies provide in vivo proof-of-concept for the involvement of the CD27-CD70 pathway in the development of the inflammatory response leading to arthritis in mice, and support a strong rationale for using anti-CD70 as a potential therapy for autoimmune and inflammatory
anti-CD70 Ab may have depleted CD70 expressing activated B and T cells.

CD70 has also been shown to be expressed on APCs. For example, a novel population of CD70-expressing APCs residing exclusively in the gut lamina propria of mice has been described (34). These cells express CD70 constitutively and are capable of presenting Ags to mediate the expansion and differentiation of Ag-specific T cells in the gut mucosa. Although gut-associated CD70-expressing APCs have not been reported at this time in humans, these data nevertheless point to potential additional opportunities to target players of the innate immune system such as APCs by using CD70-directed immunotherapy. In addition to our data, those of others also support the use of CD70-directed therapy in autoimmune diseases and inflammatory conditions. For example, anti-CD70 treatment was also effective at inhibiting inflammatory bowel disease in a mouse model (56). Anti-CD70 treatment in this model was effective in prevention and reversal of colitis as well as in reduction of key Th1 cytokines associated with colitis. Likewise, blocking anti-CD70 Ab was also shown to inhibit Ig secretion by B cells induced by SLE patient T cells in vitro (37) and inhibit the onset of experimental autoimmune encephalomyelitis (32) and cardiac allograft rejection in vivo (33).

Although the exact nature of the inflammatory process in RA is unclear, significant progress in the understanding of the pathogenesis of RA has offered new opportunities for innovative therapies. Therapies targeted against molecules that are directly linked with the pathology of RA provide us with the means to develop more specific and efficacious therapeutics while avoiding the toxicity seen in some existing therapeutics. In this study, we have identified an important in vivo role for CD70 in CIA, and provide evidence for the efficacy of anti-CD70 Ab therapy in this model. Thus, therapeutic approaches designed to block CD27-CD70 interaction or deplete CD70 expressing immune cells may provide an additional means to treat autoimmune and inflammatory diseases and to prevent graft rejection. These strategies may include the use of blocking or depleting anti-CD70 Abs, development of small molecule antagonists, or recombinant soluble proteins that inhibit CD27-CD70 interaction. Alternatively, a strategy to block CD27 signaling events could also be developed. Although the results presented here reinforce the case for testing of anti-CD70 Abs in humans, the importance of CD70 in the pathology of RA and other inflammatory diseases can only be confirmed by the resolution of inflammatory disease following CD70-targeted therapy in the clinic.

Acknowledgments
We acknowledge the animal facility staff at Seattle Genetics for their expert help in maintaining our animal colony. We thank Martha Anderson, Dana Chace, Kim Kissler, Ivan Stone, Kacy Williams, and Gerald Nelsen for excellent technical help.

Disclosures
All authors are paid employees of Seattle Genetics, Inc.

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