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Ectopic interleukin-5 receptor expression promotes proliferation without development in a multipotent hematopoietic cell line

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SUMMARY

The interleukin-5 (IL-5) receptor is a heterodimer that consists of an IL-5 specific α subunit and a common βc chain that is shared with the receptors for granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL-3). In contrast to IL-5, which acts mainly as an eosinophil lineage specific factor in vivo, IL-3 and GM-CSF stimulate the survival, proliferation and development of various hematopoietic cell lineages and also multipotent progenitor cells. IL-5 has little effect on the survival or proliferation of the multipotent stem cell line FDCP-Mix A4 but does promote some eosinophil development. To investigate whether the lineage specificity of IL-5 is due to the restricted expression of the IL-5 receptor α subunit we transfected the FDCP-Mix A4 cells with a retroviral vector containing this α subunit. The ectopic expression of the IL-5 receptor α subunit in the FDCP-Mix cells did not increase the observed eosinophilic development but did stimulate survival and proliferation of the transfected cells when IL-5 was added. IL-5 thus acts like IL-3 in these cells, promoting proliferation and survival. The results suggest that IL-5, whilst having a capacity to promote proliferation, does not influence eosinophilic lineage commitment in these multipotent cells. The results further argue that the observed lineage specificity of IL-5 is probably due to factors in addition to the restricted expression of the IL-5 receptor α subunit.

Key words: Interleukin 5, Stochastic, Multipotent, Hematopoietic

INTRODUCTION

The development and functional activity of mature hematopoietic cells is regulated by a complex set of interactions involving either direct cellular contact or the action of soluble factors such as cytokines on hematopoietic cells (Ogawa, 1993; Yoder and Williams, 1995). The survival and proliferation of primitive hematopoietic cells depends on these extracellular stimuli. It is less clear whether the self-renewal or lineage commitment of stem and progenitor cells are also determined by the action of cytokines, or whether extracellular stimuli simply provide the appropriate mitogenic stimuli to allow primitive cells to proliferate and develop after stochastic mechanisms have determined their fate (Fairbairn et al., 1993; Cross et al., 1997). Data from the erythropoietin receptor/erythropoietin knockout mice elegantly argue that primitive erythroid cells can certainly be formed without the influence of erythropoietin (Wu et al., 1995). These and other data suggest that the differentiation of multipotent cells is a stochastic process, representing a consolidation of a randomly primed genetic program in the stem cells (Hu et al., 1997). However, data from more developmentally restricted cells, such as granulocyte macrophage colony forming cells, indicate that growth factors may indeed be involved in lineage commitment (Heyworth et al., 1994; Metcalf and Burgess, 1982; Whetton et al., 1994).

Cytokine receptors function as oligomeric complexes of either identical or different chains (for a review see Heldin, 1995). In single subunit receptors the subunit both binds and signals, whereas in multi-subunit receptors the different subunits may perform specific functions such as ligand binding or signal transduction. For example, the high-affinity receptors for granulocyte macrophage colony stimulating factor (GM-CSF), interleukin 3 (IL-3) and interleukin 5 (IL-5) consist of two distinct subunits, α and β (Gearing et al., 1989; Kitamura et al., 1991; Tavernier et al., 1991). The α subunits are specific to each cytokine and bind their specific ligand with low affinity, but form heterodimers with a second shared β subunit, resulting in the formation of a high-affinity receptor. Both the α and β subunits are required for signal transduction. The shared β subunit provides a molecular basis for the functional redundancy of these cytokines. In humans only one β subunit

exists, whereas in mouse there are two, one specific for IL-3 (β_{IL3}) and the other equivalent to the human βc that is shared by all three cytokines (Itoh et al., 1990). The two murine β subunits have a high degree of sequence homology (91%) and are also homologous to the human βc (56%) (Gorman et al., 1992). GM-CSF and IL-3 act on a wide variety of hematopoietic cells to stimulate their proliferation and development into cell types including macrophages, neutrophils and eosinophils, whilst IL-5 has a rather more lineage-restricted action, stimulating production of mainly eosinophils (Metcalf and Nicola, 1995; Sanderson, 1992). At present it is unclear whether or not the differential effects of IL-3, GM-CSF and IL-5 are a reflection of the target cell, an expression of the appropriate α subunit, the conformation of the αβ dimer, or of both specific and common signals mediated by the α and β subunits respectively.

The α receptor subunits have a short cytoplasmic domain (55 amino acids) with a small, membrane-proximal, conserved region. In contrast the β subunit has a relatively large cytoplasmic domain (440 amino acids). The cytoplasmic domains have no homology with known signaling molecules, but ligand binding to the receptor induces rapid tyrosine phosphorylation of numerous signaling molecules through receptor association with JAK kinases. Thus a number of other signaling pathways and transcription of nuclear factors are activated (Mui and Miyajima, 1994). The mechanism by which the receptors transduce the signals leading to proliferation and development are not completely understood but deletion mutation studies have revealed that there are several distinct regions in the cytoplasmic domain of the βc (Kinoshita et al., 1995; Sakamaki et al., 1992; Smith et al., 1997) which are involved and that the α subunit (Mire-Sluis et al., 1995; Takaki et al., 1994) is also required (for a review see Bagley et al., 1997). However the exact role of the α and β heterodimers in the development of cytokine-specific and common signals is still largely unknown.

To unravel the complexity of the receptor signals a relatively pure population of progenitor cells, which retain the capacity to self-renew and differentiate, depending on the culture conditions, is required. The FDCP-Mix A4 cells represent such a system in that they are a cytokine-dependent, karyotypically normal, multipotent cell line (Heyworth et al., 1990; Spooncer et al., 1986). FDCP-Mix cells can be induced to proliferate and develop by altering the conditions in which the cells are cultured: GM-CSF promotes neutrophil/macrophage development, G-CSF promotes neutrophil formation and Epo promotes erythroid development. IL-3, however, can promote the continued self-renewal and maintenance of the multipotent phenotype of the FDCP-Mix cells. In soft gel CFC-Mix assays FDCP-Mix cells not only form mature macrophages, neutrophils and erythroid cells but also megakaryocytes and eosinophils. Previously we have shown that the ectopic expression of the GM-CSF cytokine gene leads to neutrophil/macrophage development of FDCP-Mix cells, an effect that can be suppressed by growing the cells in high concentrations of IL-3 (Just et al., 1991). This and other evidence suggests that IL-3 and GM-CSF have differential effects on multipotent FDCP-Mix cells, leading to self-renewal and neutrophil/macrophage development, respectively, despite activating receptor complexes with a common β subunit. Thus far little consideration has been given to the third member of this receptor subfamily, the IL-5 receptor α subunit. Previous evidence from transgenic mice (Nishinakamura et al., 1996; Takagi et al., 1995) suggests that IL-5 can stimulate proliferation of primitive hematopoietic cells when they express the IL-5α receptor subunit. However, the effect of IL-5α on the commitment of primitive progenitor cells requires further investigation in a more controlled system such as that offered by the FDCP-Mix cells.

In this paper we examine the effects of IL-5 on native FDCP-Mix cells and use the ectopic expression of the α subunit of the IL-5 receptor to determine the effects of IL-5 on self renewal, proliferation and development of the FDCP-Mix cells.

MATERIALS AND METHODS

Maintenance of FDCP-mix cells

FDCP-Mix clones were maintained in Fischer's medium supplemented with preselected horse serum (HS, 20% v/v) and IL-3 (2% v/v). The source of IL-3 used was conditioned medium from a myeloma cell line that expresses the murine IL-3 (equivalent to 200 units/ml IL-3) (Karasuyama and Melchers, 1988). In these culture conditions the FDCP-Mix cells maintain a primitive phenotype. The cells were subcultured twice a week to a cell concentration of 6-8×10^4/ml and maintained at 37°C in 5% CO_2 in air. Viable cell counts were determined using Trypan Blue exclusion.

Transfection of the FDCP-Mix cells

FDCP-Mix clone A4 cells were infected with defective retrovirus using the myeloproliferative sarcoma virus-based vector, pM5 (Laker et al., 1987), carrying either the neomycin phosphotransferase gene alone (pM5neo) or the neomycin phosphotransferase gene and the murine IL-5 receptor α subunit (pM5 IL-5Rα). The cDNA for the murine IL-5 receptor α subunit (Tavernier et al., 1991) was cloned into the *Eco*RI site of pM5neo.

The two constructs were transfected into GP+E86 (Markowitz et al., 1988b) packaging cells by lipofection (using lipofectamine™, Gibco BRL) as per the manufacturer's instructions. Following selection with G418 at 1 mg/ml, transfected GP+E86 cells were mixed with GP-env Am12 cells (Markowitz et al., 1988a). After 72 hours the GP-env Am12 were selected using 200 μg/ml hygromycin B. The target FDCP-Mix cells were co-cultured with nearly confluent, irradiated (30 Gy, caesium^{137}) GP-env Am12 cells. The FDCP-mix cells were added at a concentration of 1-2×10^5 cells/ml in Fischer's medium supplemented with HS (20% v/v), IL-3 (1000 units/ml) and polybrene (2 μg/ml). After 48 hours the FDCP-mix cells were removed and diluted to 1-2×10^5/ml with fresh medium supplemented with IL-3 and G418 at 1 mg/ml. 4 days after starting selection with G418, dead cells were removed by centrifugation on a metrazamide density gradient. The remaining cells were expanded in the presence of 1000 units/ml IL-3 and maintained at a cell density of 1-5×10^5/ml; G418 was maintained at 0.9 mg/ml and cells were cultured at 37°C.

The polyclonal, retrovirally infected FDCP-Mix populations were cloned in semi-solid medium in the presence of IL-3. Cells were plated at 3×10^3 to 3×10^4 cells/ml in Iscove's modified Dulbecco's medium supplemented with HS (20%), IL-3 (200 units/ml), BSA (1% w/v) and agar (0.33% w/v). After 10 days well spaced colonies were picked into 100 μl Fischer's medium supplemented with HS (20%), IL-3 (1000 units/ml) and G418 (0.9 mg/ml) and gradually expanded in volume. IL-3 was reduced to 200 units/ml when stable growth was achieved. Clones were frozen in liquid nitrogen as soon as possible and fresh isolates were recovered for experiments every 3 months. The procedure from initiation of infection of FDCP-Mix cells to freezing the clones took 2 months.

Cell staining procedures

Cells were prepared for staining using a Shandon cytospin 2 (>5×10^4 cells/slide at 1000 rpm for 5 minutes). For analysis of cells of the granulocyte and macrophage lineages cytospin preparations were stained with May-Grunwald-Giemsa. Cells were stained with Luxol-fast-blue for the identification of eosinophils (Johnson and Metcalf, 1980).

Measurement of DNA synthesis

DNA synthesis was measured using a ^{3}H-thymidine incorporation assay. Cells were washed free of growth factors and resuspended at 2×10^5 cells/ml in a final volume of 100 μl with the appropriate growth factors in a 96-well plate. The plates were incubated at 37°C under 5% CO_2 in air for 18 hours. 1 μCi of ^{3}H-thymidine per well was added and the cells incubated for a further 4 hours. DNA was harvested using a automated cell harvester (Automash 2000, Dynatech), and the level of ^{3}H-thymidine incorporation was assessed by scintillation counting.

Growth stimuli

Purified native or recombinant growth factors were employed in all the experiments described. The concentrations used and specific activities of the cytokines employed were as described (Heyworth et al., 1990). Recombinant IL-5 used in all experiments was prepared as described (Van der Heyden et al., 1991). Irradiated long-term marrow culture stromal cell layers were prepared as previously described (Spooncer et al., 1993).

Analysis of IL-5 receptor expression

Cells were washed free of growth factors and serum and resuspended in ice-cold PBS at 1×10^6 cells/100 μl. 30 μl samples were taken for analysis of IL-5 receptor expression by immunolabelling and flow cytometry as follows: (1) cells only (autofluorescent control), (2) cells with 10 μl of anti-rat IgG coupled to FITC (DAKO, Bucks, UK) (non-specific control), (3) cells with 10 μl of rat anti IL-5 receptor antibody (Van der Heyden et al., 1991) and 10 μl of secondary antibody as in (2) (specific binding). In each case the final volume was made up to 50 μl with cold PBS. The tubes were incubated on ice for 30 minutes and then made up to a final volume of 500 μl before analysis of FITC fluorescence, associated with the cells, was carried out using a FACS Vantage flow cytometer (Becton Dickinson).

For each cell line or clone the data was represented as a histogram of cell number against fluorescence intensity. Histograms generated in specific binding conditions were compared to the relevant non-specific control using Kolmogorov Smirnov (KS) statistics in order to determine whether there was a statistically significant population of cells showing specific binding of the primary, IL-5 receptor antibody, to the cells.

RESULTS

The effects of IL-5 on FDCP-Mix cell proliferation and development

IL-5 is known to stimulate the formation of eosinophils in liquid cultures of bone marrow cells (Yamaguchi et al., 1988). The effect of IL-5 on the survival, proliferation and development of FDCP-Mix cells was therefore assessed in liquid culture.

When IL-5 was added to FDCP-Mix cells in the absence of other cytokines some enhancement of cell survival was recorded over a 24 hour period, but all the cells eventually died within 48 hours (see Fig. 1). In a similar manner to some primitive cells isolated from in vivo sources, the FDCP-Mix cells are also known to respond to combinations of cytokines to undergo differentiation and development. For this reason the ability of IL-5 to act in a combinatorial fashion with other cytokines to stimulate eosinophil production was assessed (Table 1). SCF is known to act synergistically with other cytokines to promote proliferation and development, but when combined with IL-5 no difference was seen, in terms of proliferation, compared to SCF alone. Similarly IL-5 combined with IL-3, or a combination of IL-3, GM-CSF and G-CSF showed no effect of IL-5 on the number of FDCP-Mix cells present in liquid culture (Table 1). However, there was an effect of IL-5 on the FDCP-Mix cells in that there was a small but significant increase in the proportion of eosinophils present when the cells were cultured in IL-5 with cytokines previously shown to promote neutrophil/macrophage development (that is IL-3 plus GM-CSF plus G-CSF). This effect was dependent on the dose of IL-5 present, with the percentage of eosinophils increasing from none being apparent to 7%±2% (mean±s.e.m., *n*=3) of the total cell number over a range of IL-5 concentrations from 0 to 50 units/ml. A photomicrograph of a typical eosinophil formed under these conditions is shown in Fig. 2.

Table 1. The effect of IL-5 on FDCP-Mix cell morphology in liquid culture

	Percentage cell morphology			
Growth factors	Blast	Mature granulocyte/ macrophage cells	Eosinophil	Fold increase in cell number
IL-5	0±0	0±0	0±0	no cells
IL-3 (10 ng/ml)	99±1	1±1	0±0	55
IL-3 (0.01 ng/ml)	73±4	27±5	0±0	8
IL-3+IL-5	24±12	70±10	6±2	11
GM-CSF	0±0	99.7±0.5	0.3±0.3	9
GM-CSF+IL-5	0±0	95±3	5±1	9
SCF	10±8	90±8	0±0	0.1
SCF+IL-5	0±0	96±1	4±0.5	0.15
GM-CSF+G-CSF	1±1	98.7±1	0.3±0.3	11
GM-CSF+G-CSF+IL-5	0±0	95±2	5±3	12
IL3+GM-CSF+G-CSF	8±4	91±4	1±1	31
IL-3+GM-CSF+G-CSF+IL-5	0±0	94±1	6±1	34

Cultures were initially set up in liquid culture at a cell density of 4x10^4 cells/ml in the growth factors indicated (essentially as described in Heyworth et al., 1990). Cell morphology was assessed after 7 days. The growth factor concentrations were, unless stated otherwise, IL-3, 0.01 ng/ml; IL-5, 50 units/ml; GM-CSF, 50 units/ml; G-CSF, 5000 units/ml; SCF, 150 ng/ml. The results shown are the mean±s.e.m. from 3 or 4 experiments.

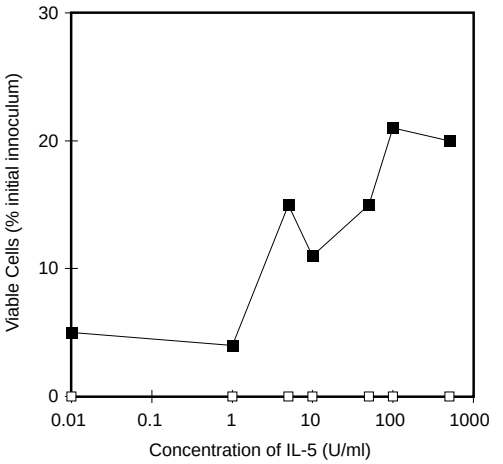

Fig. 1. The effect of IL-5 on the survival of FDCP-Mix cells. The FDCP-Mix cells were cultured with a range of IL-5 concentrations as indicated for 48 hours. The initial cell density was 4×10^4 cells/ml. Viable cell counts were determined at 24 (■)and 48 (□) hours by Trypan Blue exclusion. The results shown are the mean of three observations; the standard deviation was less than 9% in all cases. The experiment shown is typical of three performed.

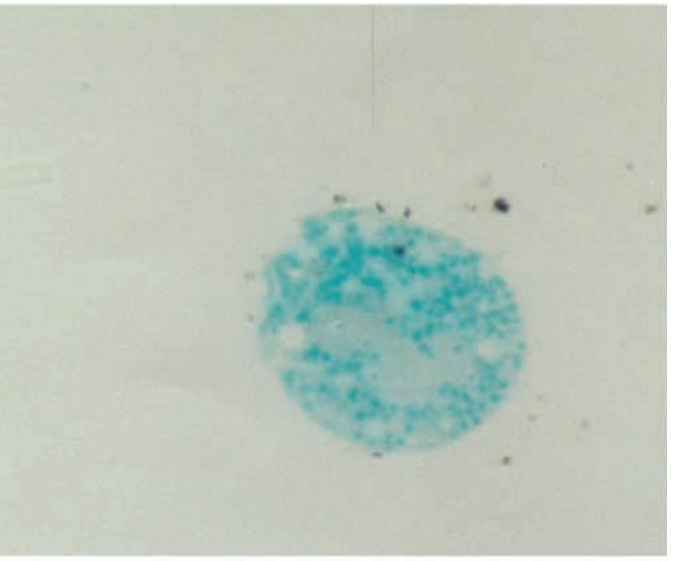

Fig. 2. A typical eosinophil, stained with Luxol-fast-blue, formed from FDCP-Mix cells in liquid culture under myeloid differentiation conditions with IL-5 present at 50 units/ml. ×100.

There was no evidence of a burst of eosinophil formation followed by their loss from culture at any point before day 7. The maximum proportion and maximum absolute number of eosinophils was always seen on day 7. To assess the ability of a discrete subpopulation of FDCP-Mix cells to respond to IL-5 by forming eosinophil-containing colonies, soft gel assays were set up where IL-5 (100 units/ml) was the sole cytokine present. No colonies or clusters were observed after 2, 5 or 7 days (data not shown).

One reason for the relatively low proportion of eosinophils seen in these cultures could be the lack of IL-5 receptors expressed on the population of FDCP-Mix cells. In support of this, flow cytometric analysis was unable to detect a population of cells expressing detectable IL-5α receptors (see Fig. 3). However, as the above experiments show that there is an eosinophilic developmental potential in FDCP-Mix cells, we decided to ectopically express the specific IL-5 receptor alpha subunit in order to determine if receptor expression is rate limiting for eosinophilic development.

Ectopic expression of the IL-5α receptor subunit on FDCP-Mix cells

Following infection of the FDCP-mix cells with the defective retrovirus containing either the neomycin resistance gene (neo) alone or the neo gene with the IL-5α receptor subunit gene polyclonal G418 resistant populations were checked for the presence of IL-5Rα by western blotting (data not shown). Subsequently clonal cell lines of the FDCP-mix cells expressing the IL-5Rα were isolated and checked for levels of receptor expression using flow cytometry. Fig. 3 shows the expression patterns of IL-5Rα in native FDCP-Mix cells, pM5-neo transfected cells and two IL-5Rα transfected clones. The immeasurably low levels of IL-5Rα expression in the native FDCP-Mix cells can be seen from the flow data, whilst the transfected clones 1 and 3 show an enhanced expression of the IL-5Rα subunit. Clone 3 can be seen to be expressing the highest level of IL-5Rα receptor at the cell surface.

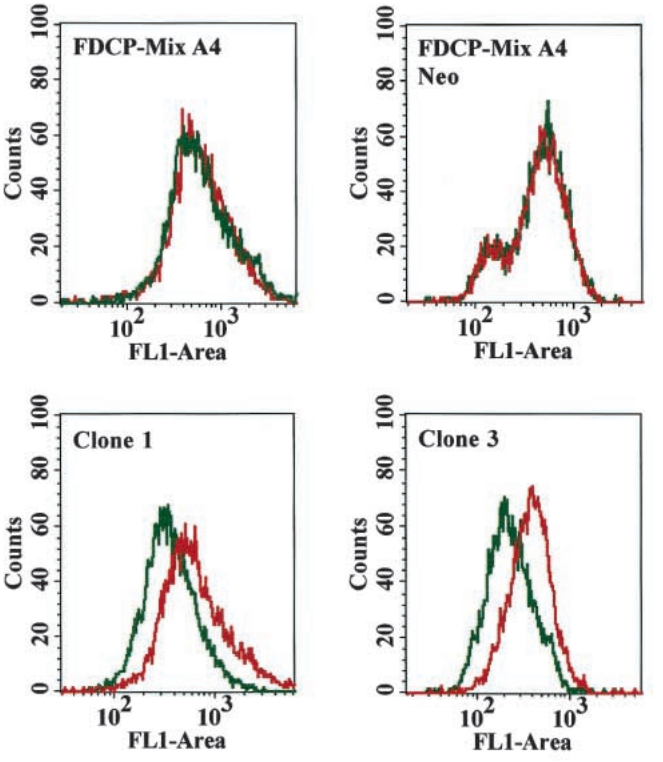

Fig. 3. Flow cytometric profiles of IL-5α receptor expression on transfected and parental FDCP-Mix cells. Cells were prepared for flow cytometric analysis as described in Materials and methods. The histograms shown represent non-specific binding of the secondary antibody alone (green) and specific binding of the IL-5Rα subunit antibody (red). The data shown are from a representative experiment of four.

The effect of increased expression of the IL-5α receptor subunit on FDCP-Mix cells

As shown above IL-5 has little effect on the survival and proliferation of FDCP-Mix cells whilst it can promote a small but significant increase in eosinophilic development of native FDCP-Mix cells. The effects of the ectopic expression of IL-5 receptor subunits on the surface of FDCP-Mix cells was therefore assessed for its effects on both proliferation and development.

Using two different transfected clones (clones 1 and 3) that were positive for IL-5Rα expression, parental FDCP-Mix cells and FDCP-Mix cells transfected with pM5-neo (FDCP-Mix neo), we assessed the level of response to IL-5 in terms of cell survival and amplification of cell number. The fact that several clones were derived from separate transfections, and polyclonal populations gave the same results, shows that these are representative experiments and not a consequence of a single rare or unrepresentative clonal population. From the data in Fig. 4 it is clear that IL-5 is incapable of promoting prolonged survival, or any increase in cell number, in FDCP-Mix cells or FDCP-Mix neo cells. In the case of the FDCP-Mix IL-5Rα clones there is, however, an increase in the numbers of cells present at high concentrations of IL-5. The maximal response to IL-5 was achieved at 50 units/ml with both cell lines expressing ectopic IL-5Rα, and clone 3 showing the greater response. When we assessed the ability of IL-5 to stimulate ^{3}H-thymidine incorporation (Fig. 5) we found that there was a small increase stimulated by IL-5 in native FDCP-Mix cells. When the FDCP-Mix IL-5Rα subunit was ectopically expressed this response dramatically increased and was comparable, in the highest IL-5Rα-expressing clone (clone 3), to the maximum value obtained with 10 ng/ml IL-3. The following values for IL-5-stimulated thymidine incorporation, expressed as a percentage of the maximum observed with IL-3, were obtained: FDCP-Mix, 10±3; FDCP-Mix neo, 12±3; FDCP-Mix IL-5Rα clone 1, 47±11; FDCP-Mix IL-5Rα clone 3, 130±35.

IL-5 could also promote the formation of colonies from the FDCP-Mix IL-5Rα transfected cells. In both instances maximal colony formation was seen at 50 units/ml IL-5. No such colonies were observed when FDCP-Mix cells or FDCP-Mix neo cells were plated with IL-5 (1-500 units/ml) in soft agar, although IL-3 (10 ng/ml) did stimulate colony formation from all three cell types (see Fig. 6). The cells present in the colonies from FDCP-Mix IL-5Rα clones 1 and 3 were predominantly blast cells; no eosinophils were present when the cultures contained between 5-500 units/ml IL-5. Thus in soft gel assays, IL-5 could induce proliferation with no apparent development to eosinophils. When compared to the maximal colony formation (seen with 10 ng/ml IL-3) the following values, as a percentage of maximum, were obtained: FDCP-Mix, 2±1; FDCP-Mix neo, 1±1; FDCP-Mix IL-5Rα clone 3, 314±54. Plainly, functional IL-5 receptors are being expressed in these cells which can bind IL-5 and signal through the cytosolic domain, and in relative terms this is short, but nonetheless essential for function (Takaki et al., 1994).

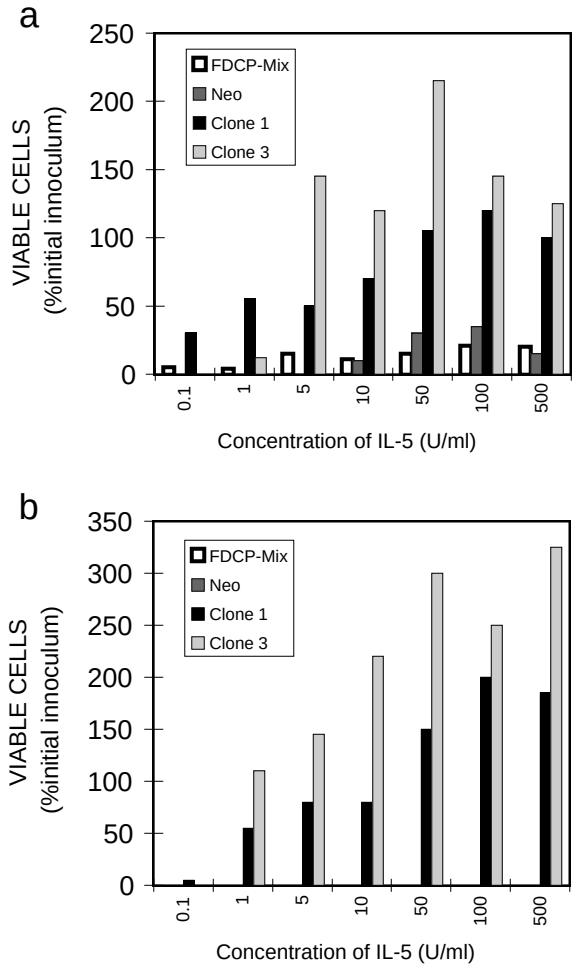

Fig. 4. The effect of IL-5α receptor expression on the survival of FDCP-Mix cells. FDCP-Mix cells, FDCP-Mix neo and IL-5Rα transfected FDCP-Mix cells clones 1 and 3 were cultured in the range of IL-5 concentrations indicated. The initial cell density was 4×10^4 cells/ml. Viable cell counts were determined at 24 (a) and 48 (b) hours by Trypan Blue exclusion. The results shown are the mean of three observations, the standard deviation was less than 9% in all cases. The experiment shown is typical of three performed.

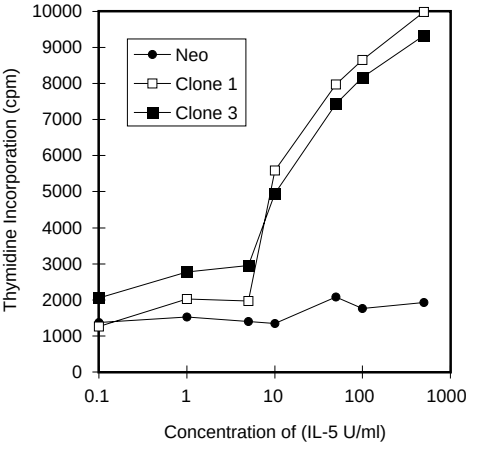

Fig. 5. The effect of IL-5α receptor expression on the proliferation of FDCP-Mix cells. Cells were washed free of growth factor and resuspended at 10^5 cells/ml. IL-5 was added at the concentrations indicated and the rate of proliferation assessed by ^{3}H-thymidine incorporation. The results shown are the amalgamated data mean of four experiments.

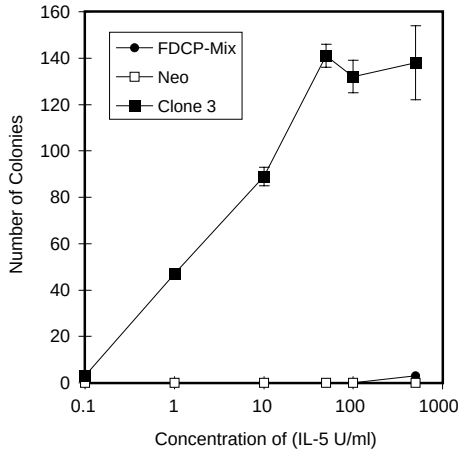

Fig. 6. Colony formation in FDCP-Mix cells expressing the IL-5α receptor. Soft agar colony forming assays were performed as described previously (Spooncer et al., 1986) in the presence of the range of IL-5 concentrations indicated, with 500 cells/plate. The number of colonies was assessed after 7 days. The results shown are the amalgamated data mean from three experiments.

Previous reports that eosinophilic development is more easily achieved in liquid cultures led us to assess the ability of FDCP-Mix cells ectopically expressing the IL-5Rα subunit to develop into eosinophils in such conditions. Table 2 shows that although increased concentrations of IL-5 can help to maintain a population with a blast cell phenotype there was no increase in the proportion of eosinophils present, although eosinophils were seen at similar frequencies as in the parental FDCP-Mix cells. To determine if there was a need to present the developing cells with IL-5 only after a specific stage of development in order to obtain eosinophils, the cells were cultured in low concentrations of IL-3 (0.01 ng/ml) plus GM-CSF and G-CSF to induce myeloid development and IL-5 was added on days 0-3 of the incubation (see Table 3). When the proportion of eosinophils was assayed on day 7 there was no eosinophil development observed in the cultures where IL-5 was 'held back'. Similarly we saw no 'burst' of eosinophil production on days 5 and 6 or days 8, 9 and 10. In short, the ectopic expression of the IL-5 receptor α subunit and the addition of IL-5 did not increase eosinophil production, but the coupling of the growth factor to its receptor could stimulate survival and proliferation (see Figs 4, 5).

To determine whether or not there was a 'missing factor' for the promotion of eosinophilic differentiation expressed on bone marrow stromal cells that is absent from our liquid cultures we incubated FDCP-Mix cell transfectants with irradiated long-term marrow culture stromal cells and IL-5. The results reflected those seen in liquid culture with no promotion of eosinophilic formation, demonstrating that there was no eosinophil development promotion factor present in the stromal cell matrix (data not shown).

DISCUSSION

In addition to possessing similar biological activities, IL-3, IL-5 and GM-CSF also exhibit marked differences. They have differential effects within in vitro assays for bone marrow progenitor cells. IL-5 promotes eosinophil development, whilst GM-CSF has a broader range of activity, promoting BFU-e, Eos-CFC, GM-CFC and CFC-Mix growth (Gasson, 1991; Metcalf and Nicola, 1995). IL-3, on the other hand, has an even broader range of responsive progenitor cell populations (Sanderson, 1992; Suda et al., 1985), including some evidence that it can act on pre-T cells (Ziltener et al., 1988). These differences in biological activity could be mediated by cellular

Table 2. The effect of IL-5 on FDCP-Mix, FDCP-Mix neo and IL-5Rα transfected FDCP-Mix cell morphology

	Percentage cell morphology			
Growth factors present	Blast	Mature granulocyte/ macrophage	Eosinophils	Fold increase in cell number
FDCP-Mix IL-5Rα Clone 3				
IL-5 100 units/ml	93±4	7±3	0±0	36
G Diff	3±2	96±3	1±1	16
G Diff plus IL-5 5 units/ml	11±4	88±4	1±0.5	18
G Diff plus IL-5 50 units/ml	31±10	69±10	0±0	27
G Diff plus IL-5 100 units/ml	52±2	48±2	0±0	24
FDCP-Mix neo				
IL-5 100 units/ml	0±0	0±0	0±0	no cells
G Diff	0±0	99±0.1	1±0.5	8
G Diff plus IL-5 5 units/ml	0±0	100±0	0±0	7
G Diff plus IL-5 50 units/ml	0±0	95±2	5±2	9
G Diff plus IL-5 100 units/ml	3±3	95±3	2±1	11
FDCP-Mix				
IL-5 100 units/ml	0±0	0±0	0±0	no cells
G Diff	6±4	92±4	2±1	21
G Diff plus IL-5 5 units/ml	2±1	96±1	2±1	19
G Diff plus IL-5 50 units/ml	4±2	91±2	5±2	23
G Diff plus IL-5 100 units/ml	14±4	83±5	3±2	17

Cells were set up in liquid culture at a cell density of 4×10^4 cells/ml as previously described. Cell morphology was assessed after 7 days. G-Diff indicates the presence of IL-3 (0.01 ng/ml), GM-CSF (50 units/ml) and G-CSF (5000 units/ml). In addition IL-5 was added at the concentrations indicated. The results shown are the mean±s.e.m. of 3-6 experiments.

Table 3. The effect of delayed addition of IL-5 on the development of FDCP-Mix and IL-5Rα transfected FDCP-Mix cells

		Percentage of cell morphology		
Cells	Day of IL-5 addition	Primitive cells	Mature granulocyte/ macrophage cells	Eosinophils
FDCP-Mix	Day 1	30±10	69±10	1±1
IL-5Rα	Day 2	29±5	70±5	1±1
Clone 3	Day 3	25±10	74±10	1±0
FDCP-Mix	Day 1	1±1	96±1	3±1
	Day 2	0±0	99±1	1±1
	Day 3	1±1	98±1	1±1

The cells were set up in liquid culture at a cell density of 4×10^4 cells/ml in myeloid differentiation conditions as described in (Heyworth et al., 1990). Cell morphology was assessed after 7 days. The cytokines present were IL-3 (0.01 ng/ml), GM-CSF (50 units/ml) and G-CSF (5000 units/ml). IL-5 was added on the days indicated at 100 units/ml. The results shown are the mean±s.e.m. from 3 experiments.

signaling differences between the cytokine-specific α subunit or β_{IL3} and β_C, or alternatively the differences may simply reflect the pattern of receptor expression. Thus the expression of specific receptors for each cytokine on specific progenitor cell populations could account for their ability to 'recruit' specific progenitor cell populations and stimulate their pre-programmed development. Alternatively, occupation of the appropriate receptor population by IL-3, GM-CSF or IL-5, respectively, could 'instruct' cells to survive, proliferate, differentiate and develop by eliciting different signals which determine cell fate.

The pattern of cell surface receptor expression on different hematopoietic cell types has been assessed by receptor/antibody staining and growth factor binding. These types of study have revealed that the IL-3α receptor has a wide distribution on hematopoietic progenitor cells whilst the IL-5α receptor appears to be more restricted (Hara and Miyajima, 1992; Hitoshi et al., 1990; Mckinstry et al., 1997).

To investigate whether or not the limited ability of FDCP-Mix to develop into eosinophils was a consequence of their restricted expression of the IL-5α receptor we ectopically expressed this receptor in the FDCP-Mix cells. When the IL-5α receptor was expressed on the FDCP-Mix cells, IL-5 was able to provide survival and proliferative stimuli. This indicates that the intracellular signals normally provided by IL-3 that promote survival and proliferation can also be elicited by IL-5. The results imply that the restricted response of normal bone marrow cells to IL-5 is probably due to the restricted expression of the IL-5Rα subunit. In the FDCP-Mix IL-5Rα-expressing cells, IL-5 had no apparent ability to induce commitment to the eosinophilic development. Furthermore, our studies show that it was not possible to induce eosinophilic development at any stage in the maturation of FDCP-Mix cells. There was no 'window' when IL-5 could 'instruct' the cells to undergo eosinophilic development. These data, coupled with the observations made on IL-5α receptor subunit transgenic mice (Nishinakamura et al., 1996; Takagi et al., 1995), indicate that IL-5 is not a deterministic signal for the development of eosinophilic progenitor cells from more primitive multipotent cells, i.e. these results suggest that the commitment to the eosinophilic lineage is a stochastic process and that IL-5 simply acts to expand a population of pre-committed eosinophilic progenitor cells. Furthermore, a qualitatively different outcome (i.e. no eosinophil differentiation) is observed when IL-5 receptor expression is increased. The quantitative increase in receptor ligand binding therefore does not directly equate to increased eosinophilic development. This fits with the idea that there is a non-linear relationship between IL-5 receptor-stimulated signal and response (i.e. eosinophil development). It is perhaps significant that in the IL-5Rα-expressing cells, IL-5 concentrations of greater than 5 units/ml never contained eosinophils; the signal from the IL-5 receptor has, perhaps reached a level which inhibits such a development process (see Table 2). This data suggests that a more complex concatenation of events is required to elicit eosinophilic development than that of IL-5Rα expression and its occupation by ligand.

Evidence from numerous cytokine knockout mice (Kopf et al., 1996; Stanley et al., 1994) would appear to indicate that there is redundancy in the hematopoietic system amongst the hematopoietic growth factors. This redundancy allows different cytokines to compensate for the absence of specific cytokines, thereby potentiating primitive cell differentiation and development. This is further supported in the conclusions drawn from a study with double-knockout mice in which the genes for both the βc receptor subunit and IL-3 are absent (Nishinakamura et al., 1996). These mice lacking the entire IL-3, GM-CSF and IL-5 cytokine system indicate that none of these three cytokines are required for steady state hematopoiesis or to react to various hematopoeitic insults. Furthermore, erythropoietin receptor 'knockout' mice (Lin et al., 1996; Wu et al., 1995) have been used to show that neither erythropoietin nor its receptor are required for erythroid lineage commitment or the proliferation and differentiation of BFU-E to CFU-E. However they are crucial for the proliferation and survival of CFU-E and their differentiation, i.e. erythropoietin is a late-acting factor in erythroid development.

The work we have reported shows that under controlled conditions, where IL-5 is presented to multipotent FDCP-Mix cells at specific stages of development, we find no eosinophilic stimulus from IL-5. Differentiation to the eosinophilic development pathway does not appear to involve IL-5 action, and the mechanism whereby FDCP-Mix cells form eosinophils remains unclear. In the non-transfected cells the addition of IL-5 could promote survival and/or proliferation/development of eosinophils, or their progeny, leading to the increase in eosinophils observed in the cultures (Table 1). How this occurs when IL-3, GM-CSF and IL-5 all share a common βc receptor subunit is currently unclear. However differences in the stoichiometry of the subunits in these receptor complexes (Woodcock et al., 1997), the presence of a specific β_{IL-3} (Itoh et al., 1990) and possible differential signalling events activated by these three cytokines (Mire-Sluis et al., 1995) within the commited eosinophil precursor, may explain these observations. Plainly, though, IL-5 receptors do not elicit an unequivocal eosinophil development signal. This is in keeping with data from Fairbairn et. al. (1993), who reported that FDCP-Mix cells can spontaneously differentiate as a consequence of enforced survival with the bcl-2 oncogene. Thus it would appear that commitment to eosinophilic development is not predetermined by expression of the IL-5

receptor but is more likely a stochastic process or some other determinative event.

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