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THE MULTIFACETED REGULATION OF INTERLEUKIN-15 EXPRESSION AND THE ROLE OF THIS CYTOKINE IN NK CELL DIFFERENTIATION AND HOST RESPONSE TO INTRACELLULAR PATHOGENS[1]

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ABSTRACT

Interleukin-15 (IL-15) is a 14- to 15-kDa member of the 4 α-helix bundle family of cytokines. IL-15 expression is controlled at the levels of transcription, translation, and intracellular trafficking. In particular, IL-15 protein is posttranscriptionally regulated by multiple controlling elements that impede translation, including 12 upstream AUGs of the 5′ UTR, 2 unusual signal peptides, and the C-terminus of the mature protein. IL-15 uses two distinct receptor and signaling pathways. In T and NK cells the IL-15 receptor includes IL-2/15Rβ and γ_c subunits, which are shared with IL-2, and an IL-15-specific receptor subunit, IL-15Rα. Mast cells respond to IL-15 with a receptor system that does not share elements with the IL-2 receptor but uses a novel 60- to 65-kDa IL-15RX subunit. In mast cells IL-15 signaling involves Jak2/STAT5 activation rather than the Jak1/Jak3 and STAT5/STAT3 system used in activated T cells. In addition to its other functional activities in immune and nonimmune cells, IL-15 plays a pivotal role in the

development, survival, and function of NK cells. Abnormalities of IL-15 expression have been described in patients with rheumatoid arthritis or inflammatory bowel disease and in diseases associated with the retroviruses HIV and HTLV-I. New approaches directed toward IL-15, its receptor, or its signaling pathway may be of value in the therapy of these disorders.

INTRODUCTION

Intercellular communications involved in immune responses are often mediated by cytokines that show a high degree of redundancy and pleiotropy. The redundancy is explained in part by the sharing of common receptor subunits among the members of the cytokine receptor superfamily (1–5). In the case of the interleukin-2 receptor (IL-2R) system, the common gamma (γ_c) subunit is shared by IL-2, IL-4, IL-7, and IL-9 (4–5). Recently, two groups simultaneously reported the recognition of an additional cytokine in this family, now known as IL-15, based on the ability of culture supernatants from two cell lines, CV-1/EBNA and the human T cell lymphotropic virus type I (HTLV-I)-associated HuT-102, to stimulate the proliferation of the cytokine-dependent murine T-cell line CTLL-2 (6–8). The active materials in the two supernatants shared many characteristics such as an apparent molecular mass of 14–15 kDa and membrane-proximal signaling components in T and natural killer (NK) cells that consist of the IL-2Rβ and γ_c subunits of the IL-2 receptor (6–14). An appropriate anticytokine antibody was used to show that the two groups identified the same interleukin, which is now termed IL-15 (15).

IL-2 and IL-15 share many features. They are both members of the 4 α-helix bundle cytokine family, they use both IL-2Rβ and γ_c for their action in T cells, and they have similar functional activities in these cells. Nevertheless, dramatic differences exist between these two cytokines in terms of their cellular sites of expression and the levels of control of their synthesis and secretion. IL-2 is produced by activated T cells and is controlled predominantly at the levels of mRNA transcription and stabilization, whereas control of IL-15 expression is much more complex, with regulation at the levels of transcription, translation, and intracellular trafficking and translocation (8, 10–11, 14–20). Furthermore, there are differences in the receptor and signaling pathways used by IL-2 and IL-15 in diverse cells (21, 22). Each cytokine has its own private receptor in T and NK cells: IL-15Rα and IL-2Rα for IL-15 and IL-2, respectively (21). IL-15 uses a novel IL-15RX receptor system and signal transduction pathway in select nonlymphoid cells including mast cells (22).

As predicted from their sharing of receptor subunits, IL-2 and IL-15 have a number of redundant functions such as induction of T-cell proliferation and the costimulation of immunoglobulin synthesis (6, 8, 23–25). However, IL-15 also

plays a pivotal role in the differentiation of NK cells from their progenitors, in the maintenance of the survival of such cells, and in their activation (26–39). IL-15 also has unique functions on nonlymphoid cells, including actions on muscle, brain microglia, and mast cells (11, 22, 40, 41).

Abnormalities of IL-15 expression have been reported in inflammatory and neoplastic diseases (42–45). In particular, abnormally high levels of IL-15 transcription and translation were observed in HTLV-I-associated diseases such as adult T-cell leukemia (ATL) and the neurological disorder tropical spastic paraparesis (TSP) (45). Furthermore, abnormalities of IL-15 expression may occur in patients with inflammatory autoimmune diseases, such as rheumatoid arthritis and inflammatory bowel disease, with IL-15 at the apex of a cascade of inflammatory factors that includes TNF-α, which induces the expression of other inflammatory cytokines and chemokines involved in the pathogenesis of these diseases (42–44). Therapeutic agents are being developed to target the receptor and signaling elements shared by IL-2 and IL-15 to provide effective treatment for such disorders (10, 46–48).

THE STRUCTURE AND GENOMIC ARCHITECTURE OF IL-15

IL-15 is a 14- to 15-kDa glycoprotein whose mature form consists of 114 amino acids (aa) (8). It has two cystine disulfide cross-linkages at positions Cys42-Cys88 (homologous to IL-2) and Cys35-Cys85, and three asparagine residues (119, 127, and 160) that in two cases are sites for N-linked glycosylation (8). IL-15 is a member of the 4 α-helix bundle cytokine family, which includes such cytokines as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9 (1, 8). The predicted folding topology of IL-15 suggests three loops connecting the four helices in an up-up down-down configuration (8). IL-15 shares no sequence homology with IL-2 or with other members of the cytokine superfamily; however, structural homology among these members is clearly conserved. There is 97% sequence identity between human and simian IL-15 and 82% sequence identity between human and porcine IL-15 (49–50).

The IL-15 gene was mapped on chromosome 4q31 (human) and to the central region of chromosome 8 (mouse) by fluorescence in situ hybridization (50). The IL-15 gene consists of nine exons (exons 1–8 and a newly discovered exon 4a) and eight introns spanning at least 35 kb. This exon-intron organization contrasts with the four exon–three intron architectural pattern observed in IL-2, IL-4, and IL-5 (51).

One form of human IL-15 mRNA contains a 5′ untranslated region (UTR) of at least 352 nucleotides (nt), a coding sequence of 486 nt, and a 3′ UTR of at least 400 nt (8). There are two alternative leader peptides, one with 48 aa and one with 21 aa (8, 19, 20, 52). In contrast to most signal peptides that are encoded in

a single or at most two exons, the IL-15 leader sequences are encoded in more than two exons (Figure 1) (19, 52). The classical long (48 aa) signal peptide associated with all secreted IL-15 is present in a 1.6 kb mRNA. It is encoded by exons 3, 4, and 5 of the human IL-15 gene (Figure 1) (50). The short 21-aa signal peptide is encoded by a 1.2-kb cDNA that lacks the elements encoded by exon 1. This signal peptide is encoded by exon 5 and by an additional 119-nt sequence inserted between exons 4 and 5 (new exon 4a) (19, 20, 52). The two signal peptides share 11 identical amino acids encoded by exon 5. The introduction of the 119 nt of exon 4a disrupts the 48-aa signal sequence by inserting a premature termination codon and then provides an alternative initiation codon with a poor Kozak context (TTCATGG) (19, 20, 52). The lack of exon 1 and the presence of a 219-nt fragment that originates from intron 1 in the 5′ UTR of the 21-aa IL-15 signal peptide transcript suggests that this isoform may be regulated transcriptionally by an intronic enhancer/promoter in intron 1. As noted below, IL-15 associated with a short 21-aa signal peptide is not secreted but rather is stored intracellularly, appearing in nuclear and cytoplasmic components.

THE MULTIFACETED REGULATORY CONTROL OF IL-15 EXPRESSION

IL-2 and IL-15 exhibit major differences in terms of their sites of synthesis and their levels of control of synthesis and secretion. IL-2 is produced by activated T cells, and its expression is regulated predominantly at the levels of mRNA transcription and message stabilization (17, 18). In contrast, Northern blot analysis indicated widespread constitutive expression of IL-15 mRNA in a variety of tissues such as placenta, skeletal muscle, kidney, lung, heart, fibroblasts, epithelial cells, and monocytes (8, 15). IL-15 mRNA could not be demonstrated by Northern blot analysis in normal resting or phytohemagglutinin-activated T cells, although the more sensitive RNase protection assay (RPA) indicated the presence of IL-15 mRNA in normal T cells obtained ex vivo and in the T-cell lines examined (8, 45).

Regulation of IL-15 Transcription

The regulation of IL-15 expression is multifaceted. Modest control occurs at the level of transcription, and a dominant control occurs posttranscriptionally at the levels of translation and intracellular trafficking. In terms of transcriptional control, freshly isolated monocytes expressed only low levels of IL-15 mRNA that was upregulated when the monocytes were activated with LPS/IFN-γ (8, 15). In addition, infection of monocytes with herpesvirus 6, herpesvirus 7, *Bacillus Calmette-Guérin* (BCG), *Mycobacterium tuberculosis*, *Toxoplasma*

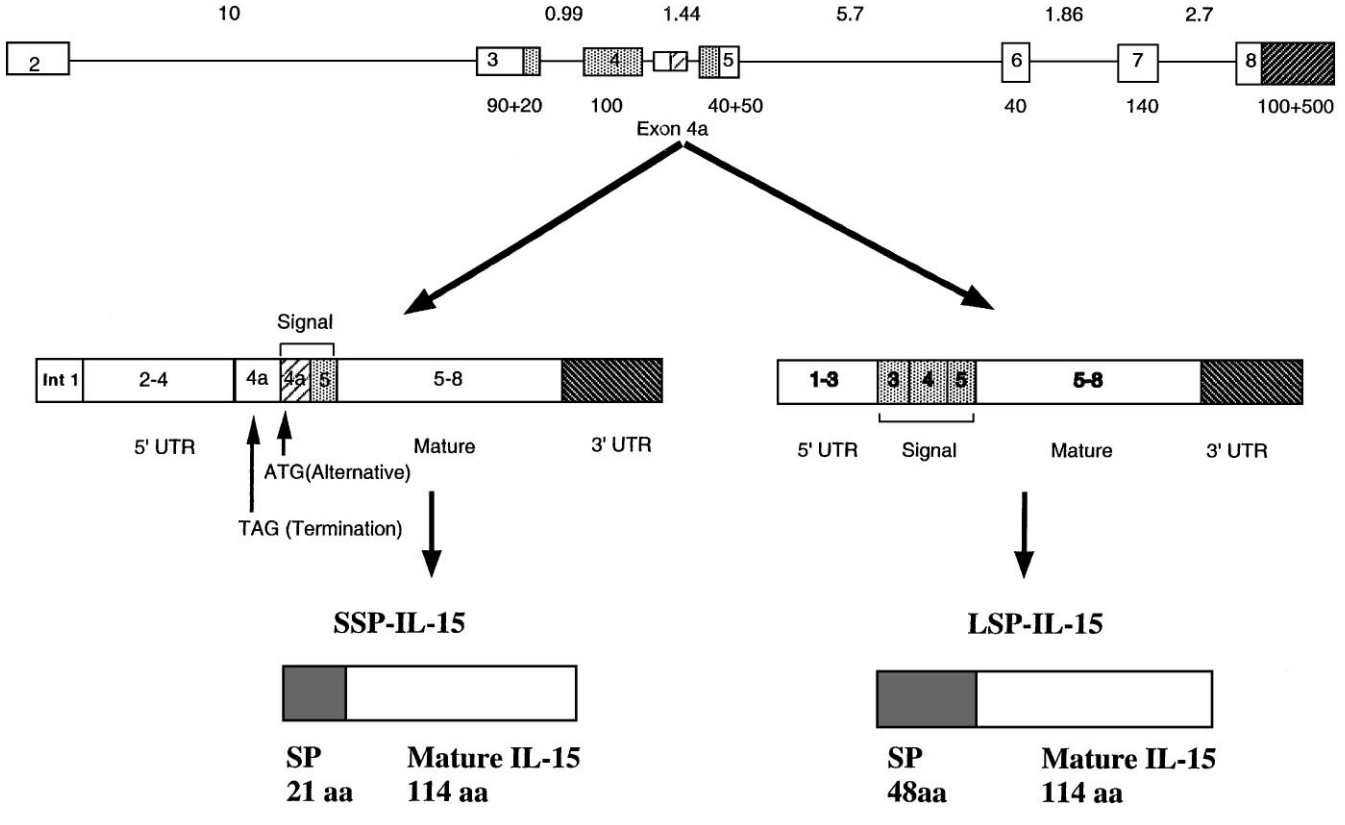

Figure 1 There are two isoforms of IL-15 that contain two leader peptides, one with 48 amino acids (aa) and one with 21 aa. The 48aa signal peptide is encoded by exons 3, 4, and 5 of the human IL-15 gene. The short 21-aa signal peptide is encoded by exon 5 and an additional 119-nt sequence inserted between exons 4 and 5 (new exon 4 a). The introduction of the 119 nt of exon 4 a disrupts the 48-aa signal sequence by inserting a premature termination codon and then provides an alternative initiation codon. LSP, long signaling peptide; SSP, short signaling peptide.

gondii, Salmonella choleraesuis, Mycobacterium leprae, Cryptococcus neoformans, or *Candida albicans* was associated with an upregulation of IL-15 mRNA expression (31, 32, 39, 53–57).

Cloning of the human and murine 5′ flanking region of the IL-15 gene has permitted the study of the mechanisms underlying the constitutive and induced expression of IL-15 mRNA (45, 58). A series of conserved motifs between mouse and human IL-15 5′ regulatory regions has been identified; these motifs include GCF, NF-κB, IRF-E, myb, γIRE, NF-IL-6, and αINF-2. The IRF response element IRF-E and NF-κB sites are involved in the induced up-regulation of IL-15 mRNA expression (45, 58). As noted below, the development of NK cells requires IL-15 induction. Mice lacking the expression of the transcription factor IRF-1 (IRF-$1^{-/-}$ mice) also exhibit a severe NK-cell deficiency (35, 36). The IRF-1 deficiency does not affect the NK-cell progenitors themselves but rather affects the function of radio-resistant cells constituting the microenvironment required for NK-cell development. IRF-$1^{-/-}$ bone marrow cells can generate functional NK cells when cultured with IL-15 (35). As noted above, within the 5′ upstream region of the mouse IL-15 gene, a 9-bp sequence, TTCACTTTC, spanning from -278 to -270 relative to the transcription initiation site, is in perfect concordance with the consensus IRF response element (IRF-E). This sequence motif binds IRF-1/2 proteins specifically. The importance of IRF-E for the activation of the IL-15 promoter was determined through the use of a series of reporter assays using IL-15 promoter deletion mutants (35, 45). These results support the view that IRF-1 in bone marrow stromal cells is pivotally involved in the up-regulation of IL-15 gene expression. The IL-15 generated acts on NK-cell precursors, stimulating their development into mature NK cells. In parallel studies, Ohteki and coworkers (36) demonstrated that IRF-1-induced IL-15 expression is important for the development of NK, NK-T cells, and intestinal intraepithelial T cells.

Another transcription factor that appears to play an important role in IL-15 transcription is NF-κB. The HTLV-I-encoded tax protein transactivates IL-15 gene transcription through this site (45). IL-15 mRNA expression is increased in HTLV-I-infected T cells and T-cell lines. Using reporter constructs bearing the 5′ regulatory region of the IL-15 gene, we found a positive correlation between HTLV-I tax protein expression and IL-15 promoter activity (45). Additionally, using a Jurkat T-cell transfectant that expressed tax under an inducible promoter, it was shown that the expression of IL-15 mRNA increased when tax was expressed. Mutations in the NF-κB motif or deletion of this sequence in the IL-15 5′ regulatory region eliminated the promoter activity in tax-transfected cells. These data represent evidence for transactivation of the IL-15 gene by the HTLV-I tax protein through an NF-κB motif in HTLV-I-transformed T cells (45).

Role of Translation in the Regulation of IL-15 Expression

IL-15 is predominantly regulated posttranscriptionally at the level of translation and translocation. In particular, although IL-15 mRNA is widely expressed constitutively, it has been difficult to demonstrate IL-15 in supernatants of many cells that express such mRNA (8, 15). Although monocytes activated with LPS/IFN-γ expressed high levels of IL-15 mRNA, the culture supernatants and cell lysates from these cells contained little or no IL-15 protein as assessed by either an IL-15-specific ELISA or a CTLL-2 proliferation assay (15). This discordance between IL-15 mRNA expression and IL-15 protein production led us to examine normal IL-15 mRNA for posttranscriptional controls, particularly for features that could inhibit IL-15 production at the level of mRNA translation. We demonstrated that IL-15 expression is posttranscriptionally regulated by multiple elements including 12 upstream AUGs of the 5′ UTR, a 48-aa signal peptide, and the C-terminus of the mature protein (15, 16).

Our initial studies focused on the 5′ UTR of IL-15 mRNA (15). In general, the 5′ UTRs of effectively translated messages are short, simple, and unencumbered by AUGs upstream of the initiation AUG (59–61). In contrast to this pattern, the 5′ UTR of IL-15 mRNA is long (at least 465 nt in mice and 352 nt in humans) and includes multiple upstream AUGs (5 in mice, 12 in humans) (8, 15). Kozak has emphasized that the presence of such AUGs in the 5′ UTR may dramatically reduce the efficiency of mRNA translation into proteins (59–61). In general, the rare mRNAs with 5′ AUG-burdened sequences include those encoding many protooncogenes, transcription factors, growth factors, receptor proteins, and signal transduction components. Among the 4 α-helix bundle cytokines, no upstream AUGs are present in the 5′ UTRs of IL-2, IL-3, IL-4, IL-5, IL-10, IL-13, or IFN-γ, but they are seen in IL-7, IL-11, IL-13, IL-2Rα, IL-2Rβ, IL-5Rα, and the IL-9Rα receptor. IL-2Rα expression is controlled at both the transcriptional and translational levels (62). Inhibition of translation by upstream AUGs has been confirmed experimentally by deletion of upstream AUGs or by site-directed mutagenesis of the AUG triplets in TGF-β3, FGF-5, and IL-7 mRNAs (63–65). Such upstream AUG codons may represent a ploy by the cell to yield poorly translated mRNAs that encode critical regulatory proteins whose efficient expression might be dangerous to the cell or the organism.

To define the effect of the upstream AUGs of the IL-15 5′ UTR on normal IL-15 mRNA translation, three IL-15 constructs were transfected into COS cells, one representing the full wild-type IL-15 mRNA with an early hairpin and 12 AUGs, the second retaining the 10 distal upstream AUGs, and the third lacking upstream AUGs (15). This latter construct produced 4- to 5-fold more IL-15 than did cells transfected with the construct retaining 10 AUGs and 12- to 15-fold more IL-15 than cells with the full wild-type construct. A number

of mechanisms have been reported to remove the 5′-UTR-AUG-mediated upstream impediments to translation. These mechanisms include ribosome shunting and internal initiation of translation, a process that bypasses the 5′ CAP-dependent scanning mechanism and translational inhibition of upstream AUGs through the use of an internal ribosome entry sequence (IRES) (66–69).

Although COS cells transfected with the expression construct lacking the 5′ UTR produced more IL-15 than did cells transfected with the wild-type construct (16), the quantity of IL-15 protein was very low (360 pg per 200,000 cells), 3 logs less than the quantity of IL-2 obtained (350,000 pg) with a comparable IL-2 construct. There were virtually equal levels of transcript for the two cytokines throughout the time-course despite the extreme disparity in protein production observed, suggesting differences in translational efficiency. Additionally, although IL-15 transcripts were readily translated in a wheat-germ in vitro translation system, they were poorly translated in a rabbit reticulocyte in vitro translation system. These data suggested that the mammalian translation systems (i.e., COS cells and rabbit reticulocytes) provided evidence for inhibitory and regulatory factors in addition to those in the 5′ UTR that interfered with efficient synthesis of IL-15.

We next examined the IL-15 mRNA for specific elements that might impede IL-15 expression and focused on the unusually long 48-aa isoform of the signal peptide (16). Because of the unusual length of this peptide, we considered the possibility that it might function as a negative regulator of IL-15 generation. To test this hypothesis we prepared expression constructs that exchanged the signal peptide coding sequences of IL-2 and IL-15 so that they were linked to the alternative mature protein coding sequence. The resulting chimeric cDNAs were transiently transfected into COS cells and the quantity of IL-15 determined. The total quantity of IL-15 generated (the sum of IL-15 retained within the cells and that secreted) increased 17- to 20-fold when the IL-15 signal peptide was replaced by that of IL-2 (16). In parallel studies, the quantity of IL-2 secreted was reduced 40- to 50-fold when COS cells were transfected with the reciprocal construct that had the IL-2 signal peptide replaced by that of IL-15. We demonstrated that the IL-15 and IL-2 protein expression differences observed could not be explained by differences in mRNA stability or by instability of the processed protein but rather were the result of a major impediment at the level of mRNA translation.

In parallel studies, Onu and coworkers (20) demonstrated that wild-type IL-15 mRNA transcription was not associated with efficient secretion of IL-15 protein. Furthermore, after replacing the IL-15 signal peptide with that of CD33, translation and secretion increased, supporting the view that IL-15 expression is controlled mainly posttranscriptionally at the levels of translation and secretion. When the IL-15 isoform with the alternative short 21-aa signal

peptide was examined, there was no secretion of IL-15 by the transfected cells. However, there was a four- to fivefold increase in translation of the isoform containing exon 4a compared to that with the long signal peptide, at least when assessed in a rabbit reticulocyte in vitro translation system (52, 70). The data support the hypothesis that the IL-15 signal peptide or its coding sequence are important factors in the negative regulation of IL-15 protein expression.

As stated by Bamford et al (16):

> The mechanisms underlying the [signal peptide]-mediated regulation of IL-15 translation have not been defined. However, with preliminary in vitro translation studies, we observed that the addition of canine microsomal membranes did not result in IL-15 chain completion and translocation into microsomes in contrast to the situation with the prototypical secretory protein, pre/prolactin, which was fully translocated and processed. . .. Therefore, a number of events or factors may be required for efficient IL-15 mRNA translation/translocation. It is possible that a translational activator(s) for chain elongation and translocation may be needed. Alternatively, a translational repressor or a stable secondary structure in the mRNA may prevent efficient IL-15 mRNA elongation and translocation. Furthermore, inefficient initiation of translation may contribute partially to the low levels of IL-15 protein generated in transfected COS cells. This stems from the observation that the start codon for the IL-15 coding sequence has a weak Kozak context (GTA ATGA). . .. In fact, modifying the start codon to a higher context (ACCATGG or GCCGCCATGA) increased IL-15 protein production four- to fivefold in transfected COS cells.

In additional studies we discovered that a third negative element may exist in the C-terminus of the IL-15 mature protein coding sequence or protein (16). Specifically for the purposes of antibody detection, we added the artificial epitope tag FLAG to the 3′ end of the IL-15 protein. We noted that the presence of FLAG increased total IL-15 protein production 5- to 10-fold, suggesting that this modification disrupted an inhibitory *cis*-element in the coding sequence of the IL-15 mature protein C-terminus. When the three IL-15 mRNA modifications (elimination of the 5′ UTR, switch of IL-15 signal peptide coding sequence with that of IL-2, and FLAG modification of the 3′ coding sequence) were combined in a single construct and introduced into COS cells, at least 250-fold more IL-15 was produced than was observed with the wild-type IL-15 construct with an intact 5′ UTR (16). These findings suggest that IL-15 mRNA, unlike IL-2 mRNA, may exist in translationally inactive pools.

Control of translation has been observed in a variety of proteins, and this regulation can occur at all levels of translation (e.g., initiation, elongation, and termination). Most mRNA-specific translational regulation has involved *cis*-acting RNA sequence elements that mediate regulation. Such regulatory sequences in the 5′ or 3′ UTR of the mRNAs and mature coding sequence have been observed in transcripts for ferritin, erythroid 5-aminolevulinate synthase, thymidylate synthase, and murine p53 (71–73). Furthermore, regulation at the level of translation has been demonstrated for the 70-kDa heat shock protein

mRNA in chicken reticulocytes IL-1β, TNF-α, TGF-β3, TGF-β1, and GM-CSF (74–75). In addition, researchers observed that one of the multiple levels of insulin biosynthesis regulation includes a glucose-dependent signal recognition particle-mediated translational arrest (76).

The studies of IL-15 indicate that the translational control of IL-15, like that of insulin, occurs at multiple distinct levels (76). The removal of these negative control mechanisms in an integrated fashion may lead to a major increase in IL-15 synthesis. The variety of negative regulatory features controlling IL-15 expression may be required because of the potency of IL-15 as an inflammatory cytokine. If indiscriminantly expressed, IL-15, with its capacity to induce the expression of TNF-α, IL-1, IFN-γ, and other cytokines and chemokines involved in the inflammatory response, could be associated with serious disorders such as autoimmune diseases (43). In terms of a more positive role for IL-15, we propose that by maintaining a pool of translationally inactive IL-15 mRNA, diverse cells may respond rapidly to an intracellular infection or other stimuli by transforming IL-15 mRNA into a form of mRNA that can be translated effectively. The IL-15 protein produced and secreted could convert T and NK cells into activated killer cells that might provide an effective host response to an invading infectious agent.

Intracellular Trafficking of IL-15

As noted above, two isoforms of human IL-15 exist. One isoform has a short 21-aa putative signal peptide, whereas the other isoform has an unusually long 48-aa signal peptide (8, 19, 20, 52, 70). In addition to their role in the regulation of IL-15 translation, these signal peptides influence intracellular trafficking of IL-15. The 21-aa IL-15 isoform is translated, but IL-15 is not secreted (19, 20, 52). Experiments using different combinations of signal peptides and mature proteins (IL-2, IL-15, and green fluorescent protein) showed that the short signal peptide regulates the fate of the mature protein by controlling the intracellular trafficking to non–endoplasmic reticulum sites such as the cytoplasm and the nucleus (52). The production of an intracellular lymphokine is not typical of other soluble interleukin systems, suggesting a biological function for IL-15 as an intracellular molecule.

The IL-15 associated with the long 48-aa signal peptide presents a more complex pattern and exists as multiple distinct molecular species within transfected COS cells. This multiplicity of protein species is partly the result of glycosylation because human IL-15 has two functional glycosylation sites. However, evidence from lysates using tunicamycin-treated IL-15-transfected cells indicates that the 48-aa signal peptide of IL-15 can be cleaved at two separate sites, yielding both partial and complete processing of the signal peptide. No evidence

of stepwise processing could be demonstrated, suggesting that the partially and fully processed forms may traffic to different cellular compartments. The form of IL-15 that retains the full signal peptide but without evidence of glycosylation was demonstrable in the cytoplasm and nucleus. The other forms entered the endoplasmic reticulum where they were glycosylated. The passage of IL-15 through the ER was much slower than that of IL-2 (16). Nevertheless, IL-15 was secreted after trafficking through the Golgi, yielding a cytokine with a fully processed signal peptide. Evidence for trafficking through the Golgi includes the inhibition of secretion by culture with brefeldin A and the endoglycosidase H–resistant nature of the secreted IL-15.

The two isoforms of IL-15 generated by usage of alternative signal peptides have different intracellular trafficking patterns. Sorting of the same protein to different cellular compartments by modifying the regulatory sequence also has been observed in other systems. Examples include proteins such as stem cell factor and Int-2, a fibroblast growth-factor-related oncoprotein (77, 78). In the case of Int-2, two different signal peptides are generated by the usage of different start codons in-frame, resulting in the alternative transport of the protein either to the secretory pathway or to the nucleus (78). The IL-15 case seems very similar to that of Int-2.

IL-15 RECEPTOR AND SIGNAL TRANSDUCTION PATHWAYS

IL-15 Type-1 Receptors in T and NK Cells

IL-15 uses two receptor and signaling pathways (7–10, 22). Cytokines such as IL-15 manifest considerable pleiotropy and redundancy controlling a wide range of functions in various cell types. The redundancy is explained in part by the sharing of common receptor subunits among members of the cytokine receptor family. Each cytokine has its own private receptor, but it usually shares one or more public receptors with other cytokines. Receptor elements are shared within the IL-2/15R system. In particular, the high-affinity IL-15R system in T and NK cells (type-1 IL-15 receptor) is made up of three distinct membrane components. Two of these components, IL-2/15Rβ and IL-2Rγ or γ_c are shared with the IL-2R system (7–9). In addition, the two cytokines have their own private α chains: IL-2Rα for IL-2 and IL-15Rα for IL-15 (21). The γ_c chain is also shared by IL-4, IL-7, and IL-9 (4, 5).

The human IL-2/15Rβ mRNA predicts a primary translation product of 551 aa (79, 80). The receptor contains a 26-aa signal peptide, and the mature human IL-2/15Rβ is composed of 525 aa with an extracellular segment of

214 aa, a hydrophobic transmembrane stretch of 25 aa, and a 286-aa cytoplasmic domain. IL-2/15Rβ is expressed constitutively by NK cells and to a lesser extent by monocytes and CD8 cells.

The human γ_c cDNA contains an open reading frame for a 369-aa residue polypeptide (81). This protein contains a 22-aa signal peptide, a 233-aa extracellular domain, a 28-aa hydrophobic transmembrane domain, and an 86-aa C-terminal cytoplasmic domain. IL-2/15Rβ and γ_c are members of the hematopoietin or cytokine superfamily of receptors that contain four conserved cysteines and the canonical WSXWS (trp-ser-X-trp-ser) motif.

A novel IL-15-specific binding protein termed IL-15Rα was identified and its cDNA cloned by Giri and coworkers (21). IL-15Rα is a type-1 membrane protein with a predicted signal peptide of 32 aa, a 173-aa extracellular domain, a single membrane-spanning region of 21 aa, and a 37-aa cytoplasmic domain. In contrast to IL-2/15Rβ and γ_c, IL-15Rα is not a member of the cytokine receptor superfamily. However, a comparison of IL-2Rα and IL-15Rα revealed the shared presence of a conserved motif known as a GP-1 motif, or a SUSHI domain (21). Another factor linking IL-2Rα and IL-15Rα is the demonstration that IL-2Rα and IL-15Rα genes have a similar intron-exon organization. Moreover, they are closely linked in both human (10q14-15) and murine genomes (chromosome 2 linked to *Vim-2* and *Spna-2*) (81). IL-15Rα binds IL-15 with a K_a of 10^{11}/M, a 1000-fold higher affinity than that of IL-2Rα for IL-2. IL-2/15Rβ in association with γ_c is able to bind IL-15 at a lower affinity (a K_a of $\sim 10^9$/M) and in select cells can transduce an IL-15 signal in the absence of IL-15Rα.

IL-15Rα has a wide cellular distribution. Its expression is observed in T cells, B cells, macrophages, and in thymic and bone marrow stromal cell lines (82). In addition, IL-15Rα mRNA is widespread in such tissues as liver, heart, spleen, lung, skeletal muscle, and activated vascular endothelial cells (21). IL-15Rα mRNA expression is increased in T cells after the addition of IL-2, an anti-CD3 antibody, or phorbol-myristate acetate (PMA) (21). Furthermore, IL-15Rα expression is augmented in macrophage cell lines after treatment with interferon-γ. Thus the widespread distribution of the IL-15Rα, IL-2/15Rβ, and γ_c elements of the IL-15R system is one of the mechanisms underlying the pleiotropy of IL-15.

IL-15 Signal Transduction Pathway in Activated T Cells

When analyzed in activated T cells, Jak3 and Jak1 were shown to be coupled functionally to the receptor systems involving γ_c, including the receptors for IL-15, IL-2, IL-4, IL-7, and IL-9 (83–85). Furthermore, the addition of IL-15 or IL-2 to such receptor-expressing T cells led to the tyrosine phosphorylation and nuclear translocation of STAT3 and STAT5 (84–85). The IL-2 and IL-15

signaling pathways in T cells also involve the phosphorylation of the *Src*-related cytoplasmic tyrosine kinases $p56^{lck}$ and $p72^{syk}$, the induction of the expression of the bcl-2 antiapoptotic protein, and the stimulation of the Ras/Raf/MAP kinase pathway leading to fos/jun activation (86).

IL-15 Uses a Distinct Type-2 Receptor/Signal Transduction Pathway in Mast Cells

IL-15 stimulates the proliferation of murine mast cell lines and normal bone marrow mast cells, whereas these cells do not respond to IL-2 (11, 22). This disparity in response to the two cytokines suggested the existence of a novel IL-15-specific receptor system in mast cells not shared by IL-2. The mast cell lines PT-18 and Mc/9 did not express mRNA-encoding IL-2Rα or IL-2/15Rβ, which explained the failure of IL-2 signaling in such cells. Furthermore, transfection of these cells with a cytoplasmic-truncated transdominant mutant form of γ_c demonstrated that IL-15-signaling in mast cells does not require this receptor element. In addition, evidence indicated that IL-15Rα is not a critical element of the mast cell IL-15R and signaling pathway.

We used ^{125}I-IL-15 in a cross-linking study with the mast cell line PT-18, using disuccinimidyl suberate to identify a possible mast cell–specific IL-15R. The IL-15/IL-15R complex in mast cells migrated approximately 75–80 kDa, implying a cytokine receptor size of 60–65 kDa. These results suggested that mast cells express a novel 60- to 65-kDa IL-15R molecule (type-2 IL-15 receptor), which was provisionally designated IL-15RX.

The lack of involvement in mast cells of the IL-2/15Rβ and γ_c chains used by IL-15 in T and NK cells and the possibility that a novel receptor (IL-15RX) is involved in IL-15 signaling in these cells prompted us to examine the membrane-proximal events of IL-15 signal transduction in mast cells to determine whether they are different from those in T cells. IL-15 addition to the mast cell line PT-18 caused the phosphorylation of Jak2 kinase rather than Jak1 or Jak3 as observed with the type-1 IL-15R in T cells (22). IL-15 also stimulated Jak2 phosphorylation in bone marrow mast cells. In further contrast to T cells, the addition of IL-15 to mast cells led to a tyrosine phosphorylation and nuclear translocation process limited to STAT5 rather than the STAT3/STAT5 activation observed with the type-1 receptor in T cells (22). These results indicate an IL-15 function distinct from that of IL-2 given that mast cells appear to use a novel IL-15 receptor and signaling pathway to stimulate the proliferation of these cells. However, the addition of IL-15 to bone marrow hematopoietic precursor cells does not result in the propagation of mast cells but rather induces NK cells (see below). Furthermore, these observations identify a second mechanism underlying the pleiotropy manifested by IL-15 in addition to the widespread tissue distribution of IL-15Rα.

BIOLOGICAL ACTIONS OF IL-15

Role of IL-15 in T and B Cell Function

As might be anticipated by their sharing of IL-2/15Rβ and γ_c receptor subunits in T, B, and NK cells, IL-15 and IL-2 have some common biological activities (6, 8, 12–14, 23–25). However, IL-15 also has unique functions, reflecting the much broader tissue distribution of its private receptor, IL-15Rα (21, 81). Furthermore, in select cells IL-15 uses a second receptor (IL-15RX) and signaling pathway not shared with IL-2 (22). Finally, the major differences between IL-2 and IL-15 in terms of their sites of synthesis and the regulation of their expression may also lead to different actions. For example, the expression of IL-15 mRNA in thymic and bone marrow stromal and epithelial cells suggests that IL-15 may play a role in the development of NK and T lymphocytes.

IL-15 stimulates the proliferation of CTLL lines; antigen-dependent T-cell clones; activated $CD4^-8^-$, $CD4^+8^+$, $CD4^+$, and $CD8^+$ cells; and dendritic epidermal T cells (6, 8, 87–91). Dendritic cells, a class of potent antigen-presenting cells, are producers of IL-15 and induce activation and chemotactic activity for Th1, the subset of helper T cells (92, 93). As IL-2 is not produced by dendritic cells, this observation suggests that IL-15 is involved in normal immune responses that are distinguishable from those involving IL-2. IL-15, like IL-2 and IL-7, appears to be involved in the development of thymic-independent gut intraepithelial lymphocytes that do not develop normally in IL-2/15Rβ- or IRF-1 deficient mice (35). IL-15 stimulates and augments the proliferation of T cells from HIV-positive individuals and of T cells derived from primary human tumor cell cultures (94, 95). Furthermore, IL-15 synergizes with IL-12 to induce proliferation of murine Th1 clones (96, 97). IL-15 addition also promotes the induction of cytotoxic lymphocyte effector cells and lymphokine-activated killer cells. The addition of IL-15 to T cells leads to the induction of their expression of IL-2Rα (CD25), IL-2Rβ (CD122), and Fas (CD95) whereas it downregulates CD27 expression (98–101). IL-15 is a chemoattractant for T cells but not B cells, monocytes, or neutrophils (25, 42). Finally, IL-15 inhibits cytokine-deprivation-induced apoptosis and apoptosis induced by anti-Fas, dexamethasone anti-CD3, or anti-IgM in activated T and B cells (102). In light of its antiapoptotic effect in CD4 cells, Kanegane & Tosato (103) have suggested that IL-15 acts as a memory-facilitating factor for helper T cells. Furthermore, IL-15 stimulates memory-phenotype $CD8^+$ cells in vivo (87).

Although IL-15 does not have an effect on resting B cells, it induces proliferation and immunoglobulin synthesis by human tonsilar B cells costimulated by PMA or by an immobilized antibody to immunoglobulin M (24). Furthermore, when used in concert with CD40 ligand (CD40L), it is an inducer of polyclonal IgM, IgG_1, and IgA but not IgG_4 or IgE (24). The effect of IL-15 on Ig secretion

can be modulated by IL-10 (increased secretion) or by IL-4 (decreased secretion) (104). The effect of IL-4 may be caused by its diversion of the shared γ_c receptor, a conclusion supported by fluorescence resonance energy transfer (FRET) analysis of receptor subunit association after cytokine addition (105). The action of IL-15 on T, B, and NK cells could be blocked by select antibodies (e.g., Mikβ1) to the IL-2/15Rβ chain (8).

IL-15 Plays a Pivotal Role in the Development, Survival, and Activation of NK Cells

NK cells are bone marrow–derived $CD2^+$, $CD16^+$, and $CD56^+$ human large granular lymphocytes (LGLs) that lack CD3 but express the ζ chain of the T-cell receptor. Observations suggest that IL-15 is important in the differentiation, survival, and function of NK cells, indeed that IL-15 may be essential for their development (26–39). For example, mice made deficient in IL-2/15Rβ by homologous recombination or through the use of an antibody to this receptor subunit are markedly deficient in NK cells (27). IL-2/15Rβ is required for the actions of IL-2 and IL-15 but is not used by other growth factors. In contrast, mice deficient in IL-2 or IL-2Rα, the private receptor used by IL-2, have a normal number of NK cells, suggesting that IL-15 may be required for NK cell development (27). Alternatively, the expression of either of these two cytokines might be sufficient for the maturation of NK cell progenitors. NK cells are also virtually absent in mice deficient in the signaling molecules required for IL-15 expression (e.g., there is no NK-cell development in IRF-$1^{-/-}$ mice) or in receptors or signaling molecules required for IL-15 action (35, 36). In particular, NK cells are markedly deficient in both human and mouse cells that do not express the normal γ_c chain used by IL-2, IL-4, IL-7, IL-9, and IL-15, whereas IL-2-, IL-4-, and IL-7- deficient mice express NK cells (106–108). Similarly, mice deficient in Jak3, which is required for IL-15 action, are also deficient in NK cells (109–110).

The role of cytokines in NK-cell development has been studied directly using in vitro stromal-independent cultures of hematopoietic precursors. IL-2 addition in the presence of other cytokines, such as IL-7 or stem cell factor, leads to NK-cell differentiation (29, 38). However, IL-15 is even more effective in inducing bone marrow progenitor differentiation into NK cells. Furthermore, it is the one factor capable of inducing $CD34^+$ $CD7^-$ bone marrow or cord blood cells to undergo such differentiation (29). In a similar way, the addition of IL-15 and to a lesser extent IL-2 to immature postnatal thymocytes or to fetal thymic organ cultures led to the development of NK cells that express the $CD3^-$, $CD56^+$, CD94 HLA Class I–specific inhibitory receptor phenotype (30). Nevertheless, in this study IL-15 alone was not sufficient for the induction of CD16 or the other HLA Class I–specific inhibitory receptors, implying that

other factors are required for the expression of these elements (30). A similar IL-15-mediated propagation of NK cells from their progenitors was observed in NK-cell-deficient IRF$^{-/-}$ mice (35). This latter observation supports the view that the defect in such mice is not in the NK-cell progenitors themselves but in the induction of the required IL-15 expression within radio-resistant bone marrow stromal cells. In various culture systems, stem cell factor, IL-7, and flt-3 ligand enhanced the IL-15-mediated expansion of NK cells (29). The data suggest that IL-15 is a dominant factor in the differentiation of NK cells from uncommitted progenitors.

In terms of NK function, resting NK cells express IL-15Rα, IL-2/15Rβ, and γ_c, which are required for a response to picamolar concentrations of IL-15 (33). IL-15 facilitates the survival of NK cells ex vivo (33). In particular, the addition of IL-15 to human blood leukocytes led to the survival of NK cells in the absence of serum for the eight-day observation period (33). This IL-15-supported survival was associated with the prevention of programmed cell death, an inhibitory action that required bcl-2 expression (33). IL-2 was modestly effective in the intradiction of cell death, whereas other cytokines that use γ_c, such as IL-4, IL-7, and IL-9, were not effective nor were the monocyte-derived factors TNF-α, IL-1β, IL-10, or IL-12. IL-15 was effective as an NK-cell chemoattractant and activator. Moreover, IL-15 synergized with IL-12 to stimulate the production by NK cells of IFN-γ, TNF-α, and GM-CSF (32). NK cytotoxicity mediated by IL-15 was induced by a variety of infectious agents such as herpesvirus 6 and herpesvirus 7 (31, 39). In the cases examined, the upregulation of NK activity by these infectious agents was markedly reduced by the addition of monoclonal antibodies to IL-15 but not by antibodies to other cytokines such as IFN-α, IFN-γ, TNF-α, TGF-β, or IL-2, suggesting that IL-15 secreted in response to the infectious agents was responsible for the observed NK-cell activation. Patients lacking NK cells are subject to multiple infections with herpesviruses (111). Moreover, NK activation induced by agents such as herpesvirus 6 and herpesvirus 7 was blocked by the addition of an antibody to IL-2/15Rβ that blocks the action of IL-15 (31, 39). This evidence supports the view that IL-15 plays a pivotal role in the development, survival, and activation of NK cells.

Role of IL-15 in the Host Defense Against Intracellular Pathogens

IL-15 as assessed by a specific ELISA assay or as an activity interpreted to be IL-15 based on inhibition by an anti-IL-15 antibody has been demonstrated in the supernatants of monocyte and macrophage preparations treated with various intracellular infectious agents (31, 32, 39, 53–57). IL-15 enhanced superoxide function and antifungal activity of human monocytes (56). Similarly,

IL-15 contributed to the anticryptococcal activity of macrophages (54). IL-15 induces IL-8 and monocyte chemotactic protein-1 production in human monocytes (112). Furthermore, although neither IL-12 nor IL-15 addition resulted in the induction of IFN-γ production by either NK cells or $\gamma\delta$ T cells, they acted synergistically on these cells, inducing the production of IFN-γ, TNF-α, and GM-CSF (53). Finally, IL-15 mRNA and protein were most highly expressed in patients with immunologically resistant tuberculoid leprosy but not in unresponsive and susceptible lepromatous patients (57). These data taken together with those demonstrating the constitutive expression of IL-15 mRNA in various tissues support our view that IL-15 may act as an "alarmin" wherein by maintaining a pool of translationally inactive IL-15 mRNA diverse cells, such as macrophages and dendritic cells, may respond rapidly to an intracellular pathogen by converting impeded IL-15 mRNA into an effectively translatable form (92, 93). Thus the IL-15 response to infectious agents, such as viruses and other intracellular organisms, may represent a critical element in the host defense against these pathogens.

IL-15 Action in Nonimmunological Cells

The broad tissue expression of mRNA encoding IL-15 and the IL-15Rα subunit suggests that IL-15 has activities beyond the immune system. An example of a nonimmunological action was observed in skeletal muscles that express IL-15 and IL-15Rα mRNA (40, 41). The addition of IL-15 to a cultured myoblast line did not induce proliferation but affected parameters associated with skeletal muscle fiber hypertrophy, especially when insulin-like growth factor levels were low, suggesting that IL-15 may be an anabolic agent that increases skeletal muscle mass (41). In another system unlike IL-2, IL-15 bound to vascular endothelial cells with a high affinity through the IL-15Rα subunit. Moreover, IL-15 promoted angiogenesis in a murine system with the induction of neovascularization of Matregel plugs after IL-15 addition (113). Mouse brain microglia and human fetal astrocytes and microglia express IL-15 mRNA and its trimeric receptor complex functionally coupled to Jak kinase activity (114, 115). The levels of IL-15 mRNA increased upon addition of IL-1β, IFN-γ, or TNF-α. IL-15 affected the functional properties of microglia such as their production of nitric oxide and their growth in culture. Thus IL-15 may participate in certain central nervous system and neuroendocrine functions previously ascribed to IL-2, which is expressed in only very minute concentrations in the central nervous system.

As discussed above, mast cells express a type-2 IL-15 receptor and signaling pathway using Jak2 that is distinct from the trimeric receptors and signaling pathways (Jak1, Jak3) used by both IL-2 and IL-15 in activated T cells (22). IL-15, but not IL-2, stimulates mast cell proliferation in vitro and ex vivo.

Moreover, the intermediate affinity type-2 IL-15 receptor may have a functional role in mast cell biology in that IL-15 is a mast cell growth and activation factor. Intestinal epithelial cells both express and respond to IL-15. IL-15 signals in T84 colonic epithelial cells in the absence of the IL-2/15Rβ chain, suggesting that IL-15 uses a receptor in these cells other than the classical type-1 trimeric IL-15 receptor (116). Finally, as noted above, in contrast to most cytokines, intracellular cytoplasmic and nuclear forms of IL-15 have been demonstrated. Such intracellular IL-15 may play a novel and as yet undefined role within the cells that produce it.

ABNORMALITIES OF IL-15 EXPRESSION IN DISEASE

Abnormalities of IL-15 in Inflammatory Autoimmune Diseases

Feldmann and coworkers have proposed that TNF-α is at the apex of a cytokine cascade that includes IL-1β, IL-6, GM-CSF, and a series of inflammatory chemokines, including Mip1α, Mip1β, and IL-8, that are intimately involved in the development and progression of rheumatoid arthritis (RA) (117). McInnes and coworkers have reported abnormalities of IL-15 in this disease and have suggested that IL-15 may precede TNF-α in the cytokine cascade (42, 43). In particular, IL-15-activated T cells can induce TNF synthesis by macrophages in RA via a cell-contact-dependent mechanism (43). They reported the presence of high concentrations of IL-15 in RA synovial fluid and showed that IL-15 is expressed by synovial-membrane-lining cells. Nevertheless, the presence of rheumatoid factor in the fluids may yield specious high estimates for IL-15 assessed by an ELISA. RA synovial fluids contain chemotactic and T-cell stimulatory activities attributable in part to IL-15. Oppenheimer-Marks and coworkers (118) demonstrated that IL-15 is produced by endothelial cells in rheumatoid tissues and that this cytokine markedly increases transendothelial migration of both CD4 and CD8 cells. Furthermore, they showed that IL-15 leads to T-cell accumulation in RA synovial tissues engrafted into severe combined immune deficiency (SCID) mice in vivo. In a parallel murine model the intra-articular injection of IL-15 induced a local tissue inflammatory infiltrate consisting predominantly of T lymphocytes (42). These data suggest that IL-15 can recruit and activate T cells into the synovial membrane, possibly contributing to the pathogenesis of RA. In support of this view, the injection of an IL-15 antagonist, the soluble form of IL-15Rα, into DBA/1 mice suppressed their development of collagen-induced arthritis (119). In summary, these reports suggest a role for IL-15 in the development of inflammatory RA and imply that antagonists to IL-15 action may have therapeutic potential in this disease.

Abnormalities of IL-15 have also been reported in other inflammatory disorders. For example, increased numbers of IL-15-expressing cells are present in the circulation of patients with active ulcerative colitis, or Crohn's disease (119). Furthermore, elevated levels of IL-15 correlated with disease activity and may reflect the degree of inflammation in the liver in type-C chronic liver disease (120). In addition, IL-15 triggers the growth of T cells in sarcoidosis through the IL-2/15Rβ/γ_c complex and may deliver proliferative signals leading to the development of the T-cell alveolitis observed in this disorder (121). Furthermore, IL-15 mRNA expression is upregulated in blood and cerebrospinal fluid mononuclear cells in multiple sclerosis (122). Finally, the observation that IL-15 stimulates mast cell proliferation suggests a potential role for this cytokine in mastocytosis (22).

IL-15 Action in Retroviral Diseases and Neoplasia

HTLV-I infects CD4 T cells and is associated with a series of diseases including ATL, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), arthritis, uveitis, inflammatory lung disease, and infectious dermatitis (123–125). HTLV-I-infected T-cells of patients with HAM/TSP expressed the HTLV-I-encoded transactivator $p40^{tax}$ and exhibited abnormal spontaneous proliferation when studied ex vivo (124). This T-cell proliferation could be inhibited partially by the addition of an antibody to IL-2 or to the IL-2-specific receptor, IL-2Rα, suggesting a role for IL-2 in this process (125). Nevertheless, the addition of Mikβ1, an antibody to the IL-2/15Rβ chain, also reduced this proliferation (125). This antibody does not inhibit the action of IL-2 on the high-affinity IL-2R but does inhibit IL-15 function, an observation that suggests a role for IL-15 in the abnormal T-cell proliferation observed in HAM/TSP. IL-15 mRNA expression is increased in HTLV-I-infected T-cells (45). By using reporter constructs bearing the 5′ regulatory region of the IL-15 gene, we observed a positive correlation between HTLV-I tax protein expression and IL-15 promoter activity in HTLV-I-infected T cells (45). We observed increased IL-15 mRNA expression not only in HAM/TSP but also in T-cell lines and ex vivo leukemic cells of patients with HTLV-I-associated ATL. Furthermore, IL-15 can replace the IL-2 signal in IL-15Rα-expressing IL-2-dependent ATL cell lines (126). Thus the production of IL-15 in this leukemia might be one factor in the constitutive activation of Jak3 observed with select lines and cells from patients with ATL (127, 128).

The production of IL-15 by the HTLV-I-associated ATL cell line HuT-102 that permitted the identification of IL-15 was especially dramatic. This phenomenon is explained by our observation that IL-15 production by the ATL line HuT-102 is associated with a human T-cell lymphotropic virus I R region/IL-15 fusion message (15). In particular, the predominant IL-15 mRNA expressed by HuT-102 cells is a chimeric RNA with a 118-nt segment of the R region

of the long terminal repeat (LTR) of HuT-102 joined to the 5′ UTR of IL-15. Normally by alternative splicing this 118-nt element of R represents the most 5′ region of several HTLV-I transcripts including those encoding tax, rex, and env. However, in HuT-102 this element derived from the R region is aberrantly spliced to the 5′ UTR of IL-15. The high-level expression of IL-15 mRNA by HuT-102 appears to result from the transcription of a large quantity of a fusion message with the IL-15 allele under the regulatory control of the HTLV-I LTR element. In addition, the introduction of the R segment eliminates over 200 nt of the IL-15 5′ UTR, including all but two of the upstream AUGs that, as indicated by our other studies, appear to behave as impediments to translation (15). Thus the effective synthesis of IL-15 protein by the ATL cell line HuT-102 appears to involve a marked increase in IL-15 mRNA transcription and translation secondary to the integration of HTLV-I provirus with a consequent production of a fusion message involving the HTLV-I R segment linked to the truncated 5′ UTR of IL-15.

IL-15 serum levels are elevated significantly in HIV-I-infected individuals (129). This cytokine enhances immune functions during HIV infection (130). Moreover, a positive correlation exists between IL-15 and serum immunoglobulin levels in this disorder, suggesting that this B-cell costimulatory cytokine may contribute to the pathogenesis of HIV-associated hypergammaglobulinemia (129).

A series of tumor cell lines have been evaluated for the expression of IL-15 mRNA by RT-PCR analysis. Several of these cell lines, including lung, ovarian, melanoma, some leukemia, osteosarcoma, and especially rhabdomyosarcoma cell lines, expressed IL-15 mRNA (19, 131). In select situations Barzegar et al (131) showed that this mRNA represented the short signal peptide IL-15 mRNA isoform. Moreover, in most cases it was difficult to demonstrate IL-15 in the culture supernatants. If IL-15 plays a role in these neoplasias, it might be through its intracellular action (131).

OPPORTUNITIES FOR THERAPY DIRECTED TOWARD IL-15, ITS RECEPTOR, OR ITS SIGNALING TRANSDUCTION SYSTEM

IL-2 is effective in the treatment of renal cell carcinoma and malignant melanoma, and in the therapy of patients with AIDS. In parallel, IL-15 was shown to help correct the impaired proliferative response of $CD4^+$ lymphocytes from HIV-I-infected individuals without the mitogenic effect of IL-2 that might also induce HIV expression (130). Thus IL-15 could provide an alternative therapeutic option in the treatment of patients with select tumors or AIDS.

The majority of therapeutic efforts involving the IL-15/IL-15R system are being directed toward inhibiting IL-15 action. The scientific basis for this approach

was discussed in part above where it was suggested that IL-15 might contribute to the pathogenesis of RA, inflammatory bowel disease, and sarcoidosis. Furthermore, select malignant cells, for example, multiple myeloma and large granular lymphocytic leukemia cells, express IL-15 receptors (132). In addition, intragraft IL-15 transcripts were increased in patients rejecting renal allografts (133). A correlation existed between IL-15 transcripts within grafts being rejected as compared to nonrejected renal allografts, suggesting that IL-15 may play a role in T- and NK-cell-mediated rejection (130). Moreover, IL-15 transcripts were present in the allografts in association with rejection of pancreatic islet allografts in wild-type mice and in IL-2 knockout mice, again suggesting that the IL-15/IL-15Rα system may be a valuable therapeutic target in organ transplantation protocols (134). Blocking the IL-15Rα with a receptor antagonist enhanced acceptance of islet cell allografts (135).

Most IL-2/15 receptor-directed therapeutic approaches have targeted the IL-2Rα chain (136–138). To exploit the difference in IL-2Rα expression between normal resting cells that do not express this receptor and IL-2Rα-expressing abnormal T cells in leukemia, select autoimmune disorders, and allograft rejection, clinical trials have been performed using unmodified murine anti-IL-2Rα, humanized antibodies, and antibodies armed with toxins and α- and β-emitting radionuclides (136–138). On the basis of two extensive randomized placebo-controlled trials, the humanized anti-IL-2Rα monoclonal antibody (Zenapax) received marketing approval by the FDA for the therapy of patients receiving renal allografts (139). Although IL-2Rα-directed therapy has met with considerable success, approaches directed toward this receptor subunit have limitations. In particular, antibodies to IL-2Rα do not inhibit the action of IL-15, a cytokine that does not use this subunit. Therefore, a number of approaches are being developed that focus on the IL-15 receptor and its signaling pathway. A diphtheria toxin IL-15 fusion protein DAB_{389} sIL-15 has been constructed that is directed toward the cytotoxic elimination of IL-15R-expressing cells (48). However, most approaches have been directed toward inhibiting IL-15 action. As noted above, the administration of the IL-15 inhibitor, the soluble IL-15Rα chain, prevented the development of murine collagen-induced arthritis (46). Furthermore, an IL-15 receptor antagonist produced by mutating glutamine residues within the C-terminus of IL-15 to aspartic acid competitively inhibited IL-15-triggered cell proliferation (135). This IL-15 mutant protein markedly attenuated antigen-specific delayed hypersensitivity responses in BALB/c mice and enhanced the acceptance of islet cell allografts (135).

Our own therapeutic approaches have focused on the cytokine receptor subunits and signaling pathways shared among multiple cytokines, including IL-15, in an effort to yield more profound immunosuppression than can be achieved by inhibition of the synthesis or action of a single cytokine such as IL-2 or by an antibody directed toward a private receptor subunit such as IL-2Rα that

binds only a single cytokine. Our initial trials used Mikβ1, an antibody directed toward IL-2/15Rβ that is shared by IL-2 and IL-15. A humanized version of this antibody prolongs renal allograft survival in cynomolgus monkeys (140). In our initial clinical trial, we evaluated this antibody in the therapy of patients with T-cell-type large granular lymphocytic leukemia associated with hematocytopenias. The monoclonal LGLs involved in this disease express IL-2/15Rβ and γ_c but not IL-2Rα (141, 142). These cells respond by proliferation and cytokine induction to the IL-15 produced by associated monocytes (132).

Additional therapeutic efforts focusing on the IL-15/IL-15R system are directed toward the development of an inhibitor of Jak3, the signaling molecule used by IL-2, IL-4, IL-7, and IL-15 as an agent to yield controlled immunosuppression. Deficiency of Jak3 in the autosomal form of severe combined immunodeficiency disease (SCID) in humans or in mice made deficient in Jak3 by homologous recombination exhibit a lack of NK cells and T- and B-cell abnormalities but do not develop disorders in nonimmunological systems, suggesting that Jak3 is a rational target to yield a controlled immunosuppression of value in the treatment of autoimmune diseases or in the prevention of allograft rejection (109, 110). In addition, Jak3 is activated constitutively in select leukemias such as HTLV-I-associated ATL cell lines (127–128). These observations suggest that drugs that inhibit Jak3 activation may be of value as immunosuppressive and antileukemic agents. In summary, our present understanding of the IL-15/IL-15R system and its signaling pathways opens new possibilities for more specific immune intervention.

CONCLUSIONS AND FUTURE DIRECTIONS

IL-15 is a 14–15 kDa member of the 4 α-helix bundle family of cytokines. In contrast to the regulation of IL-2, which is controlled at the level of message transcription and stabilization, the regulation of IL-15 is much more complex with multifaceted controls at the levels of message transcription, message translation, and protein translocation and secretion. IL-15 is regulated in part at the level of transcription induced in association with infection of monocytes by intracellular pathogens. This upregulation of IL-15 mRNA expression involves both an IRF-1/IRF-E response element and an NF-κB signaling pathway. Nevertheless, IL-15 is controlled predominantly at the level of translation and translocation. IL-15 mRNA includes a number of elements that impede its translation. In particular, the 5′ AUGs of human IL-15 mRNA are burdened with 12 upstream AUGs that interfere with effective IL-15 translation. Furthermore, the 48-aa signal peptide and a *cis*-acting negative regulatory element in the C-terminus of the mature protein impede translation. The removal of these

negative control mechanisms in an integrated fashion may give rise to a major increase in IL-15 synthesis. The broad array of negative regulatory features controlling IL-15 expression may be required because of the potency of IL-15 in inducing the expression of TNF-α, IL-1, IFN-γ, and other inflammatory cytokines and chemokines that if indiscriminantly expressed would be associated with serious disorders such as autoimmune inflammatory diseases. In terms of a more positive role for IL-15, we propose that by maintaining a pool of translationally inactive IL-15 mRNA, diverse cells might respond rapidly to an intracellular infection by transforming IL-15 mRNA into a transcript that can be effectively translated.

Despite our progress in understanding the multifaceted control of IL-15 translation, many questions must be answered before we can understand the molecular mechanisms underlying this translational control. There is considerable precedence for an impediment to translation manifested by AUGs upstream of an authentic initiation codon as seen with IL-15. Some of these transcripts may not use CAP-dependent scanning mechanisms to initiate translation; rather they may recruit ribosomes to an internal ribosome entry site (IRES), bypassing the impediments associated with the upstream 5′ UTRs. Infections by intracellular organisms may lead to the induction of specific cytoplasmic proteins that bind to such a putative IRES, thereby facilitating translation. The mechanisms underlying the signal peptide coding sequence– or mature protein coding sequence–mediated regulations of IL-15 translation also have not been defined. A translational activator such as a specific RNA-binding protein might have to be produced to facilitate chain elongation and translocation. A more likely possibility given the effective translation of IL-15 mRNAs in the wheat-germ but not mammalian systems is that a mammalian translational repressor or a stable secondary structure in the IL-15 mRNA might be present that until released prevents efficient elongation of the translated message or translocation of the protein generated. Precedence for a required release from translational repressors before effective translation is possible is provided by transcripts for ferritin, erythroid 5-aminolevulinate synthase, and thymidylate synthase.

IL-15 uses two distinct receptor and signaling pathways. In T and NK cells the type-1 IL-15 receptor includes IL-2/15Rβ and γ_c subunits, which are shared with IL-2, and an IL-15-specific receptor subunit, IL-15Rα. However, mast cells respond to IL-15 using another receptor system (type-2) that does not share elements with the IL-2R system but uses a novel 60- to 65-kDa IL-15RX element. IL-15 signaling involves activation of Jak1 and Jak3 as well as STAT3 and STAT5 in T and NK cells, whereas in mast cells IL-15-signaling through its specific IL-15RX receptor leads to Jak2 and STAT5 activation. The future molecular cloning of the gene encoding IL-15RX, the type-2 IL-15 receptor,

would be of value in defining the tissue distribution of this receptor, and in delineating its involvement in mast cell and other cell biology.

The IL-15 signal peptides are not only involved in the regulation of IL-15 translation but also direct its intracellular trafficking. Two isoforms of human IL-15 exist: one with a short (21-aa) signal peptide and another with a longer (48-aa) signal peptide. The IL-15 linked to the short signal peptide and some of that associated with the longer signal peptide is not secreted but is stored intracellularly, appearing in the nucleus and cytoplasmic components. Production of an intracellular lymphokine is not typical of other soluble interleukin systems. The possibility that IL-15 has biological functions as an intracellular molecule should be explored. IL-15 and IL-2 share some biological activities including the induction of T-cell proliferation, the activation of cytotoxic effector cells, the costimulation of immunoglobulin synthesis by B cells, and the activation of monocytes. In addition, IL-15 appears to play pivotal roles in the differentiation of NK cells from their progenitors, the maintenance of their survival, and their activation. Furthermore, IL-15 acts on an array of nonimmunological cells including mast cells, skeletal muscle cells, and microglia. The generation of an IL-15 knockout mouse that is now under way should assist in the definition of the unique nonredundant IL-15 functions.

Abnormalities of IL-15 expression caused by P40 tax–mediated transactivation of IL-15 have been demonstrated in abnormal T cells in HTLV-I-associated ATL and in TSP/HSM. Abnormalities of IL-15 expression may also be involved in the pathogenesis of inflammatory autoimmune disorders such as RA and inflammatory bowel disease. The clinical application of new therapeutic agents that target IL-15 or the receptor and signaling elements shared by IL-2 and IL-15 may provide a new perspective for the treatment of such disorders.

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