Differential regulation of the expression of interleukin-2 receptor γ -chain during the *in vitro* differentiation of human myeloid cells

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The common γ -chain (γ_c) is a shared component of cell-surface receptors for the interleukins- 2, -4 and -7, and possibly others. We studied its expression in cells and cell lines of myeloid origin and found ubiquitous presence of γ_c mRNA in all cells examined. Differential regulation of γ_c expression was observed in myeloid cell lines induced to differentiate *in vitro*. In K-562 erythromyeloid cells, a sharp rise in the levels of γ_c mRNA and protein accompanied megakaryocytic, but not erythroid, differentiation.

Surface binding of interleukin-2, as well as the transcripts for cognate receptor chains, were scarcely detectable in K-562 cells, whereas a significant increase in the binding of granulocyte-macrophage colony-stimulating factor specifically occurred during their megakaryocytic maturation. Our data indicate that expression of γ_c is a common feature of human myeloid cells, and suggest that its expression may be a requirement for human myelopoiesis.

INTRODUCTION

The discovery of the multimeric nature of many cytokine receptors has profoundly modified the established view of this family of molecules. In particular, it now appears increasingly evident that ligand-induced association of multiple receptor chains is usually required for efficient intracellular signalling [1–10]. Distinct cytokine receptor complexes have been demonstrated to utilise common subunits [1–8] and to share similar signal-transduction pathways comprising receptor-associated kinases [11–14] and a family of transcription factors that are directly activated by these kinases [15–18]. Such extreme molecular redundancy is likely to represent the structural basis for the very complex, yet fine, regulation of multiple cell functions exerted by haemopoietins.

The interleukin-2 receptor (IL2R), a typical oligomeric cytokine receptor complex, is composed of at least three polypeptide chains, namely α (p55), β (p75) and γ (p64) [19,20]. These subunits associate upon binding to interleukin-2 (IL-2) and mediate the biological effects of this cytokine. p64 alone does not show significant IL-2-binding ability, but is necessary for generation of high-affinity IL-2 binding sites, internalization of the receptor–ligand complex and signal transduction [21,22]. Different combinations of IL2R chains can be expressed on the cell surface, which bind IL-2 with low, intermediate or high affinity; signal transduction appears to require at least the simultaneous presence of both β - and γ -chains, and the dimerization of their cytoplasmic domains [23,24]. Nonsense mutations within the coding sequence of the IL2R γ gene are associated with X-chromosome-linked severe combined immunodeficiency (XSCID) [25]. The severe impairment of intrathymic maturation of T-cells in XSCID patients could not be entirely explained by the lack of functional IL-2Rs, thereby raising the question as to whether p64 might be a component of multiple

receptor complexes. This hypothesis was confirmed by the demonstration that IL2R γ is indeed associated with the receptors for at least two other interleukins, namely IL-4 and IL-7 [26–29]. The term ' γ -common chain' (γ_c) rather than 'IL2R γ ' has therefore been proposed to designate this molecule [27], and will be used henceforth in the present paper.

The association of p64 with receptors for 'lymphoid' cytokines, as well as its involvement in XSCID, are compatible with a prominent role in the control of the development and functions of the immune system; relatively little is known, however, about its distribution and properties in non-lymphoid cells. Here we show that expression of γ_c is ubiquitous in cells of myeloid origin and not always associated with that of related cytokine receptor chains. We also document a major lineage-specific up-regulation of the levels of γ_c during the megakaryocytic differentiation of K-562 cells and the myelomonocytic differentiation of HL-60 cells. Our results lend support to the notion that modifications in the expression of this molecule may represent a biologically relevant phenomenon associated with specific steps of myelopoietic differentiation and suggest its potential interaction with additional haemopoietin receptor complexes.

MATERIALS AND METHODS

Cell culture and stimulation

All cell lines were cultured in RPMI 1640 medium (Flow) containing 10 % foetal-calf serum (FCS; Hyclone), 100 units/ml of penicillin, 100 μ g/ml of streptomycin and 2 mM glutamine (Flow). The granulocyte-macrophage-colony-stimulating-factor (GM-CSF)- and IL-3-dependent GF-D8 cells [30] were maintained in the presence of 30–50 ng/ml of recombinant GM-CSF (Boehringer-Mannheim). The differentiation-inducing

Abbreviations used: ATRA, all-*trans*-retinoic acid; γ_c , γ common chain; GM-CSF, granulocyte-macrophage colony-stimulating factor; GM-CSFR, GM-CSF receptor; IL-2, interleukin-2; IL-4, interleukin-4; IL-7, interleukin-7; IL2R, IL-2 receptor; IL4R, IL-4 receptor; IL7R, IL-7 receptor; MPO, myeloperoxidase; PBMNC, peripheral-blood mononuclear cells; CBMNC, umbilical-cord blood mononuclear cells; RT-PCR, reverse transcription-PCR; PMA, phorbol 12-myristate 13-acetate ('TPA'); XSCID, X-chromosome-linked severe combined immunodeficiency; FCS, foetal-calf serum; PHA-P, phytohaemagglutinin-P; SCF, stem-cell factor; PE, phycoerythrin; FITC, fluorescein isothiocyanate; MoAb, monoclonal antibody; GAPDH, glyceraldehyde-phosphate dehydrogenase.

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agents, namely phorbol 12-myristate 13-acetate (PMA; 'TPA'), all-*trans*-retinoic acid (ATRA), ionomycin, sodium butyrate and bovine haemin, were all from Sigma. For stimulation of differentiation, KG-1 cells were cultured in the presence of PMA (10^{-7} M) alone or in combination with ionomycin ($1.6 \mu\text{g/ml}$), HL-60 were treated with PMA (10^{-8} M) or ATRA (10^{-6} M), and K-562 with PMA (10^{-8} M), sodium butyrate (1 mM) or haemin (5×10^{-6} M). For inhibition of protein synthesis, cycloheximide (Sigma) was used at $10 \mu\text{g/ml}$. Mononuclear cells from peripheral blood of healthy volunteers (PBMNC) were isolated by Ficoll-Hypaque (Pharmacia) density-gradient centrifugation, and cultured for 3 days in the presence of $1 \mu\text{g/ml}$ phytohaemagglutinin-P (PHA-P; Sigma). Monocytes were purified from PBMNC by adherence to plastic tissue-culture dishes (Falcon). Granulocytes were isolated by Ficoll-Hypaque density-gradient centrifugation (where they migrated to the bottom of the gradient) following preliminary removal of erythrocytes by sedimentation in 1% Dextran at 37°C for 1 h. For RNA preparation, cells were lysed in 3 ml of guanidinium thiocyanate buffer and subjected to phenol/chloroform extraction as described by Chomczynsky and Sacchi [31].

Haematopoietic progenitor cells were isolated from human umbilical-cord blood using a five-step protocol: (i) incubation of whole blood with 1% Dextran (Sigma) for 45–60 min at 37°C , for removal of erythroblast and erythrocytes; (ii) isolation of cord-blood mononuclear cells (CBMNC) by Ficoll-Hypaque density-gradient centrifugation; (iii) adherent cell depletion by incubation in plastic culture dishes for 2 h at 37°C ; (iv) low-density CBMNC purification by discontinuous Percoll gradient (Pharmacia) [relative density (d) ≈ 1.060]; (v) capture of CD34⁺ progenitors in CD34-coated MicroCELLector flasks (Applied Immune Science). This strategy typically yielded cell fractions containing ≥ 65 –85% CD34⁺ progenitors, as assessed by flow cytometry and *in vitro* clonogenic assays. Viable contaminant cells accounted for 5–20% of the fractions and included erythroblasts, T- and B-lymphocytes and, rarely, monocytes and granulocyte-like cells. CD34⁺ cells were cultured in Iscove's-modified Dulbecco's medium (Flow) containing 20% FCS, stem-cell factor (SCF; Sigma; 10 ng/ml), interleukin 3 (IL-3; Boehringer-Mannheim; 10 units/ml) and GM-CSF (Boehringer-Mannheim; 50 units/ml); cells were then lysed in 100–200 μl of guanidinium thiocyanate buffer and subjected to RNA extraction as described above. Prior to ethanol precipitation, $10 \mu\text{g}$ of glycogen was added to each sample as a carrier.

PCR primers and molecular probes

The following PCR primers were used in the present study (FWD and REV are forward and reverse oligonucleotide respectively):

γ_c :	FWD(649–672):	5' GTGTGGATGGGCAGAAACGCTAC 3'
	REV(969–947):	5' CAGCCAGTCCCTTAGACACACCA 3'
GAPDH:	FWD(282–301):	5' CACCATCTTCCAGGAGCGAG 3'
	REV(653–634):	5' TCACGCCACAGTTTCCCGGA 3'
CD34:	FWD(811–830):	5' TGACTCAGGGCATCTGCCTG 3'
	REV(1206–1225):	5' CTTTCTCCTGTGGGGCTCCA 3'
MPO	FWD (1069–1086):	5' CCCAATGACCCCCGCATC 3'
	REV (1387–1369):	5' TGTCCCTGCCAGGAAGCA 3'
IL2R β :	FWD(1278–1302):	5' ACTTACGACCCCTACTCAGAGGAAG 3'
	REV(1594–1571):	5' GGTGGCTGAAAATCCACCAGGTCT 3'
IL4R:	FWD(2291–2310):	5' AGCCTGGGCAGTGGCATTGT 3'
	REV(2600–2581):	5' GGGTCTGGCTTGAAGCTCTGA 3'
IL7R:	FWD(573–592):	5' CCAGCACAAAGCTGACACTC 3'
	REV(955–936):	5' CACCCTATGAATCTGGCAGTC 3'

(MPO is myeloperoxidase, GAPDH is glyceraldehyde-phosphate dehydrogenase and CD34 is a surface antigen expressed by haematopoietic progenitor cells). The amplimers for IL2R α mRNA were purchased from Clontech.

Templates generated by reverse-transcription (RT)-PCR as detailed below were subjected to ten PCR cycles in the presence of $40 \mu\text{Ci}$ of [α - ^{32}P]dATP (Amersham). Labelled probes were separated from non-incorporated [α - ^{32}P]dATP on G-50 columns (Pharmacia). *Taq* polymerase was purchased from Polymed.

RT-PCR and Northern-blot analysis of cellular mRNA

cDNA was synthesized from 2 μg of total cellular RNA (or from one-tenth of the RNA from CD34⁺ cells) using Superscript reverse transcriptase (BRL); 2.5 μM random hexamers (Boehringer-Mannheim) ($0.5 \mu\text{M}$ for CD34⁺ RNA) were used as primers. cDNA aliquots were amplified in 30–40 cycles of PCR using the primers described in a Robocycler thermal cycler (Stratagene). All amplimers were complementary to sequences contained in the coding region of each gene and designed in order to obtain similar T_m values ('melting' temperature) and PCR products of comparable size. After analytical digestion with specific restriction enzymes to verify the faithfulness of amplification, PCR products were purified from agarose gels and used as PCR templates for preparation of molecular probes. For RT-PCR mRNA analysis, cDNAs were subjected to a reduced number of PCR cycles (18 for GAPDH and γ_c , 24 for IL2R α , IL2R β , IL4R and IL7R, and 30 for CD34), electrophoresed on agarose gels and, after denaturation in alkali, transferred on to Nytran filters (Schleicher and Schuell). Filters were incubated at 80°C for 2 h, hybridized overnight at 65°C with 10–30 μCi of radiolabelled probe, extensively washed and autoradiographed with X-ray film (Kodak). For Northern-blot analysis, $10 \mu\text{g}$ of total cellular RNAs were electrophoresed on 1.5% agarose/formaldehyde gel, transferred on to Nytran membranes and processed as described for RT-PCR.

Cytokine-receptor analysis by flow cytometry

Cells were washed twice in $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free PBS and resuspended at $5 \times 10^6/\text{ml}$. A $15 \mu\text{l}$ portion of cell suspension was incubated for 45 min at 4°C with $35 \mu\text{l}$ of phycoerythrin (PE)-labelled streptavidin (as a negative control), IL-2 or GM-CSF (Fluorokines; R&D), washed twice in 5 ml of PBS, and analysed with a FACScan flow cytometer (Becton-Dickinson). For detection of p55 on the cell surface, a fluorescein isothiocyanate (FITC)-labelled monoclonal antibody (MoAb) was used (anti-CD25; Becton-Dickinson); the control was an FITC-labelled mouse IgG_{2a} (Becton-Dickinson). An anti-p75 MoAb was kindly provided by Dr. W. E. Greene (Gladstone Institute, San Fran-

cisco, CA, U.S.A.), and used in indirect immunofluorescence with a FITC-labelled goat anti-mouse IgG antiserum (Becton-Dickinson). As a control, FITC-labelled goat anti-mouse IgG antiserum was used.

Western blot

Cells were lysed in a buffer containing 50 mM Tris/HCl, pH 7.4, 140 mM NaCl, 0.5% Nonidet P40 and proteinase inhibitors (Boehringer-Mannheim); nuclei and cell debris were removed by centrifugation at 1500 *g* for 10 min; postnuclear supernatants were admixed 1:1 with 2 $\times$ sample buffer [70 mM Tris/HCl (pH 6.8)/7 mM EDTA/15% sucrose/2% SDS/50 mM di-thiothreitol], boiled and electrophoresed in 12 or 14% polyacrylamide/SDS gels. The proteins were transferred on to nitrocellulose filters by electroblotting, incubated with the R878 antibody (see below; kindly provided by Dr. W. J. Leonard, National Institutes of Health, Bethesda, MD, U.S.A.) and then with a goat anti-rabbit antibody conjugated to horseradish peroxidase (Sigma). Binding was revealed by a chemiluminescent reaction (ECL; Amersham).

RESULTS AND DISCUSSION

Ubiquitous expression of γ_c transcript in human myeloid cells

So far, the expression and functional features of γ_c have been characterized mainly in lymphoid cells, although the presence of this molecule in myeloid cell types has been reported [32–35]. This prompted us to extensively analyse the expression of γ_c gene in a variety of human cells of myeloid origin.

Preliminary Northern blot and RT-PCR experiments (Figure 1) revealed relatively large amounts of γ_c mRNA in peripheral-blood granulocytes and monocytes and in the myeloid cell lines U-937, HL-60, KG-1, K-562 and GF-D8 (Figure 1a), as well as in umbilical-cord-blood-derived haematopoietic progenitors, expressing the progenitor-specific CD34⁺ antigen, at various stages of cytokine-induced differentiation (Figure 1b). In contrast, the non-haematopoietic cells lines HeLa, HepG2, Hep3B and A1251 contained no detectable transcript, as assessed by both Northern blot and RT-PCR (results not shown). On the basis of such a specificity of expression, it could be conjectured that p64 might be required in all haematopoietic cells, including immature and mature myeloid cells. In CD34⁺ cells, no dramatic changes in the amounts of γ_c transcript appeared to be induced by stimulation with haemopoietins (Figure 1b), suggesting that continual presence of p64 could be important throughout the maturation of immature progenitors. However, since cytokine receptors are usually subjected to stringent tissue- and developmental-specific regulation, it is conceivable that transient variations of the expression of p64 may take place within specific stages of differentiation of individual myelopoietic lineages.

Regulation of γ_c gene expression during differentiation of myeloid cell lines

A suitable model to investigate this possibility is represented by the human leukaemia-derived cell lines KG-1 [36], HL-60 [37] and K-562 [38]. These cells resemble myeloid progenitors in respect of well-defined morphological, phenotypical and molecular criteria [39] and can be induced to differentiate *in vitro* towards different lineages upon treatment with a variety of chemicals [39–44]. As shown in Figure 2, stimulation of monocytic differentiation of KG-1 cells with PMA or PMA + ionomycin (Figure 2a) caused the progressive decrease of the CD34 transcript as previously reported [40], but did not noticeably affect the levels of γ_c mRNA. In HL-60 (Figure 2b),

Figure 1 Expression of γ_c mRNA in human myeloid cells

(a) Northern blot. Samples (10 μ g each) of total cellular RNA were electrophoresed, blotted, hybridized and autoradiographed as described in the Materials and methods section. Ubiquitously expressed GAPDH was used as an internal control. The two bands corresponding to γ_c transcripts (1.8 and 3.5 kb) are indicated by arrowheads. The additional band with higher molecular mass in the lanes where a strong signal is present probably reflects hybridization to mRNA molecules trapped within, and co-migrating with, the 28 S rRNA. (b) RT-PCR. CD34⁺ cells were purified and stimulated with a combination of SCF, IL-3 and GM-CSF; human PBMNC were stimulated for 3 days with PHA-P; KG-1 were treated for 12 h with PMA and ionomycin. RNA extraction and RT-PCR were performed as described in the text. The amounts of cDNA to be amplified were normalized on the basis of preliminary glyceraldehyde-phosphate dehydrogenase (GAPDH) amplifications. Abbreviations: d, day; C, control; P/I, PMA + ionomycin.

differentiation along the monocytic or granulocytic lineage induced by PMA or ATRA respectively was associated with drastic down-regulation of the MPO gene expression as described in [41], and accompanied by a moderate, but significant, increase in the abundance of γ_c mRNA. In K-562 (Figure 2c), megakaryocytic differentiation in response to PMA resulted in a conspicuous accumulation of the γ_c transcript, a peak being apparent after about 6–12 h. Haemin and butyrate, which promote erythroid maturation, failed to produce any detectable effect. Stimulation of γ_c gene expression in K-562 and HL-60 cells appears to be a differentiation-related event rather than a mere response to PMA, as it did not occur in KG-1, although these cells are responsive to phorbol esters (Figure 2a). Accumulation of γ_c mRNA in PMA-stimulated K-562 is independent from protein synthesis. As shown in Figure 2(d), cycloheximide enhanced the stimulatory effect of PMA, resulting in continual accumulation of the transcript up to 24 h. When used alone, cycloheximide induced a modest, but detectable, increase in the amounts of γ_c mRNA. Preliminary experiments suggest that the up-regulation of γ_c expression in K-562 cells is mediated mainly via post-transcriptional stabilization of the mRNA, similar to that observed in peripheral-blood monocytes in response to cytokines [33].

Figure 2 Regulation of γ_c gene expression during differentiation of human myeloid cell lines

RNAs were extracted from cells treated with the different stimulants for the indicated times, electrophoresed, blotted, and hybridized with radiolabelled probes as described in the Materials and methods section. Only the major (1.8 kb) γ_c mRNA band is shown. (a) K-562; (b) HL-60; (c) K-562; (d) effect of inhibition of protein synthesis on γ_c expression in K-562 cells; cells were treated for the indicated times with PMA, PMA + cycloheximide (CHX) or cycloheximide alone. Northern-blot analysis was as described in the text. Abbreviation: d, day.

Regulation of p64 protein expression

The correlation between expression of γ_c mRNA and protein was investigated by Western blotting with the antibody R878 [28]. This antibody, raised against a peptide within the cytoplasmic domain of p64, has been reported to recognize a doublet of approx. 50 kDa and one band of 65 kDa [34]. Under our conditions, probably owing to the different gels used, the 50 kDa doublet was not resolved and only two major bands of 50 and 65 kDa were apparent. In K-562 (Figure 3a), both forms were greatly induced by PMA, but not by butyrate or haemin. In KG-1 (Figure 3b) the comparatively high steady-state levels of γ_c were not modified by PMA. In HL-60 (Figure 3c), expression of p64 was mildly enhanced in response to both PMA and ATRA, but the latter appeared to induce a preferential increase in the 50 kDa band(s). p64 must be highly glycosylated, since its carbohydrate-free amino acid sequence accounts for a molecular mass of less than 40 kDa, and six potential N-glycosylation sites are present in its extracellular domain [21]. The 50 kDa form(s) of γ_c may represent either an immature precursor or an additional mature form of the protein bearing alternative glycosylation. The down-regulation of the larger γ_c band in ATRA-treated HL-

Figure 3 Expression of p64 in human myeloid cell lines

Cell extracts were prepared as detailed in the Materials and methods section; approx. 20 μ g of protein extract/sample was electrophoresed in 12% (a and c)- or 14% (b)-polyacrylamide/SDS gels, transferred on to nitrocellulose filters, incubated with the R878 antibody and bands revealed by the chemiluminescent method described in the text. (a) K-562; (b) KG-1; (c) HL-60. Abbreviations: P, PMA; H, haemin; B, sodium butyrate; R, ATRA; d, day; C, control. The positions of the molecular-mass (M) markers (expressed in kDa) are reported on the left-hand side.

60 (Figure 3c) tends to support the second possibility. The existence of multiple forms of cell-surface γ_c , generated via post-translational modification, might be related to different biological properties of these molecules. Further investigation is underway to establish the nature and subcellular localization of the different bands.

The data from Northern and Western blots delineate a very tight correlation between γ_c mRNA and protein expression, and consistency in their variations, in all cell lines. This suggests that all cells where the transcript for γ_c is found also express the protein. In agreement with some of our observations, Nakarai et al. recently reported the presence of γ_c transcript in all lymphoid and myeloid cell lines examined, but not in the non-haematopoietic ones [35]. However, in flow-cytometric assays those authors failed to detect cell-surface expression of p64 on most of the myeloid cell lines, including K-562, HL-60 and MO7, and found only weak positivity on KG-1 and U-937. In contrast, our Western-blot experiments (Figure 3) clearly demonstrate detectable, albeit relatively low, expression of γ_c in unstimulated HL-60, and a considerably higher amount of p64 in KG-1, consistently with the relative mRNA abundance. It cannot be excluded in principle that, in these cell lines, the transport of γ_c

Figure 4 (a) Expression of the mRNAs for γ_c and related receptor chains in K-562 cells and (b, c) modulation of cytokine receptors on K-562 cells in response to differentiation-inducing agents

(a) RNAs were extracted from PMA-, sodium butyrate- and haemin-treated K562 (left) or from PHA-stimulated PBMNC (right); RNAs were reverse-transcribed and amplified as described in the Materials and methods section. (b, c) Cells were treated with the relevant chemicals for the indicated times, incubated with PE-labelled cytokines or FITC-labelled MoAbs and analysed by flow cytometry as described in the Materials and methods section. (b) Binding of MoAbs to α - and β -chains of IL2R and of PE-IL-2 to untreated or PMA-treated K-562; (c) binding of PE-labelled GM-CSF to K-562 stimulated with PMA or sodium butyrate. The ordinate shows relative cell numbers, whereas the abscissa shows the intensity of fluorescence. White areas represent the binding of MoAbs and PE-labelled cytokines; black areas indicate the values of negative controls (FITC-IgG or PE-streptavidin). Abbreviations: u, untreated; but., sodium butyrate; d, day.

to the cell surface may be totally or partially impaired, resulting in intracellular retention of p64. However, the discrepancy between our data and those of Nakarai et al. is most likely accounted for by a number of alternative reasons linked to: (i) different affinities of the antibodies; (ii) steric hindrance by other molecules at the membrane level in the flow-cytometric assays; (iii) differential glycosylation; the monoclonal used by Nakarai et al. [35], which reacts with the extracellular domain of p64, does not apparently immunoprecipitate the 50 kDa γ_c band(s) from lysates of [35 S]methionine-labelled cells. This antibody might be specific for a carbohydrate-containing epitope present only in the 65 kDa band, which could also explain the weak positivity of KG-1 where the lower form of γ_c appears to be predominant (Figure 3b). In addition, IL-2 is known to induce strong proliferative response in the multiple-factor-dependent MO7 cells [45], indicating that they must express γ_c on their surface. This further supports the notion that the apparent lack of expression of p64 in myeloid cells, reported by Nakarai et al. [35], is probably due to intrinsic limitations of the detection system used.

Parallel up-regulation of GM-CSF binding and of p64 in K-562 cells

In addition to being a subunit of the IL2R, γ_c has been shown to be a component of IL4R and IL7R [26–29]. None of the mRNAs for the known γ_c -related chains could be detected in K-562 cells by Northern blotting. In RT-PCR experiments (Figure 4a) the IL4R cDNA was faintly visible in PMA-treated K-562, whereas those for IL2R α - and β -chain, and for IL7R, were not detectable. Likewise, both p55 and p75 chains, as well as IL-2 binding, were undetectable, by flow cytometry, on the cell surface of K-562 (Figure 4b), whereas a marked increase in the binding of PE-labelled GM-CSF occurred in these cells following PMA treatment (Figure 4c). Such an increase reached maximal values at 18 h after treatment, and its pattern was reminiscent of the γ chain expression profile. Butyrate (Figure 4c) and haemin (results not shown) did not affect GM-CSF binding. The parallel up-regulation of γ_c and GM-CSFR during the megakaryocytic differentiation of K-562 may be trivial, or it may reflect the co-ordinate regulation of cell-specific repertoires of haemopoietin receptors. Intriguingly, however, GM-CSF has been found to cross-compete for the binding of IL-2 to its receptor in the factor-dependent megakaryocytic cell line MO7 [45]. Moreover, a considerable increase in both GM-CSFR and p64 also occurred during the monocytic differentiation of the GM-CSF- and IL-3-dependent cell line GF-D8 (G. Morrone, unpublished work). Although the profile of activation of receptor-associated Jak kinases in response to GM-CSF appears to be distinct from that induced by IL-2 and IL-4 [46,47], it would be tempting to speculate that, at least in some cell types, γ_c may interact or cross-talk with the GM-CSF receptor complex. However, the data presently available are only circumstantial, and direct evidence will be required in order to corroborate this possibility.

Conclusions

In the present paper we demonstrate that relatively abundant expression of γ_c mRNA is a common feature of mature and immature cells of myeloid origin, while being completely absent in non-haematopoietic cells. In human myeloid cell lines, γ_c expression can be regulated in response to differentiation-inducing agents, possibly independently from the other related receptor chains so far identified. Taken together, our data lend support to the notion that the presence of γ_c may be a general

requirement for the control of survival, growth and/or differentiation of myeloid cells. The present state of the knowledge indicates that p64 serves as a common functional subunit of the receptors for IL-2, IL-4 and IL-7 [21,26–29]. Although myeloid cells are known to respond to IL-2 [33,34], IL-4 [48,49] and IL-7 [50,51], the better-documented action of these factors is on cells of the lymphoid lineage. Several lines of evidence suggest that additional cytokines act through p64 [4,6,27,28,52,53], and it is possible that more will be added to this number. The widespread expression of γ_c in the myeloid compartment may reflect its interactions with receptors for a variety of haemopoietins that concertedly contribute to the control of myelopoiesis. In this context, availability of p64 may be a limiting factor for the generation of such receptor complexes. Modifications of γ_c expression in different phases of cell maturation and/or activation could modulate this availability, thereby affecting the sensitivity of the cells to different cytokines. Further insight into the biological properties of γ_c will be provided by immunological and biochemical studies; the K-562 and HL-60 cell lines, where the expression of this protein appears to be modulated in a lineage-specific fashion, may prove a valuable system for investigating the role of p64 in human myelopoiesis and understanding the significance and the molecular aspects of its regulation.

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REFERENCES

- Miyajima, A., Hara, T. and Kitamura, T. (1992) *Trends Biochem. Sci.* **17**, 378–382
- Kitamura, T., Sato, N., Arai, K. and Miyajima, A. (1991) *Cell* **66**, 1165–1174
- Tavernier, J., Devos, R., Cornelis, C., Tuypens, T., Van der Heyden, J., Fiers, W. and Plaetnick, G. (1991) *Cell* **66**, 1175–1184
- Kishimoto, T., Akira, S. and Taga, T. (1992) *Science* **258**, 593–597
- Baumann, H., Ziegler, S. F., Mosley, B., Morella, K. K., Pajovic, S. and Gearing, D. P. (1993) *J. Biol. Chem.* **268**, 8414–8417
- Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N. Y. and Yancopoulos, G. D. (1993) *Science* **260**, 1805–1808
- Zurawski, S. M., Vega, F., Jr., Huyghe, B. and Zurawski, G. (1993) *EMBO J.* **12**, 2663–2670
- Kishimoto, T., Taga, T. and Akira, S. (1994) *Cell* **76**, 253–262
- Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T. and Kishimoto, T. (1993) *Science* **260**, 1808–1810
- Miyajima, A., Kitamura, T., Harada, N., Yokota, T. and Arai, K. (1992) *Annu. Rev. Immunol.* **10**, 295–331
- Stahl, N. and Yancopoulos, G. D. (1993) *Cell* **74**, 587–590
- Argetsinger, L. S., Campbell, G. S., Yang, X., Withuhn, B. A., Silvennoinen, O., Ihle, J. N. and Carter-Su, C. (1993) *Cell* **74**, 237–244
- Withuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. and Ihle, J. N. (1993) *Cell* **74**, 227–236
- Lerner, A. C., David, M., Feldman, G. M., Igarashi, K., Hackett, R. H., Webb, D. S. A., Sweitzer, S. M., Petricoin, E. F., III and Finbloom, D. (1993) *Science* **261**, 1730–1733
- Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. I., Davis, S., Withuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N. and Yancopoulos, G. D. (1994) *Science* **263**, 92–95
- Schindler, C., Kashleva, H., Pernis, A., Pine, R. and Rothman, P. (1994) *EMBO J.* **13**, 1350–1356
- Darnell, J. E., Jr., Kerr, I. M. and Stark, G. R. (1994) *Science* **264**, 1415–1420
- Lamb, P., Kessler, L. V., Suto, C., Levy, D. E., Seidel, H. M., Stein, R. B. and Rosen, J. (1994) *Blood* **83**, 2063–2071
- Taniguchi, T. and Minami, Y. (1993) *Cell* **73**, 5–8
- Minami, Y., Kono, T., Miyazaki, T. and Taniguchi, T. (1993) *Annu. Rev. Immunol.* **11**, 245–267
- Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. and Sugamura, K. (1992) *Science* **257**, 379–382
- Asao, H., Takeshita, T., Ishii, N., Kumaki, S., Nakamura, M. and Sugamura, K. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4127–4131
- Nakamura, Y., Russel, S. M., Mess, S. A., Friedmann, M., Erdos, M., Francois, C., Jacques, Y., Adelstein, S. and Leonard, W. J. (1994) *Nature (London)* **369**, 330–333
- Nelson, B. H., Lord, J. D. and Greenberg, P. D. (1994) *Nature (London)* **369**, 333–336
- Noguchi, M., Huafang, Y., Rosenblatt, H. M., Filipovich, A. H., Adelstein, S., Modi, W. S., McBride, O. W. and Leonard, W. J. (1993) *Cell* **73**, 147–157
- Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K. and Sugamura, K. (1993) *Science* **262**, 1874–1877
- Noguchi, M., Nakamura, Y., Russel, S. M., Ziegler, S. F., Tsang, M., Cao, X. and Leonard, W. J. (1993) *Science* **262**, 1877–1880
- Russel, S. M., Keegan, A. D., Harada, N., Nakamura, Y., Noguchi, M., Leland, P., Friedmann, M. C., Miyajima, A., Puri, R. K., Paul, W. E. and Leonard, W. J. (1993) *Science* **262**, 1880–1883
- Kondo, M., Takeshita, T., Ishii, N., Higuchi, M., Nakamura, M., Sudo, T., Nishikawa, S. I. and Sugamura, K. (1994) *Science* **263**, 1453–1454
- Rambaldi, A., Bettoni, S., Tosi, S., Giudici, G., Schirò, R., Borleri, G. M., Abbate, M., Chiaffarino, F., Colotta, F., Barbui, T. and Biondi, A. (1993) *Blood* **81**, 1376–1383
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Voss, S. D., Hong, R. and Sondel, P. M. (1994) *Blood* **83**, 626–635
- Bosco, M. C., Espinosa-Delgado, I., Schwabe, M., Gusella, G. L., Longo, D. L., Sugamura, K. and Varesio, L. (1994) *Blood* **83**, 2995–3002
- Bosco, M. C., Espinosa-Delgado, I., Schwabe, M., Russell, S. M., Leonard, W. J., Longo, D. L., Sugamura, K. and Varesio, L. (1994) *Blood* **83**, 3462–3467
- Nakarai, T., Robertson, M. J., Streuli, M., Wu, Z., Ciardelli, T. L., Smith, K. A. and Ritz, J. (1994) *J. Exp. Med.* **180**, 241–251
- Koeffler, H. P. and Golde, D. W. (1978) *Science* **200**, 1153
- Collins, S. J., Gallo, R. C. and Gallagher, R. E. (1977) *Nature (London)* **270**, 347–349
- Lozzio, C. B. and Lozzio, B. B. (1975) *Blood* **45**, 321–334
- Lübbert, M., Hermann, F. and Koeffler, H. P. (1990) *Blood* **77**, 909–924
- Satterthwaite, A. B., Borson, R. and Tenen, D. G. (1990) *Blood* **75**, 2299–2304
- Sagoh, T. and Yamada, M. (1988) *Arch. Biochem. Biophys.* **262**, 599–604
- Andersson, L. C., Jokinen, M. and Gahmberg, C. G. (1979) *Nature (London)* **278**, 364–365
- Rovera, G., O'Brien, T. G. and Diamond, L. (1979) *Science* **204**, 868–870
- Breitman, T. R., Selonick, S. E. and Collins, S. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2936–2940
- Kanakra, Y., Sugahara, H., Mitsui, H., Ikeda, H., Furitsu, T., Yagura, H., Kitayama, H., Kanayama, Y. and Matsuzawa, Y. (1993) *Cancer Res.* **53**, 675–680
- Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y.-Q., Blake, T. B., Shibuya, K., Ortaldo, J. R., McVicar, D. W. and O'Shea, J. J. (1994) *Nature (London)* **370**, 151–153
- Withuhn, B. A., Silvennoinen, O., Miura, O., Lai, K. S., Cwik, C., Liu, E. T. and Ihle, J. N. (1994) *Nature (London)* **370**, 153–157
- Broxmeyer, H. E., Lu, L., Cooper, S., Tushinski, R., Mochizuki, D., Rubin, B. Y., Gillis, S. and Williams, D. E. (1988) *J. Immunol.* **141**, 3852–3862
- Rennick, D., Jackson, J., Yang, G., Wideman, J., Lee, F. and Hudack, S. (1989) *Blood* **73**, 1828–1835
- Jacobsen, F. W., Rusten, L. S. and Jacobsen, S. E. W. (1994) *Blood* **84**, 775–779
- Fahlman, C., Blomhof, H. K., Veiby, O. P., McNiece, I. K. and Jacobsen, S. E. W. (1994) *Blood* **84**, 1450–1456
- Giri, J. G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K. H., Kumaki, S., Namen, A., Cosman, D. and Anderson, D. M. (1994) *EMBO J.* **13**, 2822–2830
- Russel, S. M., Johnston, J. A., Noguchi, M., Kawamura, M., Bacon, C. M., Friedmann, M., Berg, M., McVicar, D., Withuhn, B. A., Silvennoinen, O., Goldman, A. S., Schmalstieg, F. C., Ihle, J. N., O'Shea, J. J. and Leonard, W. J. (1994) *Science* **266**, 1042–1045