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Chronic CD70-Driven Costimulation Impairs IgG Responses by Instructing T Cells to Inhibit Germinal Center B Cell Formation through FasL-Fas Interactions

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CD70 provides costimulation that enhances effector T cell differentiation upon binding of its receptor, CD27. During chronic immune activation, CD70 is constitutively expressed on activated immune cells, and this induces T cell-driven disruption of neutralizing Ab responses via an unknown mechanism. We used CD70-transgenic mice to investigate the effect of constitutive expression of CD70 on T cell-dependent B cell responses. CD70 induced up-regulation of the B cell follicle homing chemokine receptor CXCR5 on T cells, enabling not only CD4 but also CD8 T cells to infiltrate the B cell follicles. CD70-transgenic mice failed to develop productive germinal center formation and displayed impaired IgG Ab responses. Defective germinal center B cell differentiation was critically dependent on CD70-mediated CD27 signaling in T cells, and involved Fas-dependent impairment of germinal center B cell differentiation. Thus, CD70-driven costimulation enables T cells to terminate B cell responses, thereby compromising durable Ab production. Our findings imply that the CD70- and CD27-driven costimulatory axis may be involved in shutdown of B cell responses before clearance of Ag. Because CD70 is expressed constitutively in chronic viral infections such as HIV-1 infection, this mechanism may also contribute to defects in humoral immunity associated with this disease. The Journal of Immunology, 2009, 183: 6442–6451.

The development of neutralizing Abs is crucial in establishing pathogen clearance after primary infection and provides immediate and long-lasting protection against reinfection with the same pathogen. Upon Ag encounter, Ag-specific B cells start proliferating and subsequently differentiate into plasma cells that generally produce specific Abs of low affinity. However, some B cells initiate the formation of germinal centers where affinity maturation of Abs takes place. Germinal centers contain follicular dendritic cells that present Ags to B cells, and follicular helper T cells that provide help through CD40L-CD40 signaling and cytokines. Ag and CD4 T cell help is limiting, thereby enabling the selective outgrowth of B cells with high affinity for Ag and differentiation of these B cells into long-lived plasma cells that produce high-affinity Abs (1–3).

Neutralizing Abs develop poorly and late after infection with chronic viruses such as hepatitis C virus (HCV)3 (4, 5) and HIV-1 (6–8) and also after experimental infection of mice with persisting strains of lymphocytic choriomeningitis virus (LCMV) (9). Several factors contribute to the inefficient induction of neutralizing Abs such as low immunogenicity of viral structures (10), low precursor frequency of virus-specific B cells (11), and induction of T cell-driven immune pathology (9, 12, 13). CD4 T cells induce polyclonal B cell activation and hypergammaglobulinemia in chronic LCMV infection at the cost of the effective generation of neutralizing Abs (12). Also, CD8 T cells are involved in down-regulation of B cell responses in chronic LCMV infection, as CD8-deficient mice, or mice in which CD8 T cells have been depleted with Abs display induction of neutralizing Ab production (9, 13). Under homeostatic conditions, B cell follicles contain very low numbers of CD8 T cells, and the function of these CD8 T cells is unclear (14). In contrast, during chronic infection, such as in HIV-1 infected patients, CD8 T cells appear to accumulate within the B cell follicles (15). This indicates that cross-talk of not only CD4 but also CD8 T cells with B cells takes place in chronic infection and that this results in shutdown of effective B cell responses.

Several diseases characterized by chronic infection or chronic inflammation, such as HIV-1 or chronic LCMV infection (16, 17), systemic lupus erythematosus (18), or rheumatoid arthritis (19), are accompanied by constitutive up-regulation of CD70 expression. CD70 is a molecule of the TNF superfamily that promotes proliferation and acquisition of effector function of T cells after binding CD27. Constitutive expression of transgenic CD70 on B cells (20), dendritic cells (21) or T cells (22) results in extensive formation and accumulation of effector T cells, in vivo. The role of chronic CD70 and CD27 signaling in the regulation of B cell responses through costimulation of T cells is unsettled. To study the impact of constitutive costimulation through CD70 and CD27 on the ability of T cells to modulate B cell responses, we used CD70-transgenic (Tg) mice. Previously, we have shown that reverse signaling through CD70 in B cells strengthens IgM Ab responses but
prevents B cells from undergoing isotype switch to IgG (23). To minimize influence of CD70-induced reverse signaling on B cells, we here made use of CD70-T cell Tg mice that specifically express CD70 on T cells. We found that CD70-driven costimulation reduced IgG but not IgM Ab responses. The pathway involved entry of effector CD4 and CD8 T cells into B cell follicles and impairment of germinal center (GC) B cell formation through a T-cell- and Fas-dependent mechanism.

Materials and Methods

Animals

Wild-type (WT), Lymy31, CD70-T cell Tg, IFN-γ−/− (24), IFN-γ−/−×CD70-T cell Tg, CD27−/− (25), CD27−/−×CD70-B cell Tg, LPR (26), and LPR×CD70-T cell Tg mice were all maintained on a C57BL/6 background. CD70-T cell Tg mice were generated by introduction of the murine CD70 gene under control of the human CD2 promoter to induce constitutive CD70 expression on T cells (22) and CD70-B cell Tg mice were generated by introduction of the murine CD70 gene under control of the human CD19 promoter to induce constitutive CD70 expression on B cells (20). IFN-γ−/− and LPR mice were obtained from The Jackson Laboratory. IFN-γ−/−×CD70-T cell Tg CD27−/−×CD70-B cell Tg and LPR×CD70-T cell Tg mice were generated by crossing CD70-T cell Tg mice or CD70-B cell Tg mice with IFN-γ−/−, CD27−/−, or LPR mice. Mice were bred under specific-pathogen-free conditions at the animal department of the Academic Medical Center (Amsterdam, The Netherlands) under institutional and national guidelines. Mice were strictly age matched within individual experiments and were between 8 and 20 wk of age at the start of the experiment.

Abs and reagents

The following mAbs from eBioscience were used: anti-CD3 (145-CD11); anti-CD4 (RM4-5 and MT4); anti-CD8 (53-6.7; 28.1a); anti-CD21/35 (7G6); anti-CD23 (B3B4); anti-CD138 (281-2); anti-CD70 (FR70); anti-GL-7 (Ly-77) and anti-B220 (RA3–6b2). Anti-CD21/35 (7G6), anti-CD23 (B3B4), anti-CD138 (281-2), anti-CD70 (FR70); anti-GL-7 (Ly-77) and anti-B220 (RA3–6b2). Anti-CD70-driven costimulation down-regulates isotype switched Ab responses. Tissue sections were mounted with Kaiser’s glycerin (Merck) and analyzed using an Olympus light microscope.

Adoptive transfer

B cells were isolated from spleen to >95% purity using anti-CD19 microbeads. Similarly, total T cells were isolated from spleen and pooled LNs (cervical, axillary, brachial, and mes LN) to >95% purity using anti-CD4 and anti-CD8 microbeads (Miltenyi Biotech). Approximately 10×10⁶ B or T cells in 200 μl of PBS were injected into the tail vein of recipient mice.

Influenza infection

Mice were intranasally infected with 10×50% tissue culture infectious dose of the H1N1 influenza A/PR8/34 strain (H1N1 subtype) and 10×50% tissue culture infectious dose of the H3N2 influenza A virus HKx31. At the indicated days after infection, mice were sacrificed, and spleen, med LN, and serum were obtained to determine levels of influenza-specific Abs or numbers of GC B cells.

Trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) immunization

Mice were immunized i.p. with 25 μg of TNP-KLH (Biosearch Technologies) in IFA. At day 14, mice were sacrificed, and serum was obtained to determine levels of TNP-specific Abs. To provide help for B cells through CD40 100 μg of agonistic anti-CD40 Abs (FGK45; Bioceros) were administered by i.v. injection at the time of immunization.

ELISA

The levels of influenza-specific and TNP-specific Abs were determined using ELISA in the serum of influenza-infected or TNP-KLH-infected mice, respectively. ELISA plates (Nunc Maxisorp flat-bottom 96-well plates; Nalgene) were coated overnight at 4°C with influenza virus or TNP-BSA (Biosearch Technologies), blocked with PBS containing 2% milk for 1 h at room temperature, and incubated with 3-fold serial dilutions of the serum of infected or immunized mice for 3 h at room temperature. IgM, IgG1, IgG2a, and IgG2b Abs directed against influenza or TNP were detected using biotinylated anti-mouse isotype-specific Abs (Southern Biotech) and alkaline phosphatase-conjugated streptavidin (Jackson Immunoresearch Laboratories). ELISA plates were developed using p-nitrophenyl phosphate (Sigma-Aldrich) and analyzed on a microplate reader (Bio-Rad) at 415 nm.

Cell culture

To examine FasL expression on CXCR5+ T cells, total splenocytes were cultured overnight at 37°C in round-bottom 96-well plates (Coming) in RPMI, 10% FCS with or without 10 ng/ml PMA (Sigma-Aldrich) and 1 μM ionomycin (Sigma-Aldrich), and FasL expression was determined using flow cytometry.

To study in vitro B cell differentiation, B cells were isolated from spleen with >95% purity using magnetic cell sorting with anti-CD19 microbeads (Miltenyi Biotech). To generate B cells with GC-like phenotype, isolated B cells were cultured for 3 days at 37°C in round-bottom 96-well plates in RPMI, 10% FCS containing 5 μg/ml LPS (Sigma-Aldrich), 5 μg/ml anti-IgM (HB88), 10 ng/ml IL-2 (Inviogen), 20 ng/ml IL-4 (R&D Systems), and/or 20 μg/ml anti-CD40 (FGK45; Bioceros).

Statistical analysis

Values are means and SEM (error bars). To analyze statistical significance, Student’s t test was used. A value of p < 0.05 was considered statistically significant.

Results

CD70-driven costimulation down-regulates isotype switched Ab responses

To study the effect of CD70-driven costimulation on T cell-dependent B cell responses, we used the influenza model of acute viral infection. WT and CD70-T cell Tg mice were infected with influenza virus and analyzed for influenza-specific Ab responses in their sera. We found that IgM responses against influenza were similar in magnitude in WT and CD70-T cell Tg mice at day 14 (Fig. 1A). In contrast, levels of influenza-specific IgG1 Abs were lower in CD70-T cell Tg mice than in WT mice at this time point (Fig. 1B). In WT mice, influenza-specific Abs of IgM isotype...
waned over time, whereas levels of isotype-switched Abs remained stable for months (Ref. 27 and Fig. 1, C and D). However, levels of influenza-specific IgG1 isotype Abs in CD70-T cell Tg mice were strongly reduced at day 28 (Fig. 1C) and almost completely absent by day 94 (Fig. 1D). IgG2a and IgG2b Ab responses were similarly impaired in CD70-T cell Tg mice compared with WT mice (unpublished data). CD70-driven costimulation also reduced levels of mucosal Abs of IgA isotype within the lungs (unpublished data). This shows that CD70 down-regulates isotype-switched Ab responses against acute viral infection with influenza, in particular at late time points, and that this occurs in the absence of reverse signaling through CD70 in B cells.

Ab responses in CD70-T cell Tg mice are not regulated through B cell numbers

CD70-B cell Tg mice that express CD70 on B cells have severely reduced numbers of peripheral B cells (20). We found that CD70-T cell Tg mice also had lower levels of follicular but not marginal zone B cells than did WT mice within the spleen. However, the reduction in follicular B cell numbers was only ∼2- to 3-fold compared with >95% in CD70-B cell Tg mice (Fig. 2A and Ref. 20). IFN-γ plays a crucial role in the reduction of B cell numbers in CD70-B cell Tg mice (20). Crossing of CD70-T cell Tg mice onto IFN-γ−/− background completely rescued B cell numbers in the spleen, demonstrating that B cell development is also blocked through an IFN-γ-dependent pathway in CD70-T cell Tg mice (Fig. 2A). To establish whether the lower numbers of B cells were responsible for the compromised Ab responses in CD70-T cell Tg mice, we infected IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice that have normal numbers of B cells with influenza. Serum levels of influenza-specific IgG1 Abs were still impaired through CD70-driven costimulation in the absence of IFN-γ (Fig. 2B). To exclude that any of the effects that we observed were related to

FIGURE 1. CD70-driven costimulation impairs IgG but not IgM responses upon acute influenza infection. WT and CD70-T cell Tg mice were infected with influenza (A/PR8/34), and serum was obtained to determine levels of influenza-specific Abs using ELISA. Influenza-specific Abs of IgM isotype were analyzed at day 14 (A) and influenza-specific Abs of IgG1 isotype were analyzed at day 14 (B), day 28 (C), and day 94 (D).

FIGURE 2. CD70 does not regulate Ab responses through reduction of B cell numbers or through blockade of CD40L-mediated T cell help. A, Absolute numbers of B cell subsets were analyzed within the spleen using flow cytometry for expression of CD21/35 and CD23 to identify CD21/35highCD23low follicular B cells and CD21/35lowCD23high marginal zone (MZ) B cells in WT and CD70-T cell Tg animals (left) and in IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice (right). B, IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice were infected with influenza (HKx31), and 14 (left) and 56 days (right) later, serum levels of influenza-specific Abs of IgG1 isotype were determined using ELISA. C, WT and CD70-T cell Tg animals (C) or IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice (D) were immunized with the T cell-dependent Ag TNP-KLH. E, WT and CD70-T cell Tg animals were also immunized with TNP-KLH in the presence of agonistic anti-CD40 Abs. Serum titers of IgG1 IgM directed against TNP were determined at day 14 after immunization using ELISA. **, p < 0.005.
differences in viral clearance, we also immunized mice with the T cell-dependent Ag TNP-KLH. Underscoring the Ab responses against influenza, also upon immunization with TNP-KLH, we observed a decrease in TNP-specific IgG1 levels that was similar for CD70-T cell Tg compared with WT mice and for IFN-γ−/− mice (Fig. 2, C and D). The formation of large GCs in the spleen of influenza-infected WT and CD70-T cell Tg mice compared with WT mice (Fig. 3A). Staining for FAS and PNA, which are both highly and specifically expressed on GC B cells (39, 40), corroborated these findings (unpublished data). GC B cell numbers were also significantly lower in the spleen and mes LN of IFN-γ−/− × CD70-T cell Tg mice compared with IFN-γ−/− mice (unpublished data).

To investigate de novo generation of GC B cells, we infected WT and CD70-T cell Tg mice with influenza and analyzed formation of CD38lowGL-7high GC B cells within the mediastinal LN (med LN), the draining LN of the lungs. The med LN contained low numbers of GC B cells before infection, but WT animals had accumulated high levels of GC B cells at day 10 after infection (Fig. 3D). As observed for the med LN, numbers of GC B cells in the spleen of influenza-infected CD70-T cell Tg mice was strongly reduced compared with WT mice (Fig. 3D). Taken together, this shows that CD70 driven costimulation impairs the differentiation of GC B cells, thereby barring the establishment of full-size GCs.

Extrinsic and not intrinsic factors regulate GC B cell development in CD70-T cell Tg mice

To examine whether the impaired formation of GC B cells was caused by an intrinsic B cell defect in CD70-T cell Tg mice, we isolated B cells from spleen and exposed them in vitro cultures
to stimuli that activate B cells and that have been shown to drive formation of GC B cells in vivo. Stimulation of B cells with anti-IgM, IL-2, and IL-4 in the presence of LPS and or anti-CD40 induces differentiation into B cells with a GC-like phenotype as identified by generation of CD38lowGL-7high B cells after 3 days of culture (41). We found that equal numbers of B cells with a GC-like phenotype developed from B cells of WT and CD70-T cell Tg mice under all culture conditions (Fig. 4, A and B). This demonstrates that B cells of CD70-T cell Tg mice do not have an intrinsic defect in their ability to differentiate into GC-like B cells in vitro, indicating that external factors are crucial for impairment of GC B cell formation in CD70-T cell Tg mice in vivo.

To examine whether B cell-extrinsic factors impair GC development in CD70-T cell Tg mice in vivo, WT B cells were adoptively transferred into WT and CD70-T cell Tg animals that were subsequently infected with influenza (Fig. 4C). Follow-up of WT donor B cells in spleen revealed that they differentiated into GC B cells upon influenza infection in WT hosts, but not in CD70-T cell Tg hosts (Fig. 4, D and E). GC B cell differentiation of WT donor B cells was equally impaired to that of host B cells in recipient CD70-T cell Tg mice (Fig. 4, D and E). This shows that CD70 driven costimulation impairs GC B cell differentiation through a B cell extrinsic rather than a B cell-intrinsic pathway.

CD70 induces formation of CXCR5+ and B cell follicle homing CD4 and CD8 T cells

Costimulation through CD70 and CD27 triggers proliferation and effector differentiation of naïve T cells. This is exemplified by enhanced numbers of effector memory (EM) CD4 and CD8 T cells in CD70-T cell Tg mice (20–22). We made use of CD70-T cell Tg mice on an IFN-γ background to study T cell infiltration into the B cell follicles, because they have a B cell compartment of normal size. Similar to CD70-T cell Tg mice, IFN-γ−/− × CD70-T cell Tg mice had more EM CD8 T cells than IFN-γ−/− mice (Fig. 5A). To determine whether EM T cells were able to interact with B cells, we analyzed expression of chemokine receptors that are involved in homing of T cells into B cell follicles. Migration into B cell follicles requires a specific profile of chemokine receptor expression, most importantly high expression of CXCR5 and low expression of CCR7 (42). We found that not only the EM CD4 T cell population but also the EM CD8 T cell population of IFN-γ−/− × CD70-T cell Tg mice had more EM CD8 T cells than IFN-γ−/− mice (Fig. 5A). To determine whether EM T cells were able to interact with B cells, we analyzed expression of chemokine receptors that are involved in homing of T cells into B cell follicles.

FIGURE 4. Differentiation of GC B cells is not intrinsically defective in CD70-T cell Tg mice. A and B, B cells of spleen from WT and CD70-T cell Tg mice were cultured for 3 days in the presence of IL-2, IL-4, and anti-IgM (depicted as medium) together with the indicated stimuli to induce differentiation into B cells with GC-like phenotype. Percentages (A) and absolute numbers (B) of GC-like B cells cultured from 0.2 × 10^6 B cells were determined using flow cytometry for CD38 and GL-7. C–E, Spleen-derived B cells of Ly5.1 WT mice were transferred into Ly5.2 WT and CD70-T cell Tg recipients. After 1 day, recipient mice were infected with influenza (HKx31), and formation of Ly5.1+ host and Ly5.1+ donor GC B cells was analyzed within the spleen using flow cytometry for expression of CD38 and GL-7 on B cells 10 days later. C, Schematic representation of the experiment is shown to study the effect of CD70 costimulation on adoptively transferred B cells upon influenza infection. D, Expression of Ly5.1 was used to analyze donor and host B cells in spleen of WT and CD70-T cell Tg mice that received Ly5.1+ WT donor B cells (left). The percentage of GC B cells within the host and donor B cell population was determined using flow cytometry for CD38 and GL-7 (right panels). E, Absolute numbers of GC B cells were determined in noninfected (left) and influenza-infected WT and CD70-T cell Tg mice (right). Host and donor-derived GC B cells are depicted separately. **, p < 0.005.
FIGURE 5. CD70-driven costimulation up-regulates CXCR5 and stimulates T cells to infiltrate B cell follicles. A, FACS plots display CD44 and CD62L expression of CD4 (top left) and CD8 T cells (top right) to visualize the percentage of CD44highCD62Llow EM T cells in IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg spleen. CXCR5 and CCR7 expression is shown of EM cells (bottom left) and CD8 T cells (bottom right) from IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice to determine the percentage of EM T cells with the potential to enter the B cell follicles. Insets depict percentage of cells within quadrant. B, The absolute number of CXCR55α5CCR7−/− EM CD4 and CD8 T cells was determined in IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice. C and D, To examine the number of CD4 and CD8 T cells that were present within the B cell follicles, immunohistochemistry was employed for B220 (red) together with either CD4 or CD8 (both in green) on spleen of IFN-γ−/− and IFN-γ−/− × CD70-T cell Tg mice. C, Representative pictures were taken; D, the number of CD4 and CD8 T cells within the B cell follicles was quantified. **, p < 0.005.

Consistent with the chemokine receptor profile, we found that more CD4 T cells were present in the B cell follicles of IFN-γ−/− × CD70-T cell Tg mice than in those of IFN-γ−/− mice (Fig. 5, C and D). CD8 T cells were virtually absent from B cell follicles of IFN-γ−/− mice, but surprisingly CD8 T cells were abundantly present in the B cell follicles of IFN-γ−/− × CD70-T cell Tg mice (Fig. 5, C and D). Thus, CD70-driven costimulation induces formation of EM T cells with the ability to infiltrate the B cell follicles. This triggers higher numbers of CD4 T cells and in particular of CD8 T cells within the B cell follicles, indicating that cross-talk between T and B cells may occur.

CD70 instructs T cells to impair formation of GC B cells

To determine whether CD70 triggered formation of B cell follicle-homing T cells through direct interactions with CD27 on T cells, we adoptively transferred WT CD4 and CD8 T cells into CD27−/− × CD70-B cell Tg mice (Fig. 6A). CD70-B cell Tg mice crossed with CD27−/− mice do not have a phenotype but are able to provide CD70-driven costimulation specifically to WT donor T cells (20). Analysis of CXCR5 expression revealed enhanced numbers of CXCR55 Tg donor CD4 and CD8 T cells in spleen and mes LN of CD27−/− × CD70-B cell Tg hosts compared with CD27−/− hosts (Fig. 6, B and C). To establish T cell entry into B cell follicles, we performed immunohistochemistry and found higher numbers of donor T cells within the B cell follicles of CD27−/− × CD70-B cell Tg hosts than within those of CD27−/− hosts (Fig. 6D). This indicates that CD70 directly acts on T cells to induce the capacity to migrate into the B cell follicles. To examine the impact on GC B cell differentiation, we analyzed the numbers of host GC B cells in spleen and mes LN of CD27−/− × CD70-B cell Tg and CD27−/− mice. Before adoptive transfer of T cells, these mice contain numbers of GC B cells comparable with those of WT animals (unpublished data). We found that CD27−/− × CD70-B cell Tg hosts in contrast to CD27−/− hosts had diminished numbers of GC B cells upon adoptive transfer of WT T cells (Fig. 6E).

This shows that CD70-induced triggering of CD27 on T cells is sufficient to impair GC B cell differentiation.

Fas mediates CD70-driven impairment of GC B cell formation

The disappearance of a large proportion of the GC B cell population in CD70 Tg mice compared with WT mice may reflect apoptosis-dependent removal. To investigate this, we determined expression of annexin V on GC B cells of WT mice and on the few remaining GC B cells of CD70 Tg mice. We observed an enhanced percentage of annexin V+ GC B cells in CD70 Tg mice compared with WT mice, indicating that they are being removed through apoptosis (Fig. 7A).

An important pathway of apoptosis that is involved in selection of GC B cells with high Ag affinity is mediated through Fas (43). GC B cells uniformly have high expression of Fas in contrast to other B cells. Fas-dependent apoptosis in GC B cells may be induced directly by FasL-mediated triggering, because the death-inducing signaling complex of Fas is preformed and fully functional in GC B cells (44, 45). To analyze whether T cells used FasL to impair differentiation of GC B cells, we crossed CD70-T cell Tg mice onto Fas-deficient LPR mice. We determined expression of FasL on CXCR55 T cells of mice with Fas-deficient background to avoid shedding of FasL after binding to Fas. Expression of FasL was low on CXCR55 CD4 and CD8 T cells, but up-regulated after 5 h of culture in the presence of PMA and ionomycin, and higher expression levels of Fasl were present on CXCR55 CD4 and CD8 T cells of LPR × CD70-T cell Tg mice than of LPR mice (Fig. 7B). To examine whether Fas was involved in impaired development of GC B cells, we analyzed GC B cell numbers in influenza-infected CD70-T cell Tg mice on WT and LPR background. Upon influenza infection high numbers of GC B cells were detected within the med LN and spleen of LPR × CD70-T cell Tg mice in contrast to CD70-T cell Tg mice (Fig. 7C). Numbers of GC B cells were restored to WT and LPR levels in LPR × CD70-T cell Tg mice (Fig. 7C). This demonstrates that under CD70-driven co-stimulation Fas impairs differentiation of GC B cells. Consistent
with rescue of GC B cell formation, we observed that at late time points upon influenza infection IgG2a isotype Abs directed against influenza were abundantly present in LPR/CD70-T cell Tg mice in contrast to CD70-T cell Tg mice (Fig. 7D). Although we found that maintenance of influenza-specific IgG2a Ab production was rescued in CD70-T cell Tg mice upon deletion of Fas, we did not observe this for Abs of IgG1 or IgG2b isotype (unpublished data). Possibly, constitutive expression of CD70 induces Th1 polarization and this triggers B cells to produce Abs of IgG2a isotype, as these have been strongly associated with Th1 responses (46). Indeed, Th1 skewing has been demonstrated as a direct consequence of CD70 triggering of CD27 (47), and moreover, effector CD4 and CD8 T cells of CD70-T cell Tg mice produce large amounts of the
Th1-polarizing cytokine IFN-γ (20, 48). Thus, CD70-driven costimulation up-regulates FasL on T cells with the ability to enter the B cell follicles, and this may enable these T cells to obstruct GC B cell formation in a Fas-dependent manner to terminate Ab responses.

Discussion

In this study, we demonstrate that CD70 negatively impacts B cell responses through induction of B cell follicle homing T cells that obstruct differentiation of GC B cells. CD70-induced triggering of CD27 on T cells resulted in up-regulation of CXCR5 and down-regulation of CCR7. This promoted infiltration of high numbers of effector memory CD4 and CD8 T cells into the B cell follicles. We showed that CD70 up-regulated FasL on T cells and enabled T cells to impair formation of GC B cells. The underlying mechanism involved FasL and Fas, which have been implicated in apoptosis of GC B cells. CD70-driven obstruction of GC B cell development was incompatible with the formation of robust IgG Ab responses, and in particular, long-term maintenance of IgG Ab responses was impaired.

In humans, CD27 is highly expressed on memory B cells and in vitro experiments have shown that triggering of CD27 on B cells drives differentiation into Ab-secreting plasma cells (49, 50). In mice, expression of CD27 on B cells is restricted to a subpopulation of GC B cells at the centroblast stage (51). It has previously been observed that GC B cell responses against influenza virus were partially dependent on CD27, although this did not have a significant impact on Ab production, somatic hypermutation, and isotype switch (51). Using adoptive transfers, it was shown that expression of CD27 on T as well as B cells positively contributed to the GC B cell response. This contrasts with our findings as we find that the introduction of constitutive expression of CD70 negatively impacts GC B cell responses against influenza. Moreover, our adoptive transfer experiments of WT T cells into CD70-B cell Tg mice on CD27−/− background also showed that expression of CD27 on T cells rather than B cells is sufficient to impair formation of GC B cells. A possible explanation for this discrepancy on GC B cell responses is the level and timing of CD70 expression that is present during influenza infection. We have previously proposed that the effect of CD70 on immune responses resembles a bell-shaped curve, because CD70 provides costimulation under transient expression, whereas it is countereffective during chronic expression (52). The CD70-driven enhancement of GC B cell responses depended on endogenous CD70 expression during influenza infection that is both low and transient (51, 53), whereas our studies were performed in CD70−T cell Tg mice that have constitutive and high CD70 expression. Thus, depending on expression levels CD70 may positively and negatively regulate GC B cell responses. This may reflect situations that occur in acute and chronic infection, respectively, because CD70 is transiently expressed in acute infection and constitutively expressed in chronic infection.

The maintenance of follicular memory CD4 T cells in contrast to other memory CD4 T cells is independent of homeostatic cytokines such as IL-7 and IL-15 and is driven by Ag that may persist for prolonged periods in B cell follicles on follicular dendritic cells (54). Ag-driven maintenance of follicular CD4 T cells may explain the effectiveness of costimulatory molecules on helper T cell responses such as the closely related TNF-like molecules OX40L, 4-1BB, and CD70. OX40L regulates influx of follicular CD4 T cells into B cell follicles through induction of CXCR5 expression, thereby enhancing GC formation and T cell-dependent Ab production (55, 56). This contrasts with the role that we observed for CD70 that induced entry of not only CD4 but also CD8 T cells within the B cell follicles. Cross-talk between follicular CD4 T cells and B cells depends on Ag-specific interactions. Cognate interactions of CD8 T cells with B cells may require cross-presentation. Indeed, the ability of cross-presentation has been attributed to B cells, enabling them to activate naïve CD8 T cells, although DCs are by far superior to B cells at cross-priming (57, 58). This may partly stem from limited expression of costimulatory molecules on B cells rather than the ability to direct Ags into the pathway of cross-presentation. Possibly, B cells cross-present Ags not only to prime CD8 T cells but also to allow Ag-specific CD8 T cells to eliminate cross-presenting B cells. Indeed, in contrast to OX40L, CD70-driven costimulation resulted in down-regulation of GCs and Ab responses. 4-1BB promoted infiltration of B cell follicles by CD4 and CD8 T cells as well, and this also triggered inhibition of T cell-dependent humoral responses (59, 60). In contrast to CD70, 4-1BB down-regulated Ab production through CD4 and CD8 T cell-mediated destruction of follicular dendritic cell networks in B cell follicles rather than through a GC B cell-dependent pathway (60). Thus, the TNF-related molecules regulate different aspects of T cell help in B cell responses to up-regulate or down-regulate T cell-dependent Ab production.

We found that CD70 increased up-regulation of FasL on T cells and that the disappearance of GC B cells in CD70−T cell Tg mice was T cell- and Fas-dependent. This indicates that CD70 instructs T cells to actively kill GC B cells through FasL and Fas. Although the involvement of the proapoptotic molecule Fas makes the induction of GC B cell apoptosis upon CD70-driven costimulation of T cells highly likely, we have not been able to directly show that GC B cells are removed through apoptosis. The Fas pathway of apoptosis is reportedly involved in the selection process of high-affinity B cell clones through the elimination of GC B cells with low Ag affinity (43), although also a role for the BH3-only proapoptotic factor Bim has been shown (61). We do not know whether costimulation through CD70 and CD27 regulates Fas-dependent selection of high-affinity B cell clones. Recently, it has become clear that Fas-dependent apoptosis of GC B cells is also of paramount importance for timely shutdown of B cell responses (62). The main problem with persistence of B cell responses is prolonged B cell-driven activation of T cells, resulting in excessive and lethal accumulation of activated T cells (62). Thus, CD70-driven costimulation may engage the Fas pathway of apoptosis to down-regulate GC B cell-dependent Ag presentation and activation of T cells. This may limit overactivation of B and T cell responses in the presence of persistent Ag, such as during chronic infection or autoimmune disease.

The phenotype of CD70−T cell Tg mice resembles the immune pathology that develops in chronic infection such as with HIV-1 or HCV and in experimental infection with chronic LCMV. Ag-driven turnover of CD8 T cells until functional exhaustion or depletion ensues occurs in chronic infection as well as in CD70-B cell Tg and CD70−T cell Tg mice (22, 63, 64). Moreover, similar to those in CD70−T cell Tg mice, B cell responses are suboptimal in mice chronically infected with LCMV and in the majority of HIV-1 and HCV patients (4–9). Expression levels of CD70 are constitutively increased during chronic infection (16, 17), indicating that CD70-driven costimulation may contribute to impairment of B cell responses. Effector CD4 and CD8 T cell responses have been shown to be involved in down-regulation of virus-neutralizing Ab responses in chronic LCMV (9, 12, 13). Also in HIV-1 infection, infiltration of B cell follicles with effector CD8 T cells has been observed, and they may be involved in destruction of LN architecture, as occurs in HIV-1-infected patients during progression to AIDS (15). Moreover, B cells in HIV-1-infected patients have a high turnover; this has been attributed at least in part to increased dysfunction. 

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susceptibility to Fas-mediated cell death (65, 66). This is compatible with events in CD70-T cell Tg mice, indicating that CD70-driven influx of effector T cells may contribute to disruption of B cell responses in chronic infection. However, there are also notable distinctions in B cell responses of chronic infection and CD70-T cell Tg mice. In chronic infection, virus-specific helper T cells stimulate naive nonspecific B cells to produce Abs, resulting in polyclonal activation of B cells and hypergammaglobulinemia of Abs that are not directed against the virus (67). We did not observe hypergammaglobulinemia in CD70-T cell Tg mice either under homeostatic conditions or upon immunization or infection with influenza (unpublished data). Thus, although constitutive expression of CD70 may contribute to some defects in B cell responses against chronic infection, it is not involved in the induction of hypergammaglobulinemia.

CD70-driven immune activation has been shown to negatively impact B cell responses in chronic LCMV infection, given that CD27−/− mice establish viral clearance through rescue of the production of neutralizing Abs (16). However, the reported mechanism involved enhanced IFN-γ and TNF-α production by CD4 T cells that triggered disruption of the B cell follicles (16). We have previously shown that CD70 in CD70-B cell Tg mice up-regulates IFN-γ production in effector T cells as well (20). This resulted in reduction of the peripheral numbers of follicular B cells through a blockade in B cell differentiation but did not completely disrupt spleen architecture in CD70-B cell Tg mice (20). Indeed, enhanced IFN-γ production is not solely responsible for CD70-driven impairment of B cell responses in CD70-B cell Tg mice, because reduced IgG production in CD70-B cell Tg mice does not normalize in the absence of IFN-γ (23). We were able to restore IgG2a production in CD70-T cell Tg mice on Fas-deficient background, indicating that CD70 may act at multiple levels to down-regulate Ab responses and that the Fas-dependent mechanism is dominant in CD70-T cell Tg mice.

Ab-inducing vaccination protocols have proven insufficient in therapeutic intervention such as in chronic infection with HIV-1 and HCV. This has fueled development of CD8 T cell-based vaccination protocols that unfortunately have been equally unsuccessful (68). The problems encountered in therapeutic intervention may arise at least in part from the inability to induce neutralizing Abs or strong CD8 T cell memory responses (69, 70). Although prime-boost strategies have recently been advocated as a powerful means to strengthen CD8 T cell memory responses (71), their use in treatment of HIV-1 infection remains to be investigated. Therapeutic vaccination may be more challenging, given that highly effective vaccines such as that against varicella zoster have a reduced success rate in HIV-1 patients (72, 73). Negative feedback of T cells on B cell responses in chronic infection potentially interferes with vaccination strategies that aim to induce B cell responses. On the other hand, CD8 T cell-inducing vaccination protocols such as the newly advanced prime-boost strategy may prove incompatible with or further reduce B cell-driven immunity, if they induce CD70-mediated GC B cell destruction. This may indicate that impairment of Ab production through B cell follicle-homing T cells holds similar promise as a mechanism in chronic infection that is applicable as a target for therapeutic intervention.

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Disclosures
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