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REVIEW

Lymphotoxin α revisited: general features and implications in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting synovial joints. Therapies blocking tumor necrosis factor-alpha (TNFα) are now routinely used in the management of RA. However, a significant number of patients with RA do not respond or develop resistance to anti-TNF therapies, and the participation of other cytokines in RA pathogenesis has been reported as well. Lymphotoxin alpha (LTα) is the closest homolog to TNFα and has been implicated in inflammation and autoimmunity since its original description in 1968. In spite of that, little is known about the role of LTα in RA or the potential of blocking this cytokine as an alternative therapeutic approach. In this review, we aim to summarize the general features of LTα and what is currently known about its participation in RA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting synovial joints. A hallmark of RA is the pseudotumoral expansion of fibroblast-like synoviocytes (FLSs), which invade and destroy the joint. Tumor necrosis factor-alpha (TNFα) plays a major role in promoting RA, and blocking this cytokine is effective for treating patients with RA [1]. However, a significant number of patients do not respond or become resistant to anti-TNF therapies; approximately 50% of the patients still receive anti-TNFs 5 years after the start of treatment [2]. The participation of other cytokines in RA has also been reported and could explain the absence of response to anti-TNFs. Often, patients treated with anti-TNFs show secondary effects such as recurrent infections [3]. Therefore, it is important to define additional therapeutic

strategies in order to better control synovial inflammation and joint destruction observed in RA. Although lymphotoxin alpha (LTα) has been associated with autoimmune and inflammatory diseases and is the closest homolog to TNFα, few data pointing to a role for LTα in RA are available [4-10]. In this review, we aim to summarize the general features of LTα and what at present is known about its role in RA.

Lymphotoxin alpha in general

LTα, formerly known as TNFβ, was originally described in 1968 as a cytotoxic factor produced by T lymphocytes after antigenic or mitogenic stimulation [11]. Later on, in 1984, human LTα was purified from a B-lymphoblastoid cell line [12,13] and its structure was determined by classic protein-sequencing methods, making LTα the first member of the TNF superfamily to be characterized [14]. TNFα was subsequently purified, and sequence comparison and receptor competition experiments revealed that these two proteins were homologous [15,16]. Indeed, LTα is the closest homolog to TNFα.

LTα and TNFα are 30% homologous in their primary amino acid sequence, but of greater significance is the observation that the regions of major sequence homology indicated a similarity in their tertiary and quaternary structures [15]. LTα is structurally similar to TNFα: LTα is a soluble homotrimer composed of 17-kDa monomers and binds to and signals specifically through TNF receptors 1 and 2 (TNFR1 and TNFR2) to exert its biological activities.

Although LTα and TNFα have many similarities, there are some distinct molecular and biological differences [17,18]. Like TNFα, LTα binds with high affinity to TNFR1 and TNFR2 [19]. However, the N-terminus of LTα, unlike that of TNFα, resembles a traditional signal peptide, making its conversion to a soluble form extremely efficient. Thus, LTα is never found at the cell surface, a unique feature among the TNF superfamily members. LTα is anchored to the cell membrane only in association with membrane-bound LTβ, as LTαβ heterotrimers [20]. LTαβ is structurally distinct from LTα and comprises two membrane-anchored heterotrimers, the predominant LTα1β2 form and a minor LTα2β1 form,

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both of which interact with the LTβ receptor (LTβR) [18,21,22]. Besides binding to TNFR1 and TNFR2, LTα binds to HVEM (herpesvirus entry mediator), a receptor discovered as an entry route for herpes simplex virus, but this binding is relatively weak [23].

LTα is expressed by CD4+ T helper type 1 (Th1) cells, CD8+ cells, natural killer (NK) cells, B cells, and macrophages [18]. LTα has specific roles in the development and function of the immune system, mainly in lymphoid organ development, organization and maintenance of lymphoid microenvironments, host defense, and inflammation [18]. However, most of the evidence pointing to these roles came from genetically deficient mice and the relevance of LTα in humans is less clear. Moreover, these mice models make it difficult to determine the relative role of LTα in these systems. This is because the *LTα* gene is closely linked to the *TNFα* and *LTβ* genes and targeting the *LTα* gene can lead to collateral damage to the neighboring genes [24]. Additionally, LTα could somehow control the expression of TNFα and the absence of LTα could interfere with the production of this cytokine. In any case, although LTα was once considered to be redundant to TNFα, the fact that the same cell types express both LTα and TNFα and that knockout mice for either cytokines can manifest different phenotypes suggest that the two cytokines have overlapping and different functions.

In regard to the development of secondary lymphoid organs, it was shown that mice deficient in LTα are completely devoid of peripheral lymphoid tissues, such as Peyer patches (PP) [25]. It has been demonstrated that LTα mediates PP formation through TNFR1 because TNFR$^{-/-}$ mice either lack or have abnormal PP whereas TNFα$^{-/-}$ mice have normal PP [26].

Several studies suggested a role for LTα in host defense against certain infections. Mice deficient in LTα are highly susceptible to *Staphylococcus aureus* infections [27]. Other studies showed the LTα requirement for granuloma formation and resistance to *Mycobacterium*, *Leishmania*, and *Plasmodium* infections in mice [28-31]. However, whether these functions are mediated by LTα, LTβ, or even TNFα is unclear. The contribution of LTα to host defense was further challenged by recently generated LTα$^{-/-}$ mice showing intact TNFα production, which allows the evaluation of LTα alone, as opposed to the earlier generated LTα$^{-/-}$ mice that showed altered expression of TNFα [32].
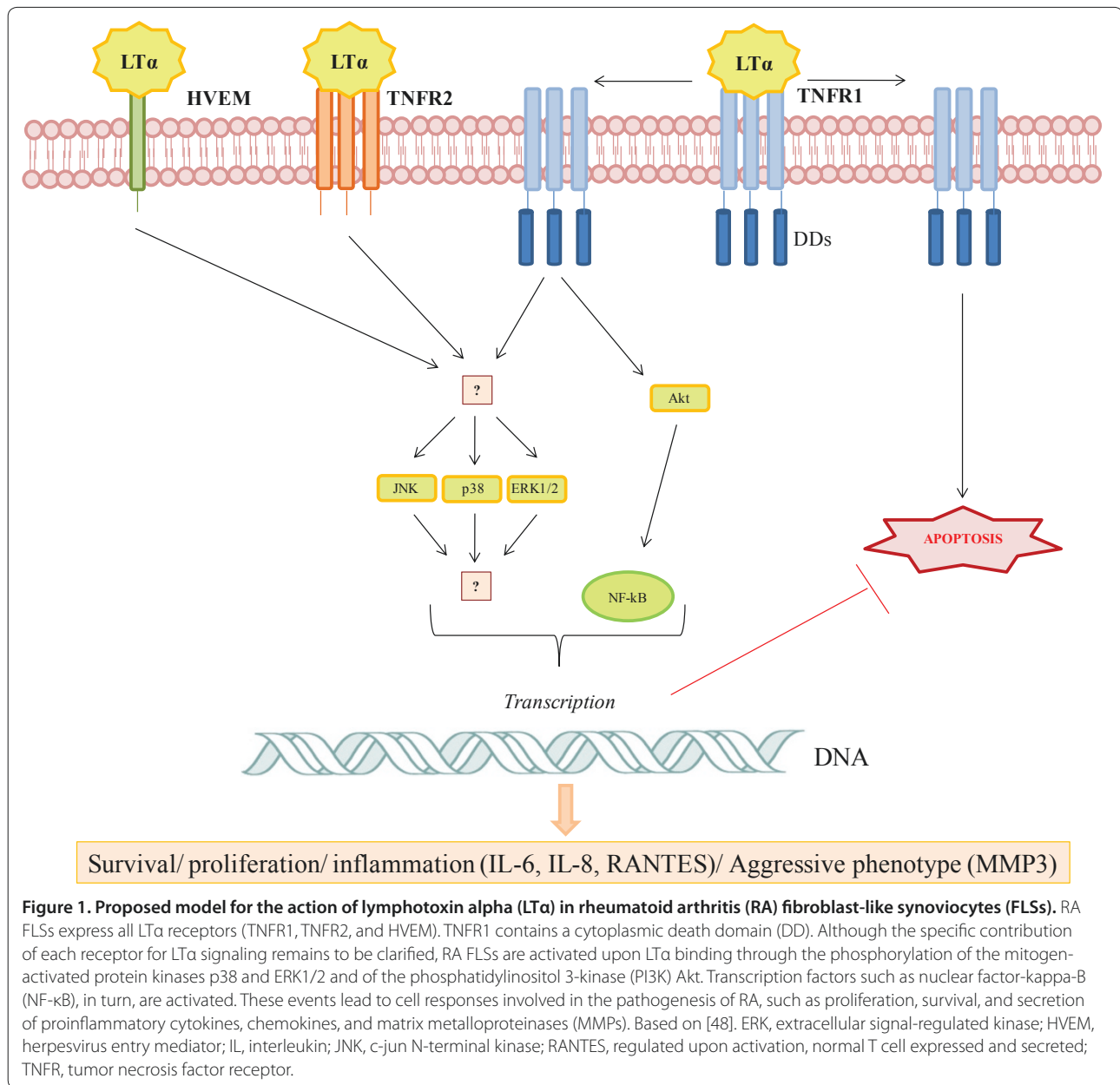
LTα has been implicated in inflammation since its initial description. LTα induces inflammation *in vivo* when expressed under the control of the rat insulin promoter (RIP) at the sites of transgene expression in the pancreas and kidney of RIPLT mice [33], and this occurs even in LTβ$^{-/-}$ mice [34], indicating that LTα alone induces inflammation. Additional data suggesting a

proinflammatory role for LTα derive from studies on experimental allergic encephalomyelitis (EAE) and show that myelin basic protein-specific T-cell clones secrete LTα [35] and that LTα$^{-/-}$ mice are resistant to inflammation and clinical signs of EAE whereas LTβ$^{-/-}$ mice can still develop EAE [36]. The mechanisms through which LTα promotes inflammation and lymphoid organ development are still poorly understood. One possibility is the induction of adhesion molecules in endothelial cells. *In vitro* studies showed that recombinant human LTα induces expression of intercellular adhesion molecule (ICAM) and E-selectin in human endothelial cells [37]. RIPLT mice overexpressing LTα exhibited a high expression of ICAM-1 and vascular cell adhesion molecule-1 in the vasculature of the inflamed pancreas and kidney independently of T or B cell-derived cytokines [38]. LTα could also contribute to inflammation by the induction of chemokines. In this manner, LTα induces the expression of RANTES (regulated upon activation, normal T cell expressed and secreted) and monocyte chemoattractant protein-1 in a murine endothelial cell line [39]. Moreover, LTα contributes to lymphatic vessel functions in steady-state conditions and induces lymphangiogenesis in inflammation through mechanisms yet to be characterized [40].

LTα is required for the differentiation of NK cells and plays a role in the recruitment and antitumor activity of mature NK cells [41-43]. When inoculated subcutaneously with syngeneic B16F10 melanoma cells, LTα$^{-/-}$ mice develop enhanced tumor growth and metastasis in comparison with wild-type littermates. This was associated with a lower number of NK cells and with slower migration of these cells from the bone marrow to peripheral organs [44]. Established, preclinical graft-versus-host disease (GVHD) models showed that LTα contributes to the development of GVHD, the most frequent complication of allogeneic transplantation [45]. Naïve and alloreactive CD4+ T cells secrete soluble LTα after T-cell receptor stimulation. LTα participates in GVHD-mediated epithelial cell apoptosis, target organ damage, and mortality and this is mediated through TNFR1 signaling [45]. These effects were not redundant to TNFα, as GVHD patients treated with TNFRFc, which cross-reacts with and blocks LTα, have outcomes different from those of patients treated with anti-TNFα monoclonal antibody, as do patients with a chronic autoimmune disease such as RA [8].

Lymphotoxin alpha in rheumatoid arthritis

The first reports suggesting a role for LTα in RA came from an analysis in patients with RA by enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction, and immunohistochemistry. It has been reported that LTα levels are elevated in the

Figure 1. Proposed model for the action of lymphotoxin alpha (LTα) in rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLSs). RA FLSs express all LTα receptors (TNFR1, TNFR2, and HVEM). TNFR1 contains a cytoplasmic death domain (DD). Although the specific contribution of each receptor for LTα signaling remains to be clarified, RA FLSs are activated upon LTα binding through the phosphorylation of the mitogen-activated protein kinases p38 and ERK1/2 and of the phosphatidylinositol 3-kinase (PI3K) Akt. Transcription factors such as nuclear factor-kappa-B (NF-κB), in turn, are activated. These events lead to cell responses involved in the pathogenesis of RA, such as proliferation, survival, and secretion of proinflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs). Based on [48]. ERK, extracellular signal-regulated kinase; HVEM, herpesvirus entry mediator; IL, interleukin; JNK, c-jun N-terminal kinase; RANTES, regulated upon activation, normal T cell expressed and secreted; TNFR, tumor necrosis factor receptor.

serum and the synovial tissue of patients with RA in comparison with the healthy controls or patients with osteoarthritis [6,46]. A relevant piece of evidence linking LTα to RA was provided by a case report describing an RA patient with no beneficial clinical effect after therapy with infliximab, a monoclonal antibody that specifically blocks TNFα. Interestingly, subsequent treatment of this patient with etanercept, a TNFR2-Fc fusion protein that also blocks LTα, resulted in clinical remission of the disease [8]. The different ligand specificities of etanercept and infliximab could account for the different outcomes of this patient after both treatments. Increased LTα expression has been shown in the synovial tissue of this

patient [8]. These data, together with the biological similarities between LTα and TNFα, suggest that resistance to TNFα blockage may occur when TNFα is not the dominant inflammatory cytokine and that LTα may play a role in the disease. An important advancement in the understanding of the participation of LTα in RA came from a study using the collagen-induced arthritis (CIA) mouse model, the most commonly used animal model for arthritis [47]. In this model, the blocking of LTα with a monoclonal antibody significantly improved the disease [47]. The main mechanism responsible for this improvement in the CIA model appears not to be the blocking of soluble LTα but the depletion of LTα expressing Th1 and

Th17 cells [47]. Still, the anti-LTα antibody applied in this study also binds to soluble LTα and inhibits its binding to a TNFR2.Ig in a competition ELISA [47]. An example of a dual functionality of an antagonist in RA is the well-established monoclonal antibody infliximab, which binds specifically to TNFα. Besides blocking secreted TNFα, infliximab can activate the complement cascade and deplete membrane-bound TNFα-expressing cells through a cytotoxic mechanism [18]. Recently, our group provided more evidence for a role of LTα in RA when we demonstrated that LTα can trigger activation (that is, proliferation and induction of an inflammatory and aggressive phenotype) of FLSs [48]. The mechanisms through which LTα activates FLSs are depicted in Figure 1, in a proposed model for the action of LTα in RA FLSs. To better evaluate the role of LTα in RA, our group analyzed LTα levels in whole sera, plasma, and synovial fluid of patients with RA, patients with osteoarthritis, and healthy controls. We were unable detect LTα reliably with the commercially available ELISA kits in these samples. However, this does not mean LTα is not expressed locally in joints of patients with RA. While it would be interesting to detect circulating LTα in synovial fluid, it would be equally or even more important to obtain *in situ* evidence of LTα expression in arthritic tissue, where it might exert effects such as those we reported on synovial fibroblasts.

Conclusions

TNFα is known to play a crucial role in RA, but several other proinflammatory cytokines have been identified to contribute to the disease as well [49]. LTα can easily be placed in the context of the RA synovium as it is secreted by CD4^{+} Th1 cells, CD8^{+} T cells, NK cells, and macrophages, cell types that are increased in the arthritic joint. The fact that LTα activates RA FLSs and thus may contribute to synovial hyperplasia suggests that LTα can also play a disease-promoting role in RA [48]. It will be important to further characterize the relevance of LTα in RA by detecting it *in vivo* in patients with RA.

Abbreviations
CIA, collagen-induced arthritis; EAE, experimental allergic encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; FLS, fibroblast-like synoviocyte; GVHD, graft-versus-host disease; ICAM, intercellular adhesion molecule; LTα, lymphotoxin alpha; NK, natural killer; PP, Peyer patches; RA, rheumatoid arthritis; RIP, rat insulin promoter; RIPLT, rat insulin promoter lymphotoxin; Th, T helper; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.

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