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Brief Conclusive Report

Interleukin-27 and interleukin-23 modulate human plasmacell functions

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ABSTRACT

This study tested the hypothesis that IL-27 and IL-23, two heterodimeric cytokines involved in physiological immune responses and immunological disorders, may function on human PC and plasmablasts. It was reported that IL-27 acts on human mature B cells, but the role of IL-27 and IL-23 in human PC remains to be established. Thus, we have asked whether these cytokines may modulate human PC functions using human PPC generated in vitro, PC isolated from tonsils, and BM. Here, we show for the first time that PC and PPC express complete IL-27R and that IL-27 exerts chemotactic properties on these cells and modulates different chemokines/chemokine receptors and secretion of IgM and IgG. Furthermore, we demonstrated that PC and PPC express both chains of IL-23R and IL-23 and exerted similar activity to IL-27 in terms of Ig production, while not inducing PC chemotaxis. These results may provide novel insight into the role of IL-27 and IL-23 in human B cell immune responses. *J. Leukoc. Biol.* 89: 729–734; 2011.

Introduction

IL-23 and IL-27 are immunomodulatory cytokines belonging to the IL-12 superfamily [1], which is composed of structurally and functionally related heterodimeric cytokines, including IL-12, IL-23, IL-27, and IL-35, involved in the promotion and/or maintenance of Th1 differentiation [1]. IL-27R contains the unique receptor subunit WSX-1 paired with the gp130 chain [2, 3], whereas the IL-23R is composed of the IL-12Rβ1 chain and the unique IL-23R chain [4]. IL-27R is expressed on human naïve and memory tonsil B cells and is up-regulated on germinal center B cells following CD40 stimulation [5]. IL-27 induces STAT-1 and -3 phosphorylation, exerts differential effects on human B cells depending on their activation/differentiation status, and induces a modest IgG1 production [6] but does not have a major effect on Ig production and class-switching [5]. In contrast, no information is available about IL-23R expression and function in human B cells.

In humans, three major subsets of PC have been isolated and are supposed to represent a gradual increase in PC maturation: early PC in tonsils, transitional PC in PB, and mature PC in BM [7]. However, PC are rare cells in vivo, representing only 1–2% of tonsil mononuclear cells and <0.5% of BM cells in healthy individuals. Thus, an in vitro-reproducible model of PB B cell differentiation into PPC, which have morphological and phenotypic features of PC, was developed [8].

Here, we asked whether IL-23 and IL-27 may function on human PPC and PC and demonstrated that both cytokines induce PPC to secrete IgM; IL-27 but not IL-23 induces specific chemotaxis of PPC and PC and modulates different chemokines/chemokine receptors; and IL-23 and IL-27 are ineffective in terms of modulation of PPC and PC proliferation and apoptosis.

MATERIALS AND METHODS

Detailed methodologies are fully described in the online **Supplemental Material**. Briefly, PPC were generated in vitro from PB samples of 18 healthy volunteers obtained after informed consent. This study was approved by the G. Gaslini Institute Ethical Committee (Genova, Italy). The PPC obtained using two procedures [9] were consistently $CD19^{+}CD20^{-}CD38^{++}CD138^{+/-}$ [8]. Tonsil PC (n=6) were sorted as $CD19^{+}$,$CD38^{bright}$,$IgD^{negative/low}$ cells or purified by positive selection using CD138-coated magnetic beads. PC from BM aspirates of three healthy donors were purified to homogeneity by positive selection using CD138-coated magnetic beads. Purity of all PPC and PC samples ranged from 90% to 95%.

Expression of both chains of IL-27R and IL-23R was analyzed on 14 PPC cell samples, six tonsil PC, and three BM PC by flow cytometry. Intracellular stainings were performed using

Abbreviations: BM=bone marrow, CD40L=CD40 ligand, MRFI=multiple risk factor intervention, PB=peripheral blood, PC=plasmacell(s), PPC=polyclonal plasmablastic cells, si=small interfering

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a cytofix/cytoperm kit and fluorochrome-conjugated anti-human CXCL9 and CXCL10 and anti-human phosphorylated STAT-1, -3, or -5. STAT signaling was studied using tonsil PC (n=2) and PPC (n=2), incubated 30 min with or without 50 ng/ml human rIL-27. The same experiments were performed using PB $CD4^+$ T cells and tonsil B cell preparations containing naïve and memory B cells as positive controls [5, 10].

ELISA for IgG, IgM, IgA, and IgG subclasses was performed using supernatants collected from $CD138^+$ tonsil PC cultured with or without IL-23 or IL-27 for 48 h or from PPC generated in vitro, treated 4 days with CD40L, IL-2, IL-4, IL-10, and CpG 2006 and for an additional 2 days with IL-2 alone or with IL-23 or IL-27; IL-6 alone or with IL-23 or IL-27; and IL-2 and IL-6 with or without IL-23 or IL-27.

Chemotaxis of PPC (n=10) and tonsil PC (n=6), in response to different concentrations of IL-23 and/or IL-27, was tested using transwell plates. In some experiments, tonsil PC (n=4) and PPC (n=4) were cultured 1 h with 100 ng/ml PTX (i.e., inhibitor of Gαi protein-coupled receptors, including chemokine receptors) and subsequently tested for chemotaxis to IL-27. Silencing of WSX-1 in five PPC samples was achieved by Silencer® select predesigned siRNA. Efficiency of WSX-1 silencing at a protein level was confirmed by flow cytometry. Chemotaxis of PPC (n=4) to CXCL12 (i.e., a conventional chemokine that binds the CXCR4 Gi protein-coupled receptor) was tested with or without PPC pretreatment with PTX. Three PPC samples transfected with siWSX1 or irrelevant siRNA were tested for chemotaxis in response to IL-27 or CXCL12. Modulation of chemokines/chemokine receptor expression was tested by a PCR array in two $CD138^+$ tonsil PC and three PPC samples cultured 36 h with or without IL-27.

RESULTS AND DISCUSSION

IL-27R and IL-23R expression in human PPC and PC

Fig. 1A shows that WSX-1 (upper panels) and IL-23R (lower panels) were expressed consistently in PPC generated in vitro (A) or PC from BM (B) and tonsil (C). A summary of all of the experiments performed was reported in Fig. 1B. PPC and PC also expressed the gp130 and IL-12Rβ1 chains (not shown and ref. [9]), indicating that these cells may be responsive to the corresponding cytokines.

Our finding that human PPC and PC expressed IL-27R is not surprising, as it has been reported that IL-27R was up-regulated during B cell differentiation and in germinal center transition to memory B cells [5, 11]. However, we did not observe significant differences in IL-27R expression within PC from tonsil, PB, and BM, which are postulated to represent the main steps of PC differentiation [7].

IL-27 and IL-23 modulate IgM and IgG secretion in human PPC and PC

The activity of IL-23 and IL-27 on PPC and PC was next investigated in terms of modulation of Ig secretion, chemotaxis, and modulation of chemokines/chemokine receptor expression, cell proliferation, and apoptosis. We demonstrated that IL-2 + IL-23 or IL-2 + IL-27 PPC treatment, compared with stimulation with IL-2 or IL-23 or IL-27 alone, caused significant up-regulation of IgM secretion (Fig. 1C, upper panel) paralleled by inhibition of IgG secretion (Fig. 1C, lower panel). Such effects were reverted when IL-6 was present for the last 2 days of culture. By contrast, the release of IgA by PPC was unaffected by IL-23 or IL-27 treatment (not shown).

The same supernatants used for the above experiments and supernatants obtained from four additional PPC samples were tested by ELISA for IgG1, IgG2, IgG3, and IgG4 secretion. These experiments revealed that IL-2 + IL-27 and more marginally, IL-2 + IL-23 PPC treatment reduced (P=0.0006, and P=0.0379, respectively) IgG1 secretion (inset in Fig. 1C, lower panel) significantly, whereas IgG2, IgG3, and IgG4 were undetectable in all experimental conditions (not shown).

Production of Ig represents the main PC function and a key step that occurs during antigen-specific immune response. Although IgM are mainly produced during primary immune responses, and IgG are the hallmark in secondary/memory responses [12], IgG and IgM are involved in complement activation and stimulation of phagocytes. Thus, considering the ability of IL-23 and IL-27 to induce IgM secretion, it is tempting to speculate that these cytokines are mainly involved in primary immune responses. Furthermore, the demonstration that IL-23 and IL-27 did not affect Ig secretion in PPC when added with IL-6 on the last 2 days of culture suggests that Ig secretion may be modulated by IL-23 and IL-27 in PPC that have not yet undergone terminal differentiation into antibody-secreting cells. This latter consideration is strongly supported by the finding that IL-23 and IL-27 had no effect on modulation of Ig secretion in $CD138^+$ tonsil PC (not shown).

Finally, we may speculate that decreased production of IgG1 driven by IL-27 and more marginally, by IL-23 parallels that of total IgG, as no detectable increase of IgG2, IgG3, and IgG4 subclass secretion was observed in these experiments. Although we cannot formally exclude that these cytokines do not affect Ig class-switching, previous studies demonstrated that IL-27 did not modulate IgG class-switching in tonsil B cells activated by anti-CD40 or *Staphylococcus aureus Cowan* [5] and regulated exclusively the production of IgG1 [6].

IL-27 induces chemotaxis and modulates expression of chemokines/chemokine receptors in human PPC and PC

The role of IL-23 and IL-27 as chemotactic molecules has never been demonstrated. Here, we provided the first evidence that IL-27, but not IL-23, induced specific chemotaxis of PPC and PC, which migrated to 100 ng/ml IL-27 (**Fig. 2B**), the optimal concentration selected, following dose-response experiments (Fig. 2C). PPC and PC chemotaxis to IL-27 was unambiguously driven by IL-27 and not induced by secondary signals, as demonstrated by the following: PTX (i.e., inhibitor of Gαi protein-coupled receptors, including chemokine receptors)-pretreated tonsil PC and PPC maintained the ability to migrate to IL-27 (Fig. 2D and E) but not to CXCL12 (Fig. 2D), and siRNA-mediated silencing of WSX-1 abolished the IL-27 (Fig. 2B and E)- but not the CXCL12-driven chemotaxis (Fig. 2E). One representative experiment proving the effi-

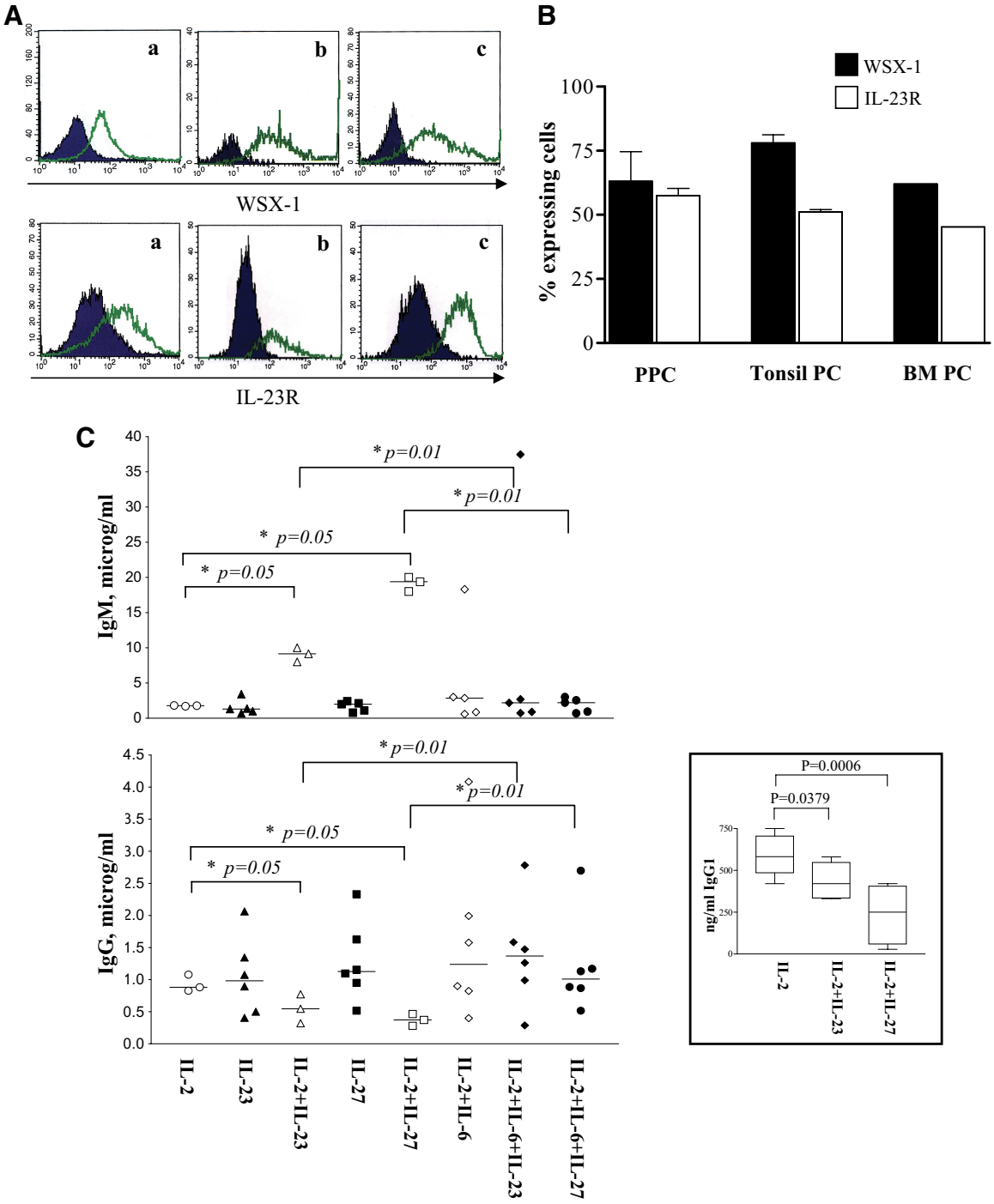

Figure 1. IL-23 and IL-27 receptor expression and function in human plasma cells. (A) WSX-1 and IL-23R surface expression in human PPC (a) or PC isolated from BM (b) or tonsils (c), as assessed by flow cytometry. Open profiles: WSX-1 or IL-23R staining; shaded profiles: isotype-matched mAb staining. One representative experiment is shown. (B) Percentages ± SD of PPC, tonsil PC, and BM PC expressing the WSX-1 and IL-23R, obtained from all of the experiments performed, are shown. (C) PPC were generated from $CD19^+$ PB cells treated 4 days with CD40L, IL-2, IL-4, IL-10, and CpG 2006. Cells were then washed and cultured for an additional 48 h with different cytokines: IL-2, IL-23, IL-27, IL-2 + IL-23, IL-2 + IL-27, IL-2 + IL-6, IL-2 + IL-6 + IL-23, and IL-2 + IL-6 + IL-27. Supernatants were collected after 48 h of culture and tested for IgM (upper panel) and IgG (lower panel) concentration by ELISA. Each plot represents a single experiment. Horizontal lines indicate medians. A *P* value <0.05 was considered significant. (Inset, lower panel) IgG1 concentration in supernatants from PPC were generated in vitro. On the last 2 days, cells were cultured with IL-2, IL-2 + IL-27, or IL-2 + IL-23. Boxes indicate values between the 25th and 75th percentiles; whisker lines represent highest and lowest values. Horizontal lines represent median values.

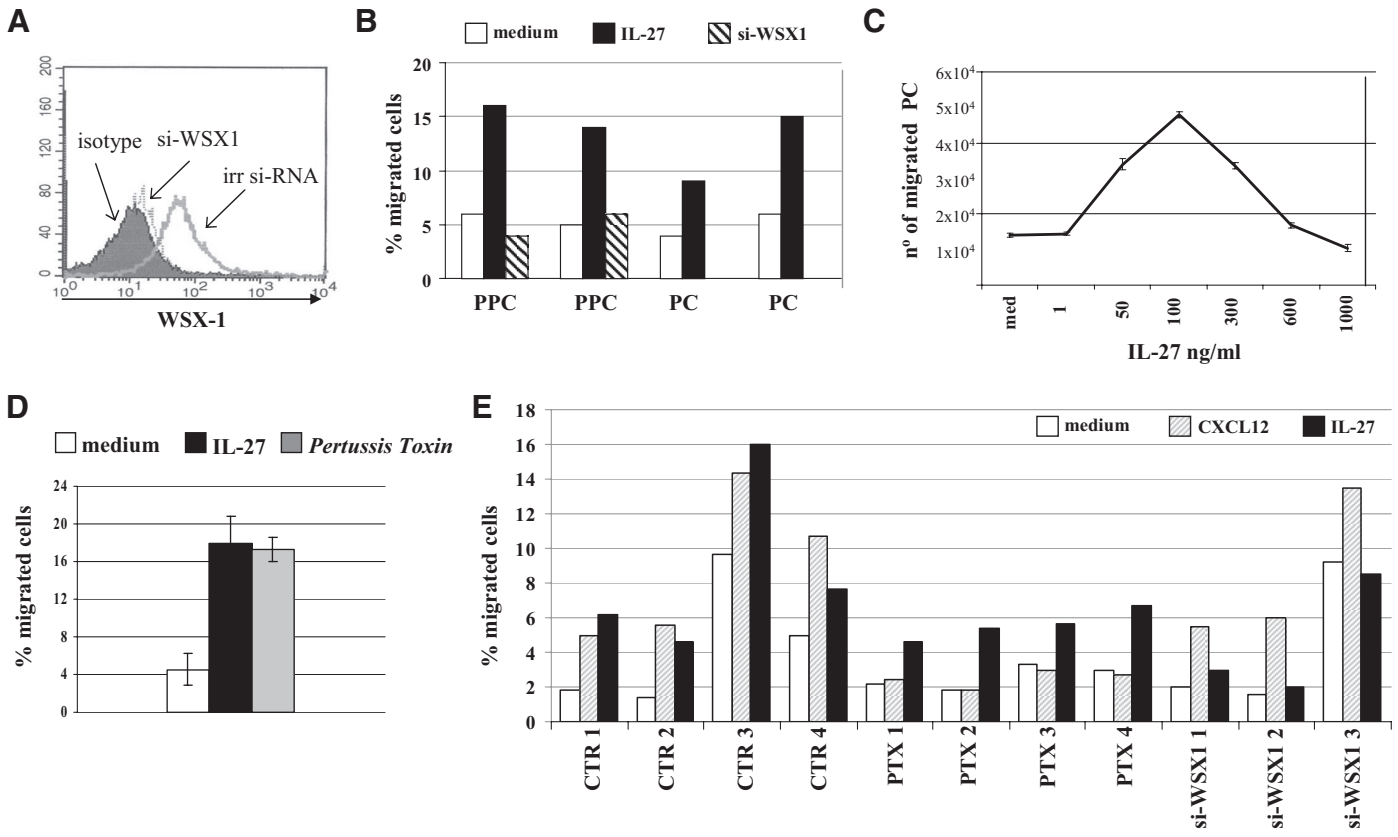

Figure 2. Chemotaxis of human plasma cells in response to IL-27. (A) WSX-1 expression in PPC transfected with siWSX1 or irrelevant (irr) siRNA. Analysis was performed by flow cytometry 48 h after transfection. One representative experiment is shown. (B) IL-27-driven chemotaxis of two PPC and two PC samples is shown. Results are expressed as percentage of migrated cells (absolute number of cells recovered in the lower chamber/absolute number of cells seeded in the upper chamber×100). (C) Dose response chemotaxis to IL-27 of tonsil PC. Pooled results from three different experiments ± SD are shown. (D) Chemotaxis of tonsil PC samples (n=4) to IL-27 following pretreatment with PTX. Open bar, PC cell chemotaxis to medium alone; black bar, PC chemotaxis in response to IL-27; gray bar, PC samples were pretreated with PTX and subsequently tested for chemotaxis to IL-27. Pooled results ± SD are shown. (E) Chemotaxis of PPC to IL-27 or CXCL12. CTR, PPC were tested for chemotactic response to medium, 300 ng/ml CXCL12 or 100 ng/ml IL-27. Four different experiments are shown. PTX, PPC were pretreated 1 h with PTX and next tested for chemotactic response to medium, CXCL12, or IL-27. siWSX1, PPC were silenced for WSX-1 expression and tested for chemotactic response to medium, CXCL12, or IL-27; three different experiments are shown.

ciency of siWSX1 to reduce the protein level of the receptor is shown in Fig. 2A.

To elucidate the signaling events underlying the chemotactic activity of IL-27, we analyzed the ability of IL-27 to induce phosphorylation of STAT-1 in tonsil PC and PPC, as it was demonstrated recently that migration of DCs in response to cytokines, such as IFN-β, was dependent on STAT-1 activation [13]. Here, we show that IL-27 specifically induced STAT-1 phosphorylation in PC (MRFI=1.47; **Fig. 3A**, top panels), suggesting that also in these cells, STAT-1 may be involved in cytokine-driven chemotaxis. Furthermore, IL-27 did not induce STAT-3 or STAT-5 phosphorylation in PC (MRFI=1 and 0.98, respectively) differently to that reported for naïve and memory tonsil B cells, here (Fig. 3A, middle panels) and by others [5]. These findings suggested that the STAT activation pathway driven by IL-27 was variable depending on the B cell subset. Similar results were obtained with two PPC samples (not shown). PB $CD4^+$ T cells, tested as positive controls [10], showed STAT-1, -3, and -5 phosphorylation upon IL-27 stimulation in the same experimental conditions (Fig. 3A, bottom panels).

Finally, we investigated whether IL-27 was able to influence the expression of chemokines/chemokine receptors in PPC and PC, thus providing an additional signal for PC and PPC migration or recruitment of other immune effector cells. As shown in Fig. 3B, IL-27 treatment caused up-regulation of CXCL9 and CXCL10 and down-regulation of different CCL chemokines, such as CCL1, CCL7, CCL8, and CCL16, in tonsil PC. Similar results were obtained in three PPC samples (not shown). CXCL9 and -10 are chemoattractant molecules for activated T and NK cells and possess potent, antiangiogenic activity through IFN-γ-dependent or -independent pathways [14–16]. However, in PC and PPC, CXCL9 and -10 modulation by IL-27 was not accompanied by induction of IFN-γ production (not shown). Flow cytometric analysis of intracellular stainings for CXCL9 and -10 in two tonsil PC samples revealed that IL-27 treatment up-regulated the percentage of $CXCL9^+$ cells (medium alone 46.44% vs. IL-27 59.58%; Fig. 3C) but not

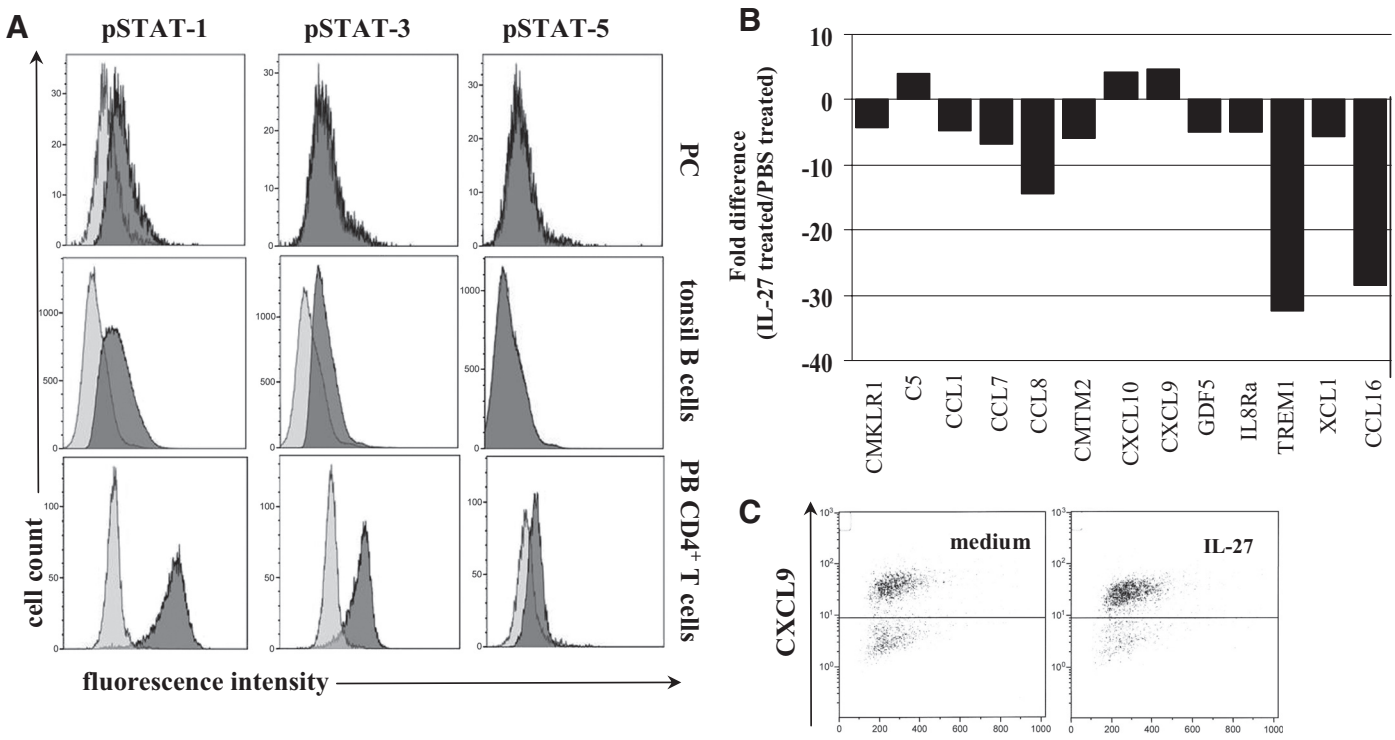

Figure 3. IL-27 signal transduction of chemokine receptors. (A) Phosphorylated (p)STAT-1 (left histograms), phosphorylated STAT-3 (middle histograms), and phosphorylated STAT-5 (right histograms) were assessed by flow cytometry in CD138$^+$ tonsil PC (top panels), tonsil B cells (middle panels), and CD4$^+$ T cells (bottom panels), stimulated 30 min with IL-27 (dark-shaded profiles) or medium (light-shaded profiles). One representative experiment is shown. (B) Gene expression profile of human chemokines/chemokine receptor genes in two CD138$^+$ tonsil PC samples cultured in the presence or absence of IL-27. Pooled results are shown. Histogram represents fold differences of individual mRNA expression between tonsil PC cultured in the presence or absence of IL-27. CMKLR1, chemokine receptor-like 1; CMTM2, chemokin-like factor MARVEL transmembrane domain-containing 2; GDF5, growth/differentiation factor 5; IL8Ra=IL-8R antagonist; TREM1, triggering receptor expressed on myeloid cells 1; XCL1, chemokine (C motif) ligand 1. (C) CXCL9 intracellular staining of CD138$^+$ tonsil PC cultured 48 h with medium alone (left panel) or with IL-27 (right panel) by flow cytometry. One representative experiment is shown.

CXCL10 (not shown). Superimposable results were obtained using two PPC preparations (not shown) or by antibody array.

Up-regulation of CXCL9 associated with down-regulation of different CCL chemokines may be relevant for the type of immune cells recruited during immune responses. Thus, IL-27 produced by APCs may function through PC and PPC by creating a chemokine gradient that attracts in inflamed, tissue-activated T, memory T, and NK cells but not neutrophils, granulocytes, and monocytes [17, 18]. In addition, other chemokines/chemokine receptors, such as CXCR4 and its ligand CXCL12, CCR2, and CD162, involved in migration and accumulation of PC in the BM [19], were not modulated by IL-27.

Taken together, our results support the concept that IL-27 and more marginally, IL-23 may play important roles in the course of a physiological-immunological response to pathogens by acting at different levels. These cytokines are produced by DCs stimulated by PAMPs through TLRs, and subsequently, IL-27 may attract plasmablast/short-lived PC; IL-27-recruited plasmablasts/short-lived PC up-regulate CXCL9 in turn, which can promote chemotaxis of activated T cells, NK cells, and memory T lymphocytes [17]; and IL-23 and IL-27 may induce short-lived PC to secrete IgM.

Although we reported that BM PC also expressed IL-23R and IL-27R, the functional relevance of these findings remains to be established.

Finally, no significant results were obtained in terms of proliferation and apoptosis (not shown), suggesting that these two cytokines do not affect expansion and survival of PPC and PC but only their immunological functions.

AUTHORSHIP

C.C. and F.M. performed experiments and analyzed data. I.A. designed research, analyzed data, and wrote the paper.

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DISCLOSURE

The authors have no conflicts of interest.

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