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Serine leucocyte proteinase inhibitor-treated monocyte inhibits human CD4$^+$ lymphocyte proliferation

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Summary

Serine leucocyte proteinase inhibitor (SLPI) is the main serine proteinase inhibitor produced by epithelial cells and has been shown to be a pleiotropic molecule with anti-inflammatory and microbicidal activities. However, the role of SLPI on the adaptive immune response is not well established. Therefore, we evaluated the effect of SLPI on lymphocyte proliferation and cytokine production. Human peripheral blood mononuclear cells (PBMC) were treated with mitogens plus SLPI and proliferation was assessed by [^{3}H]thymidine uptake. The SLPI decreased the lymphocyte proliferation induced by interleukin-2 (IL-2) or OKT3 monoclonal antibodies in a dose-dependent manner. Inhibition was not observed when depleting monocytes from the PBMC and it was restored by adding monocytes and SLPI. SLPI-treated monocyte slightly decreased MHC II and increased CD18 expression, and secreted greater amounts of IL-4, IL-6 and IL-10 in the cell culture supernatants. SLPI-treated monocyte culture supernatant inhibited the CD4$^+$ lymphocyte proliferation but did not affect the proliferation of CD8$^+$ cells. Moreover, IL-2 increased T-bet expression and the presence of SLPI significantly decreased it. Finally, SLPI-treated monocyte culture supernatant dramatically decreased interferon-γ but increased IL-4, IL-6 and IL-10 in the presence of IL-2-treated T cells. Our results demonstrate that SLPI target monocytes, which in turn inhibit CD4 lymphocyte proliferation and T helper type 1 cytokine secretion. Overall, these results suggest that SLPI is an alarm protein that modulates not only the innate immune response but also the adaptive immune response.

Keywords: inflammation; innate immunity; macrophages/monocytes; proteases; serine leucocyte proteinase inhibitor; T cells; T helper type 1

Introduction

Human serine leucocyte proteinase inhibitor (SLPI) is an 11 700 molecular weight cationic non-glycosylated serine protease inhibitor constitutively produced in mucosal surfaces such as airway epithelium and human uterus, but it can also be secreted by inflammatory cells.[1,2] The protease inhibitory site of SLPI resides in the C-terminal domain and inhibits cathepsin G, trypsin, chymotrypsin, chymase, tryptase and mainly neutrophil elastase.[2] The major function of SLPI is to inhibit inflammation by blocking the proteolytic activity of serine proteases released by leucocytes and also through down-modulating several cytokines up-regulated via lipopolysaccharide such as tumour necrosis factor-α, monocyte chemoattractant protein-1 and interleukin-6 (IL-6).[2–4] However, it is well established that SLPI anti-inflammatory activity is also mediated by inhibition of proteolytic degradation of inhibitor of κB

Abbreviations: CS, culture supernatant; PBMC, peripheral blood mononuclear cells; SLPI, serine leucocyte proteinase inhibitor.

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(IκB) and the activation of the transcription factor nuclear factor-κB (NF-κB) in monocytes.[5,6] Moreover, SLPI knockout mice show impaired cutaneous wound healing with increase activation of local transforming growth factor-β (TGF-β).[7] Other relevant functions described for SLPI, which are independent of its anti-protease properties, include its antimicrobial activity against fungi, viruses and several bacterial strains.[2,8–12] Recently, we have also reported that SLPI constitutes a pattern recognition receptor for mycobacteria that not only kills bacteria but facilitates their phagocytosis by macrophages.[13] However, the role of SLPI in adaptive immunity has been seldom tackled. Samsom *et al.*[14] proposed that SLPI expression in dendritic cells located in cervical lymph nodes contributes to mucosal tolerance. Furthermore, SLPI regulates class switching by dampening IgG and IgA class switching recombination without affecting survival and B-cell proliferation.[15] However, splenic B cells from $SLPI^{-/-}$ mice have higher proliferation rates and produce higher levels of IgM.[16] Recently, it was suggested that SLPI produced by dendritic cells can play an important role in dampening specific immune activation.[17]

The high expression of SLPI in mucosa and different microenvironments such as in tumours[18] may modify the phenotype and function of heterogeneous and plastic resident cells. For example, cells of the myelomonocytic lineage and even lymphocytes might be SLPI target cells. Therefore, the aim of the present study was to examine the ability of SLPI to modify the phenotype and function of monocytes and study the role of these monocytes in the activation and proliferation of lymphocytes. Our results strongly suggest that human SLPI mainly affects the monocyte cytokine pattern secretion and inhibits lymphocyte activation, proliferation and T helper type 1 (Th1) differentiation.

Materials and methods

Reagents

Recombinant human SLPI (rhSLPI) was cloned and expressed as described previously.[19] Briefly, *Escherichia coli* strain BL21 CodonPlus (DAE)-RIL (Novagen, EMD Biosciences, Inc., Darmstadt, Germany) transformed with the SLPI-pET22b+ expression vector (Novagen, EMD Biosciences, Inc., Darmstadt, Germany) were induced with 1 mM isopropyl-β-D-thio-galactoside (3 hr, 28°), harvested and sonicated. The rhSLPI was purified with a Ni-NTA resin column as described by the manufacturer (Quiagen, Valencia, CA). To evaluate the activity of rhSLPI, the trypsin inhibitory activity was assayed with the colorimetric substrate *N*-succinyl-Ala-Ala-Pro-Phe q-nitroanilide (Sigma-Aldrich, Saint Louis, MO), and the absorbance was monitored at 405 nm in a microplate reader. Before using SLPI in *in vitro* experiments, eluted fractions were purified with a polymyxin B column. Contamination with lipopolysaccharide was < 0·1 EU/μg protein as determined by the *Limulus* amoebocyte lysate assay. Human recombinant IL-2, IL-4, IL-6, IL-10 and interferon-γ (IFN-γ) were purchased from Peprotech (Rochy Hill, NJ).

Monoclonal antibodies

A number of monoclonal antibodies (mAb) that recognize antigens present on leucocytes were used *in vitro*. These included mAb anti-CD14 (MΦP9, IgG2b), anti-CD54 [IgG1, R-phycoerythrin (R-PE)], anti-MHC I (IgG1, R-PE), anti-CD86 (FUN-1, IgG1), anti-MHC II (IgG2a, R-PE), anti-CD4-PE (Clone SK3), anti-CD8-PE (Clone RPA-T8), anti-CD8-FITC (Clone HIT8a) and anti-CD25-FITC (Clone 2A3) which were purchased from BD Pharmingen (San Diego, CA). Isotype control mouse IgG1 (R-PE) and IgG2a (FITC) were purchased from Caltag (San Francisco, CA). Anti-CD18 (Ts1/18, IgG1) and OKT3 mAb were purchased from American Type Culture Collection (Rockville, MD).

Leucocyte isolation and harvesting of monocyte-derived culture supernatant

After informed consent, human peripheral blood was drawn and collected into EDTA plus acid citrate dextrose of healthy donors between 09.00 and 11.00 hr. The peripheral blood mononuclear cells (PBMC) were isolated by centrifugation of blood through a Ficoll-Hypaque (Pharmacia LKB Biotech., Piscataway, NJ) gradient.[20] Monocytes and lymphocytes were purified from PBMC with magnetic beads (Invitrogen Dynal AS, Oslo, Norway) by $CD14^+$ and $CD14^-$ selection, respectively. Leucocyte purification was verified by flow cytometry. The $CD4^+$ and $CD8^+$ lymphocytes were purified from PBMC by cell sorting as follows: 90×10^6 PBMC were incubated for 20 min on ice with 70 μl anti-CD4-PE and 70 μl anti-CD8-FITC antibodies. After washing, $CD4^+$ and $CD8^+$ were isolated using the cell sorter FACSAria™ II (Becton Dickinson, Mountain View, CA).

Monocyte culture supernatant (CS) was generated from purified monocytes. These cells (5×10^4/well) were incubated for 24 hr in RPMI-1640/10% fetal calf serum (FCS) with or without SLPI (4 μg/ml). Then, cells were thoroughly washed to remove SLPI. Afterwards, cells were incubated in RPMI-1640 with 10% FCS for another 24 hr (37°). Finally, monocyte CS was harvested, centrifuged (300 ***g***, 10 min), and immediately dispensed in aliquots and frozen at −70°.

Cell proliferation assay

The PBMC or lymphocytes were cultured in RPMI-1640/10% FCS for 5 days, during which they were pulsed with [^{3}H]thymidine (1 μCi/well, specific activity 5 mCi/mmol;

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PerkinElmer, Life Sciences, Boston, MA) for the final 18 hr. Cells were harvested using a multi-well cell harvesters and thymidine incorporation was measured with a beta-counter.

Flow cytometry analysis

Cell characterization was performed by direct and indirect immunofluorescence as previously described.[21] Cells were incubated with mAb at optimal concentrations (5–10 μg/ml) for 45 min. For indirect immunofluorescence experiments, cells were treated with R-PE-conjugated goat anti-mouse immunoglobulin (DAKO, Glostrup, Denmark) for 45 min. For negative controls, cells were labelled with irrelevant isotype-matched primary mAb. Fluorescence intensity was analysed using a FACStar Plus (Becton Dickinson, BD Biosciences, San Diego, CA) and dead cells were excluded by gating with propidium iodide.

Cytokine determination

Levels of IFN-γ, IL-4, IL-6 and IL-10 were measured using the BD cytometric bead array kit (BD Biosciences, San Diego, CA), following the manufacturer's instructions.

Western blot

For PAGE and Western blot analysis, 10^5 PBMC/well were treated with IL-2 and SLPI (4 μg/ml) for 2 hr. Then, cells were incubated with lysis buffer (PBS containing 5 mM EDTA, 1% nonidet-P40, 0·5% sodium deoxycholate, 0·1% SDS, 142·5 mM KCl, 5 mM $MgCl_2$, 10 mM HEPES, pH 7·2, 0·2 mM PMSF, 0·1% aprotinin, 0·7 μg/ml pepstatin and 1 μg/ml leupeptin) and incubated for 1 hr on ice. After centrifugation (12 000 ***g***, 15 min, 4°), the detergent-containing supernatant was removed and protein concentration was quantified using the Micro BCA™ Protein Assay Reagent kit (Pierce, Rockford, IL). Samples were then boiled in SDS-sample buffer containing dithiothreitol (0·2 M) (Sigma-Aldrich) and analysed by electrophoresis on a 6% polyacrylamide gel in the presence of SDS. Following electrophoresis, gels were transferred to nitrocellulose membrane and subjected to Western blot analysis using enhanced chemiluminescence (Amersham Corp., Little Chalfont, UK) with a polyclonal antibody against T-bet (Clone 4b10, IgG1; Santa Cruz Biotechnology, Santa Cruz, CA) and goat anti-rabbit IgG (Chemicon International, Temecula, CA).

Statistical analysis

Analysis of variance and post-hoc Dunnett's Multiple Comparisons test and Student–Newman–Keuls Multiple Comparisons Test were used for statistical analysis of the data, as indicated in the legend of each figure.

Results

SLPI-treated monocytes inhibit human lymphocyte proliferation

The effect of SLPI on lymphocyte proliferation was examined. For this, PBMC were treated with OKT3 mAb or IL-2 in the presence or not of SLPI. Figure 1(a) shows that SLPI significantly inhibited the cell proliferation induced by OKT3 mAb. The same inhibition was observed when cells were treated with IL-2 (Fig. 1b). The effect of SLPI on PBMC proliferation was dose dependent for both IL-2 and OKT3 mAb stimuli (Fig. 1c). Whether SLPI was affecting cell proliferation directly on lymphocytes or through monocytes was analysed next. The PBMC were depleted of $CD14^+$ cells. Then, $CD14^-$ mononuclear cells were treated with IL-2 and SLPI. Figure 1(d) shows that SLPI was not able to modify $CD14^-$ mononuclear cell proliferation. However, a significant decrease in cell proliferation was observed when those cells were cultured in the presence of monocytes ($CD14^+$ cells) and SLPI (Fig. 1d).

SLPI modulates monocyte expression of surface molecules and cytokines

The next step was to evaluate the ability of SLPI to modify the expression of monocyte surface molecules and cytokine release. Figure 2(a) shows that SLPI-treated monocytes, slightly decreased MHC II and increased CD18 expression; whereas no differences were observed in the expression of CD86, CD54 and MHC I. Moreover, SLPI-treated monocytes showed increased secretion of IL-4, IL-6 and IL-10 in cell culture supernatants (Fig. 2b).

SLPI-treated monocyte CS impair $CD4^+$ cells proliferation

Cell–cell interaction or soluble factor(s) produced by SLPI-treated monocytes may be responsible for mediating the inhibition of lymphocyte proliferation. As SLPI-treated monocytes showed increased secretion of IL-4, IL-6 and IL-10 in cell culture supernatants, we wanted to analyse whether these cytokines were able to inhibit mononuclear cell proliferation. The PBMC were treated with IL-2 plus recombinant IL-4, IL-6 and IL-10 at concentrations found in SLPI-treated monocyte CS (Fig. 2b). Figure 3(a) shows that cell proliferation was inhibited mainly by IL-4, whereas the combination of all cytokines was not able to further increase the inhibition. This result strongly suggested that SLPI-treated monocyte CS might be able to inhibit lymphocyte proliferation. To verify this hypothesis, we examined the effect of SLPI-treated monocyte CS on CD14-depleted PBMC proliferation. It is important to mention that SLPI was not present in the CS, because

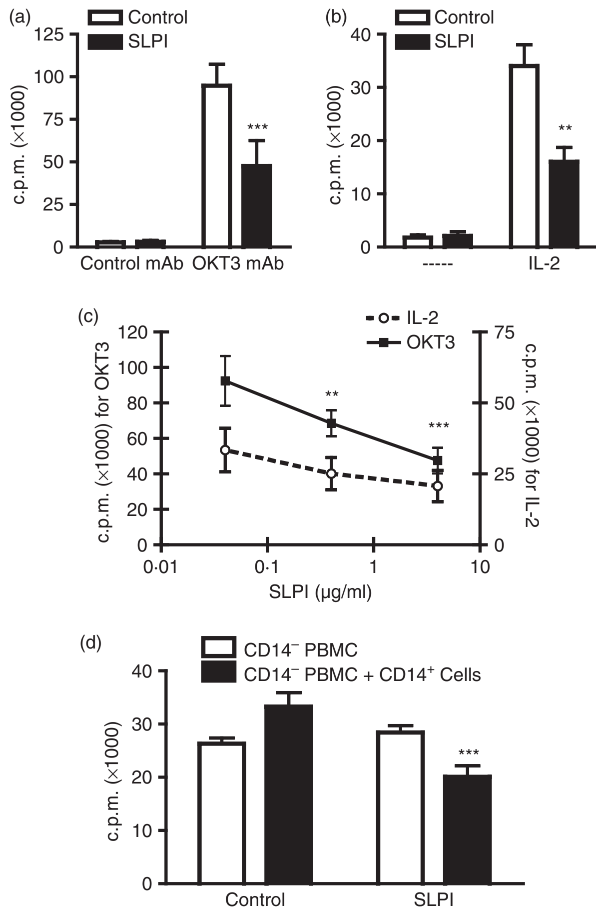

Figure 1. Serine leucocyte proteinase inhibitor (SLPI) inhibits human lymphocyte proliferation. Peripheral blood mononuclear cells (PBMC; 10^5 cells/well) were cultured for 5 days in the presence of well-coated OKT3 monoclonal antibody (mAb) (a, c) or 8 ng/ml interleukin-2 (IL-2) (b, c). Cultures were supplemented with or without SLPI (4 µg/ml). (c) SLPI dose–response on human PBMC proliferation induced by IL-2 or OKT3 mAb. Cultures were supplemented with or without SLPI (0·04, 0·4 or 4 µg/ml). (d) Proliferation of CD14-depleted PBMC. CD14-depleted PBMC (10^5/well) were cultured in the presence or not of 10^4 $CD14^+$ cells. Furthermore, cells were incubated (for 5 days) with or without SLPI (4 µg/ml) in the presence of IL-2 (8 ng/ml). Proliferation was measured by [^{3}H]thymidine incorporation during the final 18 hr of culture. Data are expressed as mean counts per min (c.p.m.) ± SEM of experiments performed in triplicate. $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ ANOVA *post hoc* Student–Newman–Keuls Multiple Comparisons Test for (a; $n = 6$), (b; $n = 6$) and (d; $n = 5$). Analysis of variance post hoc Dunnett Multiple Comparisons Test for (c; $n = 7$ for IL-2 and $n = 4$ for OKT3).

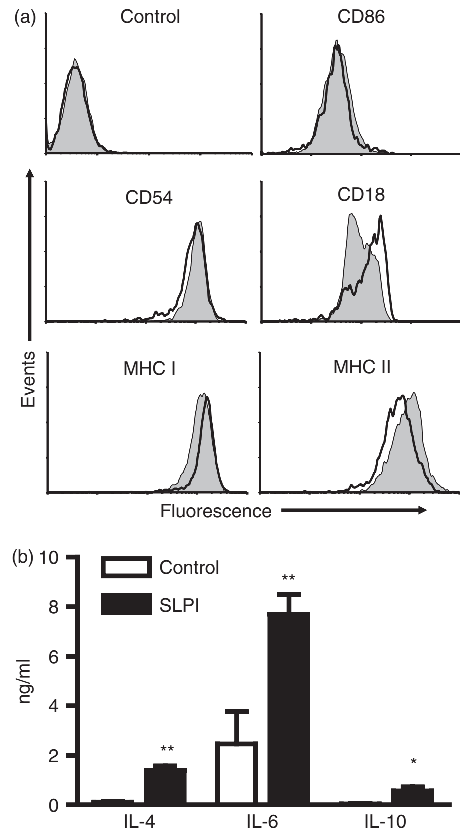

Figure 2. Serine leucocyte proteinase inhibitor (SLPI) modifies the phenotype and cytokine secretion of monocyte. (a) Expression of CD86, CD54, CD18, MHC class I and class II in monocytes treated with SLPI (4 µg/ml) for 18 hr. Data are expressed in the form of a fluorescence histogram overlay depicting the staining of cells treated with (thick solid line) and without (shaded curve) SLPI. The figure shows a representative experiment of three independent experiments. (b) Cytokine production after stimulation of monocytes with SLPI. Monocytes were treated with SLPI (4 µg/ml, 24 hr). Then, cells were thoroughly washed. Afterwards, cells were incubated (RPMI-1640/10% FCS) for another 24 hr and the concentration of cytokines in the supernatants was determined using a cytometric bead array kit. Data represent the mean ± SEM of three experiments. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$; analysis of variance post hoc Dunnett Multiple Comparisons Test.

SLPI-treated cells were thoroughly washed before the CS generation, as described in the Materials and methods. Figure 3(b) shows that SLPI-treated monocyte CS, but not untreated, decreased the CD14-depleted PBMC proliferation. This result suggests that soluble factors released by SLPI-treated monocytes mediate the inhibition on cell proliferation by targeting $CD14^-$ mononuclear cells.

Previous results with OKT3 as a mitogen (Fig. 1a), suggested to us that T lymphocytes were the target cells for the effect mediated by SLPI-treated monocyte. Moreover, PBMC incubated with OKT3 mAb for 48 hr increased the number of CD4 lymphocytes expressing CD25 (Fig. 3c). The presence of SLPI in the culture significantly decreased the number of $CD4^+$ $CD25^+$ (Fig. 3c) but not $CD8^+$ $CD25^+$ lymphocytes (data not shown). These results suggest that SLPI may affect CD4 lymphocyte activation. To confirm whether CD4 T lymphocyte subsets were the target for SLPI-treated monocytes, CD4 and CD8 lymphocytes were isolated by cell sorter and cell proliferation was examined in the presence of IL-2 and SLPI-treated

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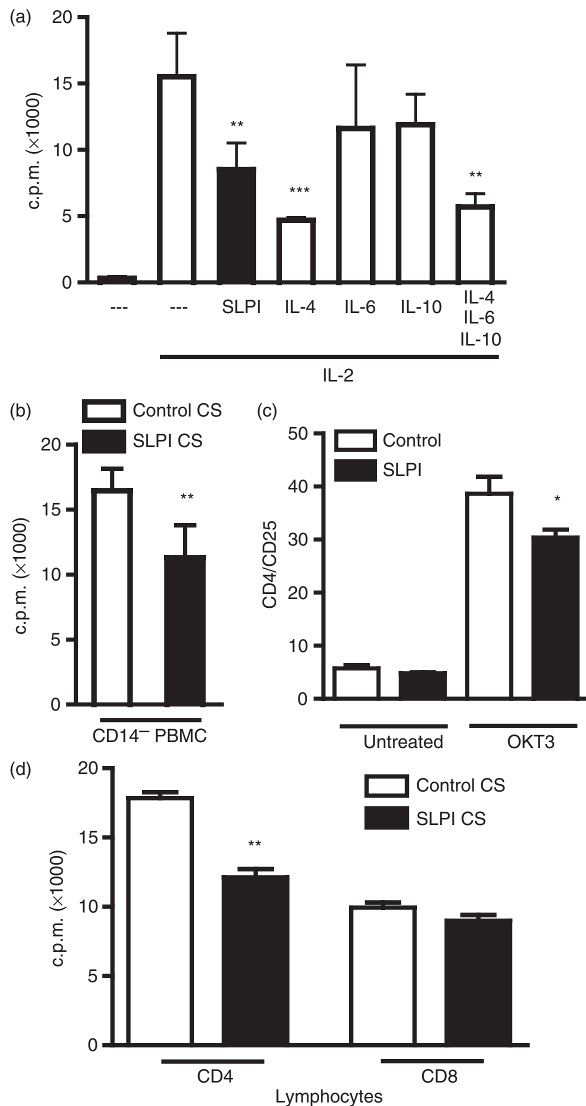

Figure 3. Serine leucocyte proteinase inhibitor (SLPI) -treated monocyte culture supernatants (CS) inhibits human lymphocyte proliferation. (a) Peripheral blood mononuclear cells (PBMC; 10^5 cells/well) were cultured, for 5 days with or without 8 ng/ml of interleukin-2 (IL-2) plus cytokines (2 ng/ml of IL-4; 10 ng/ml of IL-6; 0·7 ng/ml of IL-10) as indicated in the figure. (b) PBMC CD14-depleted cells were cultured for 5 days in the presence of IL-2 (8 ng/ml) and with either SLPI-treated or untreated (control) monocyte CS. (c) PBMC were stimulated for 48 hr with OKT3 in the presence of 4 μg/ml SLPI. Afterwards, cells were harvested and stained with monoclonal antibody (mAb) against CD4 and CD25. Cells were then analysed by flow cytometry. Bars represent the mean ± SEM of four independent experiments. (d) $CD4^+$ cells (5×10^4 cells/well) or $CD8^+$ cells (5×10^4 cells/well) were cultured for 5 day in the presence of IL-2 (8 ng/ml) and with either SLPI-treated or untreated (control) monocyte CS. Proliferation was measured as described in the Materials and methods. Data are expressed as mean counts/min (c.p.m.) ± SEM of six experiments for (a) and (b) and three experiments for (c) and (d). $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ and $^{***}P < 0{\cdot}001$, analysis of variance post hoc Dunnett Multiple Comparisons Test for (a), and Student's *t*-test for (b), (c) and (d).

monocyte CS. Figure 3(d) shows that SLPI decreased $CD4^+$ lymphocyte proliferation but did not affect the proliferation of $CD8^+$ cells. These results confirmed that the soluble factors released by SLPI-treated monocytes impair CD4 lymphocyte activation and proliferation.

SLPI-treated monocyte CS inhibit Th1 cytokine patterns

The $CD4^+$ T cells can differentiate into different lineages of T helper cells with distinct biological functions. To examine whether SLPI-treated monocytes might modify the differentiation of Th1 cells, we analysed the expression of nuclear factor T-bet. The PBMC were treated with IL-2 in the presence or not of SLPI. Figure 4(a,b) shows that IL-2 increased T-bet expression and the presence of SLPI significantly decreased it. Moreover, we analysed the expression of cytokines present at the end of the proliferation assay of CD14-depleted PBMC treated with monocyte CS; i.e. the cytokines present in the supernatant of proliferation assays of the Fig. 3(b). Figure 4(c) shows that IFN-γ was the main cytokine in the supernatant of the CD14-depleted PBMC proliferation assay. However, when this proliferation assay was performed in the presence of SLPI-treated monocyte CS, the secretion of IFN-γ decreased whereas it increased the level of IL-4, IL-6 and IL-10.

Discussion

The SLPI inhibits lymphocyte T-cell activation, proliferation and Th1 differentiation through interaction with monocytes. SLPI and other alarm anti-proteases, constitute key mediators for the innate immune response. They are secreted at the site of inflammation and present anti-inflammatory activity.[22] This anti-inflammatory activity has been demonstrated in several organs, including, the eye, lung, liver, brain and joint inflammation models.[23–28]

Monocytes/macrophages are important components of the innate immune system, displaying pro-inflammatory as well as anti-inflammatory effects depending on the stimulus sensed.[29–31] Among the effects of SLPI on monocytes already described, it has been shown that SLPI can suppress prostaglandin E_2 production, matrix metalloproteinases 1 and 9,[32] and can act through an effect on NF-κB, either directly, by binding NF-κB consensus region of target genes,[6] or indirectly by preventing the degradation of IκB, the canonical inhibitor of NF-κB.[5] Similarly, we have also observed that SLPI inhibited IL-2-induced IκB degradation on PBMC (data not shown). This SLPI-mediated monocyte/macrophage anti-inflammatory effect has been shown to further reduce eosinophil and neutrophil recruitment, histamine release, NF-κB activation and production of anti-inflammatory cytokines.[23,24,33–35] Because cells of the myeloid lineage (macrophages, monocytes, dendritic cells) are instrumental in the activation of

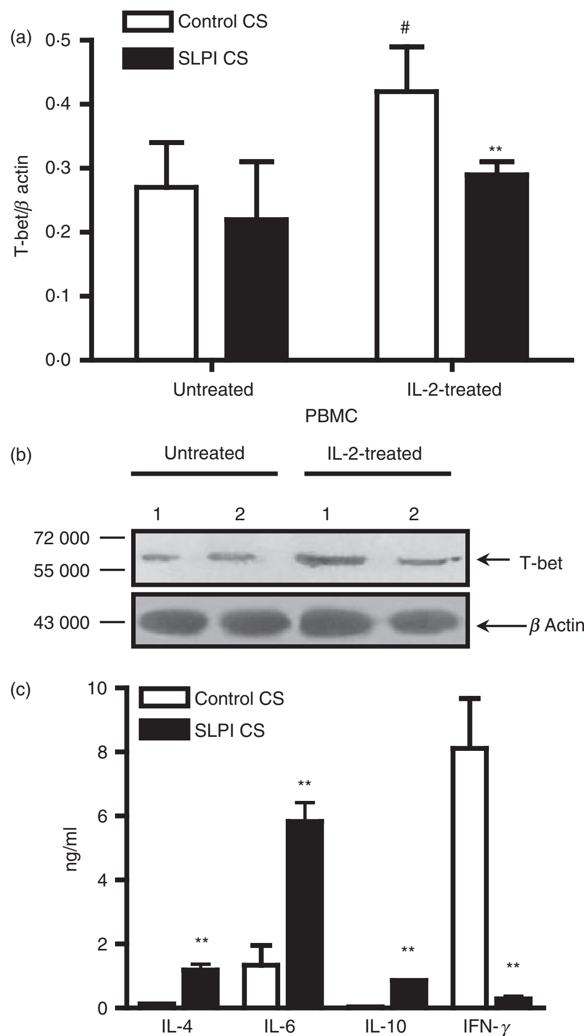

Figure 4. Serine leucocyte proteinase inhibitor (SLPI) -treated monocyte culture supernatant (CS) inhibits T helper type 1 (Th1) cytokine secretion and increases Th2 cytokine expression. (a) T-bet expression. 10^5 peripheral blood mononuclear cells (PBMC)/well were either treated or untreated with interleukin-2 (IL-2; 8 ng/ml) and SLPI (4 μg/ml) for 5 days. Then, cells were incubated for 1 hr on ice with a lysis buffer. Cell lysates with equivalent amounts of protein (17 μg/lane) were run on 12% SDS–PAGE. Western blots were performed with a polyclonal antibody against T-bet. Three different polyacrylamide gels were scanned, densitometry was performed and results were expressed as mean ± SEM of the ratio T-bet/βactin. Control = 1; SLPI = 2. Markers migration are shown on the left side, with a molecular weight for T-bet of 63 000. $^{\#}P < 0{\cdot}05$ compared with untreated PBMC; $^{**}P < 0{\cdot}01$ compared with IL-2-treated, analysis of variance post hoc Student–Newman–Keuls test. (b) A representative Western blot experiment of (a) is shown. (c) Expression of IL-10, IL-6, IL-4 and interferon-γ (IFN-γ) in the CS of CD14-depleted PBMC proliferation assay. CD14-depleted PBMC (10^5 cells/well) were treated with IL-2 and with either SLPI-treated or untreated monocyte CS for 5 days. Cytokines were measured in the CS at the end of the proliferation assay with a cytometric bead array. $^{**}P < 0{\cdot}01$ analysis of variance post hoc Student–Newman–Keuls test.

adaptive immune responses, we studied here the effect of SLPI on this process. We show that SLPI has no direct effect on lymphocytes because proliferation was not affected by incubating SLPI with CD14-depleted PBMC. However, SLPI acted on IL-2-stimulated and OKT3-stimulated PBMC and this effect was reproduced when $CD14^{-}$ mononuclear cells were incubated with monocytes in the presence of SLPI. For practical reasons, we have not examined whether a direct interaction between SLPI-treated monocytes and T cells mediates the inhibitory effect on lymphocyte proliferation, but we are confident that monocyte-treated SLPI act on lymphocytes in a paracrine fashion because the suppressive effect of SLPI-treated monocytes was present in their supernatant. Therefore, SLPI targets monocytes, which in turn release soluble factors, probably cytokines, which mediate the inhibition of lymphocyte proliferation. In fact, SLPI-treated monocytes release IL-4, IL-6 and IL-10 in cell CS. However, only IL-4 (at the same concentration found in the SLPI-treated monocyte CS) was able to reproduce the effect of SLPI on PBMC proliferation. Importantly, we showed that the soluble factors released by SLPI-treated monocytes target CD4 and not CD8 T cells and that these effects were accompanied by a selective CD25 decrease on CD4 T cells.

Although our results showed a clear effect of SLPI on monocyte-driven inhibition of CD4 T-cell proliferation, it was deemed important to further characterize the type of immune response involved. Indeed, it is known that inflammatory monocytes are recruited and differentiate into macrophages at the site of the inflammatory lesion[36,37] and that elicited macrophages are able to acquire distinct phenotypes and activities when under the stimulation of different stimuli. For instance, classical activation, alternative activation, innate activation and deactivation have been described.[37] Classical activation can be induced by treating macrophages with IFN-γ and lipopolysaccharide.[37,38] These cells present high microbicidal activity and produce pro-inflammatory cytokines and they are associated with cellular immunity. Alternative activation results from supplementing macrophages with IL-4 or IL-13 and is associated with tissue repair and humoral immunity.[37] Innate activation is mediated in culture by ligation of Toll-like receptors and is associated with microbicidal activity and pro-inflammatory cytokine production.[37] Finally, deactivation is induced by IL-10 or TGF-β, or by ligation of inhibitory receptors such as CD200 receptor or CD172a, and is associated with anti-inflammatory cytokine production and reduced MHC class II expression.[37] A more recent review by Mosser and Edwards[38] suggested a different grouping of macrophage populations based on host defence, wound healing and immune regulation activities. Hence, they coined the expression 'classically activated' to designate the effector macrophages that are produced during cell-mediated immune responses, with enhanced microbicidal

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or tumoricidal activity and secretion of pro-inflammatory cytokines; 'wound-healing macrophages' (formerly named as alternative activated macrophages) to designate macrophages that help in the production of the extracellular matrix and produce IL-4; and finally, the 'regulatory macrophages' that produce IL-10 and TGF-β, down-regulate IL-12 and present high levels of co-stimulatory molecules.[38]

It is difficult to ascertain in which category SLPI-treated monocytes belong: however, considering that monocytes treated with SLPI produce IL-4, IL-6 and IL-10 and slightly decrease MHC class II but do not produce TGF-β (data not shown) or modify the surface expression of CD86, we can tentatively assimilate SLPI-treated monocytes to either 'deactivated', 'alternatively-activated', 'wound healing' or 'regulatory' macrophages. It is probable that SLPI-treated monocytes may bias the latter to differentiate *in situ* in mucosa or tumour sites towards an 'alternatively activated state/wound healing macrophages' by producing anti-inflammatory/type 2 cytokines (as measured here with increased levels of IL-6, IL-10, IL-4) ultimately leading to an inhibition of lymphocyte proliferation and Th1 differentiation (as measured here with reduced levels of T-bet and IFN-γ). However, we cannot rule out the differentiation of SLPI-treated monocytes into regulatory macrophages. The difficulty in classifying SLPI-treated monocytes may reflect the existence of a broader range of activation states with complex phenotypes.[38]

In line with the relative 'tolerogenic' state described here for SLPI-treated monocytes, it has recently been shown that dendritic cells expressing SLPI in response to TLR ligand (but not CD40[17]) conferred mice with a tolerogenic phenotype upon immunization with a model antigen, because SLPI-deficient mice exhibited higher levels of antigen-specific CD4 and CD8 T cells, compared with wild-type mice.[17] This study suggested that the highly SLPI-expressing dendritic cells present at the mucosal lymph nodes play an important role in dampening specific immune activation,[17] as further analysed in the present work.

In conclusion, in addition to its 'classical' role as an inhibitor of inflammatory cells extra-cellular proteases (e.g. neutrophil elastase), we show here that SLPI has a major role in dampening down inflammatory and adaptive immune responses. Furthermore, we describe for the first time that human SLPI may act through monocytes and skew lymphocytic responses towards a type 2 immune response.

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Disclosures

None of the authors have any conflict of interest with any of the content of this manuscript.

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