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Natural Splicing of Exon 2 of Human Interleukin-15 Receptor α-Chain mRNA Results in a Shortened Form with a Distinct Pattern of Expression*

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We report the existence of eight different interleukin-15 receptor α-chain (IL-15Rα) transcripts resulting from exon-splicing mechanisms within the IL-15Rα gene. Two main classes of transcripts can be distinguished that do or do not (Δ2 isoforms) contain the exon 2-coding sequence. Both classes were expressed in numerous cell lines and tissues (including peripheral blood lymphocytes) at comparable levels and could be transcribed in COS-7 cells, and the proteins were expressed at the cell surface. Both receptor forms displayed numerous glycosylation states, reflecting differential usage of a single *N*-glycosylation site as well as extensive *O*-glycosylations. Whereas IL-15Rα bound IL-15 with high affinity, Δ2IL-15Rα was unable to bind IL-15, thus revealing the indispensable role of the exon 2-encoded domain in cytokine binding. A large proportion of IL-15Rα was expressed at the nuclear membrane with some intranuclear localization, supporting a potential direct action of the IL-15·IL-15Rα complex at the nuclear level. In sharp contrast, Δ2IL-15Rα was found only in the non-nuclear membrane compartments, indicating that the exon 2-encoded domain (which is shown to contain a potential nuclear localization signal) plays an important role in receptor post-translational routing. Together, our data indicate that exon 2 splicing of human IL-15Rα is a natural process that might play regulatory roles at different levels.

Interleukin (IL)[1]-15 is a cytokine that was discovered through its capacity to replace IL-2 in supporting the growth of the murine IL-2-dependent CTLL cell line (1, 2). Both cytokines belong to the short α-helical cytokine family (3). Unlike IL-2 mRNA, whose expression is restricted to activated T cells, IL-15 mRNA is expressed by a variety of tissues and cell types, including monocyte/macrophages, kidney epithelial cells, keratinocytes, fibroblasts, nerve cells, placenta, skeletal muscle, and heart (1, 4, 5). IL-15 can replace IL-2 in most of its activities in the lymphoid system, including T cell chemotaxis (6), induction of proliferation and cytotoxicity of activated T cells (1) and natural killer cells (2, 7), and costimulation of B cell proliferation and immunoglobulin synthesis (8). This redundancy was shown to be due in part to the sharing, within their high affinity receptors, of common transducing subunits, the IL-2 receptor (IL-2R) β- and γ-chains (9). Cytokine specificity is conferred by short receptor chains, IL-2Rα and IL-15Rα (10–13). Unlike IL-2Rβ and IL-2Rγ, these two α-chains are not members of the hematopoietin receptor family, but instead contain, in their extracellular parts, structural domains previously defined as protein-binding motifs (called the "sushi domain"; also known as the glycoprotein-I motif or short consensus repeat) present in some complement and adhesion molecules (14). IL-2Rα contains two such domains, whereas IL-15Rα contains only one. In contrast to IL-2Rα, which alone binds IL-2 with low affinity (K_d = 10 nM) (15), IL-15Rα on its own binds IL-15 with high affinity (K_d = 10 pM) (13). IL-2Rβ and IL-2Rγ build up a functional receptor of intermediate affinity (K_d = 1 nM) competitively shared by both cytokines, and association of the α-chains leads to the formation of cytokine-specific functional high affinity ternary (αβγ) receptor complexes (K_d = 10 pM) (16, 17).

IL-15Rα mRNA are expressed in T cells, B cells, and macrophages as well as thymic and bone marrow stromal cell lines. Like IL-15 mRNA, IL-15Rα messages are also expressed in various nonimmune cell types and tissues, including liver, skeletal muscle, heart, and lung (12, 13), suggesting that the IL-15 system may operate at multiple levels within and beyond the immune system. Accordingly, IL-15 has already been described as an anabolic agent on skeletal muscle (18) and to stimulate intestinal cell proliferation (19). IL-15 also stimulates the proliferation of murine mast cell lines, but this effect is mediated through a novel, as yet unidentified, receptor not involving IL-15Rα or IL-2Rβ and IL-2Rγ (20).

Three isoforms of IL-15Rα mRNA have been described that result from alternative splicing of exon 3 and/or alternate usage of exon 7 or 7′ (12). These isoforms are equally capable of binding IL-15. In this paper, we show the existence of a novel type of IL-15Rα mRNA lacking exon 2. The IL-15-binding capacities, intracellular routing, and biochemical features of the IL-15Rα proteins encoded by these different transcripts in COS-7 cells are compared.

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[1] The abbreviations used are: IL, interleukin; IL-2Rα, IL-2Rβ, and IL-2Rγ, interleukin-2 receptor α-, β-, and γ-chains, respectively; IL-15Rα, interleukin-15 receptor α-chain; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); NLS, nuclear localization signal.

EXPERIMENTAL PROCEDURES

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was extracted from various human cell lines and tissues using guanidinium thiocyanate/phenol as described (21). Total RNAs from human liver, brain, and small intestine were purchased from CLONTECH (Basingstoke, United Kingdom). Reverse transcription and PCR amplifications were performed as described previously (22). For PCR, the cycling conditions were as follows: denaturation for 1 min at 94 °C; annealing for 1 min at 66 °C (E1/E7), 68 °C (E1/E7′), 52 °C (E4/p3BGH), 55 °C (β5′/β3′), or 60 °C (5E4.1/E7.2); and elongation for 1 min at 72 °C (30 cycles). The following primers were used: E1, 5′-AGTCCAGCGGTGTCCTGTGG; E7, 5′-TCATAGGTGGTGAGAGCAGT; E7′, 5′-TCAACAGACGCTTCCCACTG; E4, 5′-GAACTCACAGCATCCGCC; p3BGH, 5′-TAGAAGGCACAGTCGAGG; β5′ (sense), 5′-CGTGCTGCTGACCGAGGCC; β3′ (antisense), 5′-TTCGTGGATGCCACAGGAC; 5E4.1, 5′-GCAGCTTCATCTCCCAG; and E7.2, 5′-TAGGTGGTGAGAGC.

Molecular Constructs

The four products resulting from RT-PCR using primers E1 and E7 were ligated into the pNoTA/T7 plasmid, leading to pNo15R, pNo15RΔ3, pNo15RΔ2, and pNo15RΔ2Δ3. PCR amplification of pNo15R with primers 5E4.1 (located in exon 4) and E7.2 enabled the elimination of the receptor stop codon. The resulting PCR product was purified and inserted into pNoTA/T7, leading to pNo15R*. *Sma*I fragments from pNo15R, pNoΔ315R, pNoΔ215R, and pNoΔ2Δ315R were inserted into *Sma*I-digested and dephosphorylated pNo15R*. The *Xba*I fragments from each construct were then inserted into the pcDNA3.1/Myc-His vector (pcDNA3mh; Invitrogen, Groningen, Netherlands), yielding pcDNA-15Rmh, pcDNA-Δ315Rmh, pcDNA-Δ215Rmh, and pcDNA-Δ2Δ315Rmh, respectively.

Cell Culture and Transfections

The U937 human histiocytic lymphoma cell line, the SAOS-2 human osteogenic sarcoma cell line, and COS-7 monkey kidney epithelial cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 medium containing 10% fetal calf serum and 2 mM glutamine (U937 and SAOS-2 cells) or Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 2 mM glutamine (COS-7 cells). The Kit 225 human T lymphoma cell line (obtained from Dr. Doreen Cantrell, Imperial Cancer Research Fund, London, UK) was cultured in RPMI 1640 medium containing 6% fetal calf serum, 5 ng/ml IL-2, and 2 mM glutamine. COS-7 cells (1.5×10^6 cells/plate) were transfected using the DEAE-dextran/chloroquine method (23) with 10 μg of each pcDNA3.1/Myc-His construct. The same plasmids (30 μg) were used to transfect Kit 225 cells by electroporation (220 V, 960 microfarads), and transfectants were selected in culture medium containing 750 μg/ml Geneticin and 5 ng/ml IL-2.

Antibodies

Rabbit polyclonal anti-histidine antibody and monoclonal antibody to the c-Myc epitope were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-p300 monoclonal antibody was from Upstate Biotechnology, Inc. (Lake Placid, NY). Biotin-conjugated affinipure goat anti-mouse antibody was from Immunotech (Marseille, France). Cy5-conjugated streptavidin was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Fluorescein isothiocyanate-conjugated affinity-purified anti-rabbit F(ab′)2 fragments were from BioAtlantic (Nantes, France).

Confocal Immunofluorescence Microscopy

Confluent COS-7 cells collected 48 h after transfection were fixed with 50% methanol and 50% acetone at 4 °C for 10 min and incubated with anti-p300 monoclonal antibody (10 μg/ml) overnight at 4 °C, with biotin-conjugated goat anti-mouse antibody (1:100 dilution) for 1 h, and with Cy5-conjugated streptavidin for 30 min at room temperature. Cells were further incubated for 1 h at room temperature with anti-histidine antibody (1:200 dilution) and with fluorescein isothiocyanate-conjugated anti-rabbit F(ab′)2 for 30 min and analyzed by scanning confocal microscopy (Leica, Rueil-Malmaison, France).

Subcellular Fractionation and Biochemical Analysis

Enzymatic Treatments—COS-7 cells were lysed in radioimmune precipitation assay buffer (50 mM Tris buffer (pH 7.5) containing 100 mM NaCl, 0.25% sodium deoxycholate, 0.1% Nonidet P-40, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). After 30 min on ice, cell lysates were clarified by centrifugation (12,000 rpm, 20 min, 4 °C). Samples (30 μg of protein) were treated either with 250 milliunits of endoglycosidase F (Roche Molecular Biochemicals, Meylan, France) according to the manufacturer's instructions or with 3 mM neuraminidase (Roche Molecular Biochemicals) plus 2 milliunits of *O*-glycosidase (Roche Molecular Biochemicals) for 18 h at 37 °C in 50 mM sodium acetate buffer (pH 6) containing 9 mM $CaCl_2$ and 150 mM NaCl.

Non-nuclear Membrane Fractions—They were prepared as described previously (22). After depletion of nuclei, the supernatant was centrifuged at $100{,}000 \times g$ for 45 min at 4 °C to separate the cytosolic fraction (supernatant) from the membrane fraction (pellet).

Nuclear Membrane Fractions—Nuclei were purified according to a previously described procedure (24). The nuclear pellet was lysed for 20 min at 4 °C in 20 mM Hepes buffer (pH 7.9) containing 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. After centrifugation (12,000 rpm, 30 min, 4 °C), the pellet containing nuclear membranes was resuspended in 50 μl of the same buffer. The protein content of each subcellular fraction was determined by the BCA method (Pierce). For immunoblot analysis, fraction samples (30 μg) were resolved by SDS-polyacrylamide gel electrophoresis under reducing conditions, transferred to polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA), and probed with anti-histidine antibody (1:1000 dilution). Detection of the antibody-antigen complexes was achieved by enhanced chemiluminescence (ECL kit, Roche Molecular Biochemicals) and exposure to X-Omat films (Eastman Kodak Co.).

Cell-surface Labeling and Immunoprecipitation

COS-7 cells were iodinated with 100 μg of IODO-GEN (Pierce) and 0.4 mCi of [^{125}I]iodine for 20 min at room temperature. Cell lysates were made in radioimmune precipitation assay buffer, cleared by centrifugation, and immunoprecipitated overnight with anti-histidine antibody (10 μg) and protein A-coupled Sepharose beads (Amersham Pharmacia Biotech, Orsay, France). Protein A-bound material was eluted with 1× Laemmli sample buffer (Bio-Rad, Ivry sur Seine, France) at 95 °C for 5 min and resolved by SDS-polyacrylamide gel electrophoresis under reducing conditions. Iodinated proteins were detected by autoradiography (PhosphorImager, Molecular Dynamics, Inc., Sunnyvale, CA).

IL-15 Binding Assays

Human recombinant IL-15 (Peprotech, Rocky Hill, NJ) was iodinated as described (25) with a specific radioactivity of ~2000 cpm/fmol. Binding experiments were carried out for 75 min at 4 °C in phosphate-buffered saline/bovine serum albumin as described previously (26) using 10^6 cells/well and increasing concentrations of labeled IL-15 in a final volume of 50 μl. Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled cytokine. Regression analysis of the binding data was accomplished using a one-site equilibrium binding equation (Grafit, Erithacus Software, Staines, UK).

IL-15Rα and Δ2IL-15Rα solubilized from transfected COS-7 cells as described above were purified by nickel-nitrilotriacetic acid (QIAGEN Inc., Courtaboeuf, France) affinity chromatography (27) according to the manufacturer's instructions. The IL-15Rα content of each preparation was analyzed by a specific enzyme-linked immunosorbent assay as described (26), in which anti-Myc antibody (0.5 μg/well) was used as coating antibody and the combination of anti-histidine antibody (0.5 μg/well) plus peroxidase-labeled anti-rabbit IgG (1:5000 dilution; Bio-Atlantic) was used as indicator. Purified receptors were incubated for 2 h at 4 °C in phosphate-buffered saline/bovine serum albumin with labeled IL-15 (2 nM) and 8 μg/ml anti-histidine antibody in a final volume of 25 μl. Protein A-Sepharose beads were then added (25 μl) for 1 h at 4 °C. Centrifugation through a layer of dibutyl phthalate (87.5%) and paraffin oil (12.5%) allowed separation of protein A-bound immunocomplexes (pellet) from unbound reactants (supernatant), and the radioactivity associated which each fraction was determined.

Proliferation Assays

Transfected Kit 225 cells were washed twice to remove IL-2 or IL-15 and starved for 1 h in RPMI 1640 medium with 6% fetal calf serum and 2 mM glutamine. Cells were then plated (5×10^4 cells/well) and cultured for 48 h in culture medium containing 750 μg/ml Geneticin and supplemented with increasing concentrations of IL-2 or IL-15. They were pulsed for 6 h with 4 μCi of [^{3}H]thymidine and harvested onto Whatman filters. Cell-associated [^{3}H]thymidine was measured using a Microbeta counter (Wallack, Turku, Finland).

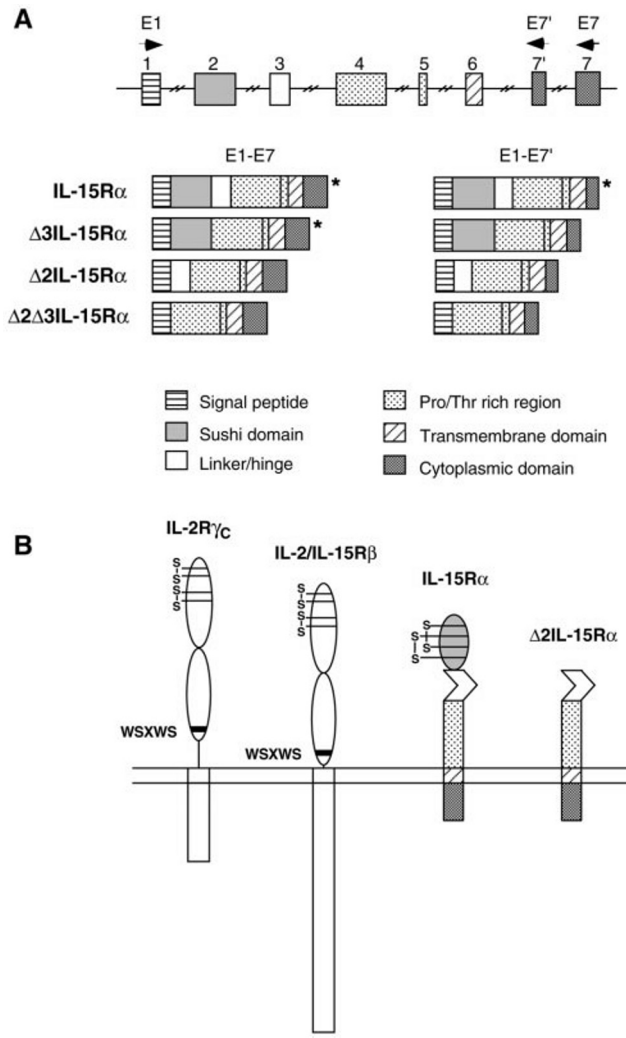

FIG. 1. **Schematic diagram of the human IL-15Rα gene, transcription products, and IL-15 receptor subunits.** *A*, the positions of oligonucleotide primers (E1, E7, and E7′) used for RT-PCR amplifications are shown by *arrows*. *Asterisks* indicate the three isoforms already described by Anderson *et al.* (12). *B*, the T cell high affinity IL-15 receptor includes IL-15Rα and the IL-2Rβ/IL-2Rγ transducing subunits. IL-2Rβ and IL-2Rγ each contain one hematopoietic receptor domain with two conserved disulfide bonds and a consensus WSXWS motif. In IL-15Rα is shown the exon 2-encoded sushi domain characterized by two interwoven disulfide bonds (C-1–C-3 and C-2–C-4). This domain is absent in Δ2IL-15Rα.

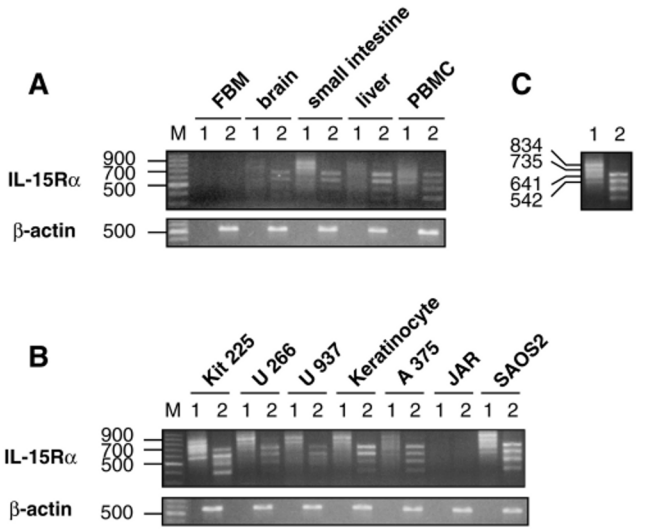

FIG. 2. **Detection of eight distinct IL-15Rα transcripts.** RT-PCR analysis of IL-15Rα was performed on different human tissues (*A*) and cell lines (*B*) as indicated using either primer pair E1/E7 (*lanes 1*) or primer pair E1/E7′ (*lanes 2*). *Lane M* shows the 100-bp DNA ladder. In *C* are shown the sizes (in base pairs) of the E1/E7 amplification products obtained from SAOS-2 cells. PCR amplification of β-actin served as internal control for the amount of RNA analyzed. *FBM*, fetal bone marrow; *PBMC*, peripheral blood mononuclear cells.

RESULTS

Characterization of New IL-15Rα Transcripts Lacking Exon 2—Different IL-15Rα isoforms using alternate C-terminal exons (exons 7 and 7′) and containing or not exon 3 have already been described (Fig. 1) (12). Using oligonucleotide primers corresponding to the N-terminal end of exon 1 (primer E1) and the C-terminal end of exon 7 (primer E7) or 7′ (primer E7′), RT-PCR amplifications were carried out on mRNAs prepared from different cell lines and tissues (Fig. 2). For each couple of primers (E1/E7 or E1/E7′), four amplification products were detected. The products from the E1/E7 amplification carried out on human peripheral blood mononuclear cells were cloned and sequenced. The two upper bands (834 and 735 bp) corresponded to full-length IL-15Rα and IL-15Rα lacking exon 3 (Δ3IL-15Rα), respectively. The two lower bands corresponded to two new mRNA species, one lacking exon 2 (641 bp; Δ2IL-15Rα) and one lacking both exons 2 and 3 (542 bp; Δ2Δ3IL-15Rα). Sequence analysis showed that exon 2 and/or exon 3 deletions did not change the reading frame (data not shown). The four products obtained from E1/E7′ amplification had sizes that were ~100 bp lower than those obtained from the E1/E7 amplification, suggesting that they corresponded to similar IL-15Rα isoforms (full-length, Δ3, Δ2, and Δ2Δ3), but using the alternate exon 7′, which is 100 bp shorter than exon 7 (12). The structures of the coding sequences of the eight mRNA isoforms are shown in Fig. 1.

These eight isoforms were detected in most of the cell lines and tissues examined (Fig. 2), except fetal bone marrow and the choriocarcinoma JAR cell line. The respective expression levels of the different isoforms varied from one cell/tissue type to another. Whereas, in most cases, the full-length and Δ3 isoforms appeared to predominate, the mRNAs corresponding to the Δ2 and Δ2Δ3 forms were as abundant as the others in a number of cell types (Kit 225 lymphoma cells and SAOS-2 osteocarcinoma cells) and even more in normal human peripheral blood mononuclear cells. The eight isoforms were also expressed by normal human T cell clones, either $CD4^+$ or $CD8^+$ (data not shown).

Biochemical Analysis of IL-15Rα and Δ2IL-15Rα Expressed in COS-7 Cells—The different IL-15Rα Myc/polyhistidine-tagged cDNA isoforms were transfected in COS-7 cells, and the expression products were analyzed by Western blotting on whole cell extracts (Fig. 3*A*). With the IL-15Rα cDNA (E7 isoform), two doublets of bands were revealed (best seen in *panel c*) at 59–62 and 39–41 kDa. Treatment with *N*-glycosidase induced a disappearance of the 62- and 41-kDa bands, leaving the 59- and 39-kDa bands unaffected, whereas treatment with *O*-glycosidase induced a disappearance of the 59- and 62-kDa bands, but not of the 39- and 41-kDa bands. Together, these results suggest that the 62- and 41-kDa bands are *N*-glycosylated forms of the 59- and 39-kDa bands, respectively, and that the 59–62-kDa bands are highly *O*-glycosylated forms of the 39–41-kDa bands.

In the case of Δ2IL-15Rα, similar results were observed. The lower band at 32.5 kDa corresponds to an unglycosylated form of the receptor. Its size is ~7 kDa lower than the corresponding 39-kDa band of IL-15Rα, in agreement with the deletion of the exon 2-encoded domain (12). The band at 35 kDa represents Δ2IL-15Rα with a carbohydrate linked to the single *N*-glycosy-

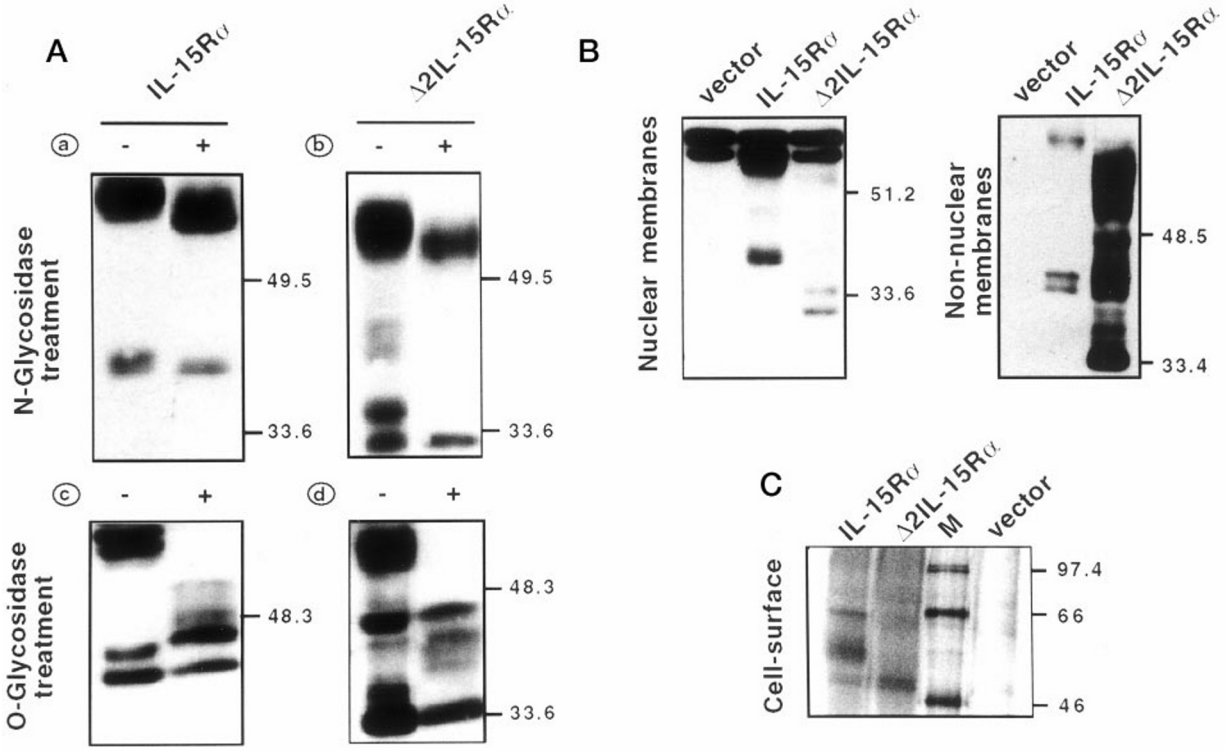

FIG. 3. **Biochemical analysis and subcellular localization of IL-15Rα and Δ2IL-15Rα in COS-7 cells.** *A*, Western blots with anti-histidine antibody on whole cell extracts. *Panels a* and *b*, IL-15Rα and Δ2IL-15Rα, respectively, before (−) and after (+) treatment with endoglycosidase F; *panels c* and *d*, IL-15Rα and Δ2IL-15Rα, respectively, before (−) and after (+) treatment with neuraminidase and *O*-glycosidase. Molecular mass standards are indicated in kilodaltons. *B*, Western blotting performed on nuclear and non-nuclear membrane fractions prepared from mock-transfected (*vector*) or IL-15Rα- or Δ2IL-15Rα-transfected cells. *C*, anti-histidine antibody- and protein A-driven immunoprecipitation of cell surface-iodinated IL-15Rα and Δ2IL-15Rα. *M*, molecular mass standards.

lation site present in IL-15Rα and retained in Δ2IL-15Rα, whereas bands at 54–57 kDa correspond to *O*-glycosylated forms of the 32.5–35-kDa bands. Some material at ~40 kDa was also observed with variable intensities and might represent intermediate states of glycosylation.

Subcellular Localization of the IL-15Rα Isoforms in COS-7 Cells—The localization of the receptor was first analyzed by confocal microscopy. As shown in Fig. 4, IL-15Rα (in *green*) was mainly found associated with the nuclear membrane, giving a ring-like pattern. By comparison, localization of the nuclear protein p300 (in *red*) was purely intranuclear. There was also some co-localization of IL-15Rα and p300 (in *yellow*), suggesting that part of the receptor was localized at the inner side of the nuclear membrane and/or in the intranuclear space. Δ2IL-15Rα behaved very differently, with an expression pattern suggesting localization within the endoplasmic reticulum, Golgi, and cytoplasmic vesicles, but not with the nuclear membrane or intranuclear space. Accordingly, it did not co-localize with p300. Other experiments (data not shown) indicated that exon 3 deletion had no influence on the localization of the receptor: Δ3IL-15Rα and Δ2Δ3IL-15Rα showed patterns similar to those of IL-15Rα and Δ2IL-15Rα, respectively.

To support these observations, Western blot analyses were carried out on subcellular fractions prepared by biochemical means (Fig. 3*B*). In the membrane fraction prepared from isolated nuclei, IL-15Rα was expressed at a much higher level than the Δ2 isoform. Conversely, Δ2IL-15Rα was predominant in the membrane fraction prepared after depletion of nuclei (non-nuclear membrane fraction), and in comparison, IL-15Rα was expressed at a much lower level in that fraction. Both unglycosylated and glycosylated forms of the receptors were observed. Examination of the cytosolic extracts did not show detectable expression of either receptor form (data not shown).

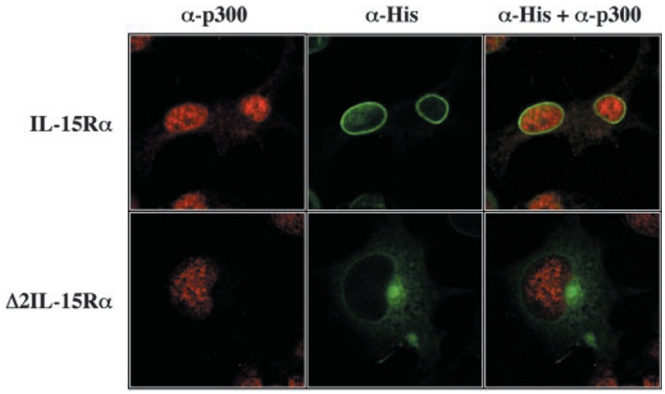

FIG. 4. **Confocal microscopic analysis of intracellular IL-15Rα and Δ2IL-15Rα localization in COS-7 cells.** Transfected cells were labeled with anti-p300 antibody (Cy5; *red*) and anti-histidine antibody (fluorescein isothiocyanate; *green*). *Red* and *green* fluorescence signals are shown separately or together as indicated. *Yellow* (*upper right*) indicates co-localization of the two antibodies.

Confocal microscopy did not reveal detectable expression of IL-15Rα or Δ2IL-15Rα at the plasma membrane. However, this could be due to low cell-surface receptor density as often observed with cytokine receptors. To analyze this point further, cells were externally labeled with [^{125}I]iodine before lysis and immunoprecipitation with anti-histidine antibody (Fig. 3*C*). Specific bands at 62 and 53 kDa were precipitated from IL-15Rα-transfected cells and a specific band at 48 kDa from Δ2IL-15Rα-transfected cells, indicating that both receptor isoforms are routed to the plasma membrane and exposed at the cell surface as glycosylated proteins.

Deletion of Exon 2 Results in a Loss of IL-15 Binding—To

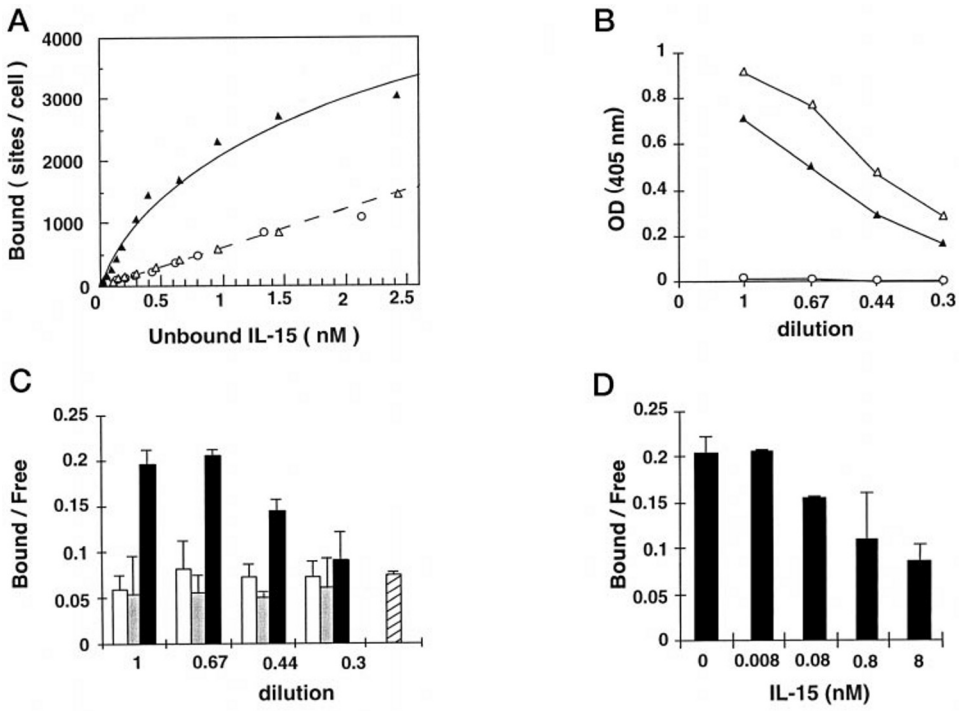

FIG. 5. **IL-15-binding capacities of IL-15Rα and Δ2IL-15Rα isoforms.** *A*, transfected cells (as indicated) were equilibrated with increasing amounts of ^{125}I-labeled IL-15, and cell-bound and unbound fractions were determined. Nonspecific binding (*dashed line*) was measured in the presence of a 100-fold excess of unlabeled IL-15. *B*, the purified receptor fractions were compared at various dilutions as indicated in an enzyme-linked immunosorbent assay using anti-Myc antibody as coating antibody and anti-histidine antibody as tracer antibody. *C*, the same fractions were analyzed for their ability to bind ^{125}I-labeled IL-15 (2 nM) using an anti-histidine antibody/protein A precipitation assay. Nonspecific immunoprecipitation was evaluated by including a 100-fold excess of unlabeled cytokine. An irrelevant rabbit antibody used instead of anti-histidine antibody served as a negative control. *D*, shown is the inhibition of the binding of ^{125}I-labeled IL-15 (2 nM) to IL-15Rα by increasing concentrations of unlabeled IL-15.

evaluate the function of the sushi domain encoded by exon 2, we first analyzed the binding of radioiodinated IL-15 to transfected COS-7 cells (Fig. 5*A*). Mock-transfected COS-7 cells did not bind IL-15, whereas cells transfected with IL-15Rα expressed ~1200 high affinity receptors (K_d = 60 pM). COS-7 cells transfected with the Δ2 isoform did not show any detectable specific IL-15 binding. As far as both receptors were expressed at the cell surface (Fig. 3*C*), these results suggested a loss of IL-15-binding capacity as a result of exon 2 deletion. To rule out the possibility that a low expression level of Δ2IL-15Rα as compared with that of IL-15Rα at the cell surface could account for the absence of detectable cytokine binding, IL-15Rα and Δ2IL-15Rα were solubilized from transfected COS-7 cells and purified by nickel affinity chromatography, and their IL-15-binding capacities were assessed. An enzyme-linked immunosorbent assay using anti-Myc antibody as coating antibody and anti-histidine antibody as tracer antibody showed similar receptor contents for the two preparations (Fig. 5*B*). Immunoprecipitation of the iodinated IL-15·receptor complex with anti-histidine antibody and protein A (Fig. 5*C*) revealed dose-dependent binding of IL-15 to IL-15Rα, whereas no IL-15 binding was found when using Δ2IL-15Rα. IL-15·IL-15Rα complex formation was competed out with unlabeled IL-15 (Fig. 5*D*). The half-maximal effect was obtained with an IL-15 concentration of ~100 pM, indicating that solubilized IL-15Rα retained a high affinity similar to that measured on intact cells.

Δ2IL-15Rα Transfection Does Not Affect IL-15- or IL-2-driven Cell Proliferation—To investigate potential regulatory effects, IL-15Rα and Δ2IL-15Rα (E7 forms) cDNAs were transfected in the human T lymphoma cell line Kit 225. RT-PCR using the E4/p3BGH primer pair showed that the corresponding transcripts were expressed at comparable levels in the IL-15Rα and Δ2IL-15Rα transfectants (data not shown). Kit 225 cells are dependent on exogenous IL-2 for proliferation (28). We also observed that this growth requirement could be replaced by IL-15, in agreement with the observation that this cell line expressed endogenous IL-15Rα mRNA (Fig. 2). Proliferation experiments (Fig. 6) showed that transfection of IL-15Rα or Δ2IL-15Rα did not change the proliferative response of Kit 225 cells. The three cell lines responded with similar dose-response curves to IL-2 or IL-15 in terms of thymidine incorporation at 48 h.

DISCUSSION

Recently, three different IL-15Rα isoforms have been described that either lack exon 3 and/or use an alternate exon 7 (exon 7′) (12). Here, by RT-PCR amplification using appropriate sets of oligonucleotide primers, we confirm the existence of these isoforms and show, in addition, the existence of novel IL-15Rα transcripts corresponding to the deletion of exon 2. This new deletion can combine with the ones already described, leading to eight different transcripts corresponding to all the possible combinations of exon 2 deletion, exon 3 deletion, and alternate usage of exon 7 or 7′. In agreement with previous reports (12, 13), IL-15Rα transcripts were found to be expressed by various cell lines and tissues. Each positive tissue or cell line expressed the eight different IL-15Rα transcripts, although at relative levels that varied from one cell/tissue type to another.

Expression of human IL-15Rα in COS-7 cells gave rise to several protein bands, in agreement with a previous report suggesting several bands for a soluble form of mouse IL-15Rα (13). We show in this study that these different products are due to alternate *N*- and *O*-glycosylations of a 39-kDa precursor. Similarly, Δ2IL-15Rα gave rise to different glycosylated bands from a 32.5-kDa precursor. The size difference between IL-

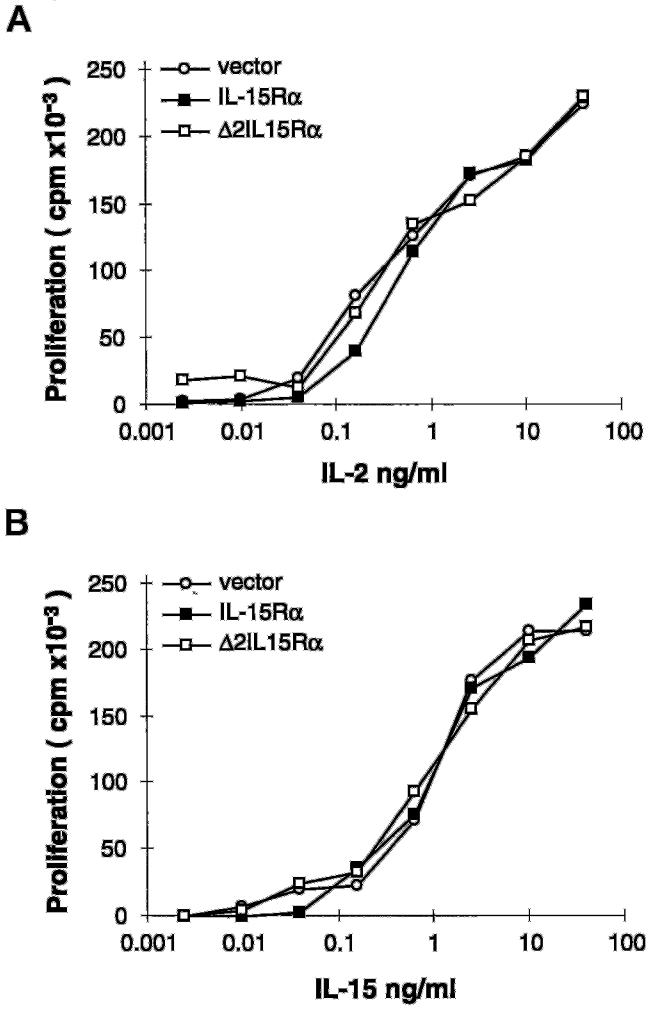

FIG. 6. **Proliferation of transfected Kit 225 cell lines.** Kit 225 cells transfected with empty vector, IL-15Rα, or Δ2IL-15Rα (as indicated) were tested for their proliferative response ([^{3}H]thymidine incorporation) to graded doses of IL-2 (*A*) or IL-15 (*B*).

15Rα and Δ2IL-15Rα precursors is in agreement with the theoretical molecular mass of the exon 2-encoded sushi domain (7329 Da). The human IL-15Rα sequence displays a single potential *N*-glycosylation site at Asn^{107} within the exon 3-encoded domain (12). Our data show that this site was used in COS-7 cells for both full-length and exon 2-deleted forms. Most of the size increases due to carbohydrate addition were the results of extensive *O*-glycosylations, which accounted for ~23 kDa in the highest molecular mass species. The extent of *O*-glycosylation of Δ2IL-15Rα was as large as in IL-15Rα, indicating that few glycosylations are associated with the exon 2-encoded domain. This is in agreement with the fact that most (74%) of the serine and threonine residues (which are potential targets for *O*-linked sugar addition) in the extracellular part of the receptor are located in the exon 3/4-encoded domains (12).

IL-15Rα extracted from transfected COS-7 cells was shown to bind IL-15 with a high affinity (K_d = 60 pM). In sharp contrast, Δ2IL-15Rα did not show any detectable IL-15-binding capacity. These results clearly demonstrate that the exon 2-encoded sushi domain is the essential element involved in IL-15 binding. They fully complement earlier work showing that exon 3-encoded linker sequence as well as exon 7/7′-encoded cytoplasmic domains were dispensable for binding and signaling (12).

IL-15Rα was expressed at the cell surface, although at low density (~1000 sites/cell). Unexpectedly, confocal immunofluorescence studies and analysis of subcellular fractions showed that most of IL-15Rα was associated with the nuclear membrane. A large proportion of this nuclear receptor was heavily *O*-glycosylated, suggesting that it was routed to the nuclear membrane through the Golgi. Some co-localization with the nuclear protein p300 was also observed, indicating that part of the receptor was inside the nucleus, possibly at the inner side of the nuclear membrane. Due to the relatively large size (~60 kDa) of the glycosylated receptor, this observation suggests that an active mechanism is involved in its nuclear translocation, rather than passive diffusion through the nuclear pores. In support of this, we found, within the human IL-15Rα sequence, the presence of a putative nuclear localization signal (NLS). This sequence consists of two clusters of polycationic residues separated by a spacer (RERYICNSGF*KRK*, amino acids 24–36) (12). Such putative NLSs have been demonstrated in the sequence of a number of ligands and receptors, including those that activate Stat transcription factors (29, 30). In this study, the possible involvement of this putative NLS in the nuclear routing of IL-15Rα is supported by the fact that the exon 2-truncated receptor (which does not contain this putative NLS motif located in the sushi domain) does not show nuclear localization. More specific deletions/mutations of this sequence are, however, required to demonstrate its role as an NLS.

Of major interest with respect to these findings is the recent demonstration that a newly identified isoform of IL-15 that uses a short (21 amino acids) signal peptide (SSP-IL-15) is not directed to the secretory pathway, but rather is stored intracellularly, appearing in the cytoplasm and nucleus (31). The other IL-15 isoform containing a long (48 amino acids) signal peptide (LSP-IL-15), in contrast to SSP-IL-15, has no nuclear localization and is directed to the secretory pathway. The two isoforms have also distinct tissue distribution and are likely to be generated by the usage of alternate promoters rather than by alternative splicing, suggesting that they might serve different roles. Our observations raise the interesting possibility that IL-15Rα, which has a high affinity on its own for IL-15, might bind SSP-IL-15 inside the cell and, through its putative NLS, translocate the cytokine-receptor complex in the nuclear compartment. Following that hypothesis, IL-15Rα might be involved in the different roles postulated for the two forms of IL-15, at the cell surface for LSP-IL-15 and at the nuclear level for SSP-IL-15.

Exon 2-truncated IL-15Rα has completely lost its capacity to bind IL-15, therefore raising the question of its biological role. Two findings suggest that it might serve some biological function: (i) a number of cell lines and tissues expressed Δ2IL-15Rα mRNAs at levels comparable to full-length IL-15Rα mRNAs; and (ii) upon transfection in COS-7 cells, Δ2IL-15Rα was expressed at the plasma membrane with similar efficiency as IL-15Rα. It is therefore possible that Δ2IL-15Rα might compete with IL-15Rα for recruitment of the transducing subunits IL-2Rβ and IL-2Rγ. Similar observations have been made in the case of IL-2Rα. A naturally occurring truncated form of IL-2Rα has been described that lacks the exon 4-encoded domain (second sushi domain) as a result of alternative mRNA splicing (32). This truncated IL-2Rα, whose function is presently unknown, was transported to the cell surface and was unable to bind IL-2. It was, however, expressed at low levels in human T cells. In this study, preliminary experiments on Kit 225 cells showed that Δ2IL-15Rα transfection did not affect IL-15- or IL-2-induced proliferation. However, a potential regulatory effect of transfected Δ2IL-15Rα in this system might have been masked by the fact that Kit 225 cells already express endogenous IL-15Rα and Δ2IL-15Rα. Additional studies addressing

signal transduction, proliferation, and apoptosis are required to determine the functional role of the Δ2IL-15Rα isoforms.

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