CHAPTER 7

Immunomodulatory Activity and Therapeutic Potential of the Filarial Nematode Secreted Product, ES-62

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Abstract

ES-62 is a protein that is actively secreted by filarial nematodes during parasitism of the vertebrate host. The molecule is able to directly interact with a number of cells of the immune system including B-lymphocytes, dendritic cells, macrophages and mast cells. Interaction appears to be dependent on complexing with TLR4 and results in modulation of the activity of a number of signal transduction molecules including MAP kinases, PI-3 kinase and NF-κB. Immunomodulatory activity of ES-62 appears to be largely due to the presence of phosphorylcholine (PC) moieties covalently attached to N-type glycans. The net effect of ES-62's interaction with the immune system is the generation of an anti-inflammatory immunological phenotype. As a consequence of this, ES-62 demonstrates striking drug-like activity in models of disease associated with aberrant inflammation, in particular those associated with autoimmunity and allergy.

Introduction

There is currently much interest amongst scientists and clinicians in the "Hygiene Hypothesis". This hypothesis claims that the increased incidence of allergic and autoimmune diseases observed in Western countries in recent decades is due to a loss of appropriate priming of the immune system by pathogens such as parasitic worms (also called helminths) during the early years of life.[1] In support of this, it has been suspected for many years that several autoimmune disorders present with reduced incidence and severity in parts of the world with high helminth load (reviewed in ref. 2). As an example, rheumatoid arthritis (RA), a T helper 1 (Th-1)-cell mediated disease associated with high levels of pro-inflammatory cytokines such as TNF-α, is reduced in helminth-endemic areas.[3,4] In addition, but more surprisingly, given that worms tend to induce strong Th-2-Type (IL-4-, IL-5- and IL-13-dependent) immune responses, there also appears to be an inverse correlation between parasite load and atopy showing that helminths also appear to suppress Th-2-biased inflammatory disorders (reviewed in refs. 4, 5). Indeed, there is ever-increasing evidence that a number of helminth species can delay or prevent the onset of a wide range of autoimmune or allergic disorders.[4,5]

Filarial nematodes represent a group of helminths that can induce particularly severe morbidity with a significant proportion of sufferers presenting with debilitating health problems including severe skin lesions, elephantiasis and several forms of eye damage each of which may lead to blindness.[6] Infection with these organisms is long-term, with individual worms surviving for up to 10 years.[7] Parasite longevity is almost certainly dependent on suppression or modulation of the host

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immune system (reviewed in refs. 8, 9), which demonstrates impairment of lymphocyte proliferative responses and polarisation of the cytokine and antibody response. With respect to the latter, there is reduced Th-1-associated, interferon-gamma (IFN-γ) and increased Th-2-associated interleukin (IL)-4 and anti-inflammatory IL-10, cytokine production and also greatly elevated levels of IgG_4, an antibody of little value in eliminating pathogens due to an inability to activate complement or bind with high affinity to phagocytic cells (a neutralising antibody). Overall therefore, the picture is of an immune response responding to infection with a somewhat suppressed (impaired lymphocyte proliferation), anti-inflammatory (increased IL-10/IgG_4 production), Th-2- (IL-4) like, phenotype. It has been speculated that in addition to promoting parasite survival, such a phenotype is conducive to host health by limiting development of the pathological lesions referred to earlier that are suspected to result from aggressive, pro-inflammatory immune responses. There is increasing evidence that immunomodulators synthesised and secreted by the nematodes may be responsible for such immune system deviation. ES-62, a secreted protein that we discovered in the rodent filarial nematode *Acanthocheilonema viteae,*[10] and subsequently found to have well conserved orthologues in human filarial nematode parasites including *Brugia malayi* and *Onchocerca volvulus*[11,12] is one such molecule.

ES-62

ES-62 is produced in cells that subtend the oesophagus by the post-infective lifecycle stages (L4 larvae and adult worms) of *A. viteae* and can be detected in the serum of the jird host, *Meriones lybicus.*[10,11] However, work on both *A. viteae* and *B. pahangi* has shown that the ES-62 gene is transcribed throughout the helminth lifecycle, although mRNA levels are considerably higher in adult worms than L3 larvae (~5% adult levels for *A. viteae*) and microfilariae (<0.2% adult levels for *A.viteae*).[12] ES-62 mRNA is translated into a protein of 62 kDa (including posttranslational modifications) that has phosphorylcholine (PC) moieties attached via an *N*-type glycan, the latter having been trimmed to the tri-mannosyl core and then substituted with between one and four *N*-acetylglucosamine residues during oligosaccharide processing (reviewed in ref. 13). The number of PC-containing glycans present on each ES-62 molecule has not been resolved to date and the number of PC groups per glycan has been shown to be variable.

ES-62 has highest sequence homology with members of the M28 peptidase family (e.g., 38% and 37% identity with mouse and human aminopeptidases) and has been shown to possess some, albeit weak peptidase activity in vitro against synthetic substrates.[14] Interestingly, the biologically active forms of many peptidases are dimeric or tetrameric[15,16] and consistent with this, gel filtration studies and sedimentation equilibrium data demonstrated that ES-62 is a tightly bound tetramer formed from dimers.[13,17,18] Furthermore, divalent cations are known to be critical for the function of many peptidases and ES-62 has a putative metal coordination motif in its sequence; indeed, a strong magnesium (Mg^{2+}) signal was detected in its atomic emission spectrum.[18] Although a function for the peptidase component of ES-62 has not yet been convincingly demonstrated, the molecule has been shown to display a variety of immunomodulatory properties, many of which have been attributed to the presence of PC. PC is a molecular pattern associated with pathogen products from a diverse range of organisms, including bacteria, fungi and protozoa, as well as filarial and gastrointestinal nematodes (reviewed in ref. 19). It enables detection of pathogens by the host (for example via antibodies or C-reactive protein), but as revealed in detail below can also function to promote pathogen survival via modulation of the host immune response.

Immunomodulatory Properties of ES-62

ES-62 is pleiotropic, being able to exert its effects on a variety of cells of the immune system including B-lymphocytes,[20-22] antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages[23-27] and mast cells.[28] Rather than acting simply in a nonspecific immunosuppressive manner, ES-62 tends to block Th-1 responses and induce anti-inflammatory type immune responses,[29,30] although it has also been shown to block Th-2 responses in a situation in which they are associated with inflammation.[28] This latter finding was only obtained recently and has led us to

conclude that our original hypothesis, namely that ES-62 is a Th-2 polarising agent is incorrect and thus to speculate that the true target of ES-62 may perhaps be a cytokine such as IL-17 (Fig. 1), which appears to act as a master regulator of both Th-1 (e.g., rheumatoid arthritis)- and Th-2 (asthma)-type inflammation.[31-33] This latter idea is currently under investigation. Specific examples of the effects of ES-62 on the mouse immune system are prevention of FcεRI-mediated mast cell degranulation,[28] inhibition of BCR-mediated B-lymphocyte proliferation,[20-22] the production of IL-10 by B1 cells,[25] reduced levels of IL-12, IFN-γ and pro-inflammatory cytokines by APCs in response to TLR ligands (BLP, LPS, CpG)[34] and the production of IgG_1 (equivalent of human IgG_4 in some aspects, e.g., inability to activate complement) rather than opsonising/complement fixing IgG_{2a} antibodies.[35]

Extensive investigation over the last fifteen years has provided a great deal of information on the mechanism of action of ES-62 and four important findings have emerged:

1. (As briefly mentioned earlier) ES-62's activity is largely dependent on its PC moiety. This belief has emerged from experiments in which normal and PC-free ES-62 were compared[35] and in which ES-62 was compared with PC-conjugated to proteins such as albumin or ovalbumin.[23,36] The take-away message is that PC-free ES-62 in general lacks ES-62's immunomodulatory activity whereas PC-conjugated proteins largely mimic it.
2. ES-62's activity is dependent on the presence of TLR4 on the target cell. This conclusion initially arose when macrophages and DCs from TLR4-KO (but not TLR2 or TLR6) mice were found to be nonsusceptible to the action of ES-62.[34] Subsequently it was found that ES-62 was largely ineffective against mouse mast cells if TLR4 levels were greatly depleted by the use of an antisense oligonucleotide approach.[28] Interestingly the interaction of ES-62 with TLR4 is distinct from that of LPS. For example, unlike LPS, ES-62 is fully active against APC derived from HeJ mice that have a mutation in the TIR domain of TLR4.[34,37]

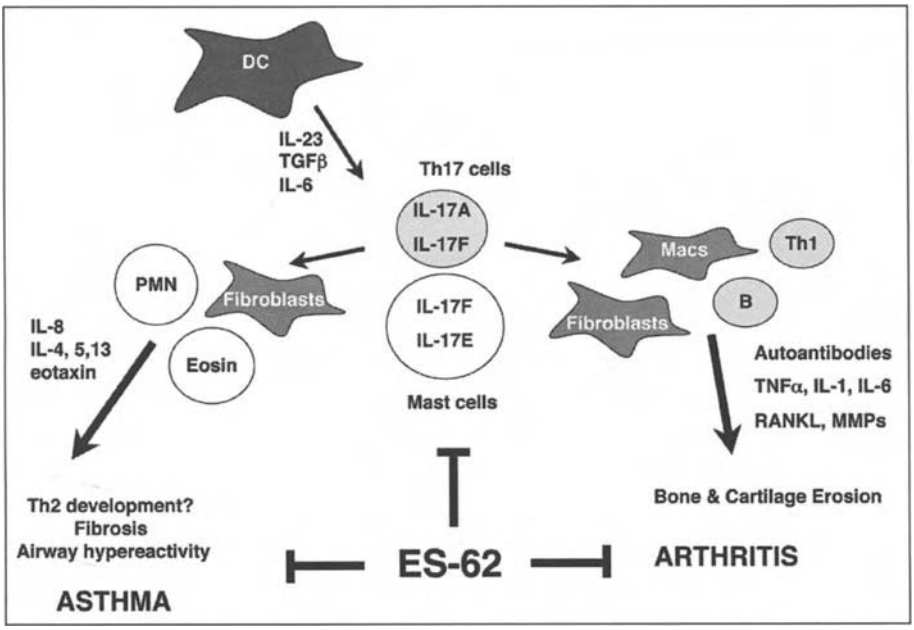

Figure 1. ES-62 may suppress inflammation by targeting IL-17 responses. IL-17 has been implicated in the pathogenesis of diseases resulting from both Th-1- and Th-2-driven inflammation. ES-62 can suppress both types of inflammation and may do this by targeting IL-17 production by mast cells and/or Th-17 cells. Macs = macrophages; MMPs = matrix metalloproteinases.

3. ES-62's activity involves modulation of signal transduction pathways. Extensive investigation has enabled us to show that exposure of cells of the immune system to ES-62 results in modulation of a number of signal transduction pathways including those dependent on PI-3 kinase, MAP kinases and NF-κB.[38,39] Modulation of such pathways can clearly explain the immunomodulatory effects of ES-62 and in some cases the mechanism of modulation has been resolved. With respect to B-lymphocytes for example, ES-62 actually activates some signalling molecules such as Erk MAP kinase resulting in the induction of negative feedback signalling such that when subsequent BCR ligation occurs, Erk activity is rapidly uncoupled by activation of the inhibitory phosphatase, PAC-1 and hence activation of the cell is prevented.[22]
4. ES-62's activity is dependent on degradation of PKC-α. This was first shown in B-lymphocytes,[20] where PKC-α is important for proliferation and hence the effect contributes to inhibition of proliferation. Subsequently it was found that ES-62 caused a similar degradation of PKC-α in mast cells. As PKC-α is necessary for PLD-coupled, sphingosine kinase-mediated, calcium mobilisation in mast cells and this pathway is necessary for degranulation, PKC-α degradation provides the molecular mechanism by which ES-62 blocks such degranulation.[28] The mechanism of ES-62-mediated PKC-α degradation has been characterised in mast cells (Fig. 2): complexing of ES-62 and TLR4 at the plasma membrane results in sequestration of PKC-α followed by caveolae/lipid raft-dependent internalisation and nonproteosomal degradation at the peri-nuclear region of the cell.[28] Sequestration of PKC-α does not take place when ES-62 is substituted by LPS, again highlighting differences between the two molecules with respect to the consequences of their interaction with TLR4.

Therapeutic Potential of ES-62

Rheumatoid arthritis (RA), as mentioned earlier, has been associated with a dysregulated Th-1 immune response in which there is excess pro-inflammatory cytokine production[40-42] within inflamed RA synovial membrane that contributes directly to cartilage/bone erosion through matrix metalloproteinase production and dysregulated chondrocyte/osteoclast function.[43,44] As our original hypothesis regarding ES-62's mechanism of action was that it inhibited Th-1-associated inflammatory responses, we investigated the prophylactic effect of ES-62 on the development of collagen-induced arthritis (CIA) in mice.[45] ES-62 significantly suppressed the severity of developing CIA and as predicted, these prophylactic effects correlated with the inhibition of collagen-specific pro-inflammatory/Th-1 cytokine production (TNF-α, IL-6 and IFN-γ) and suppression of collagen-specific IgG_{2a} antibodies. Therapeutic administration of ES-62 after CIA became clinically detectable also showed significant reduction in arthritis progression compared with vehicle-treated controls in terms of the number of subsequently recruited arthritic joints and reduced progression of articular swelling in the initially inflamed joint. Indeed, progressive articular inflammation and destruction was significantly suppressed by the nematode product.[45,46] Together these data indicate that ES-62 therapeutically and potently suppressed inflammatory CIA even when treatment was commenced after the onset of clinically detectable disease, a key predictor used in developing cytokine-targeting therapies in RA.[47] Finally, to further investigate the therapeutic potential of ES-62 in humans, we performed parallel studies on primary cultures from RA synovial fluid and membrane and compared their capacity for cytokine release in the presence or absence of ES-62. Significant suppression of LPS-induced TNF-α and IL-6 was observed in the presence of ES-62, clearly indicating that the latter can modify critical pro-inflammatory pathways ex vivo in disease relevant tissues.[45]

The idea that ES-62 activity was only directed against Th-1 type-inflammatory diseases was questioned when it was found to be active in a mouse model of allergy—ovalbumin-induced airway hypersensitivity.[28] As mentioned earlier, allergy is of course dependent on a Th-2 type of immune response and indeed since these data were obtained we have also witnessed a failure of ES-62 to modulate the activity of some Th1-inducing molecules.[48] In the airway hypersensitivity model,

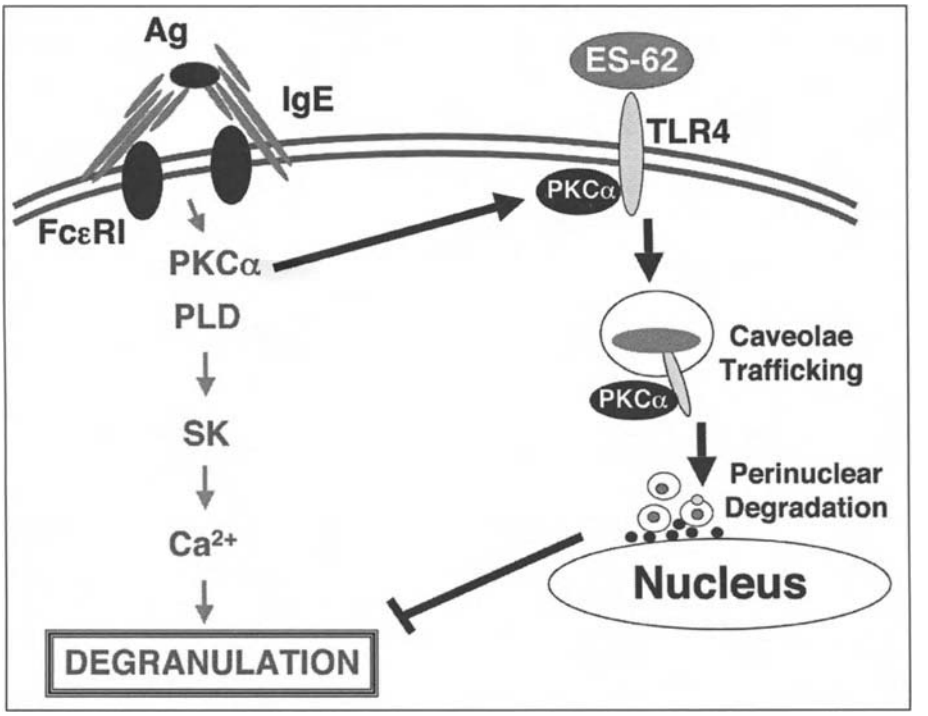

Figure 2. Filarial nematode-derived ES-62 induces hyporesponsiveness of mast cells by disrupting FcεRI signalling. Antigen (Ag)-driven ligation of IgE bound to FcεRI induces mast cell degranulation and generation of pro-inflammatory mediators and cytokines by triggering a signalling cascade involving PKCα-recruitment of the phospholipase D (PLD)-coupled, sphingosine kinase (SK)-mediated calcium mobilisation pathway. ES-62 directly blocks mast cell activation by subverting TLR4 signalling such that it results in sequestration and trafficking of PKCα for perinuclear degradation by a caveolae/lipid raft, proteosomal-independent mechanism. Such sequestration of PKCα uncouples FcεRI-mediated mast cell responses.

ES-62 was found to reduce peri-bronchial inflammation and mucosal hyperplasia, inhibit eosinophila and prevent release of the signature cytokine required for airway inflammation development, IL-4. ES-62 was also found to be active in a second model of Type I hypersensitivity, immediate-type hypersensitivity to oxazolone in the skin.[28] In this model, ES-62 targeted inflammation as shown by a reduction in ear swelling and this was correlated with inhibitory effects on mast cell activation. Subsequent in vitro analysis revealed that ES-62 directly prevented mast cell degranulation and release of mediators of allergy such as leukotrienes, prostaglandins and pro-inflammatory cytokines induced via ligation of FcεRI. It is our understanding that ES-62 is the first pathogen product described to directly inhibit mast cell effector function in this manner.

The Future

ES-62 is a large and hence immunogenic molecule whose posttranslational addition of PC is dependent on a nematode-specific pathway.[49] For these reasons, it is not suitable for use as drug. However, small PC-based derivatives offer a viable alternative. Towards this end we have shown that small PC-containing molecules (e.g., PC-glycans) can mimic some of the activities of ES-62 in vitro.[50] The next step will be to demonstrate that the same is true with respect to ES-62's anti-inflammatory activity in vivo.

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