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Expression of IL-3 Receptor in Testis

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Interleukin-3 (IL-3) is a potent growth factor for hematopoietic cells. IL-3 transmits its signal through a specific cell surface receptor which consists of a primary binding α subunit (IL-3Rα) and a signaling β subunit that is shared by the IL-5 and GM-CSF receptors. Here, we describe the expression of the α and β subunit of the IL-3 receptor in mouse testis and testicular cell lines both at nucleic acid and protein levels. Our data indicate that IL-3Rα is expressed on the cell surface of testicular Leydig cells and a Leydig cell tumor cell line I-10. Expression of the β subunit mRNA was only faintly detected in testis, but not in I-10 cells. Immunoelectron microscopy has revealed that IL-3Rα is present on cell membrane and microvilli.

Hematopoiesis is regulated by a complex network consisting of hematopoietic cells, stromal cells and soluble polypeptide factors known as cytokines. These cytokines are produced by various types of cells, either constitutively or inducibly in response to hematologic or immunologic stimulation, and exert varying effects (1). Among those cytokines, interleukin-3 (IL-3), IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF) are major hematopoietic cytokines that are secreted mainly by activated T cells. Although these three cytokines show no significant amino acid sequence homology, they share a number of similarities in their structures as well as their functions (2, 3). Cloning of the receptor genes and reconstitution of the high-affinity receptors for these cytokines have provided a molecular explanation for their common biologic functions (3, 4), i.e. the receptors consist of a cytokine specific α subunit that binds each specific cytokine and a common β subunit that transduces signals. As the three receptors share the same β subunit, they induce virtually identical signals in the same cells. Interestingly, there are two homologous β subunits for the IL-3 receptor in the mouse, i.e. one is the common β subunit among IL-3, IL-5, and GM-CSF receptors and the other one (IL-3Rβ) is specific for IL-3, although these two β subunits are 91% identical at the amino acid level. The IL-3 specific one is absent in the man.

Although the receptors for these cytokines have no intrinsic tyrosine kinase, they interact with a member of the Jak tyrosine kinases. Upon binding of a cytokine, the Jak kinase associated with the receptor is activated and then phosphorylates tyrosine residues of a latent cytoplasmic transcription factor STAT (signal transducer and activator of transcription). Activated STATs are then translocated to the nucleus where they bind to their specific target DNA sequence and regulate gene expression. Currently seven members of the STAT family have been molecularly cloned.

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While these cytokines function mainly in blood cells, there are several reports that describe the expression of IL-3 and GM-CSF as well as their receptors in nonhematopoietic tissues such the brain (5-8). Recently, several STATS including STAT1, 3, 4 and 5 were found to be expressed in testis (9, 10, Mui A.L.-F. Personal communication). These findings suggest that cytokines may play a role in the testis. As IL-3 and GM-CSF activate STAT5 (11, 12), we have examined the expression of these cytokine receptors in testis and found that the IL-3 receptor α subunit (IL-3Rα) is expressed in testicular Leydig cells and a Leydig cell tumor cell line I-10.

MATERIALS AND METHODS

Mouse and cell lines. BALB/c male mice were purchased from Jackson Laboratory. OTT-1 cell line was kindly provided by R. Hawley(13). TM3, TM4, and I-10 cell lines were obtained from ATCC. OTT-1 cells were cultured in RPMI1640 medium with 10% fetal calf serum (FCS) and IL-3. TM3 and TM4 cells were maintained in a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium suppemented with 5% horse serum (HS) and 2.5% FCS. I-10 cells were maintained in Ham's F-10 medium with 15% HS and 2.5% FCS.

RNA isolation and Northern analysis. RNA blot hybridization was performed according to a standard method(14). Briefly, Poly(A)$^+$ RNA prepared from mouse testis and various cell lines were subjected to electrophoresis through 1.0% agarose gel containing formaldehyde, and transferred to nylon membranes. The membranes were then hybridized with the probes labeled with [α-^{32}P]dCTP using T7 Quick Prime kit (Pharmacia Biotech, Alameda, CA), washed, and used to expose Kodak films. The probes for IL-3Rα, IL-3Rβ, GM-CSFRα, and IL-5Rα were prepared as a 1.4 kb EcoRI/NotI fragment from pSUT-1, a 3.6 kb EcoRI/XbaI fragment from pAIC2A, a 1.8 kb SmaI/SpeI fragment from pBSmuGMRa71, and a 1.0 kb HindIII/PstI fragment from pIL-5R.8, respectively(15-18).

Immunohistochemistry. Tissue specimens were fixed in 4% paraformaldehyde and embedded in OCT compound. Four-micrometer sections were prepared for staining with hematoxylin-eosin (HE) and for immunostaining for IL-3Rα, IL-3Rβ, and IL-5Rα using avidin-biotin complex immunoperoxidase technique. Briefly, sections were treated with 3% hydrogen peroxidase in absolute methanol for 30 min after blocking of endogenous biotin. After treatment with normal goat serum (DAKO, Carpinteria, CA), the sections were incubated with anti-IL-3Rα (5B11), anti-IL-3Rβ (9D3), or anti-IL-5Rα (H7) (19) for 1 hr at room temperature, and then incubated sequentially with biotinylated anti-rat immunoglobulin G antibody (Vector Laboratories, Burlingame, CA) for 30 min, and with streptavidin-peroxidase complex (DAKO) for 30 min.. The peroxidase reaction was developed with 0.06% 3-amino-9-ethylcarbazole in 0.05 M Tris buffer (pH7.7) containing 0.03% hydrogen peroxidase. Hematoxylin was used for counterstaining.

Immunoelectron microscopy. Mice were fixed by transcardial perfusion of cold periodate-lysine-paraformaldehyde solution, and then the testes were removed. They were snap-frozen by n-hexane precooled in dry ice-acetone. Frozen sections (5 μm thick) were cut in a cryostat, taken onto a glass slide, and stained by the indirect method with anti-IL-3Rα (5B11) or anti-IL-3Rβ (9D3), as the first antibody. After immunostaining, the sections were postfixed with 1% glutaraldehyde and 2% osmium tetroxide solutions, and then dehydrated in graded ethanol and embedded in Quetol 812 (Nishin EM, Tokyo, Japan). They were detached from the slides after polymerization of the resin. Ultrathin

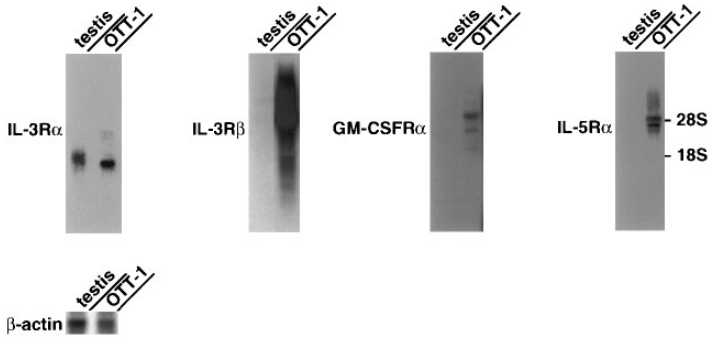

FIG. 1. Northern blot analysis of IL-3Rα, IL-3Rβ, GM-CSFRα, and IL-5Rα in mouse testis. mRNAs (2μg) from the testis were electrophoresed on a 0.8% formaldehyde/agarose gel. After transfer to nitrocellulose membrane, the blot was hybridized with ^{32}P-labeled cDNA for IL-3Rα, IL-3Rβ, GM-CSFRα, IL-5Rα, and β-actin, and after washing, analyzed by autoradiography.

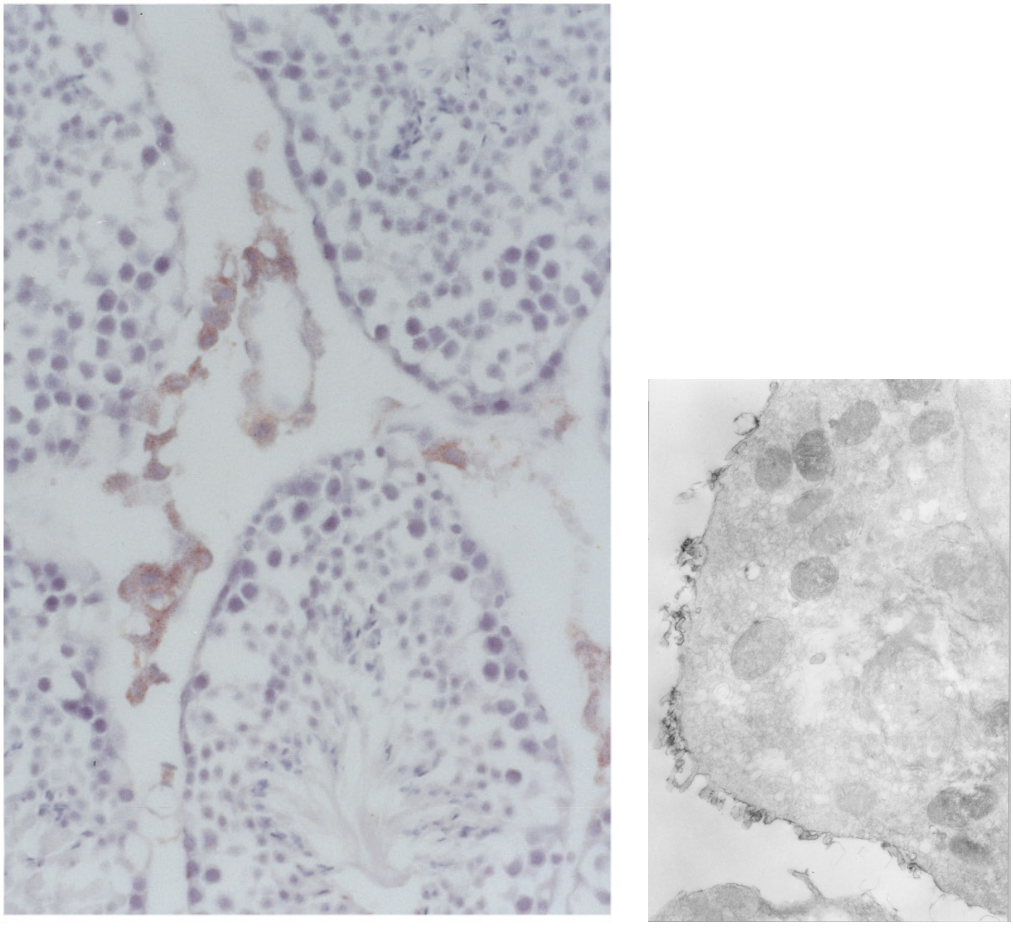

FIG. 2. Immunolocalization of IL-3Rα using monoclonal anti-IL-3Rα antibody 5B11 in an avidin-biotin immunoperoxidase procedure by light (*left*) and electron (*right*) microscopy. Microvilli and cell membrane are heavily stained. (*Left*) Immunoperoxidase reaction, counterstained with hematoxylin. $\times$400. (*Right*) $\times$35,000.

sections of individual samples were cut with an ultramicrotome (Ultracut N, Reichert-Jung, Austria) and examined in a transmission electron microscope (JEM-1200EX, JEOL, Tokyo, Japan).

Flow cytometric analysis. Expression of IL-3Rα, IL-3Rβ, and IL-5Rα on various types of mouse cell lines was analyzed by flow cytometry. Briefly, 10^6 cells were suspended in 200 μl of ice-cold PBS containing 10% FCS and 0.02% sodium azide (washing buffer), and incubated with anti-IL-3Rα (5B11), anti-IL-3Rβ (9D3), or anti-IL-5Rα (H7) monoclonal antibodies for 1 hr at 4°C. The cells were washed three times in the washing buffer, and incubated with FITC-conjugated goat anti-rat IgG antibody for 30 min at 4°C. They were washed three times, and analyzed with a FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Anti-trinitrophenol monoclonal antibodies (Pharmingen, San Diego, CA) were used as an isotype control for the first step staining.

RESULTS AND DISCUSSION

Expression of the IL-3 Receptor in Testis

We first examined mRNA expression of IL-3Rα, IL-3Rβ, GM-CSFRα, and IL-5Rα in testis by Northern blot analysis. As shown in Fig. 1, a descrete 1.8 kb band was detected by the IL-3Rα probe in mRNA obtained from OTT-1 cells and a band with the same size as well as several longer transcripts were found in mouse testis. A faint 4.6 kb band hybridizing to the

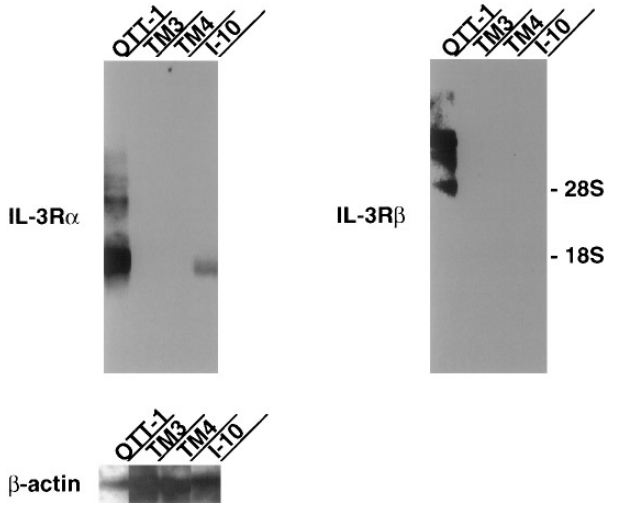

FIG. 3. Northern blot analysis of IL-3Rα and IL-3Rβ in some cell lines. mRNAs (5μg) from the testis were electrophoresed on a 0.8% formaldehyde/agarose gel. After transfer to nitrocellulose membrane, the blot was hybridized with ^{32}P-labeled cDNA for IL-3Rα, IL-3Rβ, and β-actin and, after washing, analyzed by autoradiography.

IL-3Rβ probe was also detected in mouse testis. In contrast, neither GM-CSFRα and IL-5Rα probe detected any transcripts in mouse testis.

An immunohistochemical method was useed to analyze the distribution of IL-3Rα- and β-subunit in sections of the testes from adult BALB/c mice. Many Leydig cells showed positive staining with the anti-IL-3Rα monoclonal antibody (Fig. 2, *left*). In contrast, no positive signal for IL-3Rβ was demonstrated in these cells (data not shown). Immunoelectron microscopy revealed that the positive signal for IL-3Rα was restricted to cell membrane and microvilli of Leydig cells (Fig. 2, *right*).

Expression of the IL-3 Receptor in Testicular Cell Line

We then examined the mRNA expression of IL-3Rα and IL-3Rβ in mouse testicular cell lines by Northern blot analysis. As shown in Fig. 3, a clear 1.8 kb band hybridizing to the IL-3Rα probe was detected in mRNA from I-10 cells, but it was not detected in TM3 and TM4 cells. No band hybridizing to the IL-3Rβ probe was detected in TM3, TM4, and I-10.

The cell lines TM3, TM4, and I-10 were examined for the expression of IL-3Rα- and β-subunit by flow cytometry using monoclonal antibodies against each subunit. As shown in Fig. 4, the Leydig cell tumor cell line I-10 showed positive signal for anti-IL-3Rα subunit antibody, but the other two cell lines were negative. No expression of IL-3Rβ subunit was detected on any cell lines tested in this study. This finding is consistent with the data of Northern blot analysis.

Expression of IL-3Ra in Leydig Cells

As described above, IL-3Rα was present in normal Leydig cells as well as Leydig cell tumor cell line I-10, but neither IL-5Rα nor GM-CSFRα were detected in those cells. β-subunit mRNA was detected in testis by Northern blot analysis, but immunohistological stainings failed to detect β-subunit protein. The testicular cells may be defective in post-transcriptional mechanism for expression of the β subunit. Alternatively, the level of the β subunit in the testis is too low to be detected by the method we employed. As neither Northern blot analysis

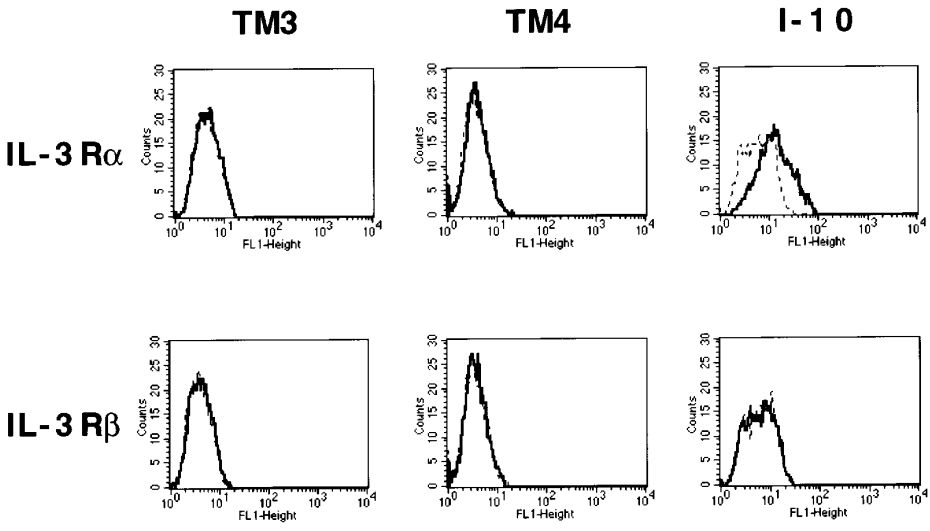

FIG. 4. Flow cytometric analysis of IL-3Rα and IL-3Rβ expression in mouse testicular cell lines. Surface expression of IL-3Rα and IL-3Rβ in mouse testicular cell lines was examined by flow cytometry by using monoclonal antibodies against IL-3Rα (5B11) and IL-3Rβ (9D3). Broken lines show staining profiles with isotype control as the primary antibody.

nor flow cytometric analysis detected the expression of the β subunit in any of the Leydig cell lines tested, the β subunit may not be present in Leydig cells.

The question then is why IL-3Rα is present in Leydig cells without the β subunit. One possibility is that IL-3Rα alone is capable of transmitting signals in these cells. Alternatively, it may interact with a unidentified subunit in the testis. To address the question we tested if IL-3 induces in the Leydig cells any biochemical events such as tyrosine phosphorylation of JAK2 kinases and STAT5 and glucose transport, which are known to be induced in response to IL-3 in hematopoietic cells. However, we failed to detect any sign of these events induced by IL-3 in I-10 cells. Signal transduction by IL-3, IL-5, and GM-CSF usually requires both α and β subunits(15, 20-22). However, it has been recently reported that the GM-CSFRα subunit alone is capable of transmitting a signal for stimulation of glucose transport(23). Although IL-3Rα and GM-CSFRα are structually related and interact with the same β subunit, our results indicate that IL-3Rα is nonfunctional in Leydig cells for at least those biochemical events we tested. Therefore, physiological role of IL-3Rα in Leydig cells remains to be elucidated.

It may be worthy to note that IL-3 was originally identified as a factor that induces the expression of 20α-hydroxysteroid dehydrogenase in splenic lymphocytes from athymic mice(24). The activity of the 20α-hydroxysteroid dehydrogenase plays a role in regulating the production of steroid hormones(25). This may suggest that IL-3 plays a role in regulation of steroidgenesis by unknown mechanism in testis.

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