IL-15: a relevant cytokine for lymphoid homeostasis and autoimmune diseases

REVIEW

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ABSTRACT

IL-15 is involved in broad effector functions of the immune response in innate immunity mediated by macrophages, neutrophils and NK cells and in acquired immunity mediated by antigen-activated T and B lymphocytes. Also, IL-15 is an important apoptosis inhibitor and acts on other non immune cells. The deregulated expression of IL-15 has been related to autoimmune and inflammatory diseases such as Rheumatoid arthritis, Multiple sclerosis, Celiac disease, Psoriasis, Sarcoidosis, and Hepatitis C. This suggests that an IL-15 antagonist could be useful for the treatment of these diseases. Recently, it was proposed that IL-15 might be superior to IL-2 in the treatment of cancer and as a component of vaccines directed against cancer or infectious agents because in contrast to IL-2, IL-15 inhibits activation-induced cell death (AICD) and promotes the persistence of memory T $CD8^+$ cells.

Keywords: interleukin-15, cytokine, IL-15 receptor, antagonist, autoimmune diseases

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RESUMEN

Interleucina-15: una citocina relevante en la respuesta inmune. La interleucina-15 (IL-15) es una citocina que está involucrada en el desarrollo y mantenimiento de células efectoras, las cuales median los mecanismos de defensa del hospedero. Se plantea que actúa en la inmunidad innata, mediada fundamentalmente por neutrófilos, monocitos/macrófagos y por células asesinas naturales (NK), y en la inmunidad adaptativa, mediada por linfocitos T y B activados por antígenos. Realiza otras funciones importantes como la de inhibir la apoptosis y actúa sobre células que no pertenecen al sistema inmunitario. La expresión incontrolada de la IL-15 se relaciona con enfermedades autoinmunes e inflamatorias como la artritis reumatoide, la esclerosis múltiple, la enfermedad celiaca, la soriasis, la sarcoidosis y la hepatitis C. Por ello, se ha sugerido el empleo de antagonistas de esta citocina en el tratamiento de esas enfermedades. Recientemente se propuso que la IL-15 pueda ser superior a la IL-2 en el tratamiento del cáncer, y como un componente en vacunas contra el cáncer y las enfermedades infecciosas. Esos criterios se basan en la función de la IL-15 en la inhibición de la muerte celular, inducida por la activación mediada por la IL-2 y en que facilita la persistencia de células T $CD8^+$ de memoria.

Palabras claves: interleucina-15, citocina, receptor de la IL-15, antagonistas, enfermedades autoinmunes

*I*ntroduction

Interleukin-15 (IL-15) was first described in 1994 simultaneously by two different research teams [1, 2]. It was characterized as a soluble factor in culture supernatants from the HuT-102 and CV-1/EBNA cell lines which stimulates the proliferation of the cytokine-dependant CTLL-2 murine T-cell line.

This cytokine is a 114-aa protein having an apparent molecular weight of 14 to 15 kDa, with two N-linked glycosylation sites (N79 and N112) at the C-terminal region. The mature polypeptide is classified as belonging to the type I cytokine family, which has a common structure defined by 4 antiparallel alpha-helices [3] and has members such as IL-2, IL-4, IL-7, IL-9, IL-13 and IL-21. Interleukin-15 produces its biological effects, whether on other cells or in an autocrine loop, by interacting with the trimeric receptor IL-15R$\alpha\beta\gamma$, formed by a α subunit unique to this receptor, a β subunit shared with the IL-2 receptor, and a γ subunit, also found in the receptors for several cytokines of this family.

The gene for human IL-15 was mapped to chromosome 4q31 [4]. Its cDNA contains 316 nucleotides on the 5' untranslated region (UTR), a coding region of 486 nucleotides, and 400 nucleotides on the 3' UTR.

The messenger RNA (mRNA) for this cytokine is ubiquitously expressed. Two isoforms for this mRNA, expressing the same mature protein but with different secretion signals, have been described [5]: One containing exon A, which introduces a stop codon and a 21 aa-long signal peptide (SSP), and another one without this exon, coding for a longer signal peptide (LSP) of 48 amino acids (Figure 1).

In a deep contrast with the wide distribution of this mRNA, its translation and the intracellular trafficking of the protein it codes for are strongly regulated. There are several putative regulatory elements in the mRNA, including 10 AUGs in the 5'UTR before the actual initiation codon, a complex secondary structure, and negative regulatory sequences within the coding region. Another regulatory element is the use of an unusually long (48 aa) signal peptide (SP), when compared with the conventional SP found in most other secreted proteins [5]. There are two hydrophobic sequences within this SP which are predicted to decrease the efficiency of translocation to the endoplasmic reticulum (ER), and the sequences surrounding its initiation codon have very low similarity to the Kozak consensus, limiting the rate of translational initiation [6].

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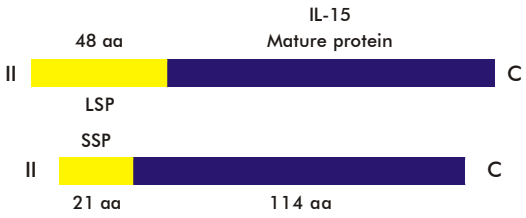

Figure 1. Isoforms of mRNA codifying for different signal peptides LSP and SSP but giving place to the same mature protein.

The LSP isoform (LSP-IL-15) of the protein enters the classical secretion pathway through the ER, after which the protein is glycosylated, the signal peptide is cleaved off, and the molecule is finally secreted to the extracellular milieu. However, the SSP isoform (21 aa signal peptide) never enters the ER and is not secreted, and located instead in the nuclear and cytoplasmic compartments. It has recently been reported that this intracellular IL-15 forms a complex with the α subunit of the IL-15 receptor (IL-15Rα) which, upon translocation to the nucleus, can inhibit the transcriptional activation of the IL-15 gene in response to LPS, and can also interfere with the binding of transcriptional factors such as IRF-3 to the IL-15 promoter [7] (Figure 2).

Another opportunity for regulation is afforded by the recently described alternative processing undergone by LSP-IL-15 after secretion to the ER and N-glycosylation, in which only 29 aa from the signal peptide are removed, as observed in experiments with LSP-GFP fusions [8]. This species is not detected in culture supernatants, and is probably retained in vesicles and later secreted or released to the intracellular compartment.

The regulatory role of the signal peptide for IL-15 has also been examined in experiments in which it was replaced by the SP from CD-33 or IL-2 [9], resulting in an increase both in the translation and secretion of the protein. These results confirmed that the levels of IL-15 are regulated mainly at the translational and secretory stages, underlining the main role played by signal peptides in the regulation and targeting of intracellular trafficking between different compartments (ER, cytoplasm, nucleus).

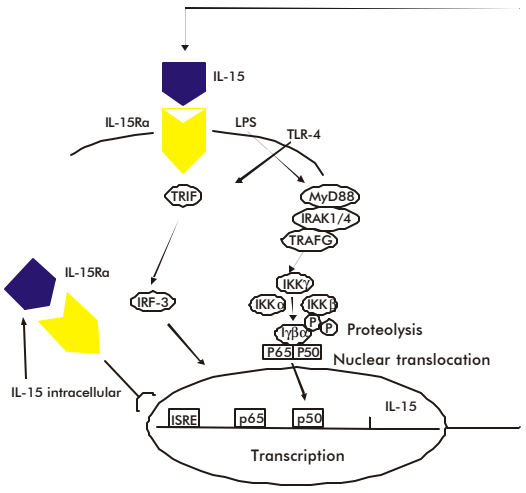

Figure 2. Hypothesis of the self-regulating mechanism of the transcriptional activation of the IL-15 gene by the intracellular complex IL-15/IL-15Rα .

In addition to the secreted and intracellular forms of IL-15, this molecule has also been found as a peripheral membrane protein in human monocytes and monocyte-derived cell lines [10]. Later work showed that the membrane-associated IL-15 is part of a high-affinity complex between this molecule and the α-chain of its receptor (IL-15Rα), which is recycled from the endosome to the cell membrane in order to maintain the steady-state levels of this cytokine [11]. However, the membrane association of IL-15 not only involves this mechanism, since a recent report has shown that IL-15 can also be found as an integral membrane protein in the cellular surface of IFNγ-activated monocytes and in the invasive prostate carcinoma cell line PC-3. In this state, IL-15 can trigger an inverse signaling event by interacting with IL-15Rα or with an anti-IL-15 antibody. This signaling involves members of the MAPK kinase family (ERK and p38) and FAK (Focal adhesion kinase), and induces the synthesis of pro-inflammatory cytokines such as IL-6, IL-8 and TNFα.

The expression of IL-15 as an integral membrane protein does not depend on the existence of subunits of the IL-15 receptor, since there is no expression of either subunit on the PC-3 cell line; and the membrane levels of IL-15 remain constant even after the treatment with acid pH or trypsin. The authors found both membrane association forms (peripherally associated to IL-15Rα and as an integral membrane protein) in IFNγ-activated monocytes, which adds a new level of complexity to the biology of IL-15 due to its potential for behaving as either a ligand or a receptor, thus triggering different signaling pathways [12].

There are few reports in the literature on the production of IL-15 by recombinant DNA technology. Our group has obtained high expression levels in the bacterium *Escherichia coli* for the mature, biologically active form of human IL-15 [13].

The IL-15 receptor

The first studies on the binding of IL-15 [I^{125}] to its receptor on a number of different cell lines evidenced the presence of high affinity (Kd 10 to 80 pM) and intermediate affinity (Kd 0.27 to 2.5 nM) interactions. Using functional studies, it was shown that IL-15 interacts physically with IL-2Rβ and IL-2Rγ, and that both subunits are essential for IL-15 signaling [2, 14]. Both subunits (IL-2Rβ and IL-2Rγ_c) belong to a superfamily of cytokine receptors. IL-2/IL-15Rβ is shared by IL-2 and IL-15, and the γ_c subunit is used for the receptors of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. The IL-2Rβ-γ_c heterodimer associates with Jak1 and Jak3, activating STAT3 and STAT5. The events triggered after ligand binding include the activation of nuclear proteins such as Myc and Fos, and the induction of Bcl-2 and Bcl-X, leading to an increase in survival or proliferation [15].

The observation that murine T-cells proliferate in response to IL-15, but simian-derived IL-15 does not

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bind the murine IL-2Rβγ complex, led to the identification of the IL-15Rα subunit, which is specific for this cytokine [16]. IL-15Rα is structurally related to the a subunit of the IL-2 receptor (IL-2Ra). Both molecules have a similar ligand binding domain (a sushi domain, common in protein-protein interactions), and relatively short intracellular regions (13 aa for IL-2Rα, 41 aa for IL-15Rα). Additionally, the IL-15Rα locus has been mapped to human chromosome 10, bands p14-p15, which is the same region containing the IL-2Rα locus. Unlike IL-2Rα, however, IL-15Rα is widely expressed in different cell types and displays high affinity towards IL-15 in the order of 10^{-11} M, which is similar to that exhibited by the trimeric IL-15Rαβγ receptor (Figure 3) [17].

A total of 8 isoforms have been described for IL-15Rα [18]. This subunit acts as a high-affinity specific receptor for IL-15, and since the complex (once formed) can function in *cis* or *trans* orientation, its components can be shared by adjacent cells. For example, IL-15Rα-associated IL-15 on the cell surface can trans-stimulate neighboring cells carrying only IL-15Rβγ through cell-cell interactions, extending the effect of the cytokine even after it has been depleted from the milieu by transendosomal recycling (Figure 4) [11]. IL-15Rα is also required for the cellular expression of IL-15, as recently evidenced using chimaeras combining IL-15-deficient and IL-15Rα-deficient mice, and controls *trans*-presentation to NK and memory CD8+ cells. The latter suggests that this is the actual physiological mechanism through which IL-15 modulates lymphoid homeostasis [19].

IL-15Rα is an effective mediator of cellular signaling in spite of its short intracytoplasmic domain. During experiments in activated B cells and Raji cells, it has been observed that IL-15Rα coprecipitates with the Syk kinase, induces cellular proliferation, and rescues from ceramide-C2-induced apoptosis [20]. The binding of IL-15 to IL-15Rα triggers the phos-phorylation of STAT3, STAT5, STAT6, Jak2 and Syk in mast cells,

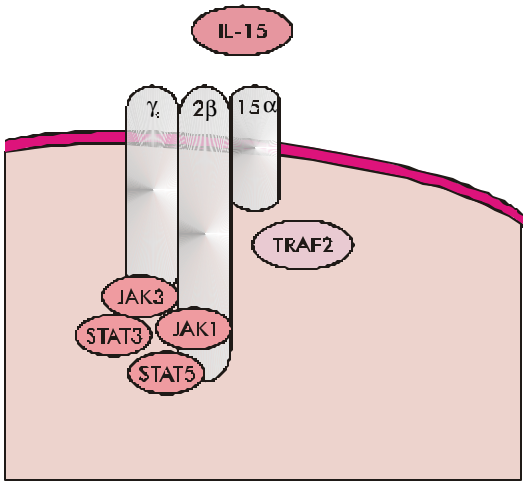
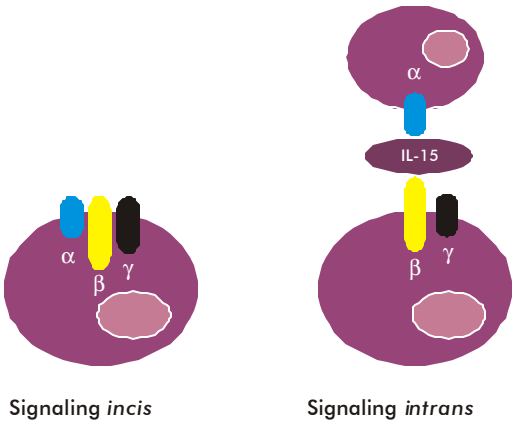


Figure 3. Trimeric receptor for IL-15 formed by IL-15Rα, IL-2/IL-15Rβ and the common γ sub-unit. Jaks 1 and 3 associated to sub-units β and γ respectivelyand TRAF2 associated to IL-15Rα.



Signalization in cis by the binding of IL-15 to a cell with the 3 receptor units and signalization in trans by the binding of the IL-15 associated to the IL-15Rα to the receptor of intermediate affinity β γ in a neighbor cell.

and is able to promote the proliferation of BA/F3 cells transfected with IL-15Rα [21, 22].

It has been reported that IL-15Rα can also be released from the cellular surface using a metalloproteinase-dependent mechanism and may be found as a soluble molecule in the extracellular medium. In this state, the protein retains its high affinity binding to IL-15 and is therefore capable of inhibiting its biological activity even at very low concentrations [23, 24].

The structure of IL-15 complexed to its receptor has not been determined, but its homology with a cytokine family that shares a well known structure of 4 anti-parallel alpha helices, and particularly with IL-2, has allowed the creation of structural models that predict residues Asp 8 and Gln 108 of the mature polypeptide as key amino acids that bind to subunits β and γ of the receptor, respectively. The same data have also been used to suggest the possible involvement of the sequence ^{21}IQATLYTESQVHP^{33} (numbered according to the sequence of the mature polypeptide) in the interaction with the α subunit of the receptor, by analogy with the binding region of IL-2 to the equivalent subunit on the IL-2 receptor [25]. On the other hand, a more recent study that mapped regions of IL-15 that interact with antibodies or the α subunit of the receptor and confirmed its findings by site-directed mutagenesis, identified sequences ^{44}LLELQVISL^{52} and ^{64}ENLII^{68} as possibly involved in binding to IL-15Rα [26].

In regard to the receptor, the latest data obtained from the elucidation by nuclear magnetic resonance (NMR) of the structure of the sushi domain (ligand binding domain) of IL-15Rα has been used to develop a model of the interaction of this molecule with IL-15 that involves a large network of ionic interactions, where most acid residues are contributed by IL-15 and most basic amino acids belong to IL-15Rα. According to the model, this type of interaction, (which has not been observed for other cytokine-receptor pairs) would explain the high affinity of the IL-15/IL-15Rα complex [27].

Functions of IL-15

IL-15 is involved in the development and maintenance of effector cells that constitute mediators for defense

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mechanisms of the host. Several lines of evidence support the notion that IL-15 is synthesized in response to infections with several different microorganisms [28-33], and it is known that it is involved in innate immunity, mainly in mechanisms involving phagocytes (neutrophils and monocyte/macrophages), as well as in "primitive immunity" (mediated by NK, NK+αβT, Tγδ and BCD5+ cells) and the adaptive immune response, mediated by antigen-activated T- and B-lymphocytes (Figure 5). It has also been shown that IL-15 promotes the maturation into highly lytic, phenotypically mature NK cells [34] of several parent populations, such as cord blood $CD34^+$ cells [35], adult $CD34^+$ bone marrow (BM) cells [36] and T/NK thymocytes [37].

There have also been efforts to elucidate the physiological roles of this cytokine using knock-out mice. Mice lacking IL-15Rα (IL-15Rα-/-), show multiple defects in effector cells involved in innate immunity, including the absence of splenic NK cells and of NK cytotoxic activity [38]. Confirming the above, IL-15-deficient mice (IL-15-/-) lack cells with an NK phenotype or function in the spleen and liver; an effect that can be reversed by the exogenous administration of IL-15 for 1 week [39].

The exogenous administration of IL-15 to normal mice increases the percentage and absolute numbers of splenic NK cells [40], and the population of NK cells is expanded in transgenic mice over-expressing murine IL-15 [41]. This *in vivo* evidence proves that IL-15 is a critical, non-redundant cytokine for the development of NK cells.

Another effect of IL-15 on NK cells is the induction of the synthesis of other cytokines and chemo-kines, and it constitutes a potent stimulus for the production of GM-CSF. Besides, its combination with IL-12 induces macrophage-activating factors such as IFNγ and TNFα [42, 43].

IL-15 also has dose-dependent effects on macrophages, inducing the production of other cytokines. Human monocytes treated with IL-15 (10 to 1000 ng/mL) synthesize IL-8 and monocyte chemo-attractant protein-1 (MCP-1), which then act on neutrophils and monocytes [10]. When used at extremely low concentrations, IL-15 suppresses the production of pro-inflammatory cytokines (TNFα, IL-6, IL-1), where as its use at high concentrations increases the production of these mediators [44].

Human neutrophils are activated by IL-15, whose effects include morphological changes and increases in phagocytosis, as well as RNA and protein synthesis [45].

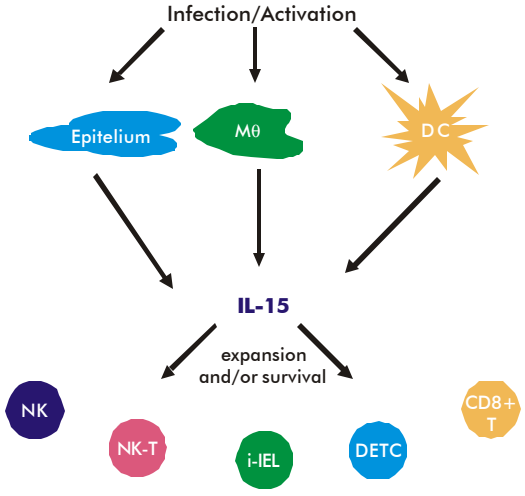
The targets for the effects of IL-15 also include other cells involved in the innate immunity response. IL-15 is essential for the growth and survival of dendritic epidermal γδT cells (DETC) after activation, and may be important in their selective localization to the skin [46]. The intestinal intraepithelial lymphocytes (TCRγδ-i-IEL) proliferate after the treatment with IL-15, which also protects them from apoptosis through positive regulation of Bcl2 [47]; and there is a two-fold reduction in the numbers of intraepithelial lymphocytes in IL-15Rα- and IL-15-deficient mice. IL-15 induces the proliferation of murine NKT cells, and their number is severely reduced in IL-2/15Rβ -/-, IRF-1 -/-, IL-15Rα -/-, and IL-15-/- mice [38, 39, 48, 49].

The expression of IL-15 by antigen-presenting cells (APC) is important during the early activation of T-cells in inflammation sites, right after binding to the T-cell receptor (TCR). Stimulation with IL-15 induces several activating antigens, such as IL-2Rα (CD25), IL-2/15Rβ (CD122), FasL (CD95), CD30, TNFRII, CD40L, CD69, and CD94/NKG2A [50-53]. Additionally, it is a potent chemo-attractant for T-cells [54], and its physiological role in the regulation of T-cell trafficking has been confirmed in IL-15Rα -/- mice, which are defective in the migration of T cells to peripheral lymph nodes.

Another role of IL-15 in the adaptive immune response is highlighted by the fact that it stimulates the proliferation of memory CD4 and CD8 (CD45RO+), and naïve CD8 (CD45RO) T-cells, with no effect on naïve CD4 cells [50]. The IL-2/IL-15Rβ receptor is highly expressed in $CD8^+CD44^{hi}$ memory T cells, and selectively stimulates this type of cells both *in vitro* and *in vivo* [55]. There is a selective deficit of $CD8^+$T cells in the thymus and periphery of IL-15Rα-/- mice; and the number of $CD8^+$ T cells with a memory phenotype in spleen and lymph nodes is reduced in IL-15-/- mice, an effect that can be reversed by the exogenous delivery of IL-15. Since the number of CD8 thymocytes in these mice is normal, it appears that IL-15 has a critical function in the expansion and survival of $CD8^+$ cells, rather than in their development.

IL-15 favors the increase in cytotoxic T lymphocyte and lymphokine-activated killer cell (LAK) numbers, particularly for the killer subpopulations induced by cytokines and the cytolytic natural killer T cells (CNK-T), whose cytotoxicity is highly effective [56].

Another very important role for IL-15 has been described: It is a potent inhibitor of apoptosis. IL-15 is a survival factor for tubular epithelial cells (TEC), and the degree of apoptosis induced by anti-Fas antibodies or actinomycin D on these cells is higher in IL-15-/- mice [57]. This cytokine also protects against



Homoestasis in Innate and Adaptative immune responses

Figure 5. The IL-15 is a pleiotropic cytosine that plays a role in the control of lymphocyte homeostasis.

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death signals induced by the TCR [58]. It has been shown *in vivo* that the cells of the liver, spleen and thymus of mice treated with an anti-Fas antibody can be rescued from apoptosis by the treatment with an IL-15-IgG2β fusion protein [59]. IL-15 inhibits apoptosis in keratinocytes as well [60], an effect which has been linked to the pathogenesis of psoriasis.

A mechanism to explain the inhibition by IL-15 of TNFa-mediated apoptosis has been proposed. In this model, the binding of IL-15 to IL-15Rα induces the subsequent binding of TRAF-2 by the intracytoplasmic domain of IL-15Rα. The affinity of this interaction is presumed to be so high as to effectively sequester TRAF-2 away from the FADD, TRADD, RIP TNFαRI complex that mediates TNFa-induced apoptosis [61] (Figure 6).

Besides the cells of the immune system, there have been other effects ascribed to IL-15. According to certain reports, it acts as an anabolic agent for muscle cells, inducing their proliferation [62, 63]. Although IL-15 is angiogenic *in vivo*, and the vascular endothelial cells express the IL-15Rαβγ mRNAs and are responsive to IL-15 [64], this cytokine does not seem to play a critical role in these functions, since IL-15-/- and IL-15Rα-/- knock-out mice show no defects in muscles, bones or blood vessels.

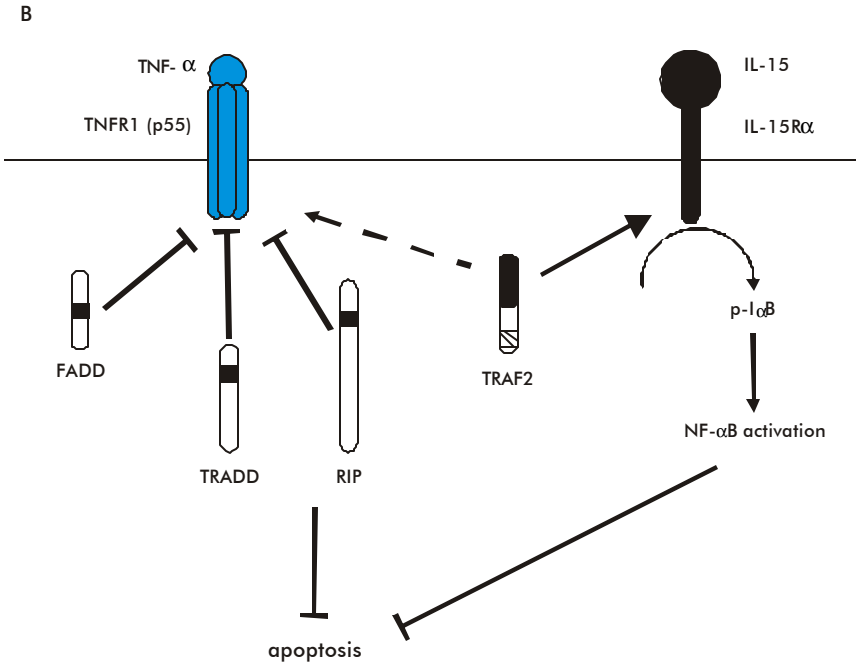

Figure 6. The IL-15 inhibits TNF mediated apoptosis. The intracytoplasmic region of the IL-15Rα sequesters TRAF2 after binding to IL-15.

Potential therapeutic uses for IL-15

There are at least two potential avenues for the therapeutic use of IL-15: the stimulation of the immune response by the exogenous administration of this cytokine, and the elimination or inhibition of IL-15 under conditions of uncontrolled expression.

The exogenous delivery of IL-15 may potentially favor the development, expansion and survival of effector cells for the immune response, in a manner not unlike that of IL-2 (which was approved for clinical use by the Food and Drugs Administration –FDA- since 1992). IL-2 has proven to be effective at low doses for enlarging lymphocyte subpopulations without showing significant toxicity [65, 66]. However, recent analyses suggest that IL-15 may outperform IL-2 for cancer treatment, as well as becoming part of vaccine formulations for the therapy or prevention of cancer and infectious diseases, due to its role in inhibiting activation-induced cell death (AICD), which is mediated by IL-2, and in facilitating the persistence of $CD8^+$ memory T-cells [67]. There is an ongoing phase-I clinical trial, still at the recruiting stage, for testing the use of IL-15 as an adjuvant on an HIV Gag-based DNA vaccine (ClinicalTrials.gov Identifier: NCT00115960).

Another therapeutic research avenue related to IL-15 is the development of antagonists for this cytokine. The deregulated expression of IL-15 has been linked to a number of autoimmune or inflammatory disorders, such as rheumatoid arthritis (RA), multiple sclerosis, celiac disease, psoriasis, sarcoidosis and hepatitis C [68-71]. In particular, there has been a long line of studies to establish the role played by IL-15 and IL-15Rα in RA, the first studies proposed that IL-15 was a modulatory factor for the disease that precedes TNFα in the pro-inflammatory cytokine cascade, acting as a T cell chemo-attractant to the synovial fluid. Specifically, it is argued that the T cells activated by IL-15 can induce the synthesis of TNFα on macrophages by a cell contact-dependent mechanism.

Supplementary evidence is provided by the high expression levels of the IL-15 mRNA in cells from the synovial fluid of RA patients [72], the detection of the production of IL-15 in the endothelial cells from RA patients, which increases the trans-endothelial migration of CD4+ and CD8+ T cells [73], and the fact that the concentration of IL-15 in the sera of patients increases with the progress of the disease [74]. The expression of IL-15 has also been detected in RA nodules, which is an extra-articular expression of this disorder that is not linked to the production of TNFα [75]. Other sources suggest that IL-15 and TNFa promote the expression of NKG2D and its ligand MIC on CD4 and CD8 T-cells, potentiating the stimulation of self-reactive T cells [76], and that IL-15 increases the expression of CD40L and the chemokine receptor CCR5 on T cells from RA patients, enhancing the release of chemokines.

A high expression of IL-15 and its receptor has also been observed in patients afflicted by psoriasis, which is a chronic inflammatory skin disorder characterized by epidermal hyperplasia, angiogenesis, the infiltration of activated T cells and the production of inflammatory cytokines. Known evidence associates dermal IL-15 with the reduction of apoptosis in keratinocytes and the activation of T cells [77, 78].

Sarcoidosis is a chronic granulomatous disorder of undetermined etiology that progressively affects a number of target organs, especially the lungs. The macrophages isolated from patients suffering from active sarcoidosis express IL-15 mRNA and the protein is found in the cytoplasm and the membrane, which does not occur in patients with inactive

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sarcoidosis or normal subjects. The CD4 T cells obtained from patients in the active stage of the disease express components of the IL-15 receptor and proliferate upon IL-15 treatment. Together, these data suggest that IL-15 is involved in the pathogenesis of sarcoidosis [79, 80].

Frequently inflammatory bowel diseases (IBD) involve either ulcerative colitis (UC) or Crohn's disease (CD) [81]. A very high percentage of the mononuclear cells from IBD patients express IL-15 during the active stage of the disease. IL-15 has been detected in the sera of patients suffering from UC in moderate to severe stages, but is not detected in normal donors or CD patients. Recent studies confirmed the synthesis of IL-15 by macrophages on the mucosa of IBD patients, and provide evidence on the modulation of T cells by IL-15 [82].

IL-15 is also involved in the pathogenesis of hepatitis C. The levels of this cytokine detected in the sera of infected patients suffering from chronic hepatitis, hepatic cirrhosis and hepatocellular carcinoma are much higher than those found in asymptomatic carriers or healthy donors [33].

Multiple sclerosis (MS) is an inflammatory disorder of the central nervous system whose origin has been attributed to autoimmune processes and the uncontrolled release of cytokines [83]. A large number of mononuclear cells expressing IL-15 mRNA has been detected in MS patients, with higher levels of IL-15 in the cases where the disease is in a chronic and progressive stage, as compared to patients in remission [84]. The levels of IL-15 in the sera of MS patients are also higher than in the sera of persons afflicted from other inflammatory or non-inflammatory neurological diseases [85].

Another clinical setting in which IL-15 is involved is organ transplant. In general, cytokines play an important role in graft rejection, since they promote the infiltration and activation of the immune effector cells of the receptor into the transplanted organ. A study focused on renal allografts found the expression of IL-15 mRNA in the 45 kidney biopsies under study, with significantly higher levels in patients that rejected the transplant [86]. The expression of both the IL-15 mRNA and the actual protein has also been observed in liver and heart transplants [87, 88].

Cases of a deregulated expression of IL-15 have been detected in some hematopoietic cell cancers as well: IL-15 is assumed to be involved in the pathogenesis of adult T cell leukemia (ATL), mainly during the infiltration phase in tissues expressing IL-15 such as skin, lungs, liver and the gastrointestinal tract [89, 90]; in cutaneous T-cell lymphoma, IL-15 promotes the growth and viability of these cells [91]. The lymphoproliferative disorders of granular lymphocytes (LDGL) are characterized by the presence of $CD3^+$ T cell or $CD3^-$ NK cell subpopulations [92] that express the 3 subunits of the IL-15 receptor, proliferate in response to IL-15 treatment, and have detectable IL-15 in the cell membrane [93].

A high percentage of IL-15 transgenic mice over-expressing the cytokine develop a fatal lymphocytic leukemia. The clinical course and symptoms of the disease are similar to those typical of patients with LGL leukemia [94, 95]. IL-15 also induces the proliferation of malignant B cells, obtained from patients suffering from chronic B-cell lymphocytic leukemia or hairy cell leukemia [96], and induces the proliferation of an acute myeloid leukemia-derived cell line known as M-O7e [97]. The cells from multiple myeloma express all the components of the IL-15 receptor, and it has been proposed that this cytokine may have an effect in their autocrine propagation [98].

IL-15 antagonists

A number of antagonist molecules that can block the effect of murine IL-15 *in vivo* have been validated in murine models. Some of them are listed as follows:

1. The extracellular region of IL-15Rα that is fused to the Fc region of human IgG_1. This fusion protein inhibits the development of collagen-induced arthritis in DBA/1 mice [99], and its administration in a murine experimental model of a completely vascularized cardiac allograft prevents rejection and induces a state of immunological tolerance [100].

2. Mutants of IL-15 at amino acids Asp 8 or Gln 108 that are involved in binding to subunits β and γ of the receptor, respectively. It has been shown that a Gln108 mutant that is fused to the Fc region of IgG2a (IL-15 mutant/Fcγ2a) inhibits IL-15-induced cellular proliferation, increases the survival of mice with pancreatic cell allografts, and effectively blocks delayed-type hypersensitivity [101]. It has also been recently reported that this fusion protein prevents rejection in a murine model of cardiac transplant [102].

3. The 404E4 antibody blocks the binding of IL-15 to the α subunit of the receptor and inhibits the proliferation of peripheral blood mononuclear cells (PBMC) when added to the cells before the treatment with cytokine [69].

4. A human antibody against the β subunit of the receptor (Hu-MiKBeta1) inhibits the stimulation of T and NK cells by IL-15 *ex vivo*, as well as the effect of IL-2 on the intermediate affinity receptor, present in NK cells and resting T lymphocytes [103].

5. The human monoclonal antibody 146B7 (AMG 714), obtained from a transgenic mouse expressing human immunoglobulins, has been effective in a murine model of skin xenotransplant with psoriasis in which it decreased the thickness of the epidermis, the infiltration of mononuclear cells, and the degree of parakeratosis and cycling keratocytes [69].

The first clinical trials with IL-15 antagonists have recently started. A phase I study with the Mikβ1 antibody in T cell large granular lymphocyte leukemia patients (T-LGT) showed no toxicity associated to the use of this drug, but unfortunately there was no clinical response to the treatment, no reduction in the number of leukemic cells, and no clinical improvement for the patients [104]. A phase I/II clinical trial evaluating the AMG 714 antibody in rheumatoid arthritis patients has also been carried out. It showed that the antibody, delivered at different dosages, provided a significant improvement for the signs and symptoms of the disease compared to the placebo [105]. This clinical trial has currently moved on to phase II/III.

Final considerations

IL-15 is an important factor, involved in both the innate and adaptive arms of the immune response.

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Unlike IL-2, which is a cytokine produced by T cells, IL-15 is present in the tissular stroma. The biology of IL-15 is complex, since it can be found as a soluble protein secreted to the extracellular milieu (involved mainly in inflammatory processes or other disorders such as leukemia and lymphoma), as a complex with IL-15Rα at the cell membrane, as an integral membrane protein, or in the cytoplasm as a soluble complex with IL-15Rα. Therefore, it is important to elucidate which of these forms is related to what disorder or physiological function. The evidence gathered from *in vitro* experiments and animal models support its potential use for the treatment of AIDS and cancer patients, as well as the use of IL-15 antagonists in autoimmune and inflammatory disorders such as RA. The current clinical trials are the first steps taken for the future clinical use of this cytokine.

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