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CD27–CD70 interactions regulate B-cell activation by T cells

(tumor necrosis factor receptor family/immunoregulation)

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ABSTRACT **CD27, a member of the tumor necrosis factor (TNF) receptor family, binds to its ligand CD70, a member of the TNF family, and subsequently induces T-cell costimulation and B-cell activation. CD27 is expressed on resting T and B cells, whereas CD70 is expressed on activated T and B cells. Utilizing transfected murine pre-B-cell lines expressing human CD27 or CD70, we have examined the effect of such transfectant cells on human B-cell IgG production and B-cell proliferation. We show that the addition of CD27-transfected cells to a T-cell-dependent, pokeweed mitogen-driven B-cell IgG synthesis system resulted in marked inhibition of IgG production, whereas the addition of CD70-transfected cells enhanced IgG production. The inhibition and enhancement of pokeweed mitogen-driven IgG production by CD27 and CD70 transfectants were abrogated by pretreatment with anti-CD27 and anti-CD70 monoclonal antibodies, respectively. In contrast, little or no inhibition of IgG production and B-cell proliferation was noted with CD27-transfected cells or either anti-CD27 or CD70 monoclonal antibody in a T-cell-independent, *Staphylococcus aureus*/interleukin 2-driven B-cell activation system. In this same system CD70-transfected cells enhanced B-cell IgG production and B-cell proliferation, and this enhancement could be gradually abrogated by addition of increasing numbers of CD27-transfected cells. These results clearly demonstrate that interactions among subsets of T cells expressing CD27 and CD70 play a key role in regulating B-cell activation and immunoglobulin synthesis.**

Ligand–receptor interactions of the tumor necrosis factor (TNF) and TNF receptor families are essential in the regulation of cell growth, differentiation, and cell death (1, 2). CD27 is a disulfide-linked 120-kDa type I transmembrane glycoprotein belonging to the TNF receptor family (1, 2) and is expressed by the majority of peripheral T cells (3–6), medullary thymocytes (5, 7), natural killer cells (8), and B cells (9). CD70, a ligand for CD27, is a type II transmembrane glycoprotein belonging to the TNF family (1, 2) and is expressed by activated T and B cells (10–14).

T-cell-dependent B-cell proliferation and differentiation require both cytokines and physical cell–cell contact (15). Various ligand–receptor interactions involved in T/B cell–cell contact are thought to play a critical role in B-cell activation, including CD54–CD2, CD5–CD72, CD28–B7, and CD40–CD40L (15, 16).

In earlier studies, we showed that the addition of either the 1A4 anti-CD27 monoclonal antibody (mAb) or recombinant soluble CD27 could inhibit T-cell-dependent pokeweed mitogen (PWM)-driven B-cell production of IgG (4, 17). Further studies indicated that CD27 was preferentially expressed on $CD45RA^+$ T cells, whereas CD70 was detected at high levels on $CD45RO^+$ T cells only after activation (18, 19). $CD4^+$-$CD45RA^+$ T cells were also shown to be capable of suppressing PWM-driven B-cell IgG production, whereas $CD4^+$ $CD45RO^+$ T cells were capable of providing helper function for B-cell IgG production (20). In support of the latter observation was the demonstration that the addition of $CD45RA^+$ T-cell clones to the PWM-driven B-cell IgG synthesis system inhibited IgG production, whereas the addition of $CD45RO^+$ T-cell clones enhanced IgG production (18). Moreover, the inhibition of B-cell IgG production by $CD45RA^+$ T-cell clones was abrogated by pretreatment with 1A4 anti-CD27 mAb (18), suggesting that CD27 was somehow involved in downregulation of IgG production by $CD45RA^+$ T-cell clones. Our more recent studies showing the differential expression of CD27 and CD70 on subsets of CD4 T cells led us to suggest that the presence of CD27 on $CD4^+CD45RA^+$ cells not only permitted interaction with CD70 on $CD4^+$-$CD45RO^+$ helper cells but also regulated the ability of these cells to activate B cells.

Given the potential importance of CD27 and CD70 in defining functionally important subsets of T cells, we have performed a series of experiments utilizing CD27 and CD70 transfectants to investigate T-cell-dependent and T-cell-independent B-cell proliferation and IgG synthesis. The studies reported here show that interactions among populations of cells expressing $CD27^+$ and $CD70^+$ play an important role in regulating B-cell activation. Moreover, the data support the view that the CD27 and CD70 antigens not only serve as markers for subsets of CD4 cells but, more importantly, are clearly involved in the helper/suppressor signal itself.

MATERIALS AND METHODS

Cells. Peripheral blood mononuclear cells were isolated from healthy donors by density gradient centrifugation and separated with 5% sheep erythrocytes into erythrocyte rosette-positive (E^+) and -negative (E^-) populations (20). E^- cells were then depleted of monocytes by adherence to the plastic surface of tissue culture dishes and further purified into B cells by negative selection with the use of mAbs (anti-CD3, anti-CD14, and anti-CD56) and goat anti-mouse IgG-coated immunomagnetic beads (Advanced Magnetics, Cambridge, MA). The B-cell population thus obtained was >95% reactive with the anti-CD20 mAb.

To develop the CD27 cDNA transfectant cells, CD27 cDNA (kindly supplied by B. Seed, Massachusetts General Hospital, Boston) (21) was subcloned into the mammalian expression vector pcDL-SRα296 (22). The murine pre-B-cell line 300-19 (23) was transfected by electroporation with CD27 cDNA containing the pcDL-SRα296 expression vector with the neomycin-resistance gene, and transfectants were selected by

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Abbreviations: SAC, *Staphylococcus aureus* cells; TNF, tumor necrosis factor; mAb, monoclonal antibody; IL, interleukin; PWM, pokeweed mitogen.

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growth in medium with the neomycin analogue G418 (Life Technologies, Gaithersburg, MD) at 1 mg/ml. Cells with high-density CD27 expression were cloned by fluorescence-activated cell sorting. Cultures of 300-19 cells transfected with CD27 cDNA, CD70 cDNA, or vector alone (24) were maintained in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum, 50 μM 2-mercaptoethanol, and gentamicin (5 μg/ml) in the presence of G418 (1 mg/ml). Both CD27 and CD70 are strongly expressed on 300-19 transfectant cells (24).

Antibodies and Reagents. mAbs reactive with CD27 (1A4; IgG1), CD3 (OKT3; IgG2a), CD14 (My4; IgG2b), CD20 (B1; IgG2a), CD56 (N901; IgG1), CD62L (TQ1, IgG1), and CD80 (133; IgM) were used and have been described elsewhere (4, 25–28). 2F11 (IgG1) is an anti-CD70 mAb produced in our laboratory by standard techniques after immunization of a BALB/c mouse (The Jackson Laboratory) with CD70-transfected 300-19 cells (24). Crossblocking studies revealed that the 2F11 mAb recognized the same or a very similar epitope of the CD70 molecule as that recognized by the known anti-CD70 mAb, HNE51 (AMAC, Westbrook, ME) (data not shown). Anti-CD54 (84H10) and anti-CD58 (AICD58) mAbs were purchased from AMAC.

PWM was purchased from Life Technologies and was used at a final dilution of 1:100. Formalinized *Staphylococcus aureus* cells (SAC) were purchased from Sigma and used at a final concentration of 1:10,000 (vol/vol). Recombinant human interleukin 2 (IL-2), a generous gift from Immunex, was used at a final concentration of 30 units/ml as described (29).

Immunofluorescence. Flow cytometric analysis was performed with the use of an Epics C flow cytometer (Coulter). Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Southern Biotechnology Associates) was used as a second antibody in this study. Isotype-matched mouse IgG or IgM (Coulter) controls were used throughout the studies, and always reacted with <5% of the cells.

Detection of IgG *in Vitro* secretion. PBMCs or purified B cells (1×10^5 per well) were cultured with PWM or SAC/IL-2, respectively, in 96-well round-bottom culture plates in 0.2 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1% sodium bicarbonate, 25 mM Hepes, and gentamicin (5 μg/ml) at 37°C in a humidified atmosphere with 5% CO_2 for 8 days. To examine the effect of transfected 300-19 cells on IgG production, various numbers of irradiated (6400 rads; 1 rad = 0.01 Gy) transfected 300-19 cells (5×10^3, 1×10^4, 2×10^4, and 3×10^4 per well) were added to the cultures at initiation. In some experiments, transfected 300-19 cells were pretreated with mAb (1A4 or 2F11) at 5 μg/ml for 30 min on ice and then added to the cultures after extensive washing. To examine the effect of anti-CD27 mAb and anti-CD70 mAb on IgG production, the 1A4 or 2F11 mAb (1 μg/ml) was added to the cultures at initiation. The culture supernatants were harvested, added to goat anti-human immunoglobulin (Southern Biotechnology Associates)-coated 96-well ELISA plates, and incubated at 4°C overnight. After supernatants were discarded and the plates were washed with 0.05% Tween 20 in phosphate-buffered saline, the bound human IgG was detected with alkaline phosphatase-labeled goat anti-human IgG (Southern Biotechnology Associates) at a dilution of 1:1500. Color detection was performed by the addition of *p*-nitrophenyl phosphate (Sigma) plus 1× Caps buffer (Sigma).

B-Cell Proliferation Assay. Purified B cells (1×10^5 per well) were cultured with irradiated (6400 rads) transfected 300-19 cells (2×10^4 per well) that were either untreated or pretreated with mAb (1A4 or 2F11). The cultures were incubated in the presence of SAC/IL-2 in a volume of 0.2 ml per well in 96-well round-bottom plates at 37°C in a humidified atmosphere with 5% CO_2 for 3 days. To examine the effect of anti-CD27 mAb and anti-CD70 mAb on B-cell proliferation, the 1A4 or 2F11 mAb (1 μg/ml) was added at the initiation of culture. After 3 days 0.5 μCi (18.5 kBq) of *methyl*-[^{3}H]thymidine was added. Eighteen hours later the cells were harvested and [^{3}H]thymidine incorporation was measured on a liquid scintillation β counter (1205 Beta plate, LKB Pharmacia).

RESULTS

Earlier studies showed that both anti-CD27 mAb (1A4) and recombinant soluble CD27 significantly inhibited IgG production in a T-cell-dependent, PWM-driven B-cell IgG synthesis system (4, 17). We now show that the addition of anti-CD70 mAb (CD27L) as well as anti-CD27 mAb also caused significant inhibition of IgG production (Table 1), again indicating that CD27–CD70 interactions are involved in T-cell-dependent B-cell activation (4, 17, 18). Since B cells can express CD27 and CD70 to a lesser degree following PWM or SAC/IL-2 activation (Fig. 1), a more detailed analysis of possible CD27–CD70 interactions was undertaken to include those involving $CD70^+$ T cells with $CD27^+$ B cells, $CD27^+$ T cells with $CD70^+$ B cells, $CD70^+$ T cells with $CD27^+$ T cells, and $CD70^+$ B cells with $CD27^+$ B cells.

To further clarify the role of CD27–CD70 interactions on B-cell activation, we undertook a series of experiments examining the effect of CD27-, CD70-, and mock-transfected cells on IgG synthesis in a T-cell-dependent, PWM-driven system. When CD27-transfected cells were added to a mixture of T and B cells and stimulated with PWM, PWM-driven IgG production was inhibited in a dose-dependent fashion (Fig. 2*A*). In this same system, however, CD70-transfected cells enhanced IgG production whereas mock-transfected cells had no effect. Although both the magnitude of the inhibition by CD27-transfected cells and enhancement by CD70-transfected cells varied among individuals tested, similar results were obtained with all donors. Moreover, both the inhibitory and enhancing effects of CD27 and CD70 transfectants on IgG synthesis could

Table 1. Effect of anti-CD70 and anti-CD27 mAbs on T-cell-independent and T-cell-dependent B-cell activation

	T-cell-dependent (PWM)		T-cell-independent (SAC/IL-2)			
	IgG production		IgG production		[^{3}H]Thymidine uptake	
Treatment	ng/ml	% inhibition	ng/ml	% inhibition	cpm	% inhibition
Medium	96		111		853	
PWM or SAC/IL-2	467		450		5081	
+ Anti-CD70 mAb	159	83	391	17	4245	20
+ Anti-CD27 mAb	144	87	379	21	3965	20
+ Anti-CD62L mAb	440	7	430	6	4962	3

Peripheral blood mononuclear cells or purified B cells (1×10^5 per well) were cultured with the anti-CD70 mAb (2F11), anti-CD27 mAb (1A4), or anti-CD62L mAb (TQ1) at 1 μg/ml in the presence of PWM (1:100 dilution) or SAC/IL-2 (1:10,000 dilution and 30 units/ml, respectively). *In vitro* secretion of IgG and B-cell proliferation ([^{3}H]thymidine uptake) were assayed as described in *Materials and Methods*. Values represent means of triplicate cultures (SD < 15% for IgG production and SEM < 15% for [^{3}H]thymidine uptake). Results are representative of three experiments.

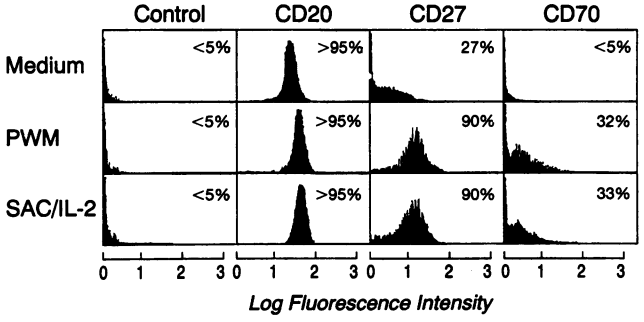

FIG. 1. Surface expression of CD20, CD27, and CD70 on purified peripheral blood B cells. Peripheral blood B cells were cultured with PWM (1:100 dilution) or SAC/IL-2 (1:10,000 dilution and 30 units/ml, respectively) for 3 days. Cells were stained with mAb (B1, 1A4, or 2F11) followed by fluorescein-labeled goat anti-mouse immunoglobulin and analyzed by flow cytometer. Percentage of positively stained cells is given in each panel.

be blocked by pretreatment of these cells with the anti-CD27 (1A4) or anti-CD70 (2F11) mAb (Fig. 2*B*). Taken together, our findings strongly support the idea that the regulatory effects observed with these transfectants are mediated by CD27 and CD70.

To determine whether CD27 transfectants interacted primarily with $CD70^+$ B cells or $CD70^+$ T cells, and whether CD70 transfectants interacted with $CD27^+$ B cells or $CD27^+$ T cells, we examined the effects of these transfectants in the T-cell-independent, SAC/IL-2-driven B-cell activation system. Addition of CD27-transfected cells in the absence of T cells resulted in little if any inhibition of B-cell IgG production and B-cell proliferation, whereas the addition of CD70 transfectants enhanced both (Fig. 3). These findings suggest that the enhancement of IgG production and B-cell proliferation by CD70 transfectants results from a direct interaction of the CD70 transfectants with $CD27^+$ B cells. Further, this observation supports the idea that CD27 on B cells, like that on T cells, can serve as a signal-transducing molecule (24). In addition, the ability of CD27 transfectants to inhibit a T-cell-dependent, but not a T-cell-independent, B-cell activation system raises the possibility that the primary effect of $CD27^+$ cells in a T-cell-dependent system is on $CD70^+$ T cells. Further support for this point of view comes from the observation that the addition of either anti-CD27 mAb or anti-CD70 mAb to the SAC/IL-2 system caused minimal inhibition of B-cell IgG production and proliferation (Table 1).

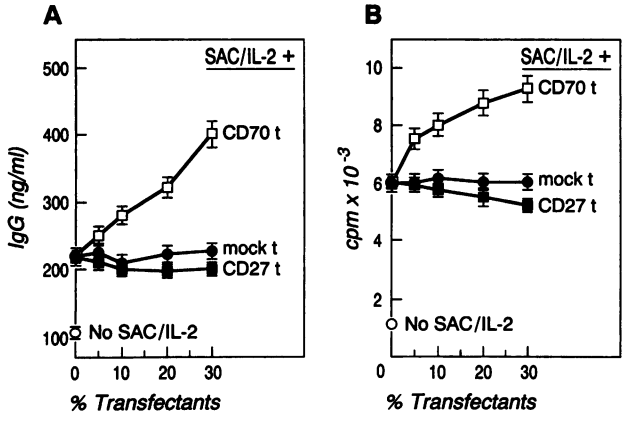

FIG. 3. Effects of CD27-, CD70-, and mock-transfected (*t*) 300-19 cells on T-cell-independent B-cell activation. Purified B cells (1×10^5 per well) were stimulated with SAC/IL-2 (1:10,000 dilution and 30 units/ml, respectively) in the absence or presence of various numbers of irradiated (6400 rads) transfected 300-19 cells (5×10^3, 1×10^4, 2×10^4, and 3×10^4 per well) for 8 days (*A*) or 4 days (*B*). IgG secretion into the culture supernatant was measured by ELISA (*A*). Proliferation ([^{3}H]thymidine incorporation) of B cells was measured during the last 18 hr of a 4-day culture (*B*). Data are expressed as the mean ± SD (*A*) or SEM (*B*), and error bars are sometimes smaller than the plot symbol.

Lastly, we examined whether CD27 transfectants could affect the ability of CD70 transfectants to enhance IgG production in the SAC/IL-2 system. Addition of increasing numbers of CD27-transfected cells gradually abrogated the enhancement of both IgG production (Fig. 4*A*) and B-cell proliferation (Fig. 4*B*) induced by CD70-transfectant cells. These findings clearly indicate that the ratio of $CD27^+$ cells to $CD70^+$ cells can regulate B-cell proliferation and differentiation. Our findings also suggest that interactions between $CD27^+$ T cells and $CD70^+$ T cells may play a key role in regulating B-cell activation and function.

DISCUSSION

In the present studies we have investigated the role of CD27–CD70 interactions on T- and B-cell function and have utilized CD27 and CD70 transfectants to modulate B-cell proliferation and IgG synthesis. Addition of CD27 transfectants to a T-cell-dependent, PWM-driven B-cell IgG synthesis system markedly inhibited IgG production, whereas addition of CD70 transfec-

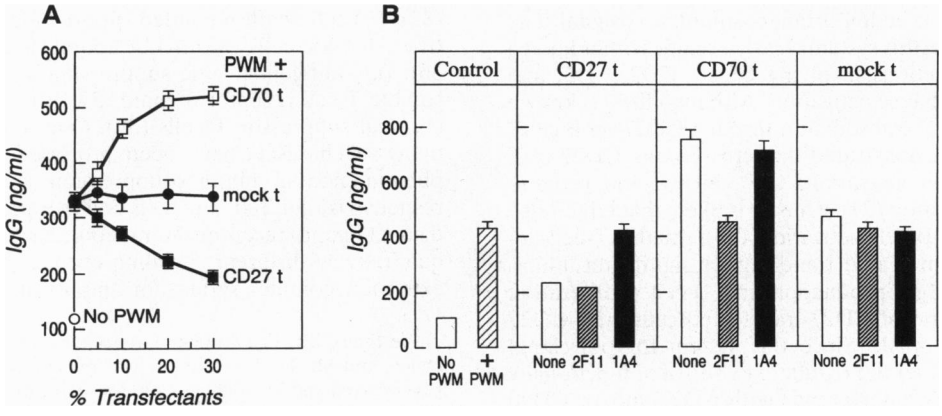

FIG. 2. Effects of CD27-, CD70-, and mock-transfected (*t*) 300-19 cells on T-cell-dependent B-cell IgG production. Peripheral blood mononuclear cells (1×10^5 per well) were stimulated with PWM (1:100 dilution) in the absence or presence of various numbers of irradiated (6400 rads) transfected 300-19 cells (5×10^3, 1×10^4, 2×10^4, and 3×10^4 per well) (*A*) or with irradiated (6400 rads) transfected 300-19 cells (2×10^4 per well) with or without pretreatment with anti-CD27 (1A4) or anti-CD70 (2F11) mAb for 8 days. (*B*) IgG secretion into the culture supernatant was measured by ELISA. Data are expressed as the mean ± SD, and error bars are sometimes smaller than the plot symbol.

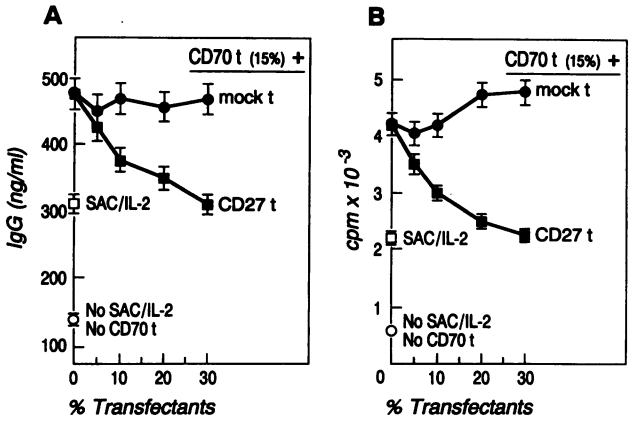

FIG. 4. Effects of CD27-transfected 300-19 cells on enhanced B-cell activation by CD70-transfected 300-19 cells in the presence of SAC/IL-2. Purified B cells (1×10^5 per well) were stimulated with SAC/IL-2 (1:10,000 dilution and 30 units/ml, respectively) and irradiated (6400 rads) CD70-transfected (*t*) 300-19 cells (2×10^4 per well) in the absence or presence of various numbers of irradiated (6400 rads) CD27- or mock-transfected (*t*) 300-19 cells (5×10^3, 1×10^4, 2×10^4, and 3×10^4 per well) for 8 days (*A*) or 4 days (*B*). IgG secretion into the culture supernatant was measured by ELISA (*A*). Proliferation ([^{3}H]thymidine incorporation) of B cells was measured during the last 18 hr of a 4-day culture (*B*). Data are expressed as the mean ± SD (*A*) or SEM (*B*), and error bars are sometimes smaller than the plot symbol.

tants enhanced IgG production. Both the inhibition of PWM-driven IgG production by CD27 transfectants and the enhancement by CD70 transfectants could be blocked by pretreatment with anti-CD27 mAb and anti-CD70 mAb. In contrast, in the T-cell-independent, SAC/IL-2-driven B-cell activation system, only minimal inhibition of IgG production and B-cell proliferation was noted following the addition of either CD27-transfected cells or anti-CD27 and anti-CD70 mAbs. Nevertheless, in this same system, CD70-transfected cells enhanced B-cell IgG production and proliferation and these effects could be gradually abrogated by addition of increasing numbers of CD27-transfected cells. These results clearly support an important role for CD27 and its ligand CD70 in these T–T and T–B cell–cell interactions.

Addition of CD70 transfectants to both the T-cell-dependent PWM and T-cell-independent SAC/IL-2 systems resulted in a marked enhancement of IgG production and B-cell proliferation (Figs. 2 and 3). These results clearly suggest that these interactions are involved in B-cell activation and, more importantly, suggest that the interaction of B-cell CD27 with CD70 can provide an important costimulatory signal. The use of transfectants in this system clearly excludes other known costimulatory interactions involving CD28, CD2, CD5, and CD40, which also enhance activation. Although little is known of the nature of signal transduction through CD27 on B cells, prior studies have demonstrated that crosslinking CD27 of T cells results in T-cell activation, Ca^{2+} influx, and protein-tyrosine phosphorylation (24). Thus, it is likely that CD27 also plays a key role in B-cell activation (30). In the T-cell-dependent PWM system (Table 1 and Fig. 2), significant inhibition of both B-cell IgG production and B-cell proliferation resulted from addition of CD27-transfected cells, anti-CD27 mAb, or anti-CD70 mAb. Since this system for polyclonal B-cell activation is under the regulatory control of functionally distinct T-cell subsets, interference with CD27 and/or CD70 could affect interactions of $CD70^+$ T cells with $CD27^+$ B cells, $CD27^+$ T cells with $CD70^+$ B cells, $CD70^+$ T cells with $CD27^+$ T cells, and/or $CD70^+$ B cells with $CD27^+$ B cells. Although each of these interactions is possible, we do not believe the evidence supports an interaction between $CD27^+$ T cells and $CD70^+$ B cells or one between $CD27^+$ B cells and $CD70^+$ B cells. In support of this conclusion is the finding that addition of CD27-transfected cells, anti-CD27 mAb, or anti-CD70 mAb minimally inhibited IgG production and B-cell proliferation in the SAC/IL-2 system. In contrast, addition of CD70-transfected cells enhanced IgG production and B-cell proliferation in both the T-cell-dependent and the T-cell-independent B-cell activation systems (Figs. 2 and 3; Table 1). Lastly, CD70 is minimally expressed and is restricted to a small fraction of activated B cells (Fig. 1). These data, taken together, support the view that interactions involving $CD70^+$ T cells with $CD27^+$ B cells and those involving $CD70^+$ T cells with $CD27^+$ T cells are likely to be more important in regulating B-cell activation than are those involving $CD27^+$ B cells with $CD70^+$ B cells or $CD27^+$ T cells with $CD70^+$ B cells.

The observation that CD27-transfected cells inhibited T-cell-dependent IgG production in the PWM system but not in the T-cell-independent SAC/IL-2 system (Figs. 2 and 3) strongly supports T–T interactions involving CD27 and its ligand, CD70. The downregulatory effect of CD27 cells on B-cell activation and immunoglobulin synthesis also suggests that T–T interactions are clearly of importance in this response. This last experiment also suggests that the extent of enhancement or suppression of B-cell activation in the SAC/IL-2 system may be regulated by the ratio of $CD27^+$ cells to $CD70^+$ cells (Fig. 4). Although the precise mechanisms by which suppression of PWM-driven B-cell IgG production occurs via CD27–CD70 interactions is still not clear, our present data suggest that $CD27^+$ T cells serve a pivotal role in regulating T-cell-dependent B-cell activation.

Earlier studies showed that $CD45RA^+$ T cells suppressed PWM-induced IgG synthesis in the presence of $CD8^+$ cells (20). Our present studies, carried out in absence of $CD8^+$ cells, suggest that $CD4^+CD45RA^+CD27^+$ cells by themselves can inhibit the productive interaction of $CD70^+$ T cells with $CD27^+$ B cells. It should be noted that the phenotype of the T helper population in our system is $CD4^+CD45RO^+CD70^+$ (18, 19). Moreover, in recent studies, we showed that $CD4^+$-$CD45RO^+CD70^+$ cells, when activated, not only bound to resting $CD27^+CD45RA^+$ T cells but also caused their subsequent activation (19). Thus, we believe that one consequence of interaction of activated helper T cells with B cells is the generation of an activated $CD4^+CD45RA^+$ population which can directly downregulate the activity of the $CD4^+CD45RO^+$-$CD70^+$ helper population.

We do not know how the present data on CD27–CD70 interactions can be incorporated into other established mechanisms for generation of suppression, which include (*i*) suppression mediated by cytokines such as TGF-β (31) or IL-10 (32); (*ii*) cell death-mediated suppression involving autoreactive cytotoxic cells (33, 34) or apoptosis via CD95 (Fas) (35, 36); (*iii*) antigen-specific suppression such as that involving soluble T-cell receptor α chain (37, 38); and (*iv*) induction of classical suppressor T cells (39). One might also ask why so many mechanisms have been proposed to account for the phenomenon of immunosuppression. Perhaps the simplest response is that T–T and T–B collaborations involve so many distinct ligand/receptor interactions, each capable of inducing qualitatively different signaling events, that one requires an extremely complex system for amplification and fine control.

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