IL-27 induces the production of IgG1 by human B cells

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ABSTRACT. It has been reported that IL-27 specifically induces the production of IgG2a by mouse B cells and inhibits IL-4-induced IgG1 synthesis. Here, we show that human naïve cord blood expresses a functional IL-27 receptor, consisting of the TCCR and gp130 subunits, although at lower levels as compared to naïve and memory splenic B cells. IL-27 does not induce proliferative responses and does not increase IgG1 production by $CD19^+CD27^+$ memory B cells. However, it induces a low, but significant production of IgG1 by naïve $CD19^+CD27^-IgD^+IgG^-$ spleen and cord blood B cells, activated via CD40, whereas it has no effect on the production of the other IgG subclasses. In addition, IL-27 induces the differentiation of a population of B cells that express high levels of CD38, in association with a down-regulation of surface IgD expression, and that are surface $IgG^{+/int}$, $CD20^{low}$, $CD27^{high}$, indicating that IL-27 promotes isotype switching and plasma cell differentiation of naive B cells. However, as compared to the effects of IL-21 and IL-10, both switch factors for human IgG1 and IgG3, those of IL-27 are modest and regulate exclusively the production of IgG1. Finally, although IL-27 has no effect on IL-4 and anti-CD40-induced Cϵ germline promoter activity, it up-regulates IL-4-induced IgE production by naive B cells. These results point to a partial redundancy of switch factors regulating the production of IgG1 in humans, and furthermore indicate the existence of a common regulation of the human IgG1and murine IgG2a isotypes by IL-27.

Keywords: cytokines, immunoglobulin, interleukin-27, B cell, isotype switching

IL-27 is a heterodimeric cytokine belonging to a family of structurally related cytokines that also includes IL-12, IL-23 and IL-6 [1]. It is produced in humans, by activated, antigen-presenting cells, such as monocytes, macrophages and monocyte-derived dendritic cells, as well as by endothelial cells [1-3]. IL-27 is composed of two chains, the EBV-induced gene3 (EBI3), a 33-kDa glycosylated protein, and a 28-kDa protein that are homologous to the p40 [4] and the p35 subunits of IL-12 [1, 5] respectively. The functional signal-transducing receptor (R) complex is composed of two chains, TCCR (also known as WSX-1) and gp130 [1, 6], the latter being a receptor component shared by several cytokines of the IL-6 family [7]. Initial studies on IL-27 in the mouse have documented the pro-inflammatory role of this cytokine that, by synergizing with IL-12 to induce the production of IFN-γ by human naïve $CD4^+$ T cells, contributes to the early differentiation of T cells into Th1 cells [1]. However, results from several studies, using functional IL-27R (WSX-1)-deficient mice, have shown an exacerbated response to a variety of challenges, indicating that IL-27 has important immunoregulatory functions *in vivo*. The latter notion is underscored by the results from two very recent studies demonstrating that IL-27 inhibits the development of IL-17-producing T cells [8, 9], highlighting the pleiotropic effects of IL-27, not only as a positive, but also negative regulator of inflammatory immune responses.

Several studies in the literature have addressed the role of IL-27 in B cells. In the mouse, IL-27 has been shown to regulate the production of Ig by B cells. IL-27 regulates the production of IgG2a, as TCCR-deficient mice were found to have reduced total IgG2a serum concentrations, whereas the levels of the other Ig isotypes were normal as compared to those in wild-type animals [10]. Furthermore, IL-27 induces IgG2a class switching in anti-CD40- or LPS-activated splenic mouse B cells, and inhibits IgG1 class switching induced by IL-4 [11]. Finally, in a model of experimentally-induced asthma, Miyazaki *et al*. have shown that ovalbumin (OVA)-challenged WSX-$1^{-/-}$ mice had increased serum IgE levels as compared with wild-type mice [12]. Although no information about the involvement of IL-27 in human Ig production has been reported, the expression of the IL-27R subunits was reported to be strongly regulated during human B cell differentiation implying that IL-27-mediated effects vary depending on the stage of B cell differentiation [13]. We have therefore analyzed, in the context of IL-27R expression by human naive and memory B cells, whether IL-27 is able to modulate Ig production.

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METHODS

Donors and cells

All human umbilical blood cells and spleen cells, used in this study, were obtained in accordance with the guidelines of the ethical committee of the Montpellier University Hospitals. Highly purified (purity > 98%) $CD19^+$ spleen B cells were obtained from human spleen fragments of healthy organ donors (Service de Chirurgie Digestive, CHU St Éloi, Montpellier, France) by positive selection using specific mAb-coated magnetic beads and a preparative magnetic cell sorter (Miltenyi Biotech, Bergisch Gladbach, Germany), as described [14]. Naïve $CD27^-sIgG^-$ and memory $CD27^+sIgG^+$ B cells were then purified following two-color staining of $CD19^+$ B cells with a PE-conjugated anti-CD27 mAb (clone M-T271, BD Biosciences, San Jose, CA, USA) and a FITC-labeled mouse anti-human surface (s) IgG mAb (BD Biosciences) and sorting of B cells, using a FACS Vantage® (BD Biosciences), according the procedure described by Scheffold *et al.* [15]. Purified naïve $CD19^+CD27^-$ B cells (purity > 98%) were also isolated from cord blood (Service Maternité, CHU Arnaud de Villeneuve, Montpellier, France) by depletion of the $CD2^+$, $CD3^+$, $CD16^+$, $CD36^+$, $CD56^+$, $CD66b^+$ cells, using the Rosettesep® procedure (StemCell Technologies, Meylan, France), according to the manufacturer's recommendations. The Epstein-Barr virus-negative Burkitt lymphoma cell line BL-2 clone 20, containing all the regulatory elements of the human Cϵ germline promoter of IgE has been described [16].

Culture conditions

Stimulation of B cells for proliferative responses or induction of Ig production was carried out as follows: naïve or memory $CD19^+$ human B lymphocytes (10^6/mL) were cultured with 1 µg/mL of the anti-CD40 mAb 89 [17], in the presence or absence of varying concentrations of recombinant (r)IL-27 (Schering Biopharma, Palo-Alto, CA, USA) in flat-bottomed, 96-well culture plates (Nunc, Roskilde, Denmark) in Yssel's medium [18], supplemented with 10% FCS, in sextuplet, in a final volume of 200 µl. For comparison, IL-21 and IL-10 (kind gifts from Dr. Don Foster, Zymogenetics, Seattle, WA, USA and Dr. Francine Brière, Schering-Plough, Dardilly, France, respectively) were added in parallel. For examining the effect of IL-27 on IgE synthesis, rIL-4 (gift from Dr Francine Brière) was added to the culture at 20 ng/mL. Proliferative responses were measured after 5 days of culture at 37° and 5% CO_2. After 12 days of incubation, culture supernatants were collected and the respective production of IgG and IgE was quantified by isotype-specific ELISA. For the determination of expression of IL-27R, as well as the state of B cell differentiation, splenocytes were cultured with an irradiated (40 Gy) CD40L-expressing mouse fibroblast L cell line, at a B cell/L cell ratio of 40:1, in the presence or absence of exogenous cytokines. The splenocytes were collected at various periods of culture and analyzed by three-color immunofluorescence and flow cytometry.

Proliferation assay

Proliferative responses were measured by thymidine incorporation by stimulated B cells. After 4 days of culture, 37 kBq of tritiated thymidine ([^{3}H]TdR, Amersham-France, Les Ulis, France) were added to the cultures for 18 h, after which the cells were harvested onto glass fiber sheets, using an automated cell harvester (Tomtec, Orange, CT, USA). Radioactivity was measured, using a microbeta Trilux scintillation counter (Wallac, Turku, Finland).

Cell surface immunofluorescence staining and FACS analysis

The purity of isolated $CD19^+$ B cells ($5x10^4$ cells per staining) was determined by flow cytometry analysis using FITC-conjugated CD2, CD3 and CD20 T and B cell-specific mAbs (BD Biosciences), whereas sorted naïve ($CD27^-sIgG^-$) and memory ($CD27^+sIgG^+$) cells were re-analyzed for control of their respective purity. The expression of IL-27R by BL2 clone 20 cells was determined by flow cytometry, following staining of the cells with the anti-human TCCR mAb (clone 191115, R&D systems Europe, Abingdon, United Kingdom) or the anti-gp130 mAb (clone AN-G30: [19]), both at 20 µg/mL, in parallel with mouse IgG2b or IgG1 (BD Biosciences), used as isotype controls, respectively, and incubation of the cells with a PE-conjugated $F_{(ab')2}$ goat anti-mouse IgG (Caltag, Burlingame, CA, USA). The expression of IL-27R by naïve and memory B cells in populations of total splenocytes was determined by three-color flow cytometry on the lymphocyte population gated on the size, and granulometry. The staining procedure was identical to that used for the BL2 cells, including incubation of the cells with mouse normal IgG1 (25 µg/mL: SouthernBiotech, Birmingham, AL, USA) to prevent subsequent non-specific binding of mAbs to the PE-conjugated goat anti-mouse IgG antibody. Finally, an anti-CD3-APC and an anti-CD27-FITC mAbs (both from BD Biosciences) were added simultaneously in order to electronically remove the $CD3^+$ cells and to identify the naive and memory B cell populations. For analysis of the IL-27R expression by purified naïve cord blood B cells, the addition of the anti-CD3-APC mAb was omitted. The expression of cell surface molecules, indicative of isotype switching and B cell differentiation, was analyzed by three-color flow cytometry, using non-separated cell preparations and combinations of an anti-CD3-APC and an anti-CD38-FITC mAbs with the following PE-labeled mAbs: anti-sIgG-PE, anti-sIgD-PE, anti-CD20-PE and anti-CD27-PE (all obtained from BD Biosciences). Cells were analyzed on a FACSCalibur using the CellQuest software (BD Biosciences).

Measurement of Ig production

IgG1, IgG2, IgG3, IgG4 and IgE secretion was determined in culture supernatants by isotype-specific ELISA, as described previously [14].

Analysis of Cϵ promoter gene activity

The BL2-clone 20 cell line was seeded at 10^6 cells/mL in a 96-well, flat-bottomed tissue culture plate (Nunc) and incubated with 1 µg/mL anti-CD40mAb cross-linked onto a goat anti-mouse IgG (1 µg/mL, Calbiochem, Burlingame,

CA, USA) and of 20 ng/mL rIL-4, in the presence or absence of various concentrations of rIL-27. Where indicated, rIFN-γ (R&D systems) was added at concentrations of 50 and 100 ng/mL. After 24, 48 and 72 h of incubation, the cells were lysed, and luciferase activity was determined using the dual-luciferase reporter assay system (Promega France, Charbonnière, France) on a Lumat luminometer (Berthold, Bad Wildbad, Germany) as described [16].

RESULTS

Naive cord blood B cells express lower levels of a functional IL-27R complex, as compared to naive and memory splenic B cells

Both naive and memory tonsilar B cells have been reported to express the IL-27R, although the latter cells do not show increased proliferation following stimulation with anti-CD40 mAb in the presence of IL-27 [13]. In order to determine whether naïve cord blood B cells are responsive to IL-27, the expression of the TCCR and gp130 chains was analyzed by immunofluorescence and flow cytometric analysis. $CD27^-$ naive cord blood B cells were found to express very low levels of both chains *(figure 1)*. By comparison, both naive and memory splenic B cells expressed high levels of TCCR, as well as gp130, although both chains were expressed at lower levels by $CD27^-$ naive B cells, as compared to $CD27^+$ memory B cells (*figure 1)*. Expression levels of both the TCCR and gp130 subunits in purified cord blood B cells was not significantly affected following stimulation of the cells via CD40, neither at 24 nor 48 h of culture *(figure 2)*. Despite the low expression levels of the IL-27R on $CD19^+CD27^-$ naive cord blood B cells, IL-27 enhanced, in a dose-dependent fashion, the proliferation of anti-CD40-stimulated cord blood B cells *(figure 3)*, indicating that they are responsive to stimulatory effects of IL-27.

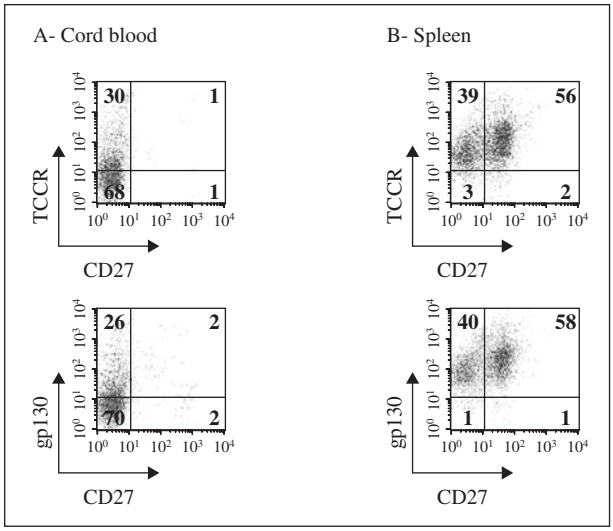

Figure 1

Naive cord blood and naive and memory splenic B cells express the IL-27R. Expression of the TCCR and gp130 chains forming the IL-27R complex in freshly isolated $CD19^+CD27^-$ naive cord blood (**A**) and splenic $CD27^-$naive and $CD27^+$ memory B cells (**B**) was analyzed by indirect and two- and three-color flow cytometry, respectively. Cell surface expression of CD27 (*x-axis*) and TCCR or gp-130 (*y-axis*) on lymphocyte-gated cells is represented by a four-decade log scale as dot-blots of correlated FITC and PE fluorescence, respectively. Quadrant markers were positioned to include > 98% of control Ig-stained cells in the lower left quadrant (not shown). For the expression of IL-27R and CD27 on splenic B cells, an anti-CD3-APC mAb was added and B cells were electronically gated on the CD3-APC-negative population. The numbers in the quadrants indicate the percentage of TCCR and/or gp130-expressing naive and memory B cells.

IL-27 acts as a specific switch factor for the production of IgG1 by human $CD19^+CD27^-$ naive B cells stimulated with anti-CD40 mAb

The capacity of IL-27 to modulate the production of IgG1, IgG2, IgG3 and IgG4 was investigated using purified human naive B cells. Therefore, splenic, naive B cell populations were separated from isotype-committed memory B cells, based on the concomitant absence of cell surface (s) CD27 and sIgG, by magnetic and double immunofluorescence flow cytometric cell sorting using an anti-sIgG-FITC and an anti-CD27-PE mAb. Reanalysis of sorted B cells showed that naïve ($CD27^-sIgG^-$) and memory ($CD27^+sIgG^+$) B cells were about equally represented and their purity after separation was ≥ 98% and ≥ 96% respectively (results not shown). Naive $CD19^+CD27^-$ B cells were activated with anti-CD40 mAb in the presence of increasing concentrations of rIL-27 and the production of Ig was analyzed by isotype-specific ELISA. In all experiments, rIL-27 significantly increased, in a dose-dependant manner, the production of IgG1 by $CD19^+CD27^-$ B cells. This enhancing effect was specific for IgG1, as rIL-27 had no clear and consistent effect on the production of IgG2, IgG3 and IgG4 *(figure 4)*. However, unlike rIL-21, a potent switch factor for the production of human IgG1 and IgG3, rIL-27 enhanced only modestly the production of IgG1 *(figure 4)* with a mean and maximal increase of 4 fold and 12 fold, respectively (results not shown). Furthermore, in contrast to the effects of IL-21, those of IL-27 were restricted to the IgG1 subclass *(figure 5)*. In order to demonstrate whether IL-27 acts as a switch factor for the induction of IgG1 production by naive B cells, these experiments were repeated using naive cord blood B cells (100% $CD19^+CD27^-$) and a single optimal dose of IL-27. rIL-27 induced, albeit at very low levels, the production of IgG1 by cord blood B cells, while not affecting the production of the other IgG sub-classes *(figure 6)*. Finally, similar results were obtained with splenic B cells stimulated by CD40L-transfected L cells (results not shown). In contrast, rIL-27 had no effect on IgG1 production by $CD19^+CD27^+$ memory B cells, stimulated with either anti-CD40 mAb or CD40L-expressing L cells (results not shown). Taken together, these results indicate that IL-27 specifically induces the production of IgG1 by naive B cells.

IL-27 induces the differentiation of naïve B cells into plasma cells

To further determine whether rIL-27 has the capacity to induce isotype switching and the subsequent B cell differentiation into plasma cells, purified splenocyte populations were cultured for varying periods of time in the presence of both anti-CD40 mAb and rIL-27. T cells were excluded by electronically gating on the population of $CD3^-$ cells

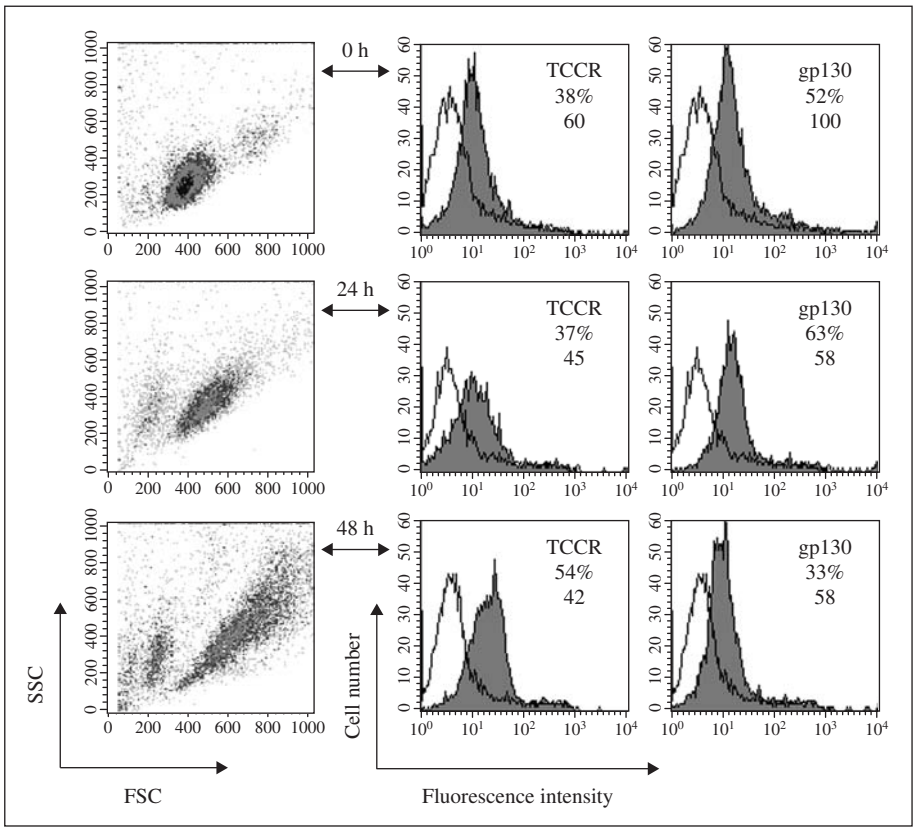

Figure 2
Activation of cord blood B cells does not result in the modulation of either TCCR or gp-130 expression. Purified cord blood B cells were activated with CD40L-expressing mouse fibroblast transfectant for 24 and 48 h, as indicated in Methods. Expression of IL-27R subunits was analyzed as mentioned in *figure 1*. Numbers represent the percentage of positive cells and mean fluorescence intensity.

and naïve ($CD19^+CD27^-sIgD^+$) or memory ($CD19^+CD27^+sIgD^+$ and $CD19^+CD27^+sIgD^-$) B cells were analyzed for changes in the expression of sIgD and CD38, indicative of isotype switching and plasma cell differentiation, respectively. $CD38^{high}$ B cells, which were either $sIgD^-$, $sIgG^{+/int}$, $CD20^{low}$ or $CD27^+$, thus representing IgG-producing committed memory B cells, were present at a low frequency (< 2%) in freshly isolated splenocytes and this latter population was not significantly increased when splenic B cells were activated with CD40L in the absence of exogenous cytokines (*figure 7*A and B). The addition of rIL-27 to CD40L-activated splenic B cells resulted in a low, but significant, increase in the percentage of B cells devoid of sIgD and expressing high levels of CD38, which reflects a down-regulation of sIgD expression on initially $sIgD^+$ naïve B cells through a mechanism of isotype switching and their subsequent differentiation into a $CD38^{high}$ plasma cell phenotype *(figure 7A)*. In parallel, the emergence of a $CD38^{high}sIgG^{+/int}$ B cell population was observed. This effect of rIL-27 was observed at day 5 (1.5%), but not at day 3 (< 0.5%) of culture, and increased over time, as higher percentages of the latter B cells were observed at day 7 (mean effective increase 5.0%) of culture *(figure 8)*. In addition, the rIL-27-induced $CD38^{high}$ B cell population was found to express, in a time-dependent manner, lower levels of CD20, as well as high levels of CD27 (*figure 7*B and 8). However, the effects of rIL-27 were modest, both in its magnitude, as well as in its timedelay, in comparison to IL-21 and IL-10, as the latter factors induced the formation of 10 to 20% of IgD^--switched B cells and plasma cell differentiated B cells by day 3 of culture.

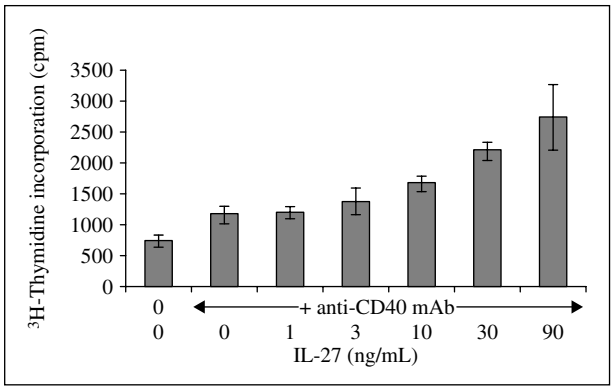

Figure 3
IL-27 induces the proliferation of anti-CD40 mAb-activated cord blood B cells. Purified cord blood B cells were activated with 1 µg/mL of the anti-CD40 mAb and increasing concentrations of rIL-27, in triplicate, for 4 days, and ^{3}H-thymidine was added for the last 18 h of culture. Values represent means ± SD of two independent experiments.

IL-27 enhances IL-4-induced IgE production by human $CD19^+CD27^-$ naïve B cells stimulated with anti-CD40 mAb and rIL-4

Next, we examined whether IL-27 has the capacity to modulate IL-4-induced IgE production by splenic and cord blood naïve B cells. Whereas IL-27 alone did not induce

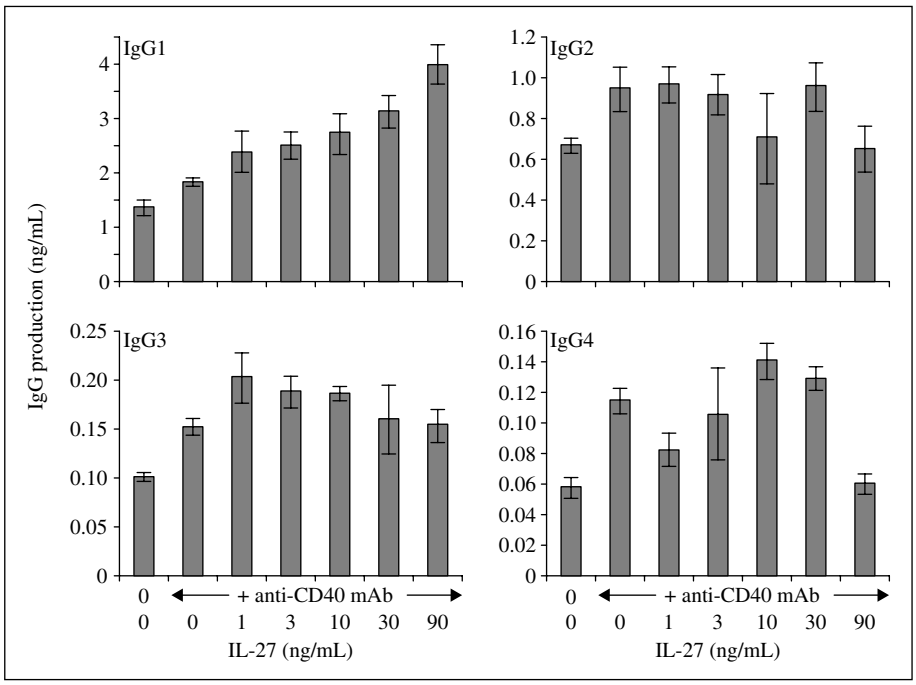

Figure 4

IL-27 increases the production of IgG1 by $CD19^+CD27^-$ naïve splenic B cells. Spleen $CD27^-sIgG^-$ naïve B cells were purified from $CD19^+$ B cells and activated with 1 µg/mL of anti-CD40 mAb in the presence or absence of increasing amounts of rIL-27, as described in Methods. Levels of each IgG subclasses were determined by isotype-specific ELISA after 12 days of culture. Values represent mean ± SD of five independent experiments, using spleen samples from three donors.

IgE synthesis directly *(figure 9)*, it strongly enhanced, in a dose-dependent fashion, IL-4-induced IgE production by both adult *(figure 9A)* and cord blood *(figure 9B)* $CD19^+CD27^-sIgD^+sIgG^-$ naïve B cells. Furthermore, in the presence of rIL-4, rIL-27 had no effect on the production of the IgG subclasses (results not shown). In order to determine whether the effect of rIL-27 on rIL-4-induced IgE production was due to a direct action on the Cϵ switch promoter activity, its effect was tested in a germline Cϵ promoter gene reporter assay. BL-2 cells, stimulated with rIL-4 in the presence of CD40L-expressing L cells, expressed both the TCCR and gp-130 subunits at their surface (results not shown), indicating their potential responsiveness to rIL-27. Stimulation of BL-2 cells with an anti-CD40 mAb and IL-4 induced a strong increase in the Cϵ reporter gene expression which was time-dependent *(figure 10A)* and which was partially inhibited by the addition of IFN-γ, used as a positive control *(figure 10B)*.

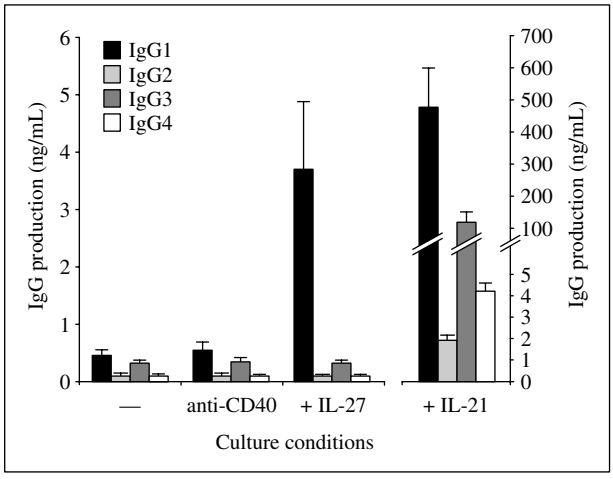

Figure 5

IL-27 has a lower capacity to enhance the production of IgG1, as compared to rIL-21. Spleen $CD27^-sIgG^-$ naïve B cells were purified from $CD19^+$ B cells and activated with 1 µg/mL of anti-CD40 mAb in the absence or presence of rIL-27 or rIL-21, both at a concentration of 10 ng/mL, as described in Methods. Levels of each IgG subclasses were determined as indicated in *figure 4*. Values represent mean ± SD of two experiments, using spleen samples from two donors.

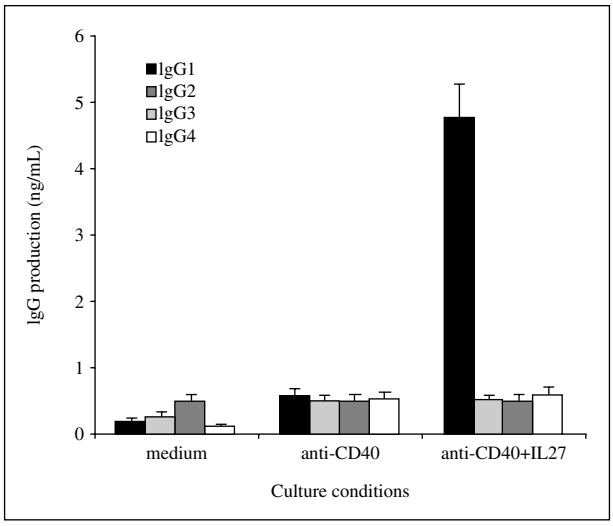

Figure 6

IL-27 induces the production of IgG1 by $CD19^+CD27^-$ naive cord blood B cells. Cord blood B cells were purified as described in Methods and activated with 1 µg/mL of anti-CD40 mAb in the absence or presence of 10 ng/mL of rIL-27. Levels of each IgG subclasses were determined as indicated in *figure 4*. Values represent mean ± SD of three experiments, using spleen samples from three donors.

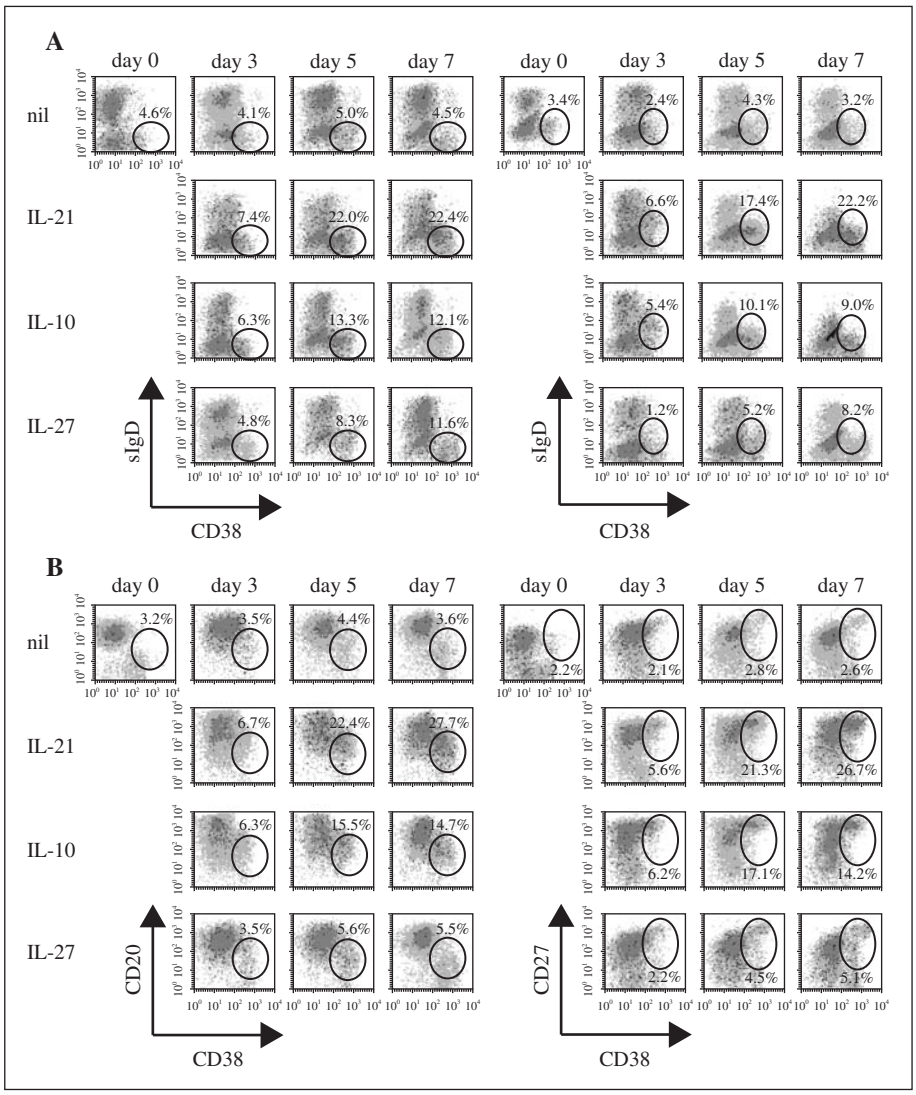

Figure 7

IL-27 induces CD19^{+}CD27^{-}sIgD^{-} naive B cells to switch and differentiate into plasma cells. Splenocytes were purified and activated with 1 µg/mL of anti-CD40 mAb in the absence or presence of rIL-10, rIL-21 or rIL-27 (each at 10 ng/mL) for 3, 5 and 7 days. Representative experiment showing cell surface expression of CD38 (*x-axis*) as compared to sIgD or sIgG (**A**) and CD20 or CD27 (**B**) (*y-axis*) on lymphocyte-gated cells is represented by a four-decade log scale as dot-blots of correlated FITC and PE fluorescence. Quadrant markers were positioned to include > 98% of control Ig-stained cells in the lower left. Data represented as indicated in legends to *figure 1*. At each of the indicated incubation periods, the cells were analyzed for the expression of CD38 and sIgD, sIgG, CD20 or CD27 by immunofluorescence and flow cytometry. Kinetics of the percentage of CD38^{high} B cells, expressing sIgD or sIgG (**A**), or expressing CD20 or CD27 (**B**), cultured in medium alone or in the presence of cytokines. The percentage of gated cells is indicated on each graph.

The addition of increasing amounts of IL-27 did not modify the expression of the reporter gene activity and furthermore, did not reverse the inhibitory effect of IFN-γ on the IL-4-mediated Cϵ gene promoter activation.

DISCUSSION

The differentiation of naive sIgM-expressing B cells into IgG, IgE or IgA producing plasma cells is a highly regulated process involving both interaction between B cell-expressed CD40 and its ligand CD154 on T cells, and the action of cytokines that determine the isotype specificity of the switched cells (reviewed in [20, 21]). In the present study, we show that IL-27 induces human naive B cells to specifically differentiate into IgG1-producing plasma cells.

It has been reported in the literature that both TCCR and gp130 subunits of the IL-27R are constitutively expressed at the surface of naive and memory human tonsillar B cells and that their expression is increased following CD40 stimulation [13]. However, in a different study the expression of TCCR mRNA by both naïve and memory tonsilar B cells was not modulated following anti-B cell receptor stimulation, either in the presence or absence of CD40 triggering or of IFN-γ [22]. The reason for this discrepancy is not clear. We extend these results by showing that both IL-27R chains are expressed on naive cord blood B cells, albeit at much lower levels than those on naive and memory splenic B cells. Furthermore, stimulation of naïve cord blood B cells with CD40 did not result in the up-regulation of TCCR and gp130 expression. The observation that IL-27 induces proliferative responses and IgG1 synthesis by naïve B cells, while not affecting memory B cells, indicates that IL-27-mediated effects are dependent on the stage of B cell differentiation, but are not correlated

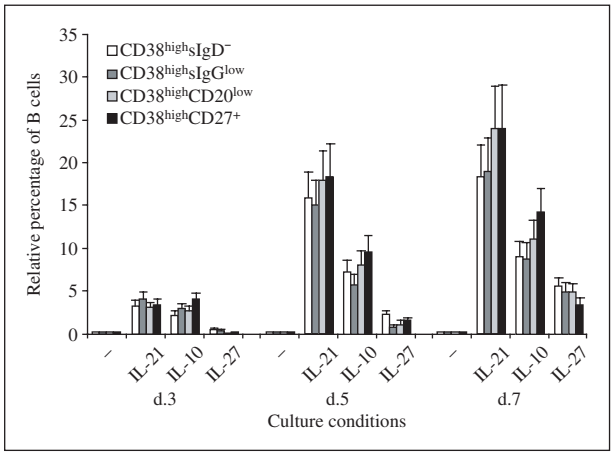

Figure 8
Kinetics of the percentage of $CD38^{high}$ $sIgD^{-}$, $sIgG^{low}$, $CD20^{low}$ or $CD27^{+}$ B cells induced by IL-27. Splenocytes were treated as described in *figure 7*. Values represent the net percentage of positive cells found in each culture condition in the presence of cytokines after subtraction of the percentage of positive cells in medium alone. Mean ± SD of three experiments, using spleen samples from three donors.

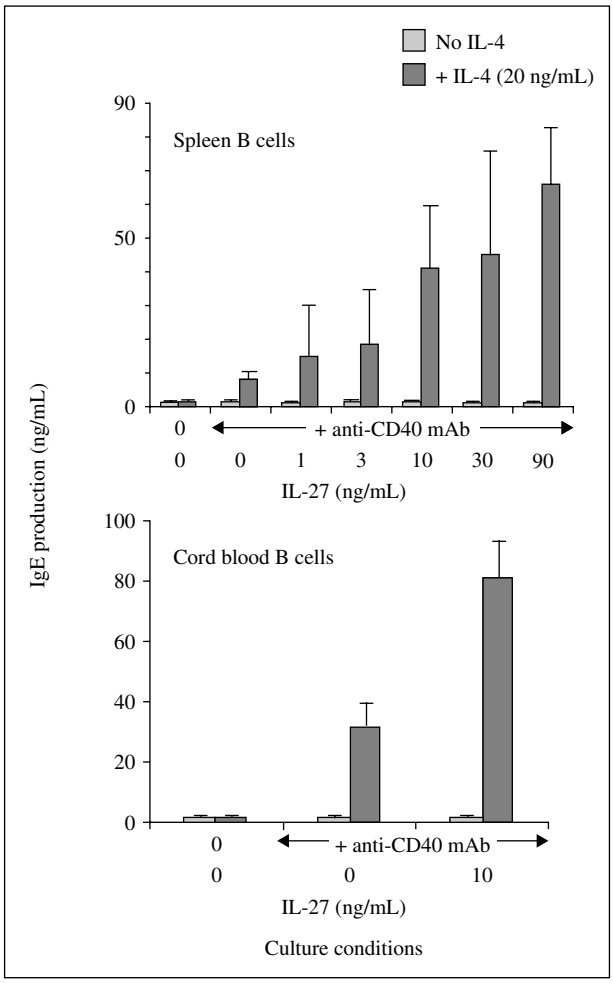

Figure 9
IL-27 enhances IL-4-induced IgE production by human $CD19^{+}CD27^{-}$ naïve B cells. $CD19^{+}CD27^{-}$ naïve spleen (**A**) and cord blood (**B**) B cells were purified and activated with 1 µg/mL of anti-CD40 mAb in the presence or absence of rIL-4 (20 ng/mL) and variable amounts of rIL-27 for 12 days. Levels of IgE were determined by isotype-specific ELISA. Values represent mean ± SD of five and three experiments using spleen or cord blood samples, respectively, each from three different donors.

with expression levels of the IL-27R, as already observed by Larousserie *et al*. [13].

Many cytokines that induce Ig production by isotype switching of naive B cells also have growth-promoting activities on committed B cells. However, the observation that IL-27 does not induce proliferative response in memory B cells, excludes the possibility that IL-27 may act by promoting the outgrowth of memory B cells. Human splenocytes reportedly contain, in addition to naive B cells, germinal center B cells. However, the Bm2' and the Bm3/Bm4 cells that form the germinal center B cell population are clearly $CD27^{+}$ [23]. Moreover, it has been shown that both human centroblasts and centrocytes express CD27, although at different levels of CD27 [24] and therefore cannot be considered to contain naive B cells. As we carried out immuno-fluorescence and cell sorting to purify naïve splenic B cells, faintly stained $CD27^{+}$ cells, representing centrocytes, were excluded from the naive, $CD27^{-}$, population. Moreover, the observation that IL-27 induced the production of IgG1 by cord blood B cells, a population that is composed exclusively of $sIgD^{+}$ naive B cells, indicates that the action of IL-27 on IgG1 secretion is the result of a switch-promoting effect of this cytokine on naive, non-committed B cells.

Naïve B cells bear, in addition to IgM, IgD at their cell surface, whereas they do not express sIgG, sIgA or sIgE. It is well accepted that the emergence, among $sIgD^{+}$ naive B cells, of $sIgD^{-}$ cells results from a switch recombination event, and consequently constitutes a signature of a switch to the production of IgG, IgA or IgE antibodies. Similarly, the acquisition of CD38 expression by activated $CD38^{-}$ naive B cells constitute a signature of their differentiation into antibody-secreting plasma cells [25]. Here, we show that the addition of IL-27 to splenocytes results in the down-regulation of sIgD expression on initially $sIgD^{+}$ naïve B cells, as well as in the concomitant emergence of a population of $CD38^{high}sIgG^{+/int}$ B cells that have not yet differentiated into plasma cells. Although we do not formally show that $CD38^{high}sIgG^{+/int}$ differentiating B cells produce IgG1, our data strongly support the notion that IL-27 may induce the production of the latter isotype by a mechanism involving the induction of isotype switching and subsequent B cell differentiation into IgG1-secreting $CD38^{high}$ plasma cells.

It has been reported previously that successful isotype switching of naive B cells is division-associated and is therefore dependent on their degree of proliferation [26]. In addition, naive B cells were found to enter the plasma cell differentiation pathway with a 30h delay as compared to memory B cells, and that the latter cells proliferate at a faster rate as compared to naïve B cells [27, 28]. The observation that IL-27 triggers the proliferation of naïve B cells only (ref. 13 and the present study), might explain the relatively modest, and time-delayed induction of $sIgD^{-}$ switched B cells and $CD38^{+}$ differentiated plasma cells, in comparison to that of IL-21 and IL-10, which are strong proliferative factors for memory and naive B cells.

Our results are in line with those showing that IL-27 regulates the production of IgG by mouse B cells. Mice deficient for the WSX-1 gene have reduced IgG2a serum concentrations, but normal levels of the other Ig isotypes, as compared to wild-type animals [10]. This finding was corroborated by the observation that IL-27 induces IgG2a

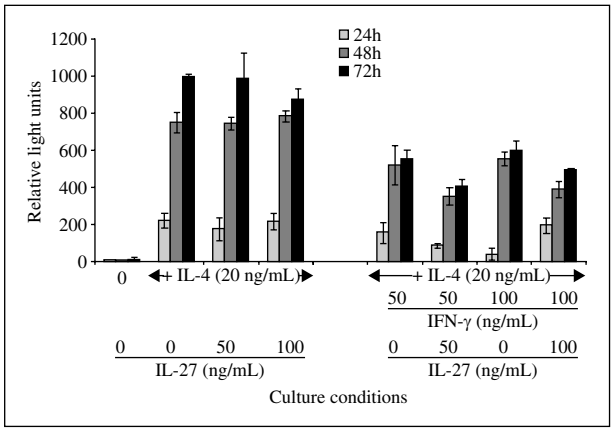

Figure 10

IL-27 does not affect IL-4-induced Cϵ switch promoter activity. The Burkitt lymphoma cell line BL-2 clone 20 was stimulated with rIL-4 (20 ng/mL), anti-CD40 mAb (1 µg/mL), crosslinked with a goat-anti mouse IgG (1 µg/mL), in the presence or absence of rIL-27 (50 and 100 ng/mL) and/or rIFN-γ (50 and 100 ng/mL) for 24, 48 and 72 h and germline Cϵ promoter activity was determined by luciferase assay. Values represent mean ± SD of two independent experiments, using spleen samples from two donors.

class switching in activated mouse B cells *in vitro* [11]. However, it is important to stress that, similar to our results, IL-27 exerts only very modest effects on Ig production in the mouse, as the magnitude of the induction of IgG2a production by activated naive B cells *in vitro* is comparable to those on IgG1 production in humans. Moreover, the IgG2a production-inducing capacity of IL-27 in the mouse is clearly inferior to that of IFN-γ, the other cytokine known to induce switching of naive mouse B cells to the production of this isotype [29], indicating that IL-27 does not play a major role in the induction of murine IgG-mediated humoral immune responses.

However, results obtained with WSX-1-deficient mice have indicated that IL-27 might regulate IL-4-dependent IgE production, as WSX-1-deficient mice showed increased serum IgE levels following allergen challenge, as compared with wild-type mice, which was associated with an increased production of Th2 cytokines in the lung, and clinical manifestation of airway responsiveness [12]. Furthermore, in a model of membranous glomerulonephritis, it has been shown that WSX-1$^{-/-}$ mice present a predominance of IgG1 in glomerular deposits, accompanied by increased IgG1 and IgE in the sera [30]. As the induction of both isotypes is under the control of IL-4, these results suggest that IL-27 might interfere with IL-4-mediated Ig production. Indeed, IL-27 was found to inhibit IgG1 class switching of anti-CD40 mAb- or LPS-activated splenic mouse B cells *in vitro*, although no data on the production of IgE were provided [11]. In humans however, IL-27 does not seem to have direct inhibitory effects on IL-4-induced IgE synthesis by naive B cells. As IL-27 did not modulate IL-4-induced Cϵ gene promoter activity, this result suggest that the observed enhancement of IL-4-induced IgE synthesis can be attributed to the growth-promoting activity of IL-27 on *de novo* differentiated IL-4-switched B cells. This conclusion is supported by the observation that IL-27 induces a stronger proliferation of naïve B cells in the presence of IL-4 (results not shown) than in its absence *(figure 5)*.

In humans, the induction of IgG1 production by naive B cells is also under the control of IL-10 and IL-21, two unrelated cytokines known for their capacity to induce isotype switching via the activation of γ1 (and γ3) germline promoters [14, 31, 32]. In addition, IL-21 has a great capacity to induce the differentiation of B cell into Ig-producing plasma cells [25]. However, as shown previously and in the present study, the effects of IL-27 are clearly inferior as compared to those of the latter two cytokines. This situation is similar in the mouse where IFN-γ is a much more potent IgG2a switch-inducing cytokine than IL-27 [11, 28]. Taken together, these results point out a redundancy as well as a hierarchy among the factors regulating isotype switching of B cells to the production of IgG1, with IL-27 having a rather marginal role in this process. Finally, although the diversification of mouse and human IgG subclasses has evolved in an independent manner [33], our results suggest that the synthesis of IgG2a in the mouse and that of IgG1 in human, characterized by similar effector functions in infectious immune responses, is not only controlled by different major regulatory cytokines, including IFN-γ in the mouse and IL-10 and IL21 in the human, but may also have conserved a common regulatory process mediated by IL-27.

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