IL-15 and IL-15R in leucocytes from patients with systemic lupus erythematosus

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Objective. To assess the functional status of the IL-15/IL-15Rα cytokine system in different leucocyte subsets from patients with systemic lupus erythematosus (SLE).

Methods. Eighteen patients with SLE (10 with inactive and eight with active disease) and 14 healthy individuals were studied. Serum levels and in vitro production of IL-15 were determined. In addition, the expression of IL-15 receptor α (IL-15Rα) and membrane-bound IL-15 was assessed and the in vitro effects of IL-15 on CD69 and CD64 expression, interferon-γ and TNF-α synthesis, respiratory burst induction and apoptosis were studied.

Results. Serum levels of IL-15 were significantly increased in inactive and active patients with SLE. Accordingly, the in vitro synthesis and release of IL-15 by monocytes in response to IFN-γ+lipopolysaccharide was significantly enhanced in SLE patients with active disease, as was the percentage of membrane-bound IL-15γδ monocytes. On the other hand, enhanced basal expression of IL-15Rα was detected in leucocytes from SLE patients, with defective induction upon stimulation with phytohaemagglutinin or phorbol myristate acetate/ionomycin. Furthermore, diminished induction of CD69 expression and interferon-γ and TNF-α synthesis by recombinant human IL-15 was detected in peripheral blood mononuclear cells from SLE, and there was defective induction of CD64 and priming for respiratory burst in neutrophils. The anti-apoptotic effect of IL-15 was diminished in leucocytes from SLE patients.

Conclusion. Our data indicate that there is enhanced synthesis of IL-15 by immune cells from SLE patients, with a poor response to this cytokine by different leucocyte subsets. This abnormal function of IL-15/IL-15Rα may contribute significantly to the pathogenesis of SLE.

Key words: Cytokines, Autoimmunity, Apoptosis, CD69, Lymphocytes.

Interleukin-15 (IL-15) is a cytokine mainly synthesized by macrophage/monocytes and other non-lymphoid cells [1, 2]. This cytokine exerts its effects through its binding to a membrane receptor that is composed of a private α chain (IL-15Rα) associated with the β chain of IL-2R (CD122), and the common γ chain of different type I cytokine receptors (γc chain, CD132) [1–3]. As expected, IL-2 and IL-15 share several effects, such as the activation of NK cells, the induction of CD8 T cell proliferation and differentiation [2, 4–6]. However, it is evident that IL-15 has additional effects, including the induction of T-cell polarization, differentiation of dendritic cells, inhibition of apoptosis of lymphoid cells, and activation of polymorphonuclear leucocytes (PMNs) [4, 7–11]. Similarly, it has been reported that this cytokine exerts a negative regulatory effect on both the proliferation of CD4 T cells and the synthesis of IL-2 [12]. Therefore, it has been widely considered that IL-15 is a pleiotropic cytokine with an important role in both innate and acquired immunity [2, 9, 10]. As stated above, the effects of IL-15 are exerted through a membrane receptor that is expressed by different leucocyte subsets and other cell types and that is apparently different from that expressed by mast cells [1–3, 13].

IL-15 synthesis is mainly regulated at a post-transcriptional level, and different stimuli, including bacterial lipopolysaccharide (LPS) and cytokines (IFN-γ, TNF-α) efficiently induce its production and release [1, 2, 14, 15]. In turn, IL-15 is able to promote the synthesis of proinflammatory cytokines (IL-1, TNF-α), a phenomenon that is important in different inflammatory conditions [2]. As in the case of TNF-α, it has been reported that there is a membrane form of IL-15 that is able to generate ‘reverse intracellular signals’ when interacting with soluble or membrane-bound receptors [16–19].

IL-15 has a key role in the pathogenesis of inflammatory and malignant diseases. Enhanced synthesis of IL-15 has been found in HTLV-1-associated conditions (adult T-cell leukaemia, myelopathy) as well as in autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), sarcoidosis, inflammatory bowel disease and multiple sclerosis [2, 20]. The effect of IL-15 on
T-cell proliferation, its anti-apoptotic activity and proinflammatory effect strongly support its important role in these conditions.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the deposition of immune complexes with an inflammatory/necrotic phenomenon in different tissues, mainly kidney, skin, blood vessels and central nervous system [21]. Multiple immune abnormalities are characteristic of this condition, including the synthesis of different autoantibodies, B-cell hyperactivity, an increased rate of lymphoid cell apoptosis, enhanced synthesis of IL-10 and defective immunoregulatory function [22–24]. In addition, T cells from these patients show a diminished in vitro response to different stimuli [anti-CD3 monoclonal antibodies (mAb), mitogenic lectins] as well as abnormalities in early cell activation events, and defective production of and response to IL-2 [25, 26].

Although IL-15 seems to have an important role in the pathogenesis of inflammatory/autoimmune conditions, there are few reports on the production and effects of this cytokine in patients with SLE. In this regard, normal and increased levels of serum IL-15 in SLE have been reported [27–31]. In addition, a protective effect of IL-15 on the apoptosis of peripheral blood mononuclear cells (PBMCs) has been also reported [32, 33]. In this work we assessed the in vivo and in vitro production of IL-15 in SLE patients. In addition, the expression of the private chain of the IL-15R and the in vitro effect of this cytokine on some important phenomena in different leucocyte subsets (mononuclear cells, monocytes, neutrophils) were also studied. Our data indicate that there is increased production of IL-15 in SLE patients, but that a defective response to this cytokine is observed despite normal basal expression of IL-15Rα.

Materials and methods

Patients

Eighteen patients with SLE (10 with inactive and eight with active disease) and 14 healthy individuals were studied. Disease activity was scored according to the MEX-SLEDAI index [34]. Active patients had a mean activity index of 10.3 (range 5.0–14.0) at the time of study, whereas all patients that were considered inactive had an index equal to or less than 2.0 (range 0–2.0) and did not have severe manifestations of the disease. All patients were female, with a mean age of 32 yr (range 16–54) and with a mean disease duration of 6.8 yr (range 0–18). Five active patients had not received glucocorticoids or immunosuppressive agents at the time of study, and the other three had not received them for at least 1 month before inclusion in the study. Four inactive patients were receiving low doses of prednisolone (<15 mg/day) but none was under immunosuppressive therapy. Fourteen healthy individuals were studied as controls. In all cases informed consent was obtained, and this study was approved by the Bioethical Committee of the School of Medicine, UASLP (San Luis Potosí, S.L.P., Mexico).

Cells

Peripheral blood samples were obtained and PBMCs were isolated with Ficoll–Hypaque cushions. Monocytes were isolated by plastic adherence after overnight incubation in complete culture medium (RPMI-1640 medium with 10% fetal bovine serum, 2 mM l-glutamine and antibiotics), and its purity was assessed by flow cytometry analysis using an anti-CD14 mAb (Becton-Dickinson, San Jose, CA, USA). PMNs were isolated from the same Ficoll–Hypaque cushions by mixing the cells at the bottom of the tube with 1.3% dextran (molecular weight 150 000; Sigma Chemical, St Louis, MO, USA), followed by sedimentation at 1 g at room temperature for 30 min. The neutrophil-enriched fraction was further purified by hypotonic lysis of erythrocytes.

Cytokine assays

Serum levels of IL-15 were quantified by enzyme-linked immunosorbent assay (ELISA) (Quantikine, R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The lower limit of detection of this assay was 3.9 pg/ml. For the measurement of in vitro production of IL-15, monocytes were stimulated with 100 U/ml recombinant human interferon-γ (rhIFN-γ) plus 5.0 µg/ml bacterial LPS (Sigma) for 48 h, and then levels of IL-15 were measured in cell-free supernatants by ELISA (R & D Systems). The in vitro synthesis and release of IFN-γ and TNF-α were determined as follows. PBMCs were stimulated or not with 50–100 ng/ml recombinant human IL-15 (rhIL-15, R & D Systems) for 48 h in complete medium, and then IFN-γ and TNF-α concentrations were measured in cell-free supernatants by ELISA (R & D Systems).

Flow cytometry analyses

The percentage of cells expressing membrane-bound IL-15 was determined as follows. Monocytes were stimulated or not with 100 U/ml rhIFN-γ plus 5.0 µg/ml LPS for 48 h, and then cells were immunostained for IL-15 using a specific mAb labelled with fluorescein isothiocyanate (FITC; IC2471F clone, R & D Systems), and analysed using a FACSCalibur flow cytometer (Becton Dickinson). Results were expressed as the mean fluorescence intensity (MFI) of positive cells.

To assess the effect of IL-15 on the expression of activation antigens, PBMCs were stimulated or not with 100 ng/ml rhIL-15 or 50 ng/ml phorbol myristate acetate (PMA; Sigma) for 24 h, and then immunostained for CD69 with the TP1-55 mAb (kindly given by Dr F. Sánchez-Madrid, Hospital de la Princesa, Madrid, Spain). In the case of CD64 expression, isolated PMNs were stimulated or not with 50 ng/ml rhIL-15 or 100 U/ml rhIFN-γ for 12 h and then immunostained using a specific mAb (Pharmingen, San Diego, CA, USA). Cells were analysed by flow cytometry and results expressed as the percentage of positive cells. To analyse the expression of the α chain of IL-15R, PBMC were stimulated or not with phytohaemagglutinin (PHA; 5.0 µg/ml; Sigma) or PMA (50 ng/ml; Sigma) plus ionomycin (1.0 µM; Sigma) for 48 h in complete medium. Then, cells were double-immunostained with a biotin-labelled anti-IL-15Rα mAb (R & D Systems) plus streptavidin–FITC (Becton Dickinson), followed by labelling with an anti-CD4 or an anti-CD8 mAb (Becton Dickinson) conjugated with phycoerythrin. Cells were also analysed by flow cytometry, and results expressed as the percentage of positive cells.

To assess the priming effect of IL-15 on the respiratory burst of PMNs, these cells were loaded with dichlorofluorescein diacetate (Sigma) and incubated in the presence or absence of 50 ng/ml rhIL-15 for 2 h in polypropylene tubes (Costar, Cambridge, MA, USA) at 37 °C in Hanks’ balanced saline solution with 0.5% bovine serum albumin. Then, cells were stimulated or not with 1.0 µM of the bacterial-derived peptide f-Met-Leu-Phe (MLP, Sigma) for 15 min and analysed by flow cytometry. Results were expressed as the MFI.

Apoptosis assays

PBMCs were stimulated or not with 50 ng/ml PMA (Sigma) plus 1.0 µM ionomycin (Sigma) for 72 h in complete medium, in the presence or absence of 100 ng/ml rhIL-15. Then, cells were fixed, stained by the terminal deoxynucleotide transferase dUTP nick end labelling (TUNEL) technique using the Apo-Direct kit (BD Pharmingen), and analysed by flow cytometry. In additional experiments, PBMCs from the same sample were stained with annexin V labelled with FITC and propidium iodide and analysed in a FACSCalibur flow cytometer.
Results

We first evaluated the in vivo production of IL-15 in SLE. An increased level of this cytokine was detected in sera from active and inactive patients compared with healthy controls (Fig. 1A, $P < 0.02$ in both cases). No significant differences were detected when active and inactive patients were compared. Accordingly, a non-significant correlation was found between disease activity and IL-15 serum levels ($r = 0.27$, $P > 0.05$). However, since active patients tended to show higher levels than patients with inactive disease, it is feasible that a significant correlation could be reached by increasing the sample size. On the other hand, no significant spontaneous production of IL-15 was seen in any group studied and cells from active patients showed enhanced release of this cytokine (Fig. 1B, $P < 0.05$ compared with controls). However, although inactive patients tended to synthesize higher levels of IL-15 than healthy individuals, no significant differences were found. Accordingly, monocytes from active SLE patients expressed higher levels of membrane-bound IL-15 compared with both inactive patients and healthy controls ($P < 0.05$ in both cases). When monocytes were activated with IFN-$\gamma$ + LPS, a significant increase in the expression of IL-15 was observed in controls and inactive patients ($P < 0.05$ in both cases, compared with non-stimulated cells; Fig. 2A). However, cells from active patients failed to increase their levels of IL-15 significantly upon stimulation with IFN-$\gamma$ + LPS.

To analyze the function of IL-15R in different leucocyte subsets from SLE patients, we first determined the expression of the private chain of this receptor. We found that fresh isolated PBMCs from patients with both inactive and active disease showed increased expression of IL-15R$\alpha$ compared with controls ($P < 0.05$ in both cases; Fig. 3A, B). When cells were activated with PHA or PMA/ionomycin, a significant increase in the number of IL-15R$\alpha$-positive cells was detected in the three groups studied (Fig. 3B). However, the percentage of lymphocytes expressing IL-15R$\alpha$ was significantly lower in inactive and active patients compared with healthy individuals ($P < 0.05$ in both cases; Fig. 3B). Further analysis showed no apparent differences between the fraction of CD4 and CD8 lymphocytes expressing IL-15R$\alpha$ in the three groups studied, and that these cell subsets exhibited a similar pattern of expression of this receptor to that observed in non-fractionated cells (Fig. 3B–D).

The response of different leucocyte subsets to IL-15 was then studied. Diminished induction of CD69 by IL-15 was seen in both inactive and active patients compared with controls ($P < 0.05$ in both cases; Fig. 4A). As we have described previously [25], similar results were observed when cells were activated with PMA. By contrast, significantly increased expression of CD69 was detected in non-stimulated cells from active and inactive patients compared with healthy individuals ($P < 0.05$ in both cases; Fig. 4A). Similarly, increased basal expression of CD64 by PMN and poor induction of this Fc$\gamma$ receptor by both IL-15 and IFN-$\gamma$ were detected in active SLE patients (Fig. 4B). Inactive patients also showed poor induction of CD64, but not increased expression in non-stimulated cells.

Additional studies on the function of IL-15R were performed. When PBMCs were stimulated with 50 ng/ml IL-15, a significant increase in IFN-$\gamma$ synthesis was observed in SLE patients and controls ($P < 0.05$ in all cases). Similar results were obtained with a higher concentration (100 ng/ml) of IL-15 (data not shown). However, the synthesis of IFN-$\gamma$ induced by IL-15 was significantly lower in SLE patients compared with healthy controls (Fig. 5A). Accordingly, non-stimulated PBMCs from SLE patients showed a lower synthesis of IFN-$\gamma$ compared with controls ($P < 0.05$ in both cases; Fig. 5A). On the other hand, the spontaneous secretion of TNF-$\alpha$ was significantly higher in PBMCs from SLE patients (Fig. 5B). In contrast, cells from healthy controls showed a five- to sevenfold increase in TNF-$\alpha$ synthesis when stimulated with rhIL-15, and a lower induction of this cytokine was observed in inactive and active patients (Fig. 5B). In addition, lower levels of this cytokine were detected in cell cultures from SLE patients stimulated with LPS compared with controls.

The anti-apoptotic effect of IL-15 was also evaluated. As has been reported [23, 24], an increased rate of apoptosis was observed in PBMCs from active and inactive patients (Fig. 5C). When PBMCs were activated with PMA/ionomycin, a high level of apoptosis was also seen in SLE patients, either with active or inactive disease. Interestingly, only in cells from healthy controls

![Fig. 1. In vivo and in vitro production of IL-15 in SLE patients.](image)
did we observe a significant anti-apoptotic effect of IL-15 (Fig. 5C). Similar results were observed when apoptosis was detected by annexin V staining (data not shown).

Finally, the function of IL-15R was studied in PMNs. As shown in Fig. 5D, the respiratory burst induced by fMLP was similar in patients and controls ($P < 0.05$ in all groups compared with basal values). As expected, IL-15 significantly increased the oxidative burst elicited by fMLP in PMNs from healthy controls ($P < 0.05$ non-treated vs IL-15 treated cells, Fig. 5D). By contrast, in cells from SLE patients this cytokine only induced a non-significant increase in the reactive oxygen species synthesis triggered by fMLP. Accordingly, the oxidative burst of cells primed with IL-15 was significantly higher in healthy controls compared with active SLE patients.
IL-15 is a cytokine mainly synthesized by non-lymphoid cells that is involved in both the adaptive immune response and natural immunity [1–3, 9]. It has been reported that this cytokine is able to induce proliferation and differentiation of B lymphocytes and CD8+ T cells and to drive the differentiation of dendritic, antigen-presenting cells [2, 6, 9, 11]. In addition, IL-15 has chemotactic activity on T cells and induces its polarization [7]. Furthermore, this cytokine activates NK cells and phagocytes, inducing the synthesis of IFN-γ and enhancing the respiratory burst, respectively [2, 10]. Finally, IL-15 inhibits CD4 T-cell proliferation and IL-2 synthesis, and exerts an anti-apoptotic effect, mainly on CD8 T cells [4, 12]. It is evident that all these effects may be of importance in the pathogenesis of SLE, which involves autoimmune, inflammatory and apoptotic phenomena [22–24].

In this work, we have found that SLE patients have increased serum levels of IL-15. This finding is in agreement with previous reports [28–31], but not with that of Gonzalez-Alvaro et al. [27], who found similar levels of IL-15 in SLE and healthy donors. However, in the latter study, the serum levels of this cytokine in the 30 SLE patients studied (9.8 ± 15.3 pg/ml) tended to be higher than in healthy controls (5.2 ± 11.6 pg/ml). Furthermore, when larger samples of individuals have been studied, even small differences in IL-15 levels between SLE patients and controls have reached statistical significance [28, 30]. Therefore, we think that it is possible to conclude that most SLE patients have increased serum levels of IL-15 and that it is very likely that this phenomenon, as discussed below, is associated with enhanced synthesis of this cytokine. Regarding SLE patients with normal or low serum levels of IL-15, it is worth mentioning that the regulation of IL-15 synthesis and its membrane expression and secretion are very complex [2], and it is feasible that genetic polymorphisms of the IL-15 gene could determine high- and low-synthesis phenotypes. This point is an interesting possibility for study.

According to serum levels, enhanced in vitro production of IL-15 and increased expression of its membrane-bound form were detected in cells from SLE patients. These data support the idea that there is increased synthesis of IL-15 in SLE, a finding that is in accordance with the enhanced production of other IFN-γ-inducing cytokines, such as IL-12 and IL-18 [28]. In addition, our findings agree with the defective production of IL-2 in SLE [26], since IL-15 exerts a down-regulatory effect on the synthesis of this cytokine [12]. However, we have found increased levels of membrane-bound IL-15 only in active patients, suggesting that there are additional factors that determine the increased levels of this cytokine in SLE. On the other hand, the poor induction of IL-15 synthesis by IFN-γ/LPS in SLE is interesting. This phenomenon could be related to the poor reactivity of the PBMCs from SLE patients [22, 26] and to polymorphisms of IFN-γ receptor gene [35]. Nevertheless, it has been reported that NK cells, B lymphocytes and PMNs from SLE patients show a normal or increased response to IFN-γ [36, 37]. Thus, the poor induction of IL-15 by IFN-γ in SLE monocytes remains an interesting point to be explored.

In contrast with the enhanced levels and production of IL-15 seen in SLE, a diminished response to this cytokine by PBMCs (as detected by CD69 expression), monocytes/NK cells (IFN-γ and TNF-α synthesis) and PMNs (CD64 expression, respiratory burst) was observed. This defective response to IL-15 is not due to diminished expression of IL-15R, and thus our data suggest that SLE leucocytes have defective function of this receptor. Since the intracellular signals generated through IL-15R have been described [1, 2], it would be of interest to assess these transduction events in cells from SLE patients. However, these studies should take into account the defective induction of IL-15Rα by different stimuli (PHA, PMA/Jo) observed by us. Although this phenomenon may be related to the poor in vitro reactivity of PBMCs from SLE patients [22, 26], the enhanced rate of apoptosis of these cells could also be involved [24]. In addition, it is evident that in vitro SLE lymphocytes are exposed to high levels of certain cytokines, such as IL-10 [38], and diminished availability of some growth factors, such as IL-2 [26]. Therefore, the behaviour of SLE PBMCs in vitro can be strongly influenced by the environmental change, a phenomenon that can account in part for the diminished response to IL-15 observed by us. Furthermore, it is evident that SLE PBMCs contain a high proportion of activated lymphocytes [23, 25] and that these cells could be refractory to further stimulation in vitro. However, we think that although all these factors may have a role in the poor responsiveness of SLE PBMCs to IL-15, our data with different cell subsets strongly suggest that there is a defective function of IL-15R in SLE leucocytes. This diminished response of SLE leucocytes to IL-15 may be of importance in the defective natural immunity detected in these patients [39]. In addition, the
decreased expression of CD69 or TNF-α synthesis in response to IL-15 may contribute to the autoimmune phenomena observed in SLE [40, 41].

Apoptosis of immune cells has a key role in the homeostasis of the immune system, and their alterations are clearly involved in the pathogenesis of autoimmune diseases. It has been widely proposed that the increased rate of lymphoid cell apoptosis in SLE may favor the autoimmune process [42]. Among the different factors that regulate the induction of apoptosis of leucocytes, IL-15 has an important role as an anti-apoptotic mediator [8]. As in other phenomena induced by IL-15, we found that cells from SLE patients showed a poor response to its anti-apoptotic effect. This finding is in disagreement with previous reports, which showed a normal or increased anti-apoptotic effect of this cytokine in SLE lymphocytes [32, 33]. However, Lorenz et al. [43] found that different type I cytokines, including IL-15, exert a poor anti-apoptotic effect on SLE lymphoblasts. The cause of these discrepancies is not apparent, but it could be related to methodological differences, sample size or the genetic background of the patients studied. In addition, it is feasible that the continuous exposure of PBMCs from SLE patients to high concentrations of IL-15 in vivo results in a diminished response to this cytokine in vitro, because these cells have already been exposed to the anti-apoptotic effect of this cytokine. Further studies are necessary to confirm this hypothesis.

In summary, we have found that leucocytes from the SLE patients included in this study show increased in vivo and in vitro synthesis of IL-15, with a decreased response to this cytokine, suggesting defective function of IL-15R. This abnormal regulation of IL-15/IL-15R may be important in the pathogenesis of SLE.

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