Responsiveness of human neutrophils to interleukin-4: induction of cytoskeletal rearrangements, de novo protein synthesis and delay of apoptosis

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Interleukin-4 (IL-4) and IL-13 are cytokines that share many biological activities. We have previously demonstrated that IL-13 affects a number of neutrophil responses, and here we extend our observations to IL-4. We present, for the first time, direct evidence for the presence of functional IL-4 receptors on human neutrophils. We report that IL-4 induces RNA synthesis in a concentration-dependent manner and, based on observations of the induction of morphological cell shape changes and spreading onto glass, we demonstrate that IL-4 activates neutrophil cytoskeletal rearrangements. We further show that IL-4 is a potent agonist of de novo protein synthesis in neutrophils, and we identify by microsequencing one of these proteins as the cytoskeletal protein actin. We were also able to demonstrate for the first time that actin is cleaved into at least two fragments of ~30 kDa (pI 5.4) and ~25 kDa (pI 5.0) in neutrophils. Finally, we report that IL-4 delays neutrophil apoptosis, as assessed by morphological observations from cytocentrifuge preparations, as well as by measurement of differences in staining by flow cytometry with both propidium iodide and Hoechst reagent. Taken together, we conclude that IL-4 is a more potent neutrophil agonist than previously believed. We discuss the possibility that the induction of the de novo synthesis of actin by IL-4 is related to the mechanism by which this cytokine delays apoptosis; in addition, the cleavage of this protein is likely to contribute to the apoptotic process.

INTRODUCTION

Interleukin-4 (IL-4) is a major immunoregulatory molecule that plays a central role in Th2-mediated immunity [1]. It can enhance immune function within various leucocyte populations, and has been shown to augment the tumour cytolytic activity of eosinophils and lymphocytes [2–14]. Considerable clinical interest has been generated by this cytokine because of its potential use as an anti-cancer agent in humans. A number of clinical trials have been initiated, all of which are in phase I [15,16]. In view of this, the necessity of fully determining all of the effects of IL-4 on host cells cannot be neglected.

We recently reported that IL-13, a cytokine known to share many biological actions with IL-4, activates a number of functions in human neutrophils, including morphological cell shape changes, tyrosine phosphorylation, RNA and protein synthesis and IL-8 production [17]. To date, only classical neutrophil responses such as phagocytosis, the respiratory burst, chemotaxis and degranulation have been the subject of investigation with IL-4 [18,19]. Results from these studies led to the conclusion that IL-4 is a weak neutrophil agonist. However, Th2-mediated inflammation was found to cause local tissue inflammation in which neutrophil infiltration was prominent [21]. Furthermore, it has been reported that IL-4 up-regulates the expression on this cell type of the type II IL-1 receptor [22], a receptor that acts as a decoy for IL-1. These latter findings clearly suggest that IL-4 may be a neutrophil agonist of greater physiological importance than previously thought.

Although there are no reported studies clearly identifying the presence of functional IL-4 receptors (IL-4Rs) on human neutrophils, it is important to note that neutrophils constitutively express the common γ chain (γc) [23–26]. This chain was shown to be one component of the IL-4R, and was found to contribute to a 2–3-fold increase in the binding of this cytokine to its receptor [27,28]. The other known component of the IL-4R is the 140 kDa IL-4Rx α chain, now referred to as CDw124 [29]. Prior to the present study, no information on the presence or absence of this IL-4R component in human neutrophils could be found in the literature. In the present study, we report that neutrophils express functional IL-4Rs, and describe novel findings on the effects of IL-4 in human neutrophils.

MATERIALS AND METHODS

Neutrophil isolation and incubation conditions

Cells were isolated from the venous blood of healthy volunteers, as previously described [17,23,30–32], by centrifugation over Ficoll/Hypaque obtained from Pharmacia Biotech Inc. All cell suspensions contained fewer than 1% monocytes, as determined by monocyte staining. Cell viability, as monitored by the ability to exclude Trypan Blue, was greater than 95% immediately after isolation and after 4, 12 and 24 h of incubation in the presence or absence of agonists. Unless otherwise specified, neutrophils were resuspended in RPMI medium supplemented with 1% (v/v) human autologous serum.

Agonists

Recombinant human IL-4 (1 × 10⁷ units/mg) was purchased from Genzyme (Cambridge, MA, U.S.A.), and recombinant human IL-2 (22 × 10⁶ units/mg) was provided by Cetus Corp. (Emeryville, CA, U.S.A.). Lipopolysaccharide (LPS; from

Abbreviations used: IL-4 (etc.), interleukin-4 (etc.); IL-4R, IL-4 receptor; GM-CSF, granulocyte/macrophage colony-stimulating factor; LPS, lipopolysaccharide; ICE, interleukin-1β-converting enzyme; Jak, Janus kinase; STAT, signal transducers and activators of transcription.

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*Escherichia coli* 0111:B4 was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF; 9 × 10⁶ units/mg) was a gift from the Genetics Institute (Boston, MA, U.S.A.). For simplicity, the recombinant human forms of IL-2, IL-4 and GM-CSF will be referred to simply as IL-2, IL-4 and GM-CSF throughout the text.

**Flow cytometry**

Freshly isolated neutrophils were preincubated with 20% (v/v) autologous serum for 30 min in order to saturate Fc sites. After washing with neutral PBS, cells were incubated with specific anti-human IL-4Rα monoclonal antibody (anti-CDw124) from Genzyme [29] or buffer for 45 min, washed, and then incubated with FITC-labelled goat anti-mouse IgG F(ab')₂ (Bio/Can Scientific, Mississauga, Ont., Canada) for 45 min. In order to prevent internalization, all steps were performed at 4 °C.

**RNA synthesis assay**

This assay was performed by measuring the incorporation of [5-³H]uridine (Amersham Corp., Oakville, Ont., Canada) into total RNA essentially as previously described [17,23,30–32]. Portions of 100 µl of a 5 × 10⁵ cells/ml suspension were incubated in 96-well microtitre plates in the presence of 1 µCi of [³H]uridine plus agonists for 4 h (optimal time point) at 37 °C in 5% CO₂. Following incubation, the cells were collected on to borosilicate glass-fibre paper by a multiple-cell culture harvester (Skatron Instruments Inc., Sterling, VA, U.S.A.), and sections of the filter corresponding to each microwell were then punched out and placed in scintillation counting vials in the presence of 4 ml of Aquasol-2 (Dupont NEN, Boston, MA, U.S.A.). All experiments were performed in triplicate.

**Microscopic observations of neutrophils**

These were performed essentially as previously reported [17,30]. Cells (5 × 10⁶ cells/ml) were incubated at 37 °C in 5% CO₂ in 96-well plates for up to 24 h in the presence of buffer, GM-CSF (65 ng/ml), IL-2 (1–4000 ng/ml) or IL-4 (1–1000 ng/ml). Morphological changes in cells were observed under light microscopy (×200), and micrographs were taken using Kodak Tmax 100 ASA film for black-and-white prints. In parallel, cells were gently loaded on to a haemocytometer and incubated for 5 min at 37 °C. Immediately after incubation, cells were examined under light microscopy and scored as spread or non-spread. The results were expressed as the percentage of spreading cells.

**Metabolic labelling of neutrophils, protein precipitation and two-dimensional gel electrophoresis**

The metabolic labelling of neutrophils (200 µl; 50 × 10⁶ cells/ml) was performed with [³⁵S]methionine and [³⁵S]cysteine (Amersham), both at 125 µCi/10⁶ cells. Cells were collected after 20 h of incubation under optimal conditions [32] as previously described [17,23,30–32] in the presence of protease inhibitors (aprotinin, 60 trypsin-inhibitory units/ml; PMSF, 1 mM; leupeptin, 0.5 µg/ml; EDTA, 200 µM). Protein precipitation was performed in Eppendorf tubes with a final concentration of 70% ethanol for 1 h at −20 °C. After centrifugation, the pellet was solubilized with lysis buffer (9.5 M urea, 2% Nonidet P-40 and 5% β-mercaptoethanol), and 10 µl portions of each fraction were placed in scintillation-counting vials with 4 ml of Aquasol-2 in order to determine the amount of radiolabelled protein loaded for the migration.

High-resolution two-dimensional gel electrophoresis was performed with intracellular neutrophil proteins by the method of O‘Farrell [35], using the Millipore Investigator 2D Electrophoresis System. This was carried out using lysates obtained from an equal number of cells (5 × 10⁶), since we are interested in the *de novo* synthesis of proteins, as we have previously documented [17,23,30–32]. First-dimension isoelectric focusing was performed using 2% Ampholytes (1:4; v/v; pH ranges 4–8 and 3–10). Gels for the second dimension were 12% polyacrylamide. The gels were dried and exposed for 3–5 days at −70 °C. Two-dimensional gel analysis of proteins was performed with a BioImage 110-S analyser (Millipore) using the 2D Gel Match Program. The two-dimensional gel analyser permitted us to compare and evaluate the intensity of each paired spot in order to confirm if the *de novo* synthesis of a particular protein is up- or down-regulated.

**Microsequencing of proteins**

Following two-dimensional gel electrophoresis, proteins were transferred on to PVDF membranes, stained with Coomassie Blue, excised and microsequenced with an Applied Biosystems gas phase sequencer model 475A with on-line phenylthiohydantoin analysis and data collection, as previously reported [30]. All the chemicals and protocols were those recommended by the manufacturer.

**Assessment of neutrophil apoptosis by cytology and flow cytometry**

Cytocentrifuge preparations of neutrophils were obtained and microsequenced with an Applied Biosystems gas phase sequencer model 475A with on-line phenylthiohydantoin analysis and data collection, as previously described [30]. The chemicals and protocols were those recommended by the manufacturer.

Cytocentrifuge preparations of neutrophils were obtained and processed as previously described using a Cyto-tek* centrifuge (Miles Scientific) [17,30]. Cells were incubated in the presence or absence of IL-4 (10 or 500 ng/ml) or GM-CSF (65 ng/ml) for 24 h in RPMI-1640 containing 10% autologous serum, and were stained with a Diff-Quick stain set (Baxter), according to the manufacturer’s instructions. Cells were examined by light microscopy at ×400 final magnification, and apoptotic neutrophils were defined as cells containing one or more characteristic darkly stained pyknotic nuclei [17,30,33,34]. An ocular lens containing 500 cells in total) for assessment of apoptotic cells. Experiments were performed with duplicate samples, and results were expressed as the percentage of cells in apoptosis.

We also evaluated apoptotic neutrophils by flow cytometry, by assessing differences in their staining with both propidium iodide and Hoechst reagents, essentially as previously described [30,36]. Cells were incubated as above. Following the 24 h incubation, aliquots of 350 µl of each cell suspension (representing...
~ 2.5 × 10⁶ cells, after 24 h) were washed twice with neutral PBS, followed by the addition of 100 μl of propidium iodide (from a 20 μg/ml solution) for 30 min on ice. Cells were then protected from light throughout the procedure. After this, the cells were treated with 950 μl of 25% ethanol and 50 μl of HO33342 (from a 112 μg/ml solution) and kept on ice for 12 h before performing FACS analysis (10000 events) using an EPIC 753 instrument (Coulter, Miami Lakes, FL, U.S.A.), as previously reported [30].

IL-8 production

In contrast with many other investigators, we measured the IL-8 concentration not only in the external milieu but also in the intracellular fraction. The measurement of IL-8 production was determined using a commercially available ELISA kit (R&D Systems), essentially as previously described [17]. Freshly isolated human neutrophils were incubated in the presence or absence of IL-4 (10 ng/ml) at 37 °C in 5% CO₂ for 20 h in a 24-well plate containing RPMI-1640 supplemented with 5% (v/v) fetal calf serum. Both supernatants and cell lysates were harvested by centrifugation and stored at −70 °C before performing ELISA.

RESULTS

Detection of IL-4Rα by flow cytometry

In previous studies, it was found that human neutrophils constitutively express the γc chain [23–26]. In order to determine if neutrophils express the complete IL-4R, we studied IL-4Rα (CDw124) expression on the surface of these cells using a specific anti-(human IL-4Rα) antibody and flow cytometry. As shown in Figure 1, we were able to detect the presence of this component on virtually all cells, since they all appeared to shift channel fluorescence intensity, with significantly different means of 111.2 ± 2.6 for IL-4Rα-positive cells and 43.4 ± 10 for negative controls (P = 0.0028, n = 3).

Figure 1 Detection of the CDw124 component of the IL-4R on the surface of human neutrophils by flow cytometry

Flow-cytometric analysis was performed with freshly isolated cells that had been incubated for 30 min with 20% (v/v) autologous serum in order to saturate the Fc sites. Cells were then incubated with buffer (Ctrl) or with anti-(human CDw124) monoclonal antibody, and then with FITC-conjugated goat anti-mouse IgG F(ab')₂, as described in the Materials and methods section. Results are means from three different donors. *P < 0.05 compared with control (Student's t test). Inset: typical results obtained with neutrophils from one donor; results on the x axis are expressed as mean log fluorescence intensity, and those on the y axis are the relative number of cells.

Figure 2 IL-4 induces morphological changes in neutrophils in vitro

Cells (5 × 10⁶ cells/ml) were incubated in the presence or absence of agonists at 37 °C in 5% CO₂ for up to 24 h in 96-well plates. (A) Results obtained after 3 h of incubation (the time point we found to be optimal). Cells were incubated with buffer (panel 1), 65 ng/ml GM-CSF (panel 2) or 100 ng/ml IL-4 (panel 3). Magnification ×130. (B) Dose–response curve of the morphological cell shape changes induced by IL-4 (○), representative of results using cells from five different donors. ●, GM-CSF-treated cells used as positive control [17,23,30].

IL-4 is a potent activator of gene expression

We have frequently used the uridine uptake assay as an indicator of gene expression in order to obtain a first indication of whether or not a particular molecule is a neutrophil activator [12,23,30–32]. By this means, we have previously identified formyl-Met-Leu-Phe, GM-CSF and tumour necrosis factor-α [31,32], and
we observed that the percentage of cells spreading on to glass was
Figure 3, after 12 h of incubation (optimal time point [30,34,37]),
cells to adhere spontaneously to glass [30,33,34]. As illustrated in
Another assay that reflects cytoskeletal activity is the ability of
observed. Such results were never obtained with IL-2 (not shown).

more recently IL-13 [17] and IL-15 [30], as neutrophil agonists
with regard to their ability to activate gene expression. Here we
show that uridine uptake was increased by IL-4 in a dose-
response fashion (n = 3). The results obtained were 432 ± 52,
592 ± 192, 2318 ± 287, 2451 ± 378 and 2489 ± 338 c.p.m. for
neutrophils incubated with 0, 1, 10, 100 and 1000 ng/ml IL-4
respectively. When compared with the agonist GM-CSF (uptake
of 2202 ± 710 c.p.m. at 65 ng/ml), IL-4 appears to be a potent
neutrophil agonist, since similar results to those with this dose of
GM-CSF were obtained with only 10 ng/ml IL-4. Thus the
10 ng/ml dose was chosen for the other experiments in the
present study.

IL-4 induces morphological changes in neutrophils
The induction of morphological cell shape changes in neutrophils is
a reflection of cytoskeletal activity. Since this function was
observed to be activated by IL-13 [17], we incubated neutrophils
with increasing concentrations of IL-4 (1–1000 ng/ml) and
observed, over time, their morphology under light microscopy.
As shown in Figure 2(A), unstimulated neutrophils remained
spherical (panel 1), whereas GM-CSF-activated (panel 2) and
IL-4-activated (panel 3) cells responded with morphological changes.
Results illustrated in Figure 2(A) were obtained after 3
h of incubation, the time point observed to be optimal [17,30],
with a concentration of 100 ng/ml IL-4, a concentration found to
induce notable morphological cell shape changes in many of
the cells (Figure 2B). When up to 4000 ng/ml IL-2 was used as a
negative control in this assay ([17,30]; results not shown),
morphological changes were not observed. Figure 2(B) shows
that the induction of morphological cell shape changes by IL-4
is a dose-dependent response. Although percentages varied
slightly between donors, a dose-response effect was always
observed. Such results were never obtained with IL-2 (not shown).

IL-4 enhances cell spreading of neutrophils on to glass
Another assay that reflects cytoskeletal activity is the ability of
cells to adhere spontaneously to glass [30,33,34]. As illustrated in
Figure 3, after 12 h of incubation (optimal time point [30,34,37]),
we observed that the percentage of cells spreading on to glass was
significantly increased by LPS (76.0 ± 3.0 %) or 10 ng/ml IL-4
(61.3 ± 3.3 %) compared with control (36.3 ± 1.9 %) and IL-2-
treated (36.7 ± 6.9 %) cells. Our results obtained with LPS agree
well with those previously published by others [33].

Induction of neutrophil protein synthesis by IL-4
Neutrophils were incubated in the presence or absence of 10 ng/ml IL-4 for 20 h in the presence of [35S]methionine and
[3H]cysteine. Cell viability remained greater than 95 % after
the 20 h incubation period, as assessed by Trypan Blue exclusion.
Before loading the gels, the radiolabelled proteins were precipi-
tated from cell lysates with 70 % ethanol, and total counts
(c.p.m.) of unstimulated and IL-4-stimulated cells were com-
pared. Differences in c.p.m. between stimulated and unstimulated
cells varied among donors (n = 5), but were always greater in the
former (results not shown). The induction of de novo protein
synthesis was analysed by two-dimensional gel electrophoresis
and fluorography. It appears that the intensity of many spots was
enhanced when the cells were stimulated with IL-4 when com-
pared with control cells. This is in agreement with the potent
effect of IL-4 on total RNA synthesis observed herein. The two-
dimensional gel analyser (using a paired–matched spots program)
revealed that 12.0 ± 5.9 % of paired–matched spots (n = 3) were
more intense in unstimulated cells, indicating that IL-4 can also
down-regulate the de novo synthesis of some proteins (results not
shown).

Identification of proteins by microsequencing
Three reproducible protein spots were selected for micro-
sequencing experiments because they were easily detected on
PVDF membranes following staining with Coomassie Blue (see
the Materials and methods section). We first microsequenced a
spot designated spot #1. By performing 29 cycles with this
material, we observed a perfect match (100 % identity) with the
human cytoskeletal protein actin. In the one-letter amino acid
code, the sequence was: M-V-G-M-G-Q-K-D-S-Y-V-G-D-E-A-
Q-S-K-R-G-I-L-T-L-K-Y-P-I-E. This sequence is common to both
the non-muscle β- and γ-actin isoforms [38]. Two other spots,
spot #2 and spot #3, were microsequenced; surprisingly,
these spots were also identified as human actin. The number of
cycles performed was seven and 27 for spots #2 and #3
respectively. Interestingly, it was recently reported that actin
cleaved by interleukin-1β-converting enzyme (ICE) into two
fragments of ~30 kDa with different isolectric points (by
two-dimensional SDS/PAGE) and one fragment of ~14 kDa [37].
However, in the present study we did not microsequence a spot
in the 14 kDa region. Densitometric analysis of the three spots
revealed that the intensity was markedly increased by IL-4
(8.8 ± 3.8, 13.3 ± 4.0 and 5.7 ± 3.0 for spots #1, #2 and #3
respectively) when compared with that in control cells (3.0 ± 1.6,
4.6 ± 1.6 and 1.7 ± 0.8 respectively).

IL-4 delays apoptosis of neutrophils
It has been demonstrated previously that various cytokines
modulate apoptosis in neutrophils [30,33,34,39], but IL-13 does
not [17]. We therefore examined whether IL-4 (at both 10 and
500 ng/ml) exerts such an effect on these cells. As shown in
Figure 4, after a 24 h incubation, IL-4 treatment delayed apop-
tosis when compared with unstimulated cells: 26.1 ± 3.8 %
(10 ng/ml IL-4), 20.0 ± 3.6 % (500 ng/ml IL-4) and 41.0 ± 5.8 %
(control) cells were in apoptosis at this time (n = 5). When
incubated with GM-CSF (known to delay neutrophil apoptosis),
10 ± 2.7 % of cells were in apoptosis (Figure 4). Of note is that
study, we cultured neutrophils at (10–20) 
affected by IL-6; in contrast, IL-6 inhibited apoptosis in
observations from cytocentrifuge preparations with those ob-
Our aim was to correlate the data obtained by microscopic
may be explained in part by differences in experimental culture
recently it was demonstrated that the number of
these cells were shown to respond to this cytokine both
ules and, moreover, it is known to be produced by neutrophils.
Since we had observed previously that IL-13 induces IL-8 production, we decided to examine the effect of IL-4 (known to share many biological activities with IL-13) on IL-8 production. Neutrophil IL-8 production was increased by IL-4 when compared with that in control cells (Figure 5). Overall, neutrophil IL-8 production was significantly increased by IL-4; on accumulating data from both the supernatant and cell lysate fractions, we observed that IL-8 production increased from 1921 to 3800 pg/ml upon stimulation with 10 ng/ml IL-4 (Figure 5). This represents an increase of almost 2-fold, greater than the increase of 1.6-fold observed on stimulation with IL-13 [17].

**Table 1** Confirmation using a flow-cytometric procedure that IL-4 delays neutrophil apoptosis

<table>
<thead>
<tr>
<th>Method</th>
<th>Addition</th>
<th>Expt. 1 (%)</th>
<th>Expt. 2 (%)</th>
</tr>
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<tbody>
<tr>
<td>Flow cytometry</td>
<td>None</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
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<td>21</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Cytospin</td>
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<td>38</td>
<td>36</td>
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<tr>
<td></td>
<td>GM-CSF</td>
<td>15</td>
<td>18</td>
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<tr>
<td></td>
<td>IL-4</td>
<td>24</td>
<td>20</td>
</tr>
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</table>

both GM-CSF- and IL-4-treated cells, as well as control cells, excluded Trypan Blue just before performing the assay (results not shown). This indicates that, under all conditions, no death by necrosis was observed. In a previous study it was reported that IL-4 had no effect on neutrophil apoptosis [39]. This discrepancy may be explained in part by differences in experimental culture conditions. Recently it was demonstrated that the number of neutrophils undergoing apoptosis may vary according to the cell concentration used [40]. Cells cultured at (1–5) × 10⁶/ml were unaffected by IL-6; in contrast, IL-6 inhibited apoptosis in neutrophils cultured at (10–20) × 10⁶/ml [41]. In the present study, we cultured neutrophils at 10 × 10⁶ cells/ml, whereas a density of 1 × 10⁶ cells/ml was used in the previous study [36].

We also evaluated neutrophil apoptosis by flow cytometry. Our aim was to correlate the data obtained by microscopic observations from cytocentrifuge preparations with those obtained using another method. We selected the flow-cytometric procedure, which was performed essentially as described previously [30,36]. The results are shown in Table 1. A good correlation was observed between the two methods, as we have previously reported [30], leading us to conclude definitely that IL-4 delays neutrophil apoptosis.

**Figure 4** IL-4 delays the apoptosis of neutrophils

Cells were incubated in the absence (Ctrl) or presence of IL-4 (10 or 500 ng/ml) or GM-CSF (65 ng/ml) for 24 h, as described in the Materials and methods section. The inset illustrates a representative cytocentrifuge preparation of neutrophils used for evaluating the percentage of apoptotic cells. Arrows indicate neutrophils that have undergone apoptosis (note the characteristic pyknotic nuclei and cell shrinkage), and arrowheads indicate normal neutrophils. Results are means ± S.E.M. of duplicates from five different donors. *P < 0.05 compared with control (Student’s t test).

**Figure 5** IL-8 production is increased by IL-4

The IL-8 concentration was measured in both the extracellular (Extra) and intracellular (Intra) fractions by ELISA 20 h after the addition of IL-4 (10 ng/ml) to neutrophil cultures, as described in the Materials and methods section. Results are means ± S.E.M. from seven different neutrophil donors. *P < 0.05 compared with control by Student’s t test; **P < 0.05 compared with control by paired non-parametric Mann–Witney test (two-tailed). Ctrl, control.

**IL-4 increases IL-8 production in human neutrophils**

Studies on IL-8 production were undertaken, since this cytokine plays a key role in the accumulation of leucocytes at sites of inflammation. In addition, IL-8 is a potent neutrophil chemotactant, it induces degranulation of neutrophil-specific granules and, moreover, it is known to be produced by neutrophils. Since we had observed previously that IL-13 induces IL-8 production, we decided to examine the effect of IL-4 (known to share many biological activities with IL-13) on IL-8 production. Neutrophil IL-8 production was increased by IL-4 when compared with that in control cells (Figure 5). Overall, neutrophil IL-8 production was significantly increased by IL-4; on accumulating data from both the supernatant and cell lysate fractions, we observed that IL-8 production increased from 1921 ± 233 to 3800 ± 580 pg/ml upon stimulation with 10 ng/ml IL-4 (Figure 5). This represents an increase of almost 2-fold, greater than the increase of 1.6-fold observed on stimulation with IL-13 [17].

**DISCUSSION**

IL-4 exerts a variety of biological activities on a large array of cell types by binding to a specific high-affinity receptor [1,2,12,14,27,28]. It is also known that cells responding to IL-4 express a relatively small number of receptors per cell [1,2]. The presence of the IL-4R on neutrophils has been proposed, since these cells were shown to respond to this cytokine both in vitro and in vivo. However, no direct evidence for the presence of functional IL-4Rs on human neutrophils was reported. In the present study we demonstrated by flow cytometry that the CD124 component of the IL-4R (previously designated IL-4Rα) is present on virtually all neutrophils. The confirmation that human neutrophils also express the γc chain [23–26], and the observation that these cells respond strongly to IL-4 (the present study), leads us to conclude that these cells express functional IL-4Rs.

We have performed experiments on the binding of IL-4 to human neutrophils by flow cytometry using freshly isolated cells incubated with biotinylated IL-4 followed by avidin/FITC
The various effects of IL-4 on neutrophils observed in the present study should alert us to the necessity of fully investigating IL-4-neutrophil interactions in the context of designing and analysing clinical trials, since both neutrophils and IL-4 play important roles in host responses. Presently, the use of IL-4 as a therapeutic agent in clinical conditions other than cancer is being contemplated. This cytokine down-regulates Th1 immunity in vitro and in animal model experiments, and its use in controlling T-cell-mediated autoimmune disease has been proposed [43]. Furthermore, IL-4 has potent monokine-suppressing activity, and its utilization as an anti-inflammatory agent in rheumatoid arthritis and inflammatory bowel disease is being considered [44]. However, neutrophils are known to exert mainly pro-inflammatory and tissue-damaging effects due to the rapid release into the extracellular milieu of oxidation compounds and host-defence proteins. How these repose of neutrophils will manifest themselves during IL-4 therapy in humans will need to be closely monitored. Furthermore, the possibility that neutrophils play other functions in host repose besides their well characterized functions in acute inflammation is starting to emerge. These cells were shown to have the ability to produce many different cytokines (including IL-8), and thus have the potential to influence other cells important in both humoral and cellular immune responses. Furthermore, although long considered to be short-lived cells, it is now well established that the life cycle of neutrophils may be considerably prolonged under the influence of a number of immunoregulatory molecules [21,30,33,34].

Our results extend the scope of the few previous studies on the effects of IL-4 on human neutrophils. The novel findings are that these cells clearly express functional IL-4Rs, and that IL-4 modulates RNA synthesis, cytoskeletal activity and actin synthesis, and delays apoptosis. Furthermore, because IL-4 markedly affects protein synthesis in neutrophils, it is likely to have a profound effect not only on this cell type but also on surrounding cells. Close monitoring of neutrophil responses will therefore be critical in ongoing and future clinical trials with IL-4, and could provide greater insight into the beneficial as well as the detrimental effects of this form of therapy in humans.

Taken together, our data indicate that, in human neutrophils, IL-4 is more potent than IL-13 in inducing various biological activities. This is not surprising, since similar results have been observed with various other cells [1,2,15]. However, we found a major different biological activity between these two cytokines: IL-4 modulates neutrophil apoptosis, whereas IL-13 does not.

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