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, CD20low, CD27high, indicating that IL-27 promotes isotype switching and plasma cell differentiation of naïve 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 naïve 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 IgG1 and murine IgG2a isotypes by IL-27.

Keywords: cytokines, immunoglobulin, interleukin-27, B cell, isotype switching
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) 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 CD24+, 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 (10^7/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% CO2. 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.

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-CD40 mAb cross-linked onto a goat anti-mouse IgG (1 μg/mL, Calbiochem, Burlingame, CA, 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 naïve 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-slgG-PE, anti-slgD-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).

Proliferation assay
Proliferative responses were measured by thymidine incorporation by stimulated B cells. After 4 days of culture, 37 kBq of tritiated thymidine (¹¹HJtdR, 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).
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 naive 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.

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 naive (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 IgG was analyzed by isotype-specific ELISA. In all experiments, rIL-27 significantly increased, in a dose-dependent 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 rIL-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). Similarly, 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 naive 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 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 three-color flow cytometry, respectively. Cell surface expression of CD27 (x-axis) and TCCR or gp130 (y-axis) on lymphocyte-gated cells is represented by a four-decade log scale as dot-blot 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.
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. CD38high 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 7A 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 CD38high plasma cell phenotype (figure 7A). In parallel, the emergence of a CD38high 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 CD38high B cell population was found to express, in a time-dependent manner, lower levels of CD20, as well as high levels of CD27 (figure 7B 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.

**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
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).

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.

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.

IL-27 induces the production of IgG1 by CD19^+CD27^− naïve 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.
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 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-blot 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.
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 naïve 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 naïve 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 naïve 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 naïve, 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 slgD− naïve B cells, indicates that the action of IL-27 on IgG1 secretion is the result of a switch-promoting effect of this cytokine on naïve, non-committed B cells.

Naïve B cells bear, in addition to IgM, IgD at their cell surface, whereas they do not express slgG, slgA or slgE. It is well accepted that the emergence, among slgD− naïve B cells, of slgD+ 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− naïve 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 slgD expression on initially slgD− naïve B cells, as well as in the concomitant emergence of a population of CD38high slgGint B cells that have not yet differentiated into plasma cells. Although we do not formally show that CD38high slgGint 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 CD38high plasma cells.

It has been reported previously that successful isotype switching of naïve B cells is division-associated and is therefore dependent on their degree of proliferation [26]. In addition, naïve 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 slgD− 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 naïve 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
IL-27 does not affect IL-4-induced Ce 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-rabbit IgG (20 ng/mL), anti-CD40 mAb (1 µg/mL), in the presence or absence of rIL-27 (50 and 100 ng/mL) and/or IFN-γ (50 and 100 ng/mL) for 24, 48 and 72 h and germine Ce promoter activity was determined by luciferase assay. Values represent mean ± SD of two independent experiments, using spleen samples from two donors.

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