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Human hematopoietic cells express two forms of the cytokine receptor common γ-chain (γc)

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ABSTRACT

Recent studies have revealed that the γ-chain of the IL-2 receptor is shared by the receptors for IL-4, IL-7, IL-9, IL-13, and IL-15, and it is therefore also referred to as the common γ-chain (γc). Mutations of γc result in X-linked severe combined immunodeficiency syndrome in humans, indicating that γc is essential for normal development and function of the immune system. We demonstrate that human hematopoietic cells express two γc transcripts differing in their carboxyl terminal coding region. One transcript is the previously reported sequence (γc-long), whereas the newly identified sequence exhibits a deletion of 72 nucleotides close to the 3'-end of the open reading frame (γc-short). This alteration predicts a loss of 24 amino acids

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Abbreviations: γc, cytokine receptor common γ-chain; RT-PCR, reversely transcribed RNA amplified by the polymerase chain reaction; IL, interleukin; IL-2R, interleukin 2 receptor; PBM, peripheral blood mononuclear cells; PHA, phytohemagglutinin.

including a conserved tyrosine residue which is shared by several members of the cytokine receptor family. The presence of these two distinct forms of γc transcripts was demonstrated by sequencing of reversely transcribed and polymerase chain reaction (RT-PCR) amplified mRNA, restriction digestion of the RT-PCR products, RNAse protection, and Northern blotting from human cell lines and human peripheral blood lymphocytes. Furthermore, the two variants were present in peripheral blood lymphocytes from both female and male donors, which rules out allelic variants since γc is a single copy gene located on the X chromosome. A truncation mutant at a site near the observed changes in γc-short has been reported by others to alter biochemical events activated by cytokines. This combined with the loss of a potential SH2 "docking" site in γc-short suggests that γc-long and γc-short may link to different signaling pathways and may play an important role in determining the cellular response to IL-2, IL-4, IL-7, IL-9, IL-13, IL-15.

Key words: *IL-2 receptor γ-chain; cytokine receptor common γ-chain; IL-2 receptor; cytokine receptors; RNA splicing.*

INTRODUCTION

Interleukin-2 (IL-2) is a T cell growth factor produced by activated T lymphocytes which, upon binding to the cell surface IL-2 receptor (IL-2R), regulates the growth and differentiation of T cells, B cells and natural killer cells[1-3]. The IL-2R complex consists of at least 3 transmembrane polypeptide chains: a 55 Kd α chain, a 72 Kd β chain, and a 64 Kd γ chain[4, 5]. IL-2Rα appears to be specific for IL-2 and is sufficient by itself to form a nonfunctional low affinity receptor. The additional presence of IL-2Rβ and IL-2Rγ are required for both intermediate and high affinity binding as well as for IL-2 initiated signal transduction[6]. IL-2Rβ and IL-2Rγ are shared by the IL-15 receptor which also likely has a separate IL-15 specific α chain[7]. In addition to the IL-2 and IL-15 receptor complexes, the γ-chain appears to be shared by receptors for IL-4, IL-7, IL-9, and IL-13[8-10]. Thus the IL-2Rγ has been redesignated as common γ-chain (γc). Germ-line mutation of γc resulting in a non-functioning γc chain underlies X-linked severe combined immunodeficiency in humans[11] and animals[12, 13]. The combinatorial interaction of γc with ligand-specific receptor chains potentially serves as a mean for transmitting different signals

to hematopoietic cells.

The mechanism by which the IL-2 receptor complex signals cells to initiate cell proliferation or differentiation is not completely characterized, particularly since the intracellular domains of the members of the receptor complex do not contain a defined enzymatic activity[14]. However, as activation of the IL-2R complex leads to a rapid increase in tyrosine phosphorylation of intracellular substrates including the β and γc chains of the complex[3, 15, 16], the IL-2R likely signals through the association with intracellular kinases. Supporting this mechanism, IL-2Rβ has been demonstrated to directly associate with LCK and JAK1, whereas IL-2Rγ chain associates with JAK3 also known as L-JAK[9, 17]. Both the IL-2Rβ and γc contain intracellular domains compatible with Box1 and Box2 domains which have been demonstrated to be essential for signal transduction for members of the cytokine receptor family[18, 19]. These domains likely play a role in the interaction of the IL-2R complex with intracellular signaling molecules including the JAK kinases[9, 17]. Furthermore, phosphorylation of the IL-2R complex generates "docking" sites for other SH2-containing intracellular signaling molecules such as phosphatidylinositol 3' kinase and SHC[19]. How these immediate early signals link to downstream events such as activation of RAS, P70S6 kinase, MAP kinases and eventually to cell proliferation remains to be delineated.

Here we report the identification of two forms of γc RNA. γc-long is identical to the sequence reported by Takeshita et al.[4] (Genbank, D11086). γc-short represents a deletion of 72 nucleotides close to the 3'-end of the open reading frame predicting a deletion of 24 amino acids. These two forms do not appear to arise from the utilization of conventional or known unconventional splice sites, rather the shorter form appears to arrise from splicing at two C-rich regions. As the alternate form of the γc includes Box1 and Box2 but lacks a conserved tyrosine at the carboxy terminus and is similar to an induced mutant which alters signaling[20], γc-long and γc-short may transmit different signals.

MATERIALS AND METHODS

Cells

The human acute leukemia T cell line, Jurkat[21]; the HLTV1 transformed human T cell line, S1T[22]; the human myelogenous leukemia line, KG1A[23]; the human leukemic monoblast cell line, U937[24]; and the human ovarian cancer cell line, HEY[25]; were maintained in RPMI 1640 (GIBCO, Grand Island, N.Y) supplemented with 10% fetal bovine serum, 2 m*M* glutamine, 50 n*M* 2-mercaptoethanol, and 10 μ*M* gentamycin (GIBCO). Human peripheral blood mononuclear cells (PBM) were isolated over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) from freshly drawn blood of normal human volunteers. Lymphocyte blasts were generated by stimulating PBM with 10 μg/ml phytohemagglutinin (PHA) in the presence of 100 U/ml IL-2 for 72 h.

RNA isolation

Total RNA was isolated by the guanidium isothiocyanate method as described[26]. Briefly, cells were pelleted and lysed in 4 *M* guanidine isothiocyanate, 25 m*M* sodium citrate, pH 7.0, 0.5%

sodium lauryl sarcosinate, and 0.1 *M* 2-mercaptoethanol. The lysates were adjusted to a concentration of 0.2 *M* sodium acetate, extracted with phenol/chloroform/isoamyl alcohol (25:24:1), then precipitated with an equal volume of isopropanol at -70℃. After washing with 75% ethanol, the RNA pellets were air dried and dissolved in DEPC-treated water.

Cloning of the RT-PCR product

Total cellular RNA was reverse-transcribed into cDNA using random hexamers and MLTV reverse transcriptase (Perkin Elmer). The resulting cDNA was then amplified by PCR with a pair of primers spanning the whole γc open reading frame of the sequence reported by Takeshita et al.[4] (Genbank, Dl1086). Primers: A= 5'-AACCAATCGATGAAGACAAGCGCCATGT-3'; and B= 5'-TGGTTAAGCTTCTACAGGACCCTGGGG-3'. The PCR product was cloned into the TA vector (Invitrogen, San Diego, CA), or into pCMV4 using restriction sites (ClaI/HindIII) included in the primers and then sequenced with a Sequenase kit (US Biochemicals, Cleveland, OH) using α-^{35}S-dATP as label.

Genomic DNA sequencing

Fragments of the genomic DNA spanning the site of deletion of the 72 amino acids in γc-short were obtained from the genomic DNA of two males and two females by PCR amplification. The PCR primers were 5'- CCAGACTACAGTGAACGACTC-3'; 5'- TTTCAGGCTTTAGGGTGT- 3'. The amplified PCR products were purified with Geneclean II (Biolabs), and then used as templates for direct sequencing using the CircumVent kit (Biolabs). Both procedures were performed as recommended by the manufacturers. The PCR primers were used as sequencing primers.

In vitro transcription

Both forms of γc were cloned into the Bluescript vector (Stratagene, La Jolla, CA) at the KpnI-SmaI or HindIII-ClaI sites of the polylinker. The vectors were linearized with NotI for γc-long and XhoI for γc-short. *In vitro* transcription was carried out by employing an *in vitro* transcription kit with T7 RNA polymerase (Stratagene). The *in vitro* transcribed products were purified and subjected to RT-PCR as described above.

RNase protection

RNase protection was performed using a ribonuclease protection assay kit (Ambion, Austin, TX) with α-^{32}p-UTP as label according to the protocol recommended by the manufacturer. The RNA probes were transcribed with T3 RNA polymerase from Bluescript vectors containing the desired sequences linearized with NcoI. The protected fragments of the RNA probes were separated on a 6% polyacrylamide gel and visualized by autoradiography.

RESULTS

Northern blot analysis of the expression of γc transcripts in SIT cells, which are IL-2 responsive, revealed a doublet with a size of approximately 1.8 kb (data not shown), indicating that there may be two forms of the γc transcript. The presence of the doublet was confirmed by densitometrical analysis which showed two peaks on a histogram (data not presented). The hybridization of the γc probe was specific as bands were absent from RNA isolated from HEY ovarian cancer cells.

To determine whether the two mRNA species observed on Northern blotting represented isoforms of γc, RT-PCR was performed with primers flanking the coding region. RT-PCR revealed two separate PCR fragments in several different hematopoi-

etic cell lines (Fig 1), but not in non-hematopoietic lineage cells such as HEY (not presented). The upper and lower bands of RT-PCR products were isolated and cloned into the pCMV4 vector utilizing ClaI and HindIII sites introduced into the primers. Sequencing of both strands demonstrated that the longer form

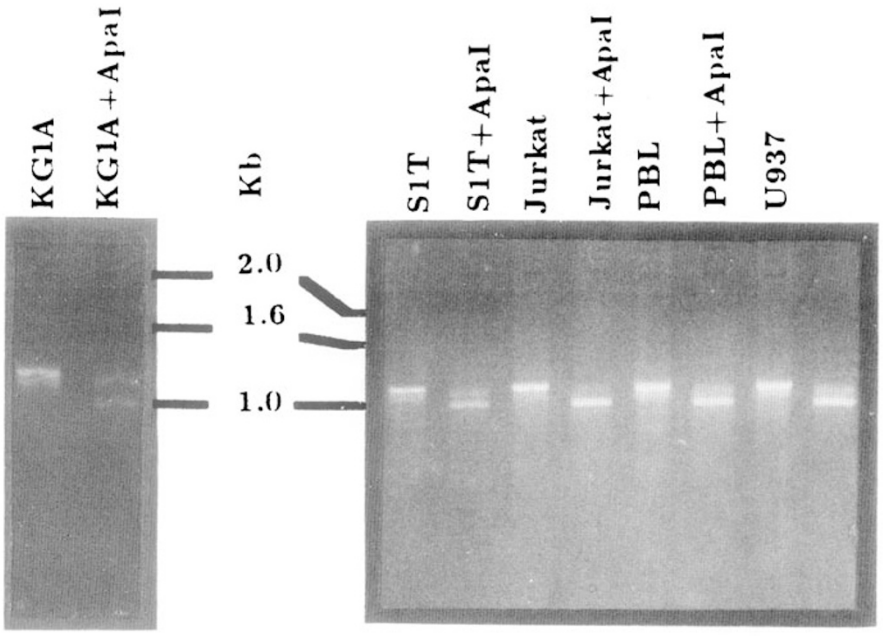

Fig 1. RT-PCR amplification of γc in different hematopoietic cells.
Total RNA (1 μg) from different cells was reverse transcribed and subjected to the polymerase chain reaction with a pair of primers spanning the open reading frame (5'-AACCAATCGATGAAGACAAGCGCCATGT-3'; 5'-TGGTTAAGCTTCTACAGGACCCTGGGG-3'). The RT-PCR products were analyzed by agarose gel electrophoresis directly and following digestion with ApaI. The PCR products appear as doublets and only the γc-long product contains an ApaI restriction site.

(γc-long) represented the previously reported sequence[4]. The shorter form (γc-short) shares the same sequence as the long, but lacks a 72 nucleotide sequence near the 3' end of the coding region (Fig 2). As there are multiple C's at both ends of the deleted fragment, the exact site(s) of the deletion can not be determined from the sequence. Nevertheless, the 72 nucleotide deletion occurs 26 to 30 nucleotides 5' to the stop codon. The γc-short cDNA predicts a protein sequence that is 24 amino acids shorter than the γc-long protein.

We have sequenced RT-PCR products from freshly isolated peripheral blood mononuclear cells from four individuals (two males and two females) as well as from two independently produced RNA samples from Jurkat T cells. In all cases,

sequencing of the intracellular domain of γc demonstrated the presence of both the long and short forms of γc (data not shown). Since the γc gene is a single copy gene located on the X-chromosome[10], the identification of both γc-long and γc-short in RNA from PBL of both male and female human subjects eliminates that possibility that γc-long and γc-short are produced by different alleles. In addition, we have examined the genomic DNA sequence spanning the deletion in γc-short by PCR amplification followed by direct sequencing of the PCR products and did not detect any difference from the published cDNA sequence or published intron-exon organization[10] that would suggest that an intron is present at this site in the genomic DNA (data not shown).

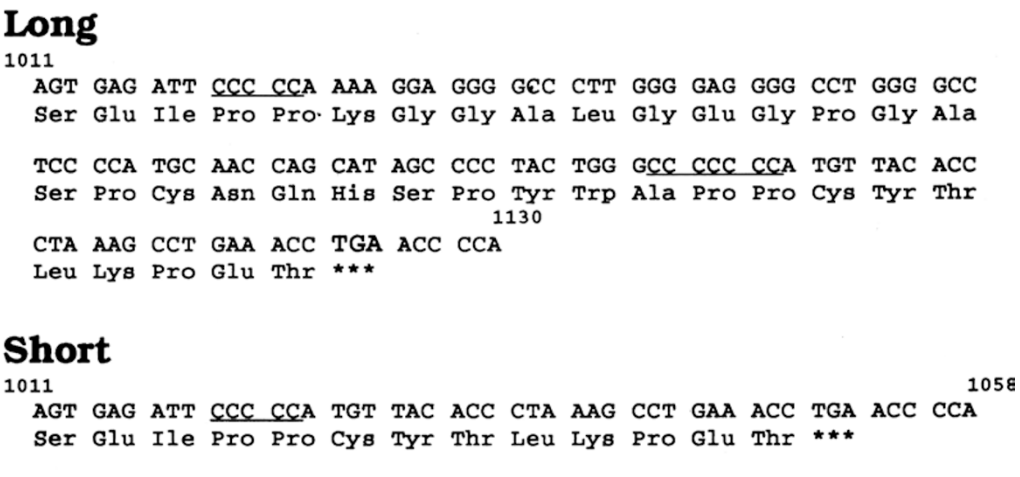

Fig 2. Comparison of the sequences of γc-long and γc-short.
RNA from Jurkat or human PBM was reverse transcribed and amplified by RT-PCR. The RT-PCR products were cloned and sequenced. γc-long has the same sequence as identified by Takeshita et al.[4] (Genbank, D11086). The cDNA sequence and the predicted amino acid sequence are shown from nucleotide 1011 to 1130 for γc-long and from 1011 to 1058 for γc-short (the nucleotide numbers correspond to Genbank D11086). The potential site of splicing is underlined at cytosine stretches and the translation stop codon is indicated by triple stars.

Examination of the γc-short cDNA sequence demonstrates the loss of two ApaI restriction sites allowing assessment of the presence of γc -short by RT-PCR followed by restriction digestion. As indicated in Fig 1, the upper of the two bands on RT-PCR, but not the lower band, was sensitive to the action of ApaI in agreement with the loss of ApaI sites in γc-short. Both bands were absent from HEY ovarian cancer cells and a γc-negative human T cell leukemia line, ED515-[27].

To confirm that γc-short did not result as a consequence of two different fragments being produced during the RT-PCR amplification of a single mRNA for the full length form of γc, we performed RT-PCR with *in vitro* produced γc-long and γc-short RNA. Bluescript vectors engineered to contain both forms of γc were in

vitro transcribed to produce a γ c-long and a γ c-short RNA. RT-PCR was then performed on the *in vitro* transcribed RNA from either γ c-long, γ c-short, or a mixture of both transcripts and this gave rise to long, short, and a mixture of long and short product respectively (Fig 3A). When the RT-PCR products were digested with NcoI which digests a site 200 bp upstream of the deletion site, we observed an invariant 831 bp fragment and smaller bands of 365 and 293 bp corresponding to γ c-long and γ c-short respectively, (Fig 3B). Thus the γ c-short product does not appear to result from RT-PCR amplification of a single γ c-long transcript.

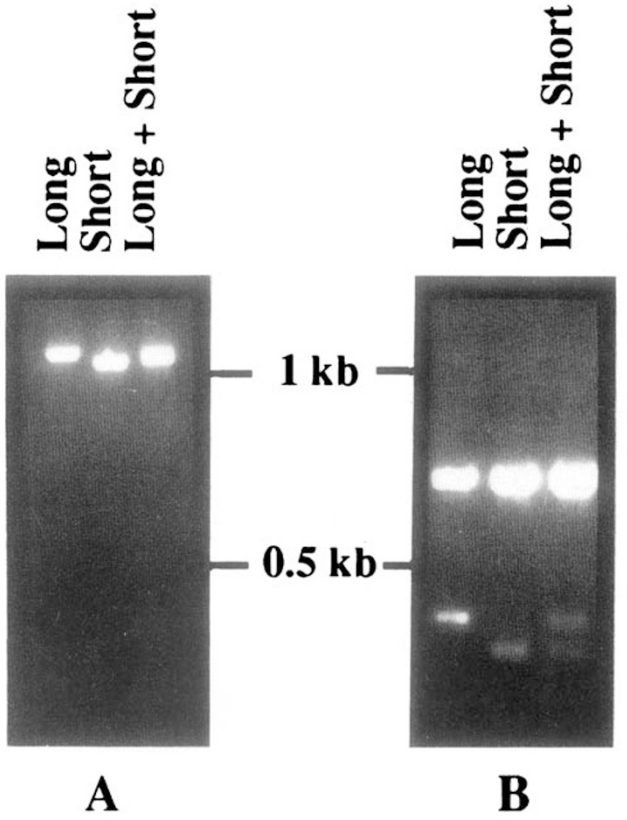

Fig 3. Fidelity of the RT-PCR procedure. The cDNA of γ c-long and γ c-short were cloned into Bluescript vector and transcribed with T7 polymerase. The *in vitro* transcribed RNA was subjected to RT-PCR as described in Fig 1. The RT-PCR product was analyzed on an agarose gel before (A) and after digestion with NcoI (B).

Both the cloning strategy and the RT-PCR analysis are dependent on the fidelity of the RT and PCR reactions. To determine whether γ c-long and γ c-short were present by an approach which is not dependent on RT or PCR, we employed an

RNase protection assay. Probe I was generated using T3 RNA polymerase from NcoI linearized Bluescript vector containing γc-long, which gave a probe of 393 bases. This probe will protect 343 bases of γc-long and 204 bases of γc-short. The 284 base- probe II was transcribed from NcoI-linearized Bluescript containing γc-short. Probe II will protect 174 bases of γc-long and 234 bases of γc-short. RNase protection analysis of RNA derived from PBL blasts and the human KG1A myelogenous leukemia cell line with both probes demonstrated the presence of the predicted bands. No bands were protected by RNA isolated from the human ovarian cancer cell line HEY (Fig 4). Therefore, we conclude that there are two forms of γc transcripts in human cells.

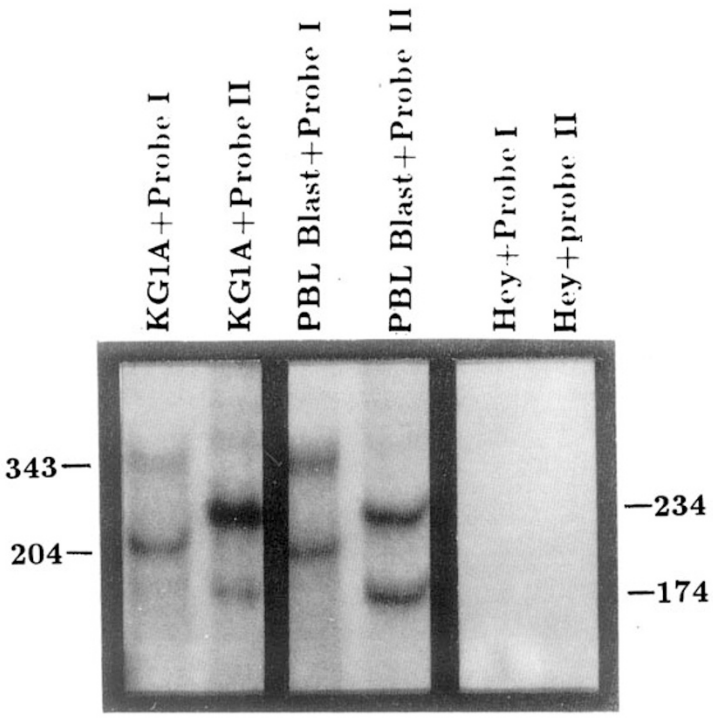

Fig 4. Ribonuclease protection assay. RNA was isolated from human KG1A myelogenous leukemia cells or PHA-stimulated human peripheral lymphocyte blasts. Probe I was generated using T3 RNA polymerase from NcoI-linearized Bluescript vector containing γc-long, which gave a probe of 393 bases spanning the 72 uucleotide deletion. Probe II was transcribed from NcoI-linearized Bluescript containing a fragment of γc-short which gave a probe of 284 bases. RNA from the human ovarian cancer cell HEY was included as a negative control.

DISCUSSION

We have demonstrated that human hematopoietic cells transcribc two forms of γc.

At present we cannot explain how these two forms arise. The published intron/exon organization of γc[10] does not show the presence of an intron which could explain the generation of two forms of γc. Genomic sequencing of the γc gene in our laboratory failed to reveal the presence of introns at the deletion sites and further did not identify consensus or known nonconsensus RNA splicing sites[28]. As there is a single copy of γc located on the X chromosome[10], the identification of γc-long and γc-short in RNA isolated from PBM from two males indicates that γc-long and γc-short do not arise from allelic variants.

The importance of tyrosine phosphorylation in creating intracellular docking sites for SH2 containing signaling molecules[14] combined with the observation that γc is highly tyrosine phosphorylated following IL2-treatment of cells[29], suggests that γc-long and γc-short may serve different functions and link to different signaling pathways. The deletion of the 72 nucleotides not only results in the loss of a conserved tyrosine residue found in the cytokine receptor family but occurs immediately downstream of the putative Box2 region of the cytokine receptor family which is required for normal function. Indeed, truncation of γc just downstream of Box2 (2 amino acids upstream/downstream of the deletion in γc-short) activates a signaling pathway different from that transduced by γc-long. The truncation mutant, expressed in L929 cells, retained the ability to increase tyrosine phosphorylation and to induce the expression of c-myc, but lost the ability to induce the expression of the c-fos and c-jun proto-oncogenes[6]. This altered effect on proto-oncogene expression could affect cell survival, proliferation, or differentiation. The function of the alternate forms of γc could be similar to the reported forms of the erythropoietin receptor, which are differentially expressed during ontogeny and, although somewhat controversial, have been implicated in differential regulation of programmed cell death of erythroid cells[5]. Thus the protein product of γc-short may alter signal transduction induced by ligation of the receptors for cytokines including IL-2, IL-4, IL-7, IL-9 IL-13 and IL-15.

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