

Mechanisms involved in interleukin-15-induced suppression of human neutrophil apoptosis: role of the anti-apoptotic Mcl-1 protein and several kinases including Janus kinase-2, p38 mitogen-activated protein kinase and extracellular signal-regulated kinases-1/2

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Abstract Interleukin-15 (IL-15) is a pro-inflammatory cytokine known as a general inhibitor of apoptosis, which possesses potential therapeutic properties. Although IL-15 was previously found to be a human neutrophil agonist, its mode of action remains unknown. Herein, we were interested in elucidating the mechanisms by which it delays neutrophil apoptosis. IL-15 was found to induce tyrosine phosphorylation events and to prevent loss of the anti-apoptotic Mcl-1 protein expression. Using different signal transduction inhibitors, we found that Janus kinase (Jak)-2, Jak-3, p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK), but not G proteins, are involved in IL-15-induced suppression of apoptosis. Furthermore, we found that IL-15 activates Jak-2, p38 MAPK and ERK-1/2, but, unlike granulocyte macrophage-colony-stimulating factor (GM-CSF), it does not activate signal transducer and activator of transcription (STAT)-5a/b. We conclude that IL-15 delays neutrophil apoptosis via several pathways, and that Mcl-1 and several kinases contribute to this. We also conclude that, unlike GM-CSF, IL-15 does not activate the Jak-2/STAT-5 pathway found to be important in neutrophil signaling.

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Key words: Inflammation; Cytokine; Anti-apoptotic; Mcl-1; Janus kinase; Signal transducer and activator of transcription; p38 mitogen-activated protein kinase; Extracellular signal-regulated kinase-1/2

1. Introduction

Interleukin-15 (IL-15) is a cytokine known to mediate its biological activity by binding to a specific cell surface receptor (IL-15R), inducing phosphorylation events in immune cells such as B and T lymphocytes, NK cells, and other non-immune cells such as the human intestinal epithelial cell line Caco-2 [1–4]. Information on phosphorylation events medi-

ated by IL-15 is presently lacking for human neutrophils. The IL-15R is composed of at least three subunits, named γ_c (CD132), IL-2R β (CD122), and the more recently identified IL-15R α [5]. The γ_c chain is shared by other receptors such as IL-2R, IL-4R, IL-7R, IL-9R and IL-21R [6–9]. In addition to the γ_c chain, both the IL-2R and IL-15R share the IL-2R β subunit. This may explain why these two cytokines possess some redundant biological actions [3,10]. IL-15 is known to utilize the Janus kinase-signal transducer and activator of transcription (Jak-STAT) pathway to mediate its effects [3]. Although there are few reports in the literature dealing with the role of the Jak-STAT pathway in neutrophils, Jak-2 appears to be preferentially involved in these cells [11–13]. IL-15 is known to recruit Jak-1/3 and STAT-3/5 in immune cells [6,10,11]. However, this has not been studied in human neutrophils. We have previously documented that IL-15 is a neutrophil agonist [14]. This cytokine induces RNA synthesis, de novo protein synthesis, phagocytosis, and delays apoptosis. More recently, IL-15, unlike IL-2, was found to induce the production of the potent neutrophil chemoattractant IL-8 and activation of NF- κ B [15]. In addition, it has been reported that IL-15 could not inhibit the ability of the plant lectin *Viscum album* agglutinin-I (VAA-I) to induce neutrophil apoptosis, and this was correlated with an inhibition of de novo protein synthesis induced by VAA-I [16].

The differential effects of IL-15 and IL-2 on human neutrophils can be explained by the fact that these cells express a high affinity IL-15R (γ_c , IL-2R β , and IL-15R α) whereas they express an IL-2R of intermediate affinity, which is lacking one component, namely, the IL-2R α (CD25) [17–19].

IL-15 is well recognized for its ability to delay apoptosis [14,20–24]. However, the mechanisms involved in this process have not been studied. In fibroblasts, it was recently demonstrated that IL-15 inhibits TNF- α -induced apoptosis by TRAF2 (TNFR associated factor 2) recruitment to the IL-15R α chain [25]. Maintenance of the anti-apoptotic Bcl-2 protein expression by IL-15 was previously demonstrated to promote NK cell survival [26]. The manner in which IL-15 delays human neutrophil apoptosis is currently unknown. It is of great importance to elucidate how IL-15 delays apoptosis, since this cytokine possesses potent pro-inflammatory activities and interesting potential therapeutic properties for humans, especially in advanced cancers [27–29].

Neutrophils are terminally non-dividing mature cells known to spontaneously undergo apoptosis without any apparent

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Abbreviations: IL-15, interleukin-15; VAA-I, *Viscum album* agglutinin-I; GM-CSF, granulocyte macrophage-colony-stimulating factor; Jak, Janus kinase; STAT, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase

stimulation. Because of their importance in the inflammatory process and because of their high cell turnover rate, an increasing number of studies have focused on the cellular and molecular biology of neutrophil apoptosis [30–32]. Pharmacological manipulation of neutrophil apoptosis is certainly of great importance for developing potential therapeutic strategies [30,31].

Among the different mechanisms involved in the regulation of neutrophil apoptosis, members of the Bcl-2 family proteins were found to exert pivotal roles. Certain members of this family act by protecting from apoptosis (Bcl-X_L and Mcl-1, Bcl-2 being absent in mature neutrophils), while others promote apoptosis (Bad, Bak, Bax, Bid) [33]. Recently, Mcl-1 has gained attention for its role in the neutrophil apoptotic rate [32–34], since it was found to be freely expressed in the cytosol and to be rapidly induced by different factors that delay neutrophil apoptosis (granulocyte macrophage-colony-stimulating factor (GM-CSF), sodium butyrate, IL-1 β , and lipopolysaccharide (LPS)), while its loss during normal aging or hypoxia was found to promote apoptosis. The role of Mcl-1 in IL-15-induced suppression of neutrophil apoptosis is presently unknown.

The present study was conducted in order to better understand the mechanism by which IL-15 delays human neutrophil apoptosis. We found that this cytokine induces tyrosine phosphorylation events in neutrophils and that two important proteins involved in IL-15-induced suppression of neutrophil apoptosis are Jak-2 and p38 mitogen-activated protein kinase (MAPK). IL-15 was also found to prevent the loss of anti-apoptotic Mcl-1 protein expression.

2. Materials and methods

2.1. Chemicals and agonists

The plant lectin VAA-I derived from *V. album* was purchased from Sigma Chemical Company (St. Louis, MO, USA). The protein tyrosine kinase inhibitor genistein and the Jak-3 specific inhibitor I were purchased from Calbiochem (La Jolla, CA, USA). The Jak-2/Jak-3 STAT-1, -3, -5a, and -5b inhibitor tyrphostin B42 (or AG490), the MEK inhibitor PD098059, the p38 MAPK inhibitor SB203580, and the G protein inhibitor *Pertussis toxin* were purchased from Sigma. IL-15 was obtained from PeproTech Inc. (Rocky Hill, NJ, USA).

2.2. Neutrophil isolation

Cells were isolated from venous blood of healthy volunteers by dextran sedimentation followed by centrifugation over Ficoll-Hypaque (Pharmacia Biotech Inc, QC, Canada), as previously described [14,16–18]. Blood donations were obtained from informed and consenting individuals according to our institutionally approved procedures. Cell viability (>98%) was monitored by trypan blue exclusion and the purity (>98%) was verified by cytology from cytocentrifuged preparations colored by Diff-Quick staining [14,16,17].

2.3. Phosphorylation events

Neutrophils (40×10^6 cells/ml in RPMI-1640) were incubated for 1, 5, 15, or 30 min at 37°C with buffer alone, with 65 ng/ml GM-CSF, or with 250 ng/ml IL-15 in a final volume of 120 μ l. Reactions were stopped by adding 125 μ l of 2 \times Laemmli's sample buffer as we have described elsewhere [16,35,36]. Aliquots corresponding to 1×10^6 cells were loaded onto 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred from gel to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Non-specific sites were blocked with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS)-Tween (25 mM Tris-HCl, pH 7.8, 190 mM NaCl, 0.15% Tween-20) for 1 h at room temperature. After washing, the membranes were incubated with monoclonal anti-phosphotyrosine UB 05-321 (1:4000) (UBI) for 1 h at room temperature. Membranes were then washed and incubated

with a horseradish peroxidase-conjugated goat anti-mouse IgG+IgM (1:10 000) (Jackson Immunoresearch Laboratories, Inc.) for 1 h at room temperature in fresh blocking solution. Membranes were washed three times with TBS-Tween, and phosphorylated bands were revealed with the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham, Pharmacia Biotech Inc). Protein loading was verified by staining the membranes with Coomassie blue at the end of the experiments.

2.4. Assessment of neutrophil apoptosis

Freshly isolated human neutrophils (10×10^6 cells/ml in RPMI-1640 supplemented with 10% autologous serum) were pre-incubated for 60 min in 24-well plates with the transduction signal inhibitors and then incubated for 23 h in the presence or absence of IL-15. Inhibitors remained in the culture. Apoptosis was evaluated by cytology as previously published [14,16]. Briefly, cytocentrifuged preparations of neutrophils (with ~ 200 μ l) were performed using a Cyto-tek[®] centrifuge (Miles Scientific, IN, USA) and processed essentially as previously documented [14,16]. Cells were examined by light microscopy at 400 \times final magnification and apoptotic neutrophils were defined as cells containing one or more characteristic darkly stained pycnotic nuclei. An ocular containing a 10×10 squares grill was used in order to count at least five different fields (>100 cells) for the assessment of apoptotic cells. Results were expressed as percentage of apoptotic cells. This assay was found to correlate well with other techniques such as fluorescein isothiocyanate (FITC)-annexin-V/PI binding [16], cell surface CD16 expression [37], and PI/Hoechst binding [14]. In the present study, we have confirmed the apoptotic rate of neutrophils by flow cytometry and observed that the percentage of FITC-annexin-V positive cells was significantly diminished in cells treated with GM-CSF (39.1 ± 4.9) or IL-15 (30.3 ± 7), when compared to untreated cells (67.2 ± 3.7) (mean \pm S.E.M., $n = 3$) (not shown).

2.5. Mcl-1 expression

Neutrophils (10×10^6 cells/ml RPMI-HEPES-P/S) were stimulated 24 h with or without agonists and were harvested and washed twice with cold phosphate-buffered saline (PBS). Whole cell lysates were prepared as above. Proteins (1×10^6 cells/well) were separated on 10% SDS–polyacrylamide gels and transferred to nitrocellulose. Membranes were blocked overnight at 4°C in TBS-Tween+3% non-fat dry milk (Carnation, Don Mills, ON, Canada). After washing, the Mcl-1 antibody (K-20 clone from Santa Cruz Biotechnology) was added at a final dilution of 1:200 in TBS-Tween+3% non-fat dry milk for 1 h at room temperature. Membranes were then washed with TBS-Tween and incubated for 1 h at room temperature with a goat anti-rabbit horseradish peroxidase (HRP) secondary antibody (Jackson Immunoresearch Laboratories) at 1:20 000 in TBS-Tween+3% non-fat dry milk followed by washes. The Mcl-1 protein was revealed with ECL and quantified using a Fluor-S MultiImager (Bio-Rad) and the Multi-Analyst version 1.1 program (Bio-Rad).

2.6. Jak-2, p38, extracellular signal-regulated kinase (ERK)-1/2 and STAT-5a/b phosphorylations

This was performed essentially as described above for the Mcl-1 expression except that a rabbit polyclonal anti-Jak-2[pYpY^{1007/1008}] (BioSource international, Camarillo, CA, USA), anti-p38[pTpY^{180/182}] (BioSource), anti-pERK-1/2 MAPK (BioSource) or anti-STAT-5a/b (Y694/Y699, Upstate Biotechnology, Lake Placid, NY, USA) phosphospecific antibody was used for the primary antibody incubation at a concentration of 0.5 μ g/ml. Phosphorylated proteins were revealed with ECL and quantified using a Fluor-S MultiImager as above. In some experiments, specific antibodies against the non-phosphorylated form of ERK-1/2 (UBI) and STAT-5b (UBI) were used.

3. Results

3.1. IL-15 prevents the loss of neutrophil Mcl-1 expression

We have previously documented that IL-15 induces suppression of human neutrophil apoptosis [14] and that it cannot inhibit VAA-I-induced apoptosis [16]. Mcl-1 was recently found to be a key element in the regulation of human neutrophil apoptosis [32–34]. As illustrated in Fig. 1, in contrast to spontaneous and to VAA-I-induced neutrophil apoptosis,

IL-15 was found to prevent the loss of Mcl-1 expression, previously detected as a doublet of 40/42 kDa or infrequently as a single band [33,34]. As expected, GM-CSF was found to prevent the loss of Mcl-1 [33,34] when compared to neutrophils undergoing spontaneous apoptosis.

3.2. Involvement of tyrosine kinases in IL-15-induced suppression of neutrophil apoptosis

Tyrosine kinases were previously found to be involved in suppressing granulocyte apoptosis [38,39]. In order to elucidate the mechanisms of IL-15-induced suppression of human neutrophil apoptosis, we investigated the role of different kinases by using several inhibitors. As illustrated in Fig. 2, the pan tyrosine kinase inhibitor, genistein, was found to inhibit the effect of IL-15 by 84%. The more specific tyrosine kinase inhibitor AG490 (Jak-2/Jak-3 STAT-1, -3, -5a, and -5b STAT

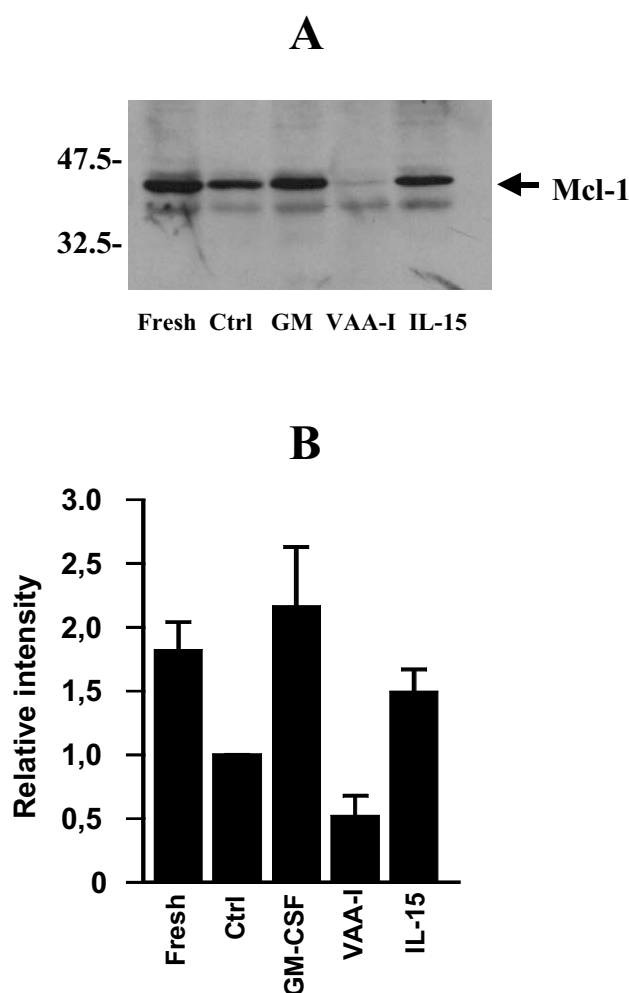


Fig. 1. Loss of neutrophil Mcl-1 expression is prevented by IL-15 but not by VAA-I. Neutrophils were incubated in the presence of agonists for 22 h and the level of expression of the anti-apoptotic p40/42 Mcl-1 protein was detected by immunoblotting as described in Section 2. A: Results are from one representative experiment out of four. Note that Mcl-1 is down-regulated in untreated 22 h aged neutrophils (Ctrl) when compared to freshly isolated cells (Fresh) and that results obtained with other treatments should be compared to lane 2 (Ctrl) and not lane 1 (Fresh). The second band is probably a degradation product, as sometimes observed by other investigators using the same anti-Mcl-1 antibody [33,34]. B: The figure represents the densitometric analysis of Mcl-1 protein ($n=4$).

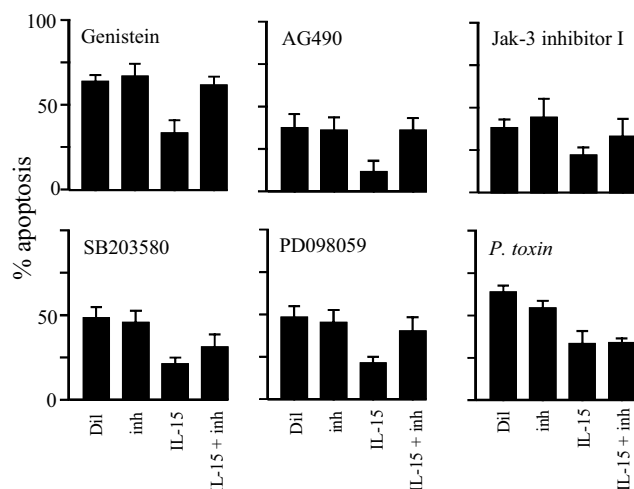


Fig. 2. Involvement of tyrosine kinases in IL-15-induced suppression of human neutrophil apoptosis. Neutrophils (10×10^6 cells/ml) were treated for 1 h in the presence or absence of inhibitors (inh) prior to an incubation of 23 h in RPMI-1640 supplemented with 10% autologous serum in the presence or absence of IL-15. Apoptosis was assessed by cytology from cytocentrifuged preparations stained with Diff-Quick as described in Section 2. Results are means \pm S.E.M. ($n \geq 3$). Dil, the diluent ($< 1\%$ dimethyl sulfoxide (DMSO) in the final volume) was used to solubilize all inhibitors except *P. toxin*, which was solubilized in water before being diluted with the buffer. The concentration of inhibitor was: 50 μ g/ml for genistein; 100 μ M for AG490; 100 μ g/ml for Jak-3 inhibitor I; 50 ng/ml for *P. toxin*; 1 μ M for SB203580; and 10 μ M for PD098059.

inhibitor), the Jak-3 specific inhibitor I, and the serine/threonine p38 MAPK inhibitor SB203580 and the MEK inhibitor PD098059, were found to inhibit this response by 100, 49, 41, and 79%, respectively. The G protein inhibitor *P. toxin* did not alter the IL-15-induced suppression of apoptosis. These results indicate that IL-15 induces suppression of neutrophil apoptosis via different kinases; in particular, Jak proteins appear to play a major role.

3.3. IL-15 induces phosphorylation events in human neutrophils

As illustrated in Fig. 3, IL-15 was found to induce tyrosine phosphorylation events in human neutrophils. Note that we have performed time-course experiment studies (1, 5, 15, 30, and 60 min), but only the results obtained after 1 min are shown for simplicity and also because the response was largely attenuated after 30 min. In two separate experiments, we also found that VAA-I could not alter the IL-15-induced tyrosine phosphorylation events (data not shown). This is not without precedent, as we have recently demonstrated that VAA-I could not alter GM-CSF-induced tyrosine phosphorylation in human neutrophils [16]. A particular protein with a M_r of ~ 120 kDa (near the molecular weight of Jaks) exhibited tyrosine phosphorylation after IL-15 stimulation.

3.4. IL-15 activates Jak-2

Based on these latter results, and because Jak-2 is known to be an important tyrosine kinase involved in general neutrophil cell physiology [11–13,38], we then decided to verify whether or not IL-15 can activate Jak-2. As illustrated in Fig. 4, IL-15 can rapidly induce tyrosine phosphorylation of Jak-2 and this was reversed by addition of genistein or the Jak-2/Jak-3 in-

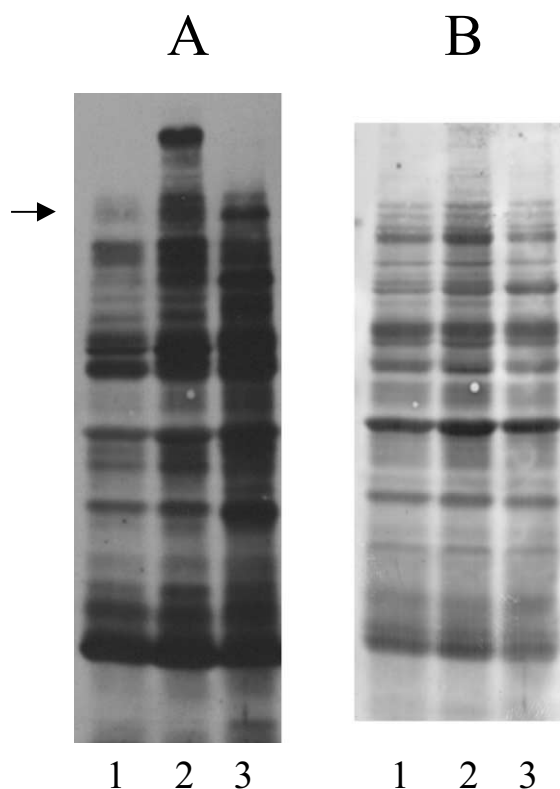


Fig. 3. Phosphorylation events in IL-15-induced neutrophils. Freshly isolated human neutrophils (40×10^6 cells/ml) were incubated with or without agonists and cell lysates were prepared before performing Western blot experiments as described in Section 2. A: Cells were treated for 1 min with buffer (lane 1), 65 ng/ml GM-CSF (lane 2), or 250 ng/ml IL-15 (lane 3). Results shown are from one experiment but are representative of those obtained from four experiments, conducted with different blood donors. The arrow indicates a 120 kDa region. B: The corresponding membrane was stained with Coomassie blue to verify the protein loading.

3.5. Activation of p38 by IL-15

The role of p38 MAPK in neutrophil apoptosis is obscure and varied according to different laboratory conditions [40–42]. Here, we were interested in answering whether or not p38 MAPK is activated by IL-15, since we have found that pre-treatment with SB203580 inhibits the effect of IL-15 (Fig. 2). As illustrated in Fig. 5, IL-15, as well as GM-CSF, was found to activate p38 MAPK. Although we have performed time-course experiments, only the results obtained after 15 min are shown.

3.6. IL-15 induces phosphorylation of ERK-1/2

According to our results (Fig. 2) and knowing that IL-15 was recently found to mediate its biological activity partly via the MAPK ERK-1 pathway in human U937 leukemia cells [43], we decided to investigate the role of ERK-1/2 in IL-15-induced neutrophils. As illustrated in Fig. 6, as GM-CSF, IL-15 was found to induce phosphorylation of ERK-1/2.

3.7. Role of STAT-5 in IL-15-induced human neutrophils

STAT-5 is an important transcription factor in human neutrophils. In particular, GM-CSF was found to activate STAT-5 in these cells [12]. We then decided to study the role of

inhibitor AG490. As expected, Jak-2 was activated by GM-CSF [11,12]. Note that, as reported by the manufacturer, we detected a few bands that may correspond to degradation products of Jak-2.

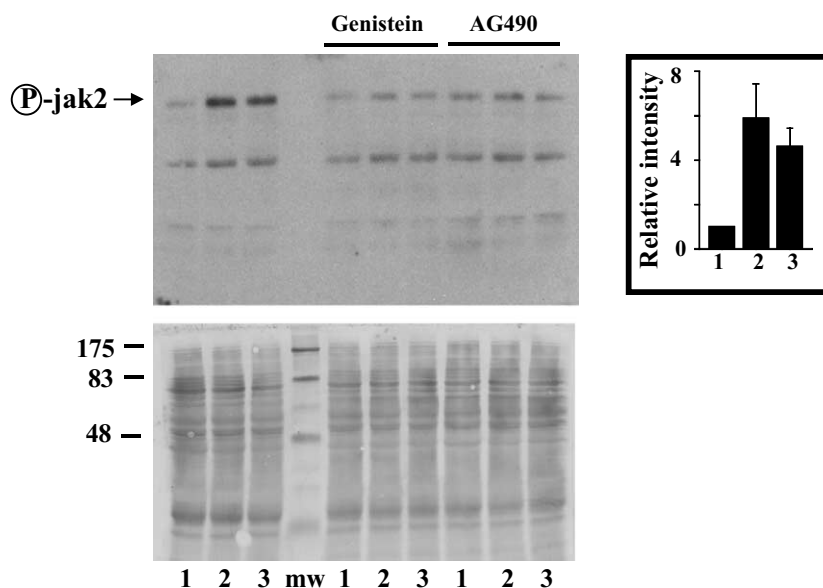


Fig. 4. IL-15 induces Jak-2 phosphorylation in human neutrophils. Cells were freshly isolated as described in Section 2 and were pre-incubated 30 min with or without inhibitors (50 μ M for genistein or 200 μ M for AG490) before being stimulated for 1 min with buffer (lane 1), 65 ng/ml GM-CSF (lane 2), or 250 ng/ml IL-15 (lane 3). Upper panel: The membrane was revealed with an anti-phosphorylated Jak-2 (p-Jak-2) antibody as described in Section 2. Bottom panel: Representation of the corresponding Coomassie blue-stained membrane indicating equivalent loading. Numbers on the left are molecular weight standards that were loaded in the fourth lane (mw). Results shown are from one experiment and are representative of those obtained from three experiments. The relative intensity of phosphorylated Jak-2 following treatment with buffer (lane 1), GM-CSF (lane 2) or IL-15 (lane 3) is indicated in the inset ($n=3$).

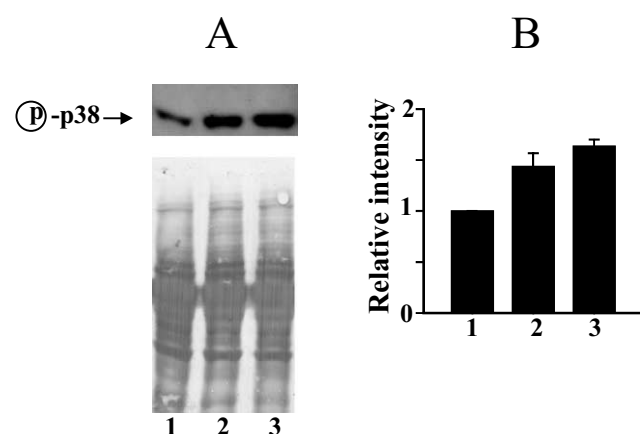


Fig. 5. IL-15 induces p38 MAPK in human neutrophils. Cells were freshly isolated as described in Section 2 and were stimulated for 15 min with buffer (lane 1), 65 ng/ml GM-CSF (lane 2), or 250 ng/ml IL-15 (lane 3). A: Upper panel illustrates the membrane that was revealed with an anti-phosphorylated p38 MAPK antibody as described in Section 2 and the bottom panel represents the corresponding Coomassie blue-stained membrane indicating equivalent loading. Results shown are from one experiment and are representative of those obtained from three experiments. B: The relative intensity of phosphorylated p38 following treatment with buffer (lane 1), GM-CSF (lane 2) or IL-15 (lane 3) is indicated in the inset ($n = 3$).

STAT-5 in neutrophils following IL-15 stimulation. As indicated in Fig. 7, IL-15, in contrast to GM-CSF, does not activate STAT-5. This suggests that IL-15 induces different cell signaling events than GM-CSF.

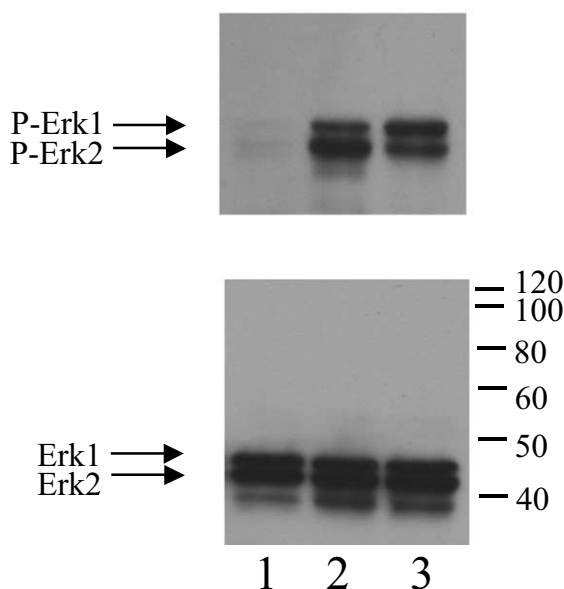


Fig. 6. IL-15 induces the phosphorylation of ERK-1/2 in human neutrophils. Cells were freshly isolated as described in Section 2 and were stimulated for 15 min with buffer (lane 1), 65 ng/ml GM-CSF (lane 2), or 250 ng/ml IL-15 (lane 3). Upper panel illustrates the membrane that was revealed with an anti-phosphorylated ERK-1/2 antibody as described in Section 2 and the bottom panel illustrates the corresponding membrane stained with the unphosphorylated form of ERK-1/2 indicating equivalent loading. Results shown are from one experiment and are representative of those obtained from three experiments. Numbers in the right are the molecular weight standards.

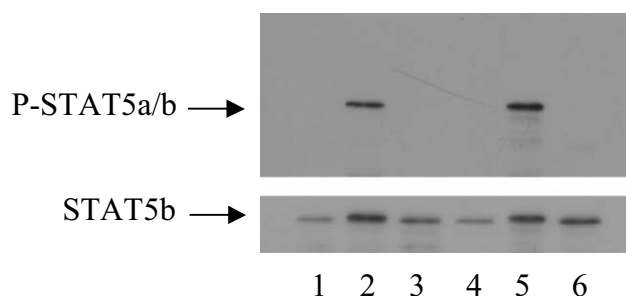


Fig. 7. IL-15 does not activate the phosphorylation of the transcription factor STAT-5 in human neutrophils. Cells were freshly isolated as described in Section 2 and were stimulated for 5 min with buffer (lane 1), 65 ng/ml GM-CSF (lane 2), or 250 ng/ml IL-15 (lane 3) or for 15 min (lanes 4–6, in the same order). The upper panel illustrates the membrane that was revealed with an anti-phosphorylated STAT-5a/b antibody as described in Section 2 and the bottom panel is the corresponding membrane stained with the unphosphorylated form of STAT-5b. Note that although the loading was higher with IL-15 (lane 6) than in control (lane 4) or GM-CSF (lane 5), the phosphorylated forms of STAT-5a/b were not detected, indicating that IL-15 does not recruit STAT-5a/b. Results shown are from one experiment and are representative of those obtained from four experiments.

4. Discussion

IL-15 is a pro-inflammatory cytokine suspected to be an important pathogenic factor in different human diseases, including immunoinflammatory disorders [44]. In this regard, high concentrations of IL-15 have been detected in the synovial fluid and in synovial membrane cells from rheumatoid patients [1,2,45,46]. In addition, the identification of IL-15 as a neutrophil agonist [12,13] together with its ability to enhance the production of TNF- α through activation of synovial T cells and the production of IL-8 by human neutrophils, support a pathogenic role of IL-15 in inflammatory disorders. Moreover, elevated levels of IL-15 have been demonstrated in peripheral blood mononuclear cells from patients with active ulcerative colitis and in alveolar macrophages from patients with active sarcoidosis, and in chronic hepatitis C [47–50].

Binding of IL-15 to its receptor is known to induce tyrosine phosphorylation in immune cells, as well as in cells of non-immune origin such as intestinal epithelial Caco-2 cells [4]. Prior to the present study, the effect of IL-15 on tyrosine phosphorylation events in human neutrophils was unknown. Our results concur with the fact that human neutrophils express the complete IL-15R composed of γ_c , IL-2R β , and IL-15R α chains [17–19], since it induces cell signaling. Jak-2 was recently found to be a candidate involved in GM-CSF-induced suppression of apoptosis by another type of granulocyte, the eosinophil [38]. In this study, we have demonstrated that, among numerous tyrosine kinases that could be involved, at least Jak-2 has been identified as contributing to IL-15-induced suppression of neutrophil apoptosis. To our knowledge, this is the first time that Jak-2 activation has been correlated with a human neutrophil function, namely apoptosis. Prior to our study, researchers have focused only on the ability of different molecules, such as GM-CSF, to phosphorylate Jak-2 [11–13]. Our experiments conducted with the panel of inhibitors suggest that IL-15 might utilize different cell signaling pathways to mediate its effects on human neutrophils.

It has been reported that both GM-CSF and anti-Fas antibodies, well known to delay and induce apoptosis respectively, did not activate p38 MAPK [40]. Others have found that at least two pathways leading to apoptosis exist in human neutrophils, one that is dependent on p38 MAPK activation and another that is independent of this kinase [42]. In addition to the Jak-STAT pathway, we found that the p38 MAPK and ERK-1/2 cascades may be involved in IL-15-induced suppression of neutrophil apoptosis. As for Jak-2, we have found that IL-15 activates p38 MAPK. The precise role of Jak-3 (and the various STATs) requires further investigation, since the specific Jak-3 inhibitor was also found to reverse IL-15-induced suppression of neutrophil apoptosis (Fig. 2). This attests to the complexity of the apoptotic process in these cells. Our results are in agreement with others demonstrating that, in human neutrophils, Jak-2 is a pivotal player in cell signaling [11–13]. Considering that tyrphostin B42 (AG490) is now recognized as an inhibitor of Jak-2/Jak-3 STAT-1, -3, -5a, and -5b [51,52], our results agree with the fact that IL-15 utilizes at least the JAK-STAT pathway to mediate its effect on neutrophils. Interestingly, we found that IL-15, unlike GM-CSF, does not utilize the JAK-2/STAT-5 pathway, since it does not induce phosphorylation of STAT-5.

We have also found that IL-15 can suppress neutrophil apoptosis by preventing the loss of Mcl-1 expression. This loss not only occurs in spontaneous apoptosis, but also during VAA-I-induced neutrophil apoptosis, as well as during co-incubation with both VAA-I and IL-15 (unpublished data). Mcl-1 was recently found to be synthesized *de novo* and linked to the inhibition of neutrophil apoptosis in human neutrophils, as demonstrated by the use of antisense oligonucleotides [34]. As previously stated, IL-15 is known to induce *de novo* protein synthesis in human neutrophils [14]. Our present results indicate that IL-15 delays neutrophil apoptosis by synthesizing at least Mcl-1 when compared with control cells incubated for 24 h. This confirms the concept that neutrophils delay their programmed cell death via the synthesis of macromolecules [53]. However, when comparing *de novo* protein synthesis of Mcl-1 with the level expressed in freshly isolated neutrophils, it appears more appropriate to state that IL-15 delays its degradation. This is in agreement with the general hypothesis proposing that factors suppressing neutrophil apoptosis increase or maintain Mcl-1 levels [32].

The results of the present study indicate that IL-15 induces phosphorylation events in human neutrophils. Knowing the cascade of events mediated by IL-15 will open new avenues for the development of potential new therapeutic strategies. One of these may be to antagonize the effect of IL-15 by using downstream inhibitors to Jaks or other related molecule(s). In this regard, the *in vivo* effects of tyrphostin B42 in acute lymphoblastic leukemia and experimental allergic encephalomyelitis (EAE), were found to reduce the incidence and severity of the disease [54–56]. Knowing how to block IL-15-induced suppression of neutrophil apoptosis will certainly be of benefit for the resolution of inflammation.

New approaches directed toward IL-15, its receptor, or its signaling pathways may be of value in the therapy of various diseases, including inflammatory disorders such as rheumatoid arthritis. Mcl-1, Jak-2, p38 MAPK, ERK-1/2 may now be considered as important potential candidates involved in IL-15-induced suppression of apoptosis.

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