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Differential Effects of IL-27 on Human B Cell Subsets

Frédérique Larousserie, Pascaline Charlot, Emilie Bardel, Josy Froger, Robert A. Kastelein, and Odile Devergne

IL-27 is a novel heterodimeric cytokine of the IL-12 family that plays an important role in the regulation of T cell responses. Its role on human B cells has not been previously studied. In this study, we show that both chains of the IL-27 receptor complex, IL-27R and gp130, are constitutively expressed at the surface of naive and memory human tonsillar B cells, and are induced on germinal center B cells following CD40 stimulation. In naive B cells, IL-27 induced strong STAT1 and STAT3 phosphorylation, whereas it induced moderate STAT1 and low STAT3 activation in memory B cells. IL-27 induced T-bet expression in naive and memory B cells only. In anti-Ig-stimulated naive or memory B cells, IL-27 also induced CD54, CD86, and CD95 surface expression. In addition, IL-27 increased proliferation of anti-Ig-activated naive B cells and of anti-CD40-activated naive and germinal center B cells, but not of CD40-activated memory B cells. These data indicate that the B cell response to IL-27 is modulated during B cell differentiation and varies depending on the mode of B cell activation. The Journal of Immunology, 2006, 176: S890–S897.

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4 Abbreviations used in this paper: EB13, EBV-induced gene 3; GC, germinal center; SAC, Staphylococcus aureus Cowan strain; PC5, PE-cyanine 5.
on the stage of B cell differentiation and on the costimulus delivered to the B cell.

Materials and Methods
Isolation of human B cells and B cell subsets
B cells were isolated from human tonsils obtained with informed consent from patients undergoing tonsillectomy at Necker Hospital. Tonsils were grinded and the cell suspension was filtered through gauze. Tonsillar mononuclear cells were then isolated by Ficoll-Paque Plus (Amersham Biosciences) density centrifugation and subjected to CD2 microbead depletion and magnetic separation by using LS columns (Miltenyi Biotec). The resulting B cell preparations were consistently from >98 to 99.5% CD19+ as assessed by FACS analysis. Naive, GC, and memory B cells were isolated by magnetic separation based on their differential expression levels of two cell surface markers, IgD and CD38 (Fig. 1). Total B cells were separated into IgD−naive B cells and IgD+ populations using anti-IgD mAb (mouse IgG2a; BD Biosciences) followed by goat anti-mouse IgG microbeads (Miltenyi Biotec) and magnetic separation by using LS columns (Miltenyi Biotec). IgD− cells were further separated into IgD−CD38− (memory B cells) and IgD+CD38+ (GC B cells) by incubation with anti-CD38-PE mAb followed by anti-PE FITC microbeads (Miltenyi Biotec) and separation by using LS columns. The negative fraction (memory B cells) was collected and the positive fraction was passed again over a MS column to remove CD38−cells from the GC cell fraction. As assessed by FACS analysis, the purity of each isolated B cell subset was >95%.

Purified B cells or B cell subsets (2 × 10^6/ml) were cultured in RPMI 1640 medium supplemented with 10% FBS, l-glutamine, and antibiotics in 10% FBS, L-glutamine, and antibiotics in 1640 medium supplemented with 10% FBS, L-glutamine, and antibiotics in IgD B cell subset was assessed by FACS analysis. As assessed by FACS analysis, the purity of each isolated B cell fraction. As assessed by FACS analysis, the purity of each isolated B cell subset was >95%.

Cell surface immunofluorescent staining and FACS analysis
Purified total B cells (5 × 10^5 to 1.5 × 10^6 per staining) were saturated in PBS containing 20% normal human serum for 20 min before incubation with specific Abs. All stainings and washes were performed in FACS buffer (PBS containing 2% FBS and 0.01% sodium azide). IL-27R, gp130, and IL-12Rβ1 were detected, respectively, with mouse anti-TCCR mAb (clone 191106, IgG2b; R&D Systems), mouse anti-gp130 mAb (clone AN-G30 (34), IgG1, developed in Hugues Gascan’s laboratory, Angers, France), and mouse anti-IL-12Rβ1 mAb (clone 2B6, IgG2a; BD Biosciences) at 10 μg/ml in parallel with the isotype control mAbs, MOPC141 (mouse IgG2a; Sigma-Aldrich) and MOPC21 (mouse IgG1, Sigma-Aldrich), followed by PE-conjugated Fab(1)γ of goat anti-mouse IgG (Beckman Coulter). IL-12Rβ2 was detected with rat anti-IL-12Rβ2 mAb (clone 2B6, IgG2a; BD Biosciences) at 10 μg/ml in parallel with a rat IgG2a isotype control (R&D Systems), followed by PE-conjugated Fab(1)γ of goat anti-rat IgG (Beckman Coulter). PE-labeled cells were then stained simultaneously with anti-CD38-PE-cyanine 5 (PC5) and anti-IL-12R-PE mAbs (BD Biosciences) to identify the different B cell subsets. For the detection of cell surface expression of CD10, CD11a, CD23, CD25, CD39, CD40, CD54, CD58, CD80, CD86, CD95, HLA-DR, and HLA class I, total B cells were stained simultaneously with PE-conjugated specific Abs (all from BD Biosciences), along with anti-CD38-PCS and anti-IL-12R-PCS Abs (BD Biosciences), CD38-FITC (BD Biosciences), and IL-12R-PC5 (DakoCytomation) Ab were used to verify the purity of total B cell or B cell subset purification. Cells were analyzed on FACSscan using CellQuest software.

Proliferation assay
For proliferation assays, B cells (2 × 10^5/well) were cultured in triplicate in 96-well plates for 3 days. Proliferation was measured by adding 0.5 μCi/well of [3H]thymidine (Amersham Biosciences) for the last 10–15 h of the third day of incubation. Cells were then collected, and [3H]thymidine incorporation was measured in a beta scintillation counter. Results are expressed in cpm (mean of triplicates ± SD). A paired t test was used for statistical analysis. A p < 0.01 was considered as significant.

Western blot analysis and ELISA
Before lysis, cells were washed in ice-cold PBS. For STAT analysis, cells were lysed for 1 h on ice in lysis buffer (1% Nonidet P-40, 50 mM Tris (pH 7.4), 150 mM NaCl, 3% glycerol, and 1.5 mM EDTA) containing protease inhibitors (1 mM PMSF, 1/μg/ml pepstatin, and 10 μg/ml leupeptin) and phosphatase inhibitors (1 mM Na3VO4 and 10 mM NaF). Cell lysis was centrifuged for 15 min at 13,000 × g and the supernatant was assayed for protein concentration using the microBCA protein assay kit (Pierce). T-bet expression was analyzed from nuclear extracts. To this end, cells were lysed for 30 min on ice in lysis buffer A (0.1% Nonidet P-40, 10 mM HEPES (pH 7.9), 10 mM KCl, and 1 mM EDTA) containing protease inhibitors as indicated above. Cell lysis was centrifuged for 15 min at 13,000 × g, and the cell pellet was subjected to a second extraction in lysis buffer B (20 mM HEPES (pH 7.9), 1 mM EDTA, 420 mM NaCl, and 10% glycerol) supplemented with protease inhibitors for 1 h on ice. Cell lysis was centrifuged for 15 min at 13,000 × g, and protein concentration of the nuclear extract was determined as indicated above. Lysates were subjected to SDS-PAGE and transferred to nitrocellulose for immunoblotting. Tyrrosine-phosphorylated STAT proteins were detected using polyclonal rabbit anti-phospho-STAT1, 2, 3, 5, and 6 Abs (1/1000 dilution; Cell Signaling Technology). STAT1 was detected with rabbit polyclonal Ab (1/1000 dilution; Cell Signaling Technology) and STAT3 was detected with mouse F-2 mAb (2 μg/ml; Santa Cruz Technology). T-bet was detected with mouse 4B10 mAb (2 μg/ml; Santa Cruz Technology). Binding of mouse or rabbit Abs was detected with HRP-conjugated mouse or HRP-conjugated protein A, respectively (Amersham Biosciences). Peroxidase reaction was developed with chemiluminescence reagents (Pierce).

Levels of IFN-γ in culture supernatants of stimulated B cells were determined by ELISA using human IFN-γ Quantikine (detection limit: 15 pg/ml; R&D Systems).

Results
Surface expression of IL-27R and gp130 on human tonsillar B cells
Previous studies (5, 33) of IL-27 receptor expression in B cells analyzed the expression of IL-27 receptor subunits at the mRNA level by RT-PCR analysis of total B cells. In this study, we investigated by FACS analysis the cell surface expression of both chains of the IL-27 receptor complex, IL-27R and gp130, on total human tonsillar B cells and on the different B cell subsets. As shown on Fig. 2, both chains were detected on unstimulated total tonsillar B cells. Interestingly, levels of IL-27R expression were very variable depending on the stage of B cell differentiation. Thus, IL-27R was readily detected on both IgD−CD38− naive B cells and IgD+CD38− memory B cells, but was barely detectable

FIGURE 1. Purification of human tonsillar B cell subsets. Subsets of naive, GC, and memory B cells were purified from tonsillar B cells by a two-step magnetic separation, based on their differential surface expression levels of IgD and CD38, as described in Materials and Methods. The purity of each fraction (≈95%), assessed by FACS analysis, is shown.
on IgD<sup>−</sup>CD38<sup>+</sup> GC B cells. In contrast, gp130 was expressed at similar low levels in all three B cell subsets. This expression profile was independent of the donor because similar data were observed using B cells from five different donors.

To investigate the effect of CD40 and surface Ig ligation on IL-27R and gp130 expression, B cells were stimulated for 1 or 2 days with anti-CD40 or anti-μ Abs (Fig. 2). Following CD40 activation, IL-27R and gp130 surface expression was increased on each B cell subset, notably on IgD<sup>−</sup>CD38<sup>+</sup> GC B cells that, once activated, displayed significant levels of both chains of the IL-27 receptor. This increased expression was already observed after 1 day of stimulation and was more pronounced after 2 days of stimulation (Fig. 2 and data not shown). Anti-μ stimulation resulted in a similar increase of IL-27R and gp130 surface expression on IgD<sup>−</sup>CD38<sup>+</sup> naive and IgD<sup>−</sup>CD38<sup>−</sup> memory B cells. Regarding IgD<sup>−</sup>CD38<sup>+</sup> GC B cells, no or very low increase of both chains was observed following 1 day of anti-μ Ab stimulation. After 2 days of anti-μ stimulation, their number was in most experiments too low to allow their analysis (data not shown). Combination of anti-CD40 and anti-μ stimulation resulted in expression levels similar to those observed with anti-CD40 Ab alone (data not shown).

Taken together, these data indicate that the expression of IL-27 receptor is highly regulated during B cell differentiation and following B cell activation.

**FIGURE 2.** Cell surface expression of IL-27R and gp130 on human tonsillar total B cells and B cell subsets. Cell surface expression of IL-27R and gp130 was analyzed on purified tonsillar B cells by indirect staining and FACS analysis, before stimulation (day 0) or after a 2-day stimulation with either anti-CD40 (0.5 μg/ml) or anti-μ Abs (10 μg/ml). To identify the different B cell subsets, cells were also stained with anti-IgD-FITC and anti-CD38-PC5 mAbs, and gated on IgD<sup>−</sup>IgM<sup>+</sup> naive B cells, IgD<sup>−</sup>IgM<sup>−</sup> IgD<sup>−</sup>CD38<sup>+</sup> GC B cells, or IgD<sup>−</sup>IgM<sup>−</sup> IgD<sup>−</sup>CD38<sup>+</sup> memory B cells. Staining with anti-IL-27R or anti-gp130 Abs is represented by a bold line, whereas staining observed with isotype control Abs is represented by a filled gray line (x-axis, log fluorescence intensity; y-axis, cell number). One representative donor of three to five tested is shown. At day 2, anti-μ-stimulated GC B cells are not represented because of the very low number of surviving GC B cells.

**IL-27 activates STAT1 and STAT3 in human tonsillar B cells**

To verify that the IL-27 receptor expressed on human tonsillar B cells is functional, we analyzed by Western blot the activation of STAT proteins in response to IL-27 stimulation. As shown on Fig. 3A, incubation of freshly purified total B cells with IL-27 resulted in strong STAT1 and STAT3 tyrosine phosphorylation. No STAT2, STAT5, or STAT6 activation was observed (data not shown).

To determine in which B cell subsets STAT1 and STAT3 are activated, we performed similar experiments using magnetically purified B cell subpopulations (Fig. 3B). A strong STAT1 and STAT3 phosphorylation was consistently observed in naive B cells. In memory B cells, a significant STAT1 phosphorylation and a weak STAT3 phosphorylation were observed. Despite of a lower total STAT1 protein level and of very low levels of IL-27R and gp130 at the cell surface, a weak phosphorylation of STAT1 and STAT3 was detected in GC B cells. Although we cannot exclude that this weak signal may be in part due to the presence of <5% contaminating naive B cells, this suggests that the low level of IL-27 receptor expression detected on GC B cells may be sufficient to induce a weak STAT activation. Thus, while STAT1 and STAT3 activation was observed in each B cell subset in response to IL-27, the extent of STAT1 and STAT3 phosphorylation was very variable depending on the B cell subset.

**IL-27 induces T-bet expression in anti-CD40 or anti-Ig activated naive and memory B cells**

In mouse CD4<sup>+</sup> and CD8<sup>+</sup> naive T cells, IL-27 induces T-bet expression in a STAT1-dependent manner (11, 25). To investigate whether IL-27 induces T-bet expression in human tonsillar B cells, total B cells were stimulated for 1 or 2 days with anti-CD40 Ab, alone or in the presence of IL-27, and nuclear extracts were analyzed by immunoblotting for T-bet expression. As shown on Fig. 4A (left panel), IL-27 increased T-bet expression in CD40-stimulated total B cells. This induction was already maximal after 1 day of stimulation. Similarly, IL-27 induced T-bet expression in total B cells stimulated for 1 day with anti-μ Ab (Fig. 4A, right panel).

Because we previously observed a significant STAT1 phosphorylation in naive and memory B cells, we analyzed T-bet induction in these two B cell subsets activated via CD40 or via their surface Ig (anti-μ Ab for naive B cells and a combination of anti-μ and anti-γ Abs for optimal stimulation of memory B cells) (Fig. 4B).

**FIGURE 3.** IL-27 induces STAT1 and STAT3 phosphorylation in human tonsillar B cells. Purified total B cells (4, purity >98%) or B cell subsets (B, purity >95% for each subset) were incubated for 15 min with RPMI 1640 containing 2% FBS in the absence (−) or presence of IL-27 (50 ng/ml). Cell lysates were then analyzed by Western blot with anti-phospho-STAT1 or anti-phospho-STAT3 Abs. Blots were subsequently analyzed with anti-STAT1 and anti-STAT3 Abs to monitor STAT expression levels. Blots were also analyzed with anti-actin Ab to verify equal total protein loading (data not shown). Data shown are representative of three independent experiments performed on two to three different tonsils.
Our results indicated that IL-27 induced significant T-bet expression in anti-CD40- or anti-μg-stimulated naive B cells (Fig. 4B, lane 3). Induction of T-bet by IL-27 was also observed in anti-CD40- or anti-Ig-stimulated memory B cells (Fig. 4B, lane 5). However, T-bet induction in these latter was weaker than the one observed in naive B cells.

T-bet has been involved in IFN-γ production by different cell types, including murine B cells (35–39). In human naive CD4 T cells, IL-27 by itself does not induce detectable IFN-γ production, but synergizes with IL-12 for its production (2). To investigate whether B cells produce IFN-γ in response to IL-27, total tonsillar B cells were stimulated for 2–4 days with anti-CD40 or anti-Ig Abs (anti-μ Ab alone or in combination with anti-γ Ab), in the absence of recombinant cytokines or in the presence of IL-27 with or without IL-12, and culture supernatants were tested by ELISA for IFN-γ production (three to four different donors were tested in each condition). In the absence of cytokines or in the presence of IL-27 alone, no IFN-γ was detected (detection limit: 15 pg/ml). In the presence of both IL-12 and IL-27, IFN-γ levels either remained undetectable or did not significantly exceed those observed with IL-12 alone (data not shown). Thus, in human B cells, IL-27, alone or in combination with IL-12, had no significant effect on IFN-γ production.

**IL-27 induces IL-12Rβ2 surface expression in anti-μ-stimulated naive B cells**

Next, we examined whether IL-27-mediated T-bet induction observed in B cells is followed by induction of IL-12Rβ2 expression, as it has been demonstrated in mouse and human CD4 T cells and in mouse CD8 T cells (8–10, 25). In parallel, we analyzed the IL-27 effect on the expression of the β1 chain of the IL-12 receptor.

By cell surface staining and FACS analysis, IL-12Rβ2 chain was undetectable in freshly isolated tonsillar B cells (Fig. 5A). Stimulation with either anti-CD40 Ab or anti-Ig Ab (anti-μ Ab alone or in combination with anti-γ Ab) failed to induce detectable expression of this chain (Fig. 5A). In contrast, IL-12Rβ1 chain was detected in unstimulated naive, GC, and memory B cells, and its expression increased on each B cell subset following anti-CD40 or anti-Ig stimulation (Fig. 5A).

When B cells were stimulated for 2 days with anti-CD40 Ab in the presence of IL-27, a weak increase of IL-12Rβ1 surface expression was consistently observed on total B cells, compared with B cells stimulated with anti-CD40 Ab alone (Fig. 5B). This induction was observed on naive B cells and occasionally on memory B cells, but not on GC B cells (data not shown). Similarly, a weak induction of IL-12Rβ1 by IL-27 was consistently observed on total and naive B cells stimulated with anti-Ig Abs (anti-μ Ab or a combination of anti-μ and anti-γ Abs). Under these conditions, no or a very weak induction was observed on memory B cells. These data were consistent among the different donors tested (five and six different donors tested for anti-CD40 and anti-Ig stimulation, respectively).

No induction of IL-12Rβ2 expression by IL-27 was observed by FACS analysis on total B cells or on B cell subsets stimulated via CD40 (12 different donors tested, Fig. 5B and data not shown). In contrast, when B cells were stimulated with anti-Ig Abs, a significant induction of IL-12Rβ2 expression by IL-27 was observed in most cases (10 of 12 different donors tested). In these cases, IL-12Rβ2 induction was observed in naive B cells, but generally not on memory B cells (Fig. 5B). On both total and naive B cells, induction of IL-12Rβ2 expression by IL-27 was heterogeneous, in that only a fraction of cells showed IL-12Rβ2 positivity (Fig. 5B).

Altogether, these data indicated that the pattern of IL-12Rβ2 induction by IL-27 did not overlay with that of T-bet induction.
Indeed, although induction of T-bet expression by IL-27 was observed in CD40-stimulated B cells, no induction of IL-12Rβ2 expression by IL-27 was detected in these cells. In particular, lack of induction of IL-12Rβ2 expression was observed using B cells from donors that were tested for T-bet induction and showed significant induction of T-bet expression by IL-27 (B cells from the same donor were used for the experiments shown in Fig. 4A, left panel, and Fig. 5B). Also, the low level of T-bet induction by IL-27 observed in anti-Ig-stimulated memory B cells was not sufficient to induce detectable induction of IL-12Rβ2 expression in this specific B cell subset.

**IL-27 induces surface expression of CD54 (ICAM-1), CD95 (Fas), and CD86 (B7.2) on anti-Ig-stimulated naive and memory B cells**

In addition to IL-12Rβ1 and β2, we investigated whether IL-27 could modulate the expression level of various cell surface molecules known to be regulated following B cell activation. Of the various B cell surface molecules analyzed (CD10, CD11a, CD23, CD25, CD39, CD40, CD54, CD80, CD86, CD95, HLA-DR, and HLA class I) only three of them, CD54, CD86, and CD95, showed consistent variation in their surface expression in the presence of IL-27. Thus, as shown in Fig. 6, IL-27 increased expression of CD54, CD86, and CD95 on anti-Ig-stimulated total B cells. This induction was observed on both naive and memory B cells, although it was weaker on the latter. Induction of CD54 and CD95 expression was already detectable after 1 day of stimulation, whereas induction of CD86 expression was generally detectable only after 2 days of stimulation. Similar induction data were observed when B cells were stimulated with a combination of anti-μ...
and anti-\(\gamma\) Abs (data not shown). However, no or very weak induction of these three molecules by IL-27 was observed in anti-CD40-stimulated B cells (data not shown). Similar data were observed using tonsillar B cells from six different donors.

**IL-27 enhances proliferation of activated naive and GC B cells**

The effect of IL-27 on B cell proliferation has not been previously investigated. To investigate a possible effect of IL-27 on B cell proliferation, total B cells were incubated for 3 days with suboptimal doses of either anti-CD40 Ab (0.1, 0.25, or 0.5 \(\mu\)g/ml) or anti-\(\mu\) Ab (2, 5, or 10 \(\mu\)g/ml) in the absence or presence of IL-27, and DNA synthesis was assessed by thymidine incorporation. In each condition, addition of IL-27 resulted in increased B cell proliferation (Fig. 7A). The proliferative effect of IL-27 was modest on CD40-stimulated B cells and more pronounced on anti-\(\mu\)-stimulated B cells. On average, proliferation of anti-CD40-stimulated B cells increased by \(>70\%\) in the presence of IL-27, whereas that of anti-\(\mu\)-stimulated B cells increased by 400\% (\(p < 0.01\)). No consistent increase was observed in anti-\(\gamma\)-stimulated B cells (data not shown). This proliferative effect of IL-27 was dependent. On anti-\(\mu\)-stimulated B cells, a positive effect of IL-27 was already detectable with 1 ng/ml IL-27, and maximal effects were observed at concentrations ranging from 50 to 100 ng/ml IL-27 (Fig. 7B and data not shown).

Next, the proliferative response of the different B cell subsets to IL-27 was analyzed using purified B cell subsets. Because the different B cell subsets responded differently to each stimulus, different suboptimal concentrations of Abs were used to stimulate each B cell subset. We observed a positive effect of IL-27 on proliferation of anti-\(\mu\)-stimulated naive B cells, as their proliferation increased by \(>400\%\) (\(p < 0.01\); Fig. 7C). In our experiments, no significant proliferative response to anti-\(\mu\)-stimulation alone was observed in GC and memory B cells, and addition of IL-27 had no significant effect (data not shown). When each B cell subset was stimulated with anti-CD40 Ab, proliferation of naive and GC B cells increased by \(\sim 100\) and \(\sim 50\%\) on average, respectively (\(p < 0.01\)). In contrast, proliferation of CD40-stimulated memory B cells was not affected by IL-27 (Fig. 7C). To determine whether the lack of response of memory B cells to IL-27 was specific to the stimulus (anti-CD40 Ab), other B cell stimuli were tested. When memory B cells were stimulated with SAC particles, results were similar to those obtained with anti-CD40 stimulation: addition of IL-27 did not increase memory B cell proliferation, while proliferation of SAC-stimulated naive B cells tested in parallel increased by \(\sim 70\%\) on average in the presence of IL-27 (\(p < 0.01\); Fig. 7D).

Only in anti-\(\gamma\)-stimulated memory B cells was a small but not statistically significant proliferative effect of IL-27 observed (data not shown).

**Discussion**

This is the first report demonstrating a role of IL-27 on human B cells. With respect to B cells, only one recent study (33) reported IL-27 effects on murine primary spleen B cells. In that former study (33), the effects of IL-27 were studied on total B cells only. One subunit of the IL-27 receptor complex, IL-27R, was detected at the mRNA level in total murine spleen B cells and was found to be expressed at similar levels in unstimulated or CD40-stimulated B cells. In murine spleen B cells, IL-27 was shown to induce STAT1 activation and IgG2a class switching in a STAT1-dependent but IFN-\(\gamma\) independent manner. The effect of IL-27 on IL-12 receptor expression, cell proliferation, or expression of B cell activation markers was not investigated (33).

Our results showed that IL-27 has direct effects on human tonsillar B cells and that the B cell response to IL-27 is regulated at multiple steps. First, surface expression of IL-27 receptor subunits is regulated during B cell differentiation and following B cell activation. Thus, whereas naive and memory B cells displayed constitutive expression of IL-27R and gp130, GC B cells exhibited barely detectable levels of IL-27R. The differential expression of IL-27R by the different B cell subsets is consistent with a previous study (40), in which gene expression during GC reaction was tracked by gene profiling. In this study, IL-27-R gene expression was found to be down-regulated by 32-fold upon differentiation of naive B cells into GC B cells and to be strongly up-regulated (21.5-fold) during the GC to memory B cell transition, to regain expression levels comparable to those observed in naive B cells. In addition, we found that expression of both chains of the IL-27 receptor increased on each B cell subset upon in vitro B cell activation via CD40 or surface Ig. These results are in line with recent findings showing that mitogenic stimulation of murine CD4 T cells through the TCR enhances IL-27 receptor surface expression (41).

Second, independently from their levels of IL-27 receptor surface expression, B cell subsets responded differently to IL-27. In most assays used in our study, naive B cells exhibited a stronger response than memory B cells to IL-27, although both subsets expressed comparable levels of IL-27 receptor surface expression. In naive B cells, a strong phosphorylation of both STAT1 and STAT3 was observed, whereas in memory B cells a moderate activation of STAT1 and a low activation of STAT3 was observed. Similar to STAT activation, a weaker induction of T-bet expression by IL-27 was observed in memory B cells than in naive B cells, regardless of the mode of B cell activation (CD40 or Ig stimulation). Also, IL-27-mediated induction of the expression of different surface molecules, such as IL-12R\(\beta1\) and \(\beta2\), CD54, CD86, and CD95, was generally more robust on naive B cells than on memory B cells. One striking difference between the two B cell subsets was their proliferative response to IL-27. A previous study (2) showed that IL-27 enhanced proliferation of naive, but not memory, CD4\(^+\) T cells stimulated by anti-CD3 and anti-CD28 Abs in the presence of anti-IL-2 Ab. In a similar manner, we found that IL-27 increased proliferation of naive B cells stimulated with anti-CD40 Ab or SAC, but not that of memory B cells. This lack of effect on memory B cells was not due to the lack of a functional IL-27 receptor, because IL-27 induced expression of T-bet and other molecules (CD54, CD86, and CD95) in these cells. In mouse naive CD4\(^+\) T cells, IL-27-induced proliferation has been shown to be STAT1 independent, whereas STAT3 was considered to be important for IL-27-induced proliferation (11). Taken together, these findings suggest that the lower level of STAT3 activation induced by IL-27 in memory B cells compared with naive B cells may be involved in the lack of proliferative effect of IL-27 observed in memory B cells in these conditions.

We showed that IL-27 induced T-bet expression in anti-CD40 or anti-Ig stimulated human tonsillar B cells. In murine B cells, T-bet has been linked to IgG2a class switching. Specifically, T-bet has been shown to be necessary for IgG2a class switching in response to T-independent stimuli such as LPS, but not T-dependent stimuli via CD40 (36, 42–44). Consistent with these data and the lower amount of serum IgG2a observed in IL-27R-deficient mice (12), IL-27 was shown to induce IgG2a class switching in a T-bet-dependent manner in LPS-stimulated murine spleen B cells (33). The role of T-bet in class switching in human B cells is unknown and the observations from murine B cells cannot be transposed to human B cells. First, IgG subclasses differ in humans and mice, and in humans the IgG2a isotype does not exist. Second, due to their very low levels of TLR4 expression, human B cells respond very poorly to LPS stimulation (45) and as a consequence isotype...
switching cannot be studied in these cells in the context of LPS stimulation. Thus, in human B cells, it was difficult to establish a direct link between T-bet, IL-27, and class switching to a specific isotype. In addition, our preliminary experiments suggest that IL-27 has no major effect on Ig class or IgG subclass production. Indeed, when culture supernatants of CD40- or SAC-activated tonsillar B cells were tested by ELISA for Ig classes (IgM, IgG, IgA, and IgE) or IgG subclasses (IgG1, IgG2, IgG3, and IgG4), no significant and consistent variation was observed when IL-27 was present during the 12-day culture (three to five different donors tested; our unpublished observations). This failure to detect IL-4 expression in CD40-stimulated B cells, no up-regulation of IL-12Rß1 chain was detected on each B cell subset in the absence of stimulation. Its expression significantly increased following CD40 or surface Ig stimulation, and showed a further increase in the presence of IL-27. In previous studies (46, 47) of IL-12 receptor expression in human tonsillar B cells, expression of both chains was analyzed by RT-PCR. IL-12Rß1 and ß2 genes were found to be constitutively expressed in each B cell subset. This constitutive expression of IL-12Rß2 gene by unstimulated tonsillar B cells contrasts with the lack of detection of IL-12Rß2 surface expression by FACS analysis. This failure to detect IL-12Rß2 by FACS is more likely to be due to a low sensitivity of the immunostaining technique compared with RT-PCR analysis than to a dissociated expression of IL-12Rß2 mRNA and protein. Indeed, freshly purified tonsillar B cells express a functional IL-12 receptor at the cell surface, as shown by their ability to respond to IL-12 (46). Also, this low sensitivity of IL-12Rß2 immunostaining may be responsible for the lack of detectable IL-12Rß2 induction in response to anti-ß plus IL-27 stimulation observed in 2 of the 12 donors tested. Indeed, when IL-12Rß2 expression was analyzed in these two donors at the mRNA level by RT-PCR, a clear induction of IL-12Rß2 mRNA in response to anti-ß plus IL-27 stimulation was observed. In contrast, in CD40-stimulated B cells, no up-regulation of IL-12Rß2 by IL-27 was observed, both at the protein and mRNA levels (Fig. 5B and unpublished observations). This selective induction of IL-12Rß2 expression by IL-27 in anti-ß-, but not anti-CD40-, stimulated B cells, indicates that this IL-27 effect depends on the mode of B cell activation, and that T-bet induction is not sufficient to induce detectable induction of IL-12Rß2 expression in CD40-stimulated B cells. Similarly, we observed an induction of CD54, CD86, and CD95 expression by IL-27 when B cells were stimulated via their surface Ig, but no or a very low induction when B cells were stimulated via CD40. This much lower IL-27 effect observed in this latter condition may be due to the already strong induction of these molecules by CD40 stimulation itself. Indeed, although both anti-Ig and anti-CD40 Abs induced expression of these molecules, the induction observed following anti-Ig stimulation was much weaker than the one observed following anti-CD40 stimulation (our unpublished observations). Our finding that IL-27 induced ICAM-1 expression on human tonsillar B cells, is in line with a study (48) published while this manuscript was in preparation, that showed induction of ICAM-1 expression by IL-27 in mouse CD4+ T cells.

Altogether, these data indicate that IL-27 has multiple effects on B cells and may play a role at different stages of B cell differentiation. Further studies are necessary to delineate the mechanisms underlying the differential response of each B cell subset to IL-27 and to investigate its possible role in B cell pathological conditions.

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Disclosures

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References
