Ligation of CD27 on Murine B Cells Responding to T-Dependent and T-Independent Stimuli Inhibits the Generation of Plasma Cells

Vanitha S. Raman, Vineeta Bal, Satyajit Rath and Anna George

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B cells can be stimulated either allogenically with the Th cell clone D10G4.1 and bone marrow-derived dendritic cells or polyclonally with LPS to proliferate and undergo terminal differentiation to Ig-secreting plasma cells in vitro. The addition of anti-CD27 to such cultures inhibits Ig secretion, and inhibition is more marked in T-dependent cultures than in T-independent cultures. Both IgM and secondary isotypes are affected, and addition of anti-CD27 even 4 days after culture initiation inhibits Ig secretion. Anti-CD27 does not affect B cell proliferation or the acquisition of activation markers by B cells, and no marked loss of B cell viability is detected in cells cultured in the presence of anti-CD27, suggesting that the inhibition of Ig secretion is not due to inhibition of early activation events or to death of activated cells in vitro. However, the presence of anti-CD27 significantly inhibits the induction of Blimp-1 and J chain transcripts, which are turned on in cells committed to plasma cell differentiation. Furthermore, mice immunized under cover of anti-CD27 make less Ag-specific IgM and IgG, but have equivalent T cell responses when compared with control mice. These data suggest that ligation of CD27, a member of the TNFR family, on the B cell surface may prevent terminal differentiation of activated B cells into Ig-secreting plasma cells. The Journal of Immunology, 2000, 165: 6809–6815.

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hen naive B cells in the periphery encounter specific Ag in the context of appropriate costimulatory molecules, they get activated and proliferate. Early clonal expansion can be quite rapid, with doubling times estimated to be as short as 6–8 h (1, 2). The expanded population has to be controlled to ensure that while enough numbers of activated cells are generated to eliminate the Ag that initiated the response, a rapid return to homeostasis is also possible. In addition, the population has to be screened to ensure that any self-reactive cells that may be generated during the process of somatic mutation do not survive (3). Therefore, B cell activation is accompanied by extensive proliferation on the one hand and with cell death on the other, and members of the TNFR family of proteins have emerged as important mediators of both events (4–14).

Activated B cells differentiate into Ig-secreting effector cells and to memory cells that are not immediately functionally active but are capable of responding to a subsequent antigenic challenge with rapid and enhanced proliferation and Ig secretion. Commitment to effector plasmablasts as well as to memory cells seems to occur in germinal centers (15, 16), but the signals that lead cells down one differentiation pathway rather than another have not yet been elucidated. TNFRs are crucial for the formation of germinal centers and they also control the spatial arrangement of B and T cells within lymphoid organs, and recent reports have shown that in various TNFR knockout mice, in which optimal interactions between B cells, T cells, and follicular dendritic cells in germinal centers do not occur, B cell responses are compromised (17–26).

CD27 is a member of the TNFR family and there is evidence to suggest, on the one hand, that it may be a potential marker for human memory B and T cells (27–30) and, on the other, that engagement of CD27 by its ligand CD70 may promote the differentiation of human memory B cells to plasma cells (31, 32). Although CD27 is expressed on a substantial proportion of human PBL, only a small proportion of murine B cells express CD27, and they are found predominantly in the marginal zone of the spleen and the germinal centers of tonsils and lymph nodes (33–35). Thus, CD27 may identify a recently activated population of cells in murine lymphoid tissues, and this raises the possibility that signaling through CD27 may influence the choice between terminal differentiation and memory cell generation in activated B cells.

We have examined the effect of ligation of CD27 on events following B cell activation using a T-dependent (TD) culture set up in the presence or absence of anti-CD27. Because CD27 and its ligand are present on activated B and T cells, we also looked for B cell-specific effects of the Ab in T-independent (TI) culture. We report that whereas CD27 engagement does not affect B cell activation, proliferation, or viability, it inhibits terminal differentiation of B cells into Ig-secreting plasma cells.

Materials and Methods

Mice

Six- to 10-wk-old C57BL/6, BALB/c, and C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME), bred and maintained in the Small Animal Facility of the National Institute of Immunology, were used for all experiments. Approval from the Institutional Animal Ethics Committee was obtained for all experimental procedures involving animals.

3 Abbreviations used in this paper: TD, T dependent; D10, D10.G4.1; DC, dendritic cell; TI, T independent; NP, nitrophenyl; PNA, peanut lectin (agglutinin); PI, propidium iodide; NP-CGG, nitrophenyl-chicken gammaglobulin.
Anti-CD27 inhibits Ig secretion in clonal TD culture. I-A<sup>b</sup> B cells were stimulated with γ-irradiated (1000 rad) D10s and DCs in the presence or absence of various Abs in Terasaki plates. One week later, culture supernatants were diluted and tested for total IgM, IgG1, and IgA by ELISA. The percentage of wells (of 60 plated for each set) that contained detectable Ig of each isotype is plotted, and one experiment, representative of three, is shown. **, IgG1 was not estimated in wells that contained CTLA-4-Ig because the CTLA-4-Ig cross-reacts with ELISA reagents. No Ig was detected in wells containing only B cells and DCs (data not shown).

**B cells**

Single-cell suspensions of splenocytes were obtained by mechanical disruption of the spleen, and RBCs were lysed by treatment of the cell pellet with Gey’s solution. For some experiments, unfractionated splenocytes were used as a source of B cells. For other experiments, B cells were enriched either by depleting T cells with anti-Thy1.2 (Y-19, a kind gift of Dr. C. A. Janeway, Jr., Yale Medical School, New Haven, CT) and complement (Cedarlane, Westbury, NY), followed by removal of plastic-adherent cells or by positive enrichment of total or IgM<sup>+</sup> B cells on magnetic columns (Miltenyi Biotec, Bergisch-Gladbach, Germany). For such separations, cells were labeled either with anti-CD19 beads (Miltenyi Biotec) or with goat anti-mouse IgM-biotin (Southern Biotechnology Associates, Birmingham, AL) or anti-B220-biotin (PharMingen, San Diego, CA) followed by streptavidin-coupled magnetic beads (Miltenyi Biotec). Purified cells were used for experiments only if they were >90% pure by flow cytometric analysis.

**T cells**

The conalbumin-specific I-A<sup>b</sup>-restricted Th2 clone, D10.G4.1 (D10), which also recognizes I-A<sup>b</sup> molecules in alloreactive fashion, (36), was maintained in Click’s medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% FBS, 2 mM l-glutamine, 0.05 mM 2-ME (all from Life Technologies, Grand Island, NY), and antibiotics (complete Click’s medium). The culture was maintained by weekly stimulation with conalbumin and γ-irradiated syngeneic splenocytes, and cells were used for assays between 10 and 14 days after the last stimulation.

**Dendritic cells (DCs)**

DCs were grown from the bone marrow of C3H/HeJ (H-2<sup>b</sup>) mice in complete Click’s medium containing 20 ng/ml of GM-CSF (PeproTech, Rocky Hill, NJ), and to get activated, mature DCs, 10 ng/ml of recombinant TNF-α (PeproTech) was added 24 h before using them in culture (37).

**B cell stimulation**

For TI B cell activation, 10<sup>5</sup> B cells or 3 × 10<sup>5</sup> whole splenocytes were stimulated with various doses of LPS (BBL-Difco, Becton Dickinson India, New Delhi, India) in 200-μl cultures. For clonal TD experiments, 1–5 I-A<sup>b</sup> B cells were alloglogously stimulated with 3000 resting D10s and 400 DCs in a final volume of 15 μl in 60-well Terasaki plates (Falcon, Franklin Lakes, NJ), and to get activated, mature DCs, 10 ng/ml of recombinant TNF-α (PeproTech) was added 24 h before using them in culture (37).

**B cell proliferation**

B cell proliferation was assessed by stimulating cells in TD or TI culture for 48 h, pulsing the wells with 0.5 μCi/well of [3H]thymidine (NEN, Life Science Products, Boston, MA) for 12–16 h and harvesting the cells onto glass fiber filtermats for liquid scintillation counting (Betaplate; Wallac, Turku, Finland). Data are expressed as mean ± SE of triplicate cultures. Ig secretion was estimated in replicate cultures stimulated for 6–8 days. Serial dilutions of culture supernatants were added to microtiter plates (Dynatech, Chantilly, VA) coated with 10 μg/ml of goat anti-mouse IgG (Southern Biotechnology Associates). Bound Ig was detected with biotinylated goat anti-mouse IgG, IgG1, or IgA (Southern Biotechnology Associates).
followed by streptavidin-HRP (Genzyme, Cambridge, MA). Hydrogen peroxide (Merck, Mumbai, India) was used as substrate, and color was read at 490 nm in a microplate reader (Sanoft, Redmond, WA). IgG amounts were calculated from a standard curve run in parallel with isotype-specific myeloma standards (Sigma). The absorbance values shown in some experiments represent values for a 1/1000 dilution of supernatant in T1 cultures and a 1/300 dilution in TD cultures.

Flow cytometry

Reagents used for single-step staining or for primary labeling were anti-B220-biotin, anti-CD24, anti-GL7, PE-anti-CD44 (all from PharMingen), fluorescein-peanut lectin (agglutinin) (PNA; Vector Laboratories, Burlingame, CA), anti-I-A^b (culture supernatant from the hybridoma 212.A1 (a gift of Dr. C. A. Janeway, Jr.), anti-Thy1.2 (Y-19 culture supernatant), and anti-Mac-1 (culture supernatant from the hybridoma M1/70.16 (gift of Dr. C. A. Janeway, Jr.)). Secondary reagents used were PE-streptavidin (PharMingen), fluorescein-mouse-anti-rat IgG (Jackson ImmunoResearch, West Grove, PA), PE-donkey anti-rat IgG (Jackson ImmunoResearch), and CyChrome-avidin (PharMingen). Between 10^5 and 10^6 cells were incubated with staining reagents in buffer containing 0.1% sodium azide and 1% FBS. All incubations were for 45 min on ice. If stained cells could not be analyzed immediately, they were fixed in 0.1% paraformaldehyde and stored at 4°C. For determination of cell viability, 5 μg/ml of propidium iodide (PI; Sigma) was added to samples before acquisition. Samples were run on an Elite ESP flow cytometer (Coulter Electronics, Hialeh, FL) or on a Btyre flow cytometer (Bio-Rad, Hemel-Hempstead, U.K.), and data were analyzed with WinMDI shareware or FlowJo software (Treestar, San Carlos, CA).

**RT-PCR**

Total RNA was isolated from cells with RNeasy kits (Qiagen, Valencia, CA) or with Trizol reagent (Life Technologies). RT-PCRs were set up with 100 ng of RNA using the Access RT-PCR System (Promega, Madison, WI), and the reaction was set up according to the manufacturer’s recommendations. Briefly, cDNA was synthesized at 48°C for 45 min, the strands were denatured at 94°C for 2 min, and the cDNA was amplified for 40 cycles (1 min at 95°C, 1 min at 55°C, and 1 min at 72°C), followed by a final elongation step of 10 min at 72°C. Primers used for J chain were: sense, 5'-ATGAAAGACCCACCTGCTTCTC-3'; J chain antisense, 5'-GT CAGGTTAGCAGAATTCGGG-3', yielding a 430-bp product. Primers used for Blimp-1 were: sense, 5'-TCCGGTCCTGGAAGTITCCA-3'; antisense, 5'-GGTGGACCTCCTCTGGAAT-3', yielding a 370-bp product. Primers used for β-actin were: sense, 5'-CGTGCCCCTCACCTAG GCACCA-3'; antisense, 5'-CCGTGTCCTTCAAGTGTTCA-3', yielding a 254-bp band. All three sets of primers were added to a single RT-PCR, and the PCR conditions were chosen to be within the optimal range of amplification for all three products. Half the PCR products were run on a 2% agarose gel, with a 100-bp ladder (Promega) for sizing the bands.

**In vivo priming and recall responses**

BALB/c mice were immunized s.c. with 100 μg of nitrophenyl-chicken gammaglobulin (NP-CGG; Biosearch Technologies, Novato, CA), emulsified in CFA (Sigma), and were given either PBS or 30 μg of anti-CD27 i.p. on the day of immunization. To assess Ab responses, mice were bled 14 days after immunization, and anti-nitrophenyl (NP) Abs were estimated by ELISA on NP-BSA-coated plates. To assess T cell priming, cells from the draining lymph nodes were cultured in vitro with serial dilutions of heat-inactivated chicken serum, and proliferation was assessed 72 h later by scintillation spectroscopy as described earlier. The proliferative responses of cells to 1/10,000 dilution of chicken serum are shown.

**Results**

**Anti-CD27 inhibits Ig secretion in TD microcultures**

Very small numbers (1–5) of naive I-A^b B cells can be stimulated in microcultures containing the alloreactive Th2 clone D10 and DCs isolated from the spleen or Peyer’s patches of mice to undergo proliferation, isotype switching to all secondary isotypes, and terminal differentiation to plasma cells (16, 38). We find that DCs that grow out from murine bone marrow cultured in the presence of GM-CSF can substitute for ex vivo DC, and we have used the system to analyze the role of various cell surface molecules in B cell activation and differentiation. Fig. 1 shows that the addition of 5 μg/ml of anti-CD27 to such supportive cultures inhibits induction of secreted IgM, IgG1, and IgA, whereas the addition of anti-CD2, anti-CD48, anti-CD54, and anti-CD86 have no effect. CTLA-4-Ig (a kind gift of Dr. E. A. Clark, University of Washington, Seattle, WA) does inhibit IgA secretion substantially, but has little effect on IgM secretion. Similar results were observed with cultures containing DCs activated overnight with 10 ng/ml of TNF-α (data not shown).

**Anti-CD27 inhibits Ig secretion in TI and TD bulk cultures**

The data in Fig. 1 represent the percentage of wells (of a total of 60) that are positive for a given isotype when very small numbers of B cells are stimulated in TD microcultures in Terasaki plates (Falcon). In the absence of anti-CD27, isotype switching does not occur in each well, although B cells are driven to IgM production in each well. Although this allows us to look at the two phenomena...
Anti-CD27 does not inhibit B or T cell proliferation. A, I-A<sup>b</sup> B cells were stimulated with 10 μg/ml of LPS in the presence of 5, 1, or 0.2 μg/ml of anti-CD27. B, I-A<sup>b</sup> B cells were stimulated with titrating numbers of γ-irradiated (1000 rad) D10s and 4000 DCs in the presence or absence of 5 μg/ml anti-CD27 (Ab). Background signals from B cells and DCs in the presence (∆) or absence (▲) of anti-CD27 are also shown. C, D10s were stimulated with titrating numbers of γ-irradiated (1000 rad) I-A<sup>b</sup> B cells in the presence or absence of 5 μg/ml of anti-CD27 (Ab). One experiment, representative of two each, is shown.

Anti-CD27 does not inhibit B or T cell proliferation

Because CD27 and its ligand CD70 are present both on activated B and T cells, we also set up TI cultures to look for B cell-specific effects of anti-CD27. As seen in Fig. 2B, B cells stimulated with 10 μg/ml of LPS for 7 days in the presence of anti-CD27 also make substantially less IgM than cells cultured in the absence of Ab, although higher amounts of anti-CD27 are required for reliable inhibition to be observed. Other hamster IgG Abs (anti-CD28, anti-CD48, anti-CD54, and anti-CD154) did not inhibit Ig secretion in such cultures (Fig. 2C), establishing the specificity of inhibition. Furthermore, Ig secretion is inhibited even if anti-CD27 is added 2 or 4 days after culture initiation (Fig. 2D, TI; Fig. 2E, TD), although secondary isotypes appear to be less affected if anti-CD27 is added at later time points.

**Anti-CD27 does not prevent acquisition of activation markers by stimulated B cells**

Signaling through TNFRs appears to be crucial for germinal center formation and for the maintenance of the correct spatial relationships between B cells, T cells, and DCs in lymphoid organs that are required for optimal humoral responses (17–26). Therefore, it is possible that anti-CD27 in culture disrupts cellular interactions and prevents optimal B cell activation. To examine this, we stained cells for the up-regulation of various activation markers 60–72 h after TD or TI stimulation. As can be seen (Fig. 3), in both TI and TD cultures, stimulated B cells up-regulate levels of CD24, MHC class II, and CD44, and the enhancement is similar whether B cells are cultured in the presence or absence of anti-CD27. Stimulated B cells also bind high levels of PNA and up-regulate GL-7 (phenotypes characteristic of B cells in germinal centers) in the TD cultures, and the up-regulation is similar in B cells stimulated in the presence or absence of anti-CD27 (Fig. 3). As expected, neither PNA binding nor GL-7 expression is induced on B cells stimulated with LPS (Fig. 3). Thus, B cell activation, including the acquisition of germinal center markers, is unaffected by ligation of CD27.

Anti-CD27 does not inhibit B or T cell proliferation

B cell activation normally leads to clonal expansion, and we considered the possibility that anti-CD27 may inhibit the proliferation of activated B cells. However, this appears not to be the case. Proliferation of B cells stimulated with LPS (Fig. 4A) or with T cells and DCs (Fig. 4B) is unaffected by the presence of anti-CD27. Moreover, Fig. 4C shows that proliferation of D10s stimulated with titrating numbers of irradiated I-A<sup>b</sup> B cells is also not affected by the presence of anti-CD27. These results indicate that ligation of CD27 has no adverse effects on B or T cell proliferation.

**Anti-CD27 does not affect B cell viability**

Because CD27 is a member of the TNFR family of proteins that also includes apoptosis-inducing receptors like CD95/Fas, and because CD27 has been shown to induce apoptosis by binding to the proapoptotic protein Siva (10), it is possible that ligation of CD27 on activated cells may lead to cell death before terminal differentiation to Ig-secreting plasma cells occurs. We tested this possibility by adding anti-CD27 to LPS-stimulated cultures at the time of culture initiation and counting the number of viable cells at various times by trypan blue exclusion. As seen in Fig. 5A, cells stimulated with LPS survive better than unstimulated cells, and there is no difference in the number of viable cells recovered from cultures stimulated in the presence or absence of anti-CD27. In another set of experiments, anti-CD27 was added at various times after culture initiation, and the number of viable cells was scored on day 8. Again, the continued presence of anti-CD27 did not affect the viability of the cultured B cells and their progeny (data not shown). Because the TD cultures contained irradiated T cells and DC, B cell viability was assessed by estimating the exclusion of PI by gated Thy1.2-negative, Mac-1-negative cells harvested at various times after culture initiation. As seen in Fig. 5B and C, the presence of anti-CD27 in culture does not affect the viability of cells at either early (24 h) or late (7 days) times. The proportion of PI-positive cells in the two groups was also similar at 48 h (data not shown). Together, our results indicate that ligation of CD27 does not induce death of activated B cells.

**Anti-CD27 prevents terminal differentiation of B cells to plasma cells**

So far, our data indicate that whereas B cells stimulated in the presence of anti-CD27 undergo normal activation and proliferation, and whereas a significant proportion of activated cells are viable at the end of 7–8 days in culture, they fail to secrete Ig. The results raise the possibility that CD27 may exert its effect by preventing terminal differentiation of activated B cells into Ig-secreting plasma cells. We tested this possibility by estimating levels of mRNA for J chain and for the Blimp-1 transcription factor, both of
which are induced in B cells committed to plasma cell differentiation, in B cells stimulated in the presence or absence of anti-CD27. Fig. 6 shows that anti-CD27 inhibits the induction of both transcripts. In the TI system, J chain induction is lower by 2-fold, and Blimp-1 induction by 5-fold. In the TD system, J chain induction is lower by 5-fold, and Blimp-1 induction by 6-fold. However, the levels of β-actin mRNA are comparable. Addition of anti-CD27 alone to cultures did not induce expression of either gene product (data not shown).

Anti-CD27 inhibits Ag-specific B cell responses in vivo

Our experimental protocols so far rely on polyclonal TI or allogenic TD stimulation of B cells and do not directly address the role of CD27 in physiologically relevant Ag-specific responses. To assess the physiological consequences of ligation of CD27 in vivo directly, we immunized mice with a nominal Ag (NP-CGG emulsified in CFA) under cover of PBS or anti-CD27. As seen in Fig. 7, mice treated with 30 μg of anti-CD27 on the day of immunization make substantially less anti-NP IgM and IgG than PBS-treated mice do. Significantly, T cells from the draining lymph nodes of both groups of mice show equivalent proliferation to NP-CGG in an in vitro recall response (Fig. 7). Thus, B cells responding to specific Ags in vivo fail to differentiate efficiently into plasma cells if CD27 is ligated.

Discussion

We have analyzed the effect of ligation of CD27, a member of the TNFR family, on B cell responses. Earlier data with knockout mice indicated that signaling through other TNFRs and their ligands affects TD responses (17, 20, 21, 22, 24, 25); therefore, we initially looked for possible effects of CD27 ligation on splenic B cells stimulated in vitro in TD culture. Ag-specific B cell responses are difficult to analyze ex vivo because of the requirement for enriching hapten-specific B cells. Very small numbers of Ag-specific B cells are recovered even from immunized mice; therefore, the approach has to be limited to microcultures (40). Another drawback with enrichment procedures is that B cells with low levels of surface Ig do not bind well, so that germline center cells that have down-regulated surface Ig are excluded. Therefore, for our TD cultures we have relied on the allogenic stimulation of I-A<sup>+</sup> B cells by the Th clone D10 in the presence of DCs. We found that the addition of anti-CD27 to clonal or bulk TD cultures inhibited Ig secretion by the B cells and that both primary and secondary isotypes were affected (Figs. 1 and 2A). Because CD27 and its ligand CD70 are present on activated B cells as well as on activated T cells, there is always the possibility that the Ab primarily affects T cells and that the effects on Ig secretion are indirect. However, a similar effect on Ig secretion was observed in TI cultures (Fig. 2B, C), suggesting that ligation of CD27 on B cells can specifically affect B cell differentiation. Interestingly, TD responses appear to be more sensitive to inhibition by Ab. Although 1 μg/ml of anti-CD27 inhibits the secretion of all isotypes in TD culture, 5 μg/ml is required for reproducible inhibition of IgM secretion in TI culture (Fig. 2B and data not shown).

Our results appear to differ somewhat from earlier reports. It has been shown, for instance, that engagement of CD27 with CD70 transfectants on human B cells stimulated with IL-4 and anti-CD40 induces IgE secretion by promoting the generation of plasma cells (31). However, the reported enhancement of IgE secretion was seen only if purified CD27<sup>+</sup> (but not CD27<sup>−</sup>) cells were used in culture, and such cells also proliferated better in the presence of the CD70 transfectant. It has also been reported that greater differentiation to plasma cells occurs in human peripheral CD27<sup>+</sup> (but not CD27<sup>−</sup>) B cells in the presence of IL-10 and CD70 transfectant (32). We have not estimated IgE in our culture supernatants, and it is possible that IgE secretion may actually be enhanced. However, CD27<sup>−</sup> cells are undetectable in murine spleen (although they are seen in the chronically activated Peyer’s patches, data not shown), and therefore our data are compatible with the data on CD27<sup>−</sup> human B cells. However, it is possible that anti-CD27 and the CD70 transfectants used in these studies may have different effects.

Most B cells responding in vivo to TI stimuli differentiate into plasma cells in the outer peri-arteriolar lymphoid sheath. However, in the response to TD Ags, while some activated cells do differentiate into plasma cells at this site, others move into follicles, where they initiate vigorous germinal center reactions. Ig secretion in both cases is a relatively late event following B cell activation. Because anti-CD27 modulates this late event significantly, we also looked for anti-CD27-mediated inhibition of early events in B cell activation. Our results indicate that at least two events that precede Ig secretion, namely, the acquisition of activation markers and the proliferation of stimulated B cells, are unaffected by anti-CD27. It has been reported that anti-CD27 can enhance the proliferative response of purified T cells to suboptimal mitogenic stimulation (39), but no such enhancement of T cell proliferation was seen in our system (Fig. 4). CD27 engagement also does not induce death of activated cells because the viability of cells cultured in the presence or absence of anti-CD27 is similar (Fig. 5). Our results are
consistent with earlier observations on the proliferation and survival of B cells from patients with chronic lymphocyte leukemia. B cells from such patients do not proliferate well to B cell receptor cross-linking or CD40 ligation, but they can proliferate almost as well as normal B cells when stimulated with activated T cells, and it has been shown that disrupting CD27-CD70 interactions during such stimulation does not affect either proliferation or survival of the B cells (41).

Because neither B cell activation nor survival were affected by the presence of anti-CD27 in culture, we looked for possible effects of anti-CD27 on the generation of plasma cells. Our results show that transcription of J chain and Blimp-1 are inhibited in cells in the presence of anti-CD27 (Ab) (for 24 h) in the presence or absence of 5 μg/ml of anti-CD27. Simultaneous RT-PCRs for J chain, Blimp-1, and β-actin were set up. The expected bands (430 bp for J chain, 370 bp for Blimp-1, and 245 bp for β-actin) are indicated. The bands were scanned and analyzed with NIH Image shareware, and the densitometric profiles are shown for the lane with (thin line) or without (thick line) anti-CD27.

References


