

Signalling mechanisms underlying subversion of the immune response by the filarial nematode secreted product ES-62

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Introduction

Filarial nematodes are arthropod-transmitted parasites of vertebrates including humans. Of the eight species known to infect humans, three – *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* – are a major cause of morbidity in the Tropics. It is currently estimated that about 150 million people are infected with one or more of these worms and a significant proportion of these suffer debilitating health problems including severe skin lesions, elephantiasis and eye damage that may lead to blindness.¹ Infection with filarial nematodes is long-term, with individual worms surviving for ~10 years.² Parasite longevity reflects suppression or modulation of the host immune system (reviewed in refs 3,4) and there is increasing evidence that such immunomodulation can be mediated by bioactive molecules secreted by the worms (reviewed in refs 5,6). In this review we will discuss the results of studies in our laboratories over the last 10 years, which have examined immunomodulation by ES-62, a secreted product of the rodent filarial nematode *Acanthocheilonema viteae*.

The immune response to filarial infection

Filarial parasites are transmitted to mammalian hosts by an arthropod vector. They develop inside the vector

Summary

Secretion of immunomodulatory molecules is a key strategy employed by pathogens to enable their survival in host organisms. For example, arthropod-transmitted filarial nematodes, which achieve longevity within the infected host by suppressing and modulating the host immune response, produce excretory–secretory (ES) products that have been demonstrated to possess immunomodulatory properties. In this review we discuss the immunomodulatory effects of the phosphorylcholine-containing filarial nematode-secreted glycoprotein ES-62 and describe the intracellular signal transduction pathways it targets to achieve these effects.

Keywords: ES-62; filarial nematodes; immunomodulation; signal transduction

from microfilariae to infective larvae, before migrating to the arthropod mouth parts for transmission to the mammalian host when the arthropod feeds. The larvae then develop into adult worms and the life-cycle is completed with the generation of microfilariae, which are ingested again in the arthropod blood-meal. A spectrum of disease states exists in areas where filarial infection is endemic (reviewed in ref. 7). Aggressive immune responses to filarial nematodes occur in some individuals, resulting in chronic pathology, such as elephantiasis. However, the majority of individuals, although having detectable microfilariae in their bloodstreams, are otherwise apparently asymptomatic and have been described as being immunologically tolerant to the parasite. As alluded to earlier, this is thought to be the result of immunomodulation to achieve a situation conducive to both parasite survival and host health. Such people often have dramatically increased levels of immunoglobulin G4 (IgG4) and interleukin-10 (IL-10), with reduced interferon- γ (IFN- γ) production.^{8–11} Levels of IgE, which may be protective against infection with filarial nematodes and other helminths, are elevated in patients with chronic pathology.⁸ In contrast, individuals with detectable microfilariae tend to have a higher IgG4 : IgE ratio. IgG4 has been shown to compete with IgE for epitope recognition and hence may limit the induction of pathology.¹²

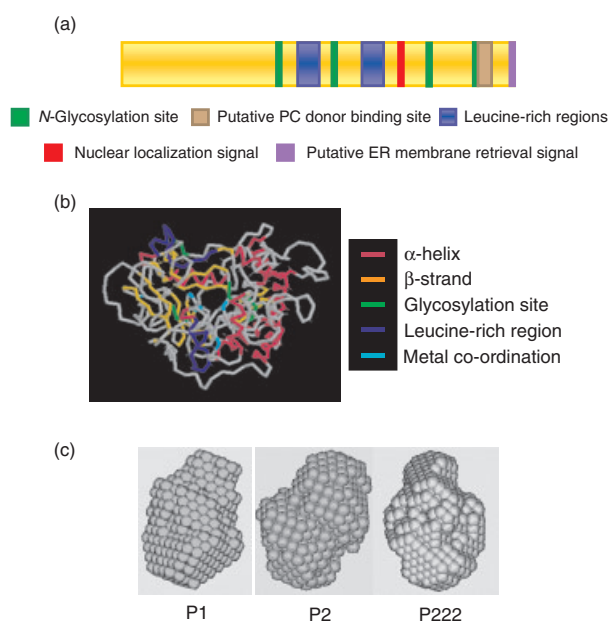


Figure 1. ES-62 structural studies. (a) The location of key residues within the ES-62 sequence, including N-glycosylation sites, a possible site for interaction with PC donors,²³ leucine-rich regions (likely to be involved in protein–protein interaction), and regions containing subcellular targeting motifs. (b) Prediction of a tertiary structure for the ES-62 monomer, obtained using DRAGON⁶⁶ indicating α -helices and β -strands, as well as glycosylation sites, leucine-rich regions and residues involved in metal ion co-ordination (ES-62 shows homology with aminopeptidases that contain a divalent cation in their active site¹⁷). (c) A low-resolution dummy atom model of the ES-62 tetramer, which is likely to be slightly elongated, obtained using DAMMIN⁶⁷ under three symmetry conditions (P1, no symmetry; P2, two-point symmetry; P222, 222-point symmetry).

ES-62, a phosphorylcholine-containing glycoprotein

ES-62 is produced by the post-infective life-cycle stages (L4 larvae and adult worms) of *A. viteae* and can be detected in the serum of infected jirds.^{13,14} However, the ES-62 gene is transcribed throughout the *A. viteae* life-cycle, although mRNA levels are considerably higher in adult worms than in L3 larvae ($\sim 5\%$ adult levels) and microfilariae ($< 0.2\%$ adult levels).¹⁵ ES-62 mRNA is translated into a glycoprotein with a molecular weight of 62 000 (including post-translational modifications) that has phosphorylcholine (PC) moieties attached via N-type glycans (reviewed in ref. 16; see Fig. 1). The number of PC-containing glycans present on each molecule is currently unknown but the number of PC groups per glycan has been shown to be variable.

ES-62 has highest sequence homology with a recently found family of aminopeptidases and carboxypeptidases (e.g. 38% and 37% identity with mouse and human aminopeptidases) and has been shown to possess some,

albeit weak, aminopeptidase activity *in vitro* against synthetic substrates.¹⁷ Interestingly, the biologically active forms of many aminopeptidases are dimeric or tetrameric^{18,19} and consistent with this, gel filtration studies and sedimentation equilibrium data demonstrated that ES-62 is a tightly bound tetramer formed from dimers.^{16,20,21} Furthermore, divalent cations are known to be critical to the function of aminopeptidases and ES-62 has a putative metal co-ordination motif in its sequence; indeed, a strong magnesium (Mg^{2+}) signal was detected in its atomic emission spectrum.²¹ Although a function for the aminopeptidase 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. 22). It enables the detection of pathogens by the host (for example via antibodies or C-reactive protein), but can also function to promote pathogen survival via modulation of the host immune response.²³

ES-62 exerts its immunomodulatory effects on a variety of cells of the murine immune system including B and T lymphocytes as well as antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages.^{23–30} Broadly, rather than acting in an immunosuppressive manner, the molecule induces a T helper type 2 (Th2)/anti-inflammatory phenotype, characterized by the production of IL-10, with reduced levels of IL-12, IFN- γ and pro-inflammatory cytokines, and IgG1 rather than IgG2a antibodies. These effects (summarized in Fig. 2) and the signalling pathways targeted by ES-62 to achieve this immunomodulation are described below.

Immunomodulation by ES-62

B-cell activation and antibody production

At high concentrations (25–50 $\mu\text{g/ml}$), ES-62 can act as a weak mitogen when incubated alone with murine splenic B cells.²⁴ However, at concentrations comparable to those found in the bloodstream of infected humans (0.2–2 $\mu\text{g/ml}$),³¹ *in vitro* stimulation with ES-62 substantially inhibits the proliferation of splenic B cells activated via the B-cell receptor (BCR).²⁴ Furthermore, the same effect is observed when such B cells exposed to ES-62 *in vivo* by release from subcutaneously implanted osmotic pumps are activated *ex vivo*.^{26,27} This inhibition appears to be direct and can be mimicked by PC alone or by PC conjugated to bovine serum albumin (BSA), indicating that the PC moiety may be responsible for this immunomodulatory effect.²⁴ Indeed, B cells from mice exposed to PC *in vivo* are hyporesponsive to BCR cross-linking compared to B cells from control animals.²³

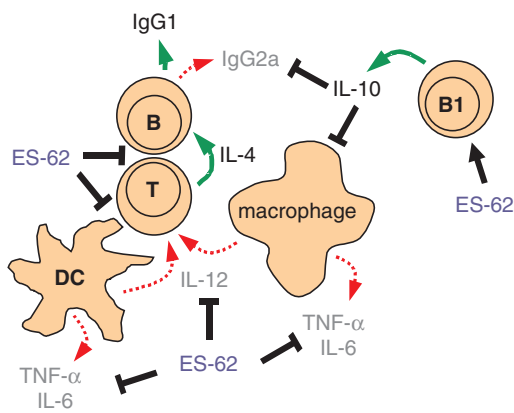


Figure 2. Immunomodulation by ES-62. ES-62 targets multiple cells of the immune system [black arrows (+) and T bars (-)] to achieve immunomodulation, broadly biasing the immune response to a Th2/anti-inflammatory response characterized by the production of low levels of IL-12, IFN- γ , TNF- α and IL-6 (red arrows), secretion of IL-4 (green arrow) by Th2 cells and production of the Th2-associated antibody isotypes IgG1 (mouse) and IgG4 (human). IL-10 production by B1 cells (green arrow) also contributes to this response. ES-62 alters costimulatory molecule expression on DCs and targets the signalling pathways triggered following cross-linking of the B- and T-cell antigen receptors (BCR and TCR), hence disrupting the responses of these cells to specific antigen.

Rather paradoxically, and despite its apparent ability to desensitize B lymphocytes, ES-62 induces an antibody response during natural infection in jirds.³² Indeed, BALB/c mice injected with ES-62 also mount an antibody response; subcutaneous injection with ES-62 induces the production of the Th2-associated isotype IgG1, but not IgG2a (Th1).²³ Moreover, this paradox is not simply found experimentally but is reflected by the literature in that many human studies reveal an indirect association between the presence of circulating filarial nematode products and levels of parasite-specific IgG1, IgG2 and IgG3 whilst the IgG4 subclass is usually found to be elevated and often to a considerable degree.⁸ One possible explanation relates to the generation of IL-4, a cytokine that would promote IgG4 production, and which is a frequently recorded feature of filariasis. We have previously shown during *in vitro* studies that IL-4 actually synergises with ES-62 to cause B-lymphocyte proliferation rather than hyporesponsiveness.²³ Thus it is possible that although ES-62 renders conventional B lymphocytes hyporesponsive *in vivo* such that their ability to produce antibody is impaired, in an environment that contains IL-4, the cells may in fact be induced to produce antibodies of the IgG4 subclasses. Such a scenario could also help explain why total, in addition to specific, levels of this subclass are greatly increased in filariasis patients. Indeed, we find experimentally that ES-62 and IL-4 are comitogenic for B cells and that such IL-4-mediated rescue appears to arise as a consequence of exposure to IL-4 pre-

venting the degradation of protein kinase C- α (PKC- α), an important enzyme in mitogenic activation of B lymphocytes, that is normally driven by ES-62.³³ In addition, the observed Th2 bias of the anti-ES-62 response is dependent on IL-4 because IL-4 knockout mice fail to produce IgG1; an IL-10-dependent role for PC in blocking the IgG2a response has also been implicated.²⁹ Thus the main effect of PC-containing molecules such as ES-62 on antibody responses during filarial nematode infection may not be so much to inhibit them as to polarize them. Consistent with this, we have recently shown that the murine antibody response to ES-62 is converted from solely IgG1 (a Th2 antibody) to mixed IgG1/IgG2a (the latter, a Th1 antibody) when the PC moiety is removed.²⁹

B1 lymphocytes, which reside in the pleural and peritoneal cavities, respond to PC-containing filarial nematode molecules by producing IL-10 and specific IgM antibody.³⁴ In contrast to the splenic B cells (B2 phenotype) described above, exposure of B1 cells to ES-62 released from osmotic pumps results in their proliferation and IL-10 production, even in the absence of further stimuli.²⁷ However, ES-62-exposed B1 cells show further proliferation and IL-10 production following subsequent *in vitro* ligation of the antigen receptor or lipopolysaccharide (LPS) stimulation. Since up to 10% of B1 cells would be expected to bind PC via their antigen receptor³⁵ the spontaneous B1-cell proliferation may simply reflect this. Interestingly, these data raise the possibility that the anti-PC IgM response frequently observed in filaria-infected humans and in animal models of filariasis (reviewed in ref. 36) may be the result of B1-cell activation rather than B2-cell activation.

Macrophage and DC activation and polarization of immune responses

In contrast to its ability to profoundly modulate B-cell responses, ES-62 exhibits only marginal direct effects on T-cell function, at least in terms of antigen/mitogen-driven proliferation and cytokine secretion. However, ES-62 polarizes T-cell responses indirectly via modulation of the maturation and function of DCs²⁸ and macrophages.^{30,37} These specialized APCs are required for the priming and activation of CD4⁺ T lymphocytes and are capable of directing the subsequent differentiation and function of T cells via both interaction with costimulatory molecules expressed on the APC surface and the secretion of cytokines. With respect to the former, ES-62 and indeed PC modulate the surface expression of costimulatory molecules to generate DCs with an immature phenotype, which are capable of driving the development of Th2 cells.^{28,38} Therefore, immature DCs exposed to ES-62 in the tissues during a natural filarial nematode infection could contribute to the Th2/anti-inflammatory phenotype observed in these infections. However, we have recently

shown that exposure to ES-62 *in vivo* can act even earlier; specifically, it can subvert the development of DC and macrophage progenitors in the bone marrow. *In vivo* exposure of bone marrow progenitors to ES-62 released from osmotic pumps biases their subsequent *ex vivo* differentiation to generate DCs and macrophages that are hyporesponsive to subsequent activation by LPS.³⁸ Thus the immunomodulatory effects of ES-62 are retained throughout the *ex vivo* development of these cells from their precursors, suggesting that the effects of ES-62 can be long-lived. Once again, these effects can be mimicked by PC-BSA/PC-ovalbumin. Production of the Th1-inducing cytokine IL-12 and the pro-inflammatory cytokines tumour necrosis factor- α (TNF- α) and IL-6 in response to pathogen molecules other than LPS, e.g. bacterial lipopeptide (BLP), as well as CpG oligonucleotides, is also significantly reduced following the exposure, either *in vitro* or *in vivo*, of macrophages and DCs to ES-62.^{30,39} Again, inhibition is also seen following *in vitro* or *in vivo* exposure to PC, either unconjugated or conjugated to ovalbumin or BSA.³⁸

Intriguingly, in spite of these anti-Th1/anti-inflammatory effects, treatment of macrophages and DCs with ES-62 alone induces low levels of IL-6, IL-12 and TNF- α production.³⁰ The precise reason for this is not known but we postulate that it may be the consequence of abortive signalling, which renders cells hyporesponsive to subsequent (or even simultaneous) stimulation with other ligands^{30,37} and interestingly is compatible with the finding that pro-inflammatory cytokines dominate the early immune response to filarial parasites.⁴⁰

Modulation of intracellular signal transduction

B-cell signalling

The best understood mechanism of ES-62 action is the disruption of B-cell activation, which results in the suppression of proliferation following BCR cross-linking. The BCR comprises a clonotypic antigen-binding component (surface immunoglobulin, sIg) and its accessory immunoreceptor tyrosine-based activatory motif (ITAM)-containing signal transducing molecules Ig α and Ig β . Ligation of the BCR triggers protein tyrosine kinase (PTK) activity, resulting in tyrosine phosphorylation of the ITAMs (reviewed in refs 41,42) and the recruitment of a number of key signal-transducing pathways implicated in cellular activation and proliferation (Fig. 3). These include the phospholipase C (PLC)- γ , phosphoinositide-3-kinase (PI-3-K) and the Ras-Erk (extracellular-regulated kinase) mitogen-activated protein kinase (MAP kinase) signalling cascades.

Our studies have shown that ES-62 selectively targets these key signalling events following BCR ligation to disrupt the activation and proliferation of B cells. For exam-

ple, it does not target the early BCR-coupled PLC- γ -mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate, which generates the second messengers, inositol trisphosphate and diacylglycerol that mobilize intracellular stores of calcium and activate protein kinase C isoforms.²⁴ Rather, ES-62 appears to selectively modulate the expression and activity of certain PKC isoforms in resting and BCR-stimulated B cells.^{24,33} Thus, whilst ES-62 selectively down-regulates the expression of the α , β , ζ , δ and $1/\lambda$ isoforms, predominantly by stimulating proteolytic degradation, it up-regulates the expression of PKC- γ and PKC- ϵ in murine splenic B cells.³³ In addition, ES-62 acts to modulate PKC signalling resulting from antigen-receptor ligation of B cells by disrupting the normal activation and nuclear translocation patterns of the PKC- α and PKC- $1/\lambda$ isoforms.³³ These data are consistent with proposals that PKC- α , - β and - $1/\lambda$ transduce key activation signals involved in the regulation of antigen-driven DNA synthesis and proliferation in B cells⁴³ such as phosphorylation of the nuclear protein lamin B and the induction and activation of NF- κ B, Fos, Egr-1 and Myc.⁴⁴⁻⁴⁸

In addition to its effects on PKC signalling, pre-exposure to ES-62 selectively inhibits BCR-mediated recruitment of key proliferative pathways, such as the PI-3-K and Ras/Erk MAP kinase cascades.⁴⁹ An intriguing feature of these results was that stimulation of B cells with ES-62 alone did not induce activation of Ras or PI-3-K despite the fact that ES-62 can induce activation of the PTKs Lyn and Syk (upstream regulators of Ras and PI-3-K) and Erk MAP kinase (downstream effector of Ras).⁴⁹ This apparent discrepancy was resolved by our finding^{49,50} that ES-62 does not mediate uncoupling of the BCR from the PI-3-K or Ras/Erk MAP kinase cascades by targeting activation of Syk or Lyn. Instead, it primes for the induction of the tyrosine phosphatase SHP-1, which negatively regulates activation via the BCR complex by dephosphorylating the Ig α / β -ITAMs, thereby preventing recruitment of the Ras/Erk MAP kinase cascade. Moreover, ES-62 recruits additional negative regulatory elements of this pathway, namely RasGAP and the dual (thr/tyr) phosphatase Pac-1, to terminate any residual coupling of the BCR to Ras and Erk activity, respectively. This multipronged mechanism provides for a rapid and profound desensitization of BCR-stimulated Erk MAP kinase signalling (Fig. 3).

ES-62 also modulates the activation of the two other major MAP kinase subfamilies, p38 and c-Jun N-terminal kinase (JNK).⁵¹ The precise targets of ES-62 in these pathways are unclear but our results, like those of others,⁵² suggest that the BCR modulates p38 and JNK signalling in a Vav- and Rac-dependent manner.⁵¹ The finding that ES-62 alone can selectively stimulate Syk, Lyn and MAP kinase activation, whilst it does not appear to modulate Ras, Rac, or PI-3-K activity^{33,49} also suggests that MAP kinases can be activated in B cells via alternat-

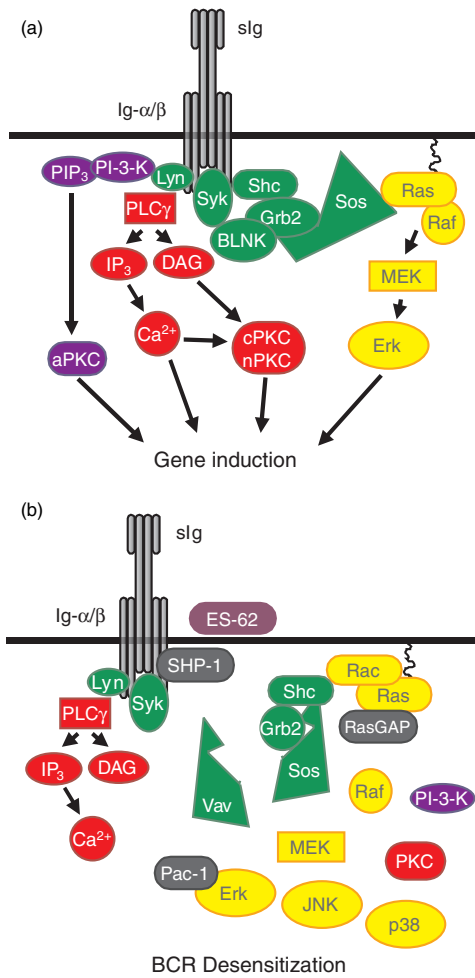


Figure 3. BCR signalling in untreated (a) and ES-62-exposed (b) B cells. Following ligation of the B-cell antigen receptor (BCR) of untreated B cells (a) the kinase, Lyn, tyrosine phosphorylates the immunoreceptor tyrosine activation motifs (ITAMs) on the accessory transducing molecules Ig- α and Ig- β resulting in the recruitment and activation of the PI-3-K and PLC- γ -signalling pathways. Whilst PI-3-K activation results in the activation of atypical PKC isoforms (aPKC), PLC- γ activation induces inositol trisphosphate (IP₃) and diacylglycerol (DAG) generation, ultimately resulting in activation of classical (cPKC) and novel (nPKC) PKC isoforms. Binding of the adaptor proteins Shc and BLNK to the phosphorylated ITAMs leads to the recruitment of the Grb2Sos complexes (Grb2 is an adaptor protein which binds Sos, a guanine nucleotide exchange factor) required for activation of the GTPase, Ras. Active Ras initiates the Erk MAP kinase cascade by binding and activating the ser/thr kinase, Raf leading to stimulation of the thr/tyr kinase MEK and consequent activation and nuclear translocation of the ser/thr kinase Erk. ES-62/PC signalling (b) disrupts BCR coupling to the PI-3-K cascade as well as targeting major negative regulatory sites in the control of the Erk, p38 and JNK MAP kinase cascades. First, ES-62 signalling promotes the BCR-activation of SHP-1 tyrosine phosphatase to prevent initiation of BCR signalling by maintaining the ITAMs in a resting, dephosphorylated state and hence prevents recruitment of the ShcGrb2Sos complexes required to activate the Ras- and Rac-MAP kinase cascades. Second, ES-62 signalling promotes the BCR-mediated recruitment of RasGAP to terminate ongoing Ras signals. In addition, ES-62 is also likely to target MAP kinase activation by down-regulating PKC isoform expression. Finally, ES-62-signalling promotes the BCR-driven association of the nuclear MAP kinase dual (thr/tyr) phosphatase, Pac-1 with Erk to terminate any ongoing Erk signals. This multipronged mechanism results in a rapid and profound desensitization of BCR coupling to the MAP kinase cascades.

ive PTK-dependent pathways. This proposal is consistent with the increasing evidence for Ras-independent pathways of Erk MAP kinase activation involving lipid second messengers and PKC.⁵³ We have not, however, found any evidence to support the proposal that ES-62 or BCR stimulates Erk MAP kinase activity via lipid second messengers derived from phosphatidylcholine-specific phospholipase C (PtdCho-PLC), PtdCho-phospholipase D (PtdCho-PLD) or sphingomyelinase-dependent pathways.⁴⁹ However, PKC- α has been reported to mediate activation of Erk MAP kinase via Raf and MAP kinase kinase (MEK) pathways^{54,55} and this is consistent not only with reports that PKC activity plays a role in coupling the BCR to ErkMAP kinases⁵⁶ but also provides a rationale for our finding that, whilst prolonged pretreatment with ES-62 acts to reduce this PKC activity, ES-62 initially up-regulates PKC- α expression.^{24,33}

T-cell signalling

Although ES-62 exerts few, if any, direct effects on antigen-/mitogen-driven proliferation or cytokine secretion, in a manner analogous to that observed for the suppression

of antigen receptor stimulation of B cells, the protein suppresses anti-CD3-induced proliferation of Jurkat T cells and also promotes concanavalin A-induced growth arrest of these cells²⁵ and this can be mimicked by PC.²³ The precise sequence of signalling events underlying these responses has not been fully elucidated, but it is clear that ES-62-mediated desensitization of TCR signalling is associated with disruption of TCR coupling to PLD, PKC, PI-3-K and Ras-Erk MAP kinase signalling but, as with B cells, not the PLC-mediated generation of inositol phosphates.²⁵ Again, PC appears to be the active moiety, because culture with PC or PC-BSA has comparable effects to ES-62 on the coupling of the TCR to PTK activation (ZAP-70, Lck and Fyn recruitment) and the Ras-Erk MAP kinase signalling cascades.^{22,23,25} These findings are consistent with an earlier report showing that PC-containing molecules of the human filarial nematode, *B. malayi*, inhibit the response of human T cells to mitogens.⁵⁷

Macrophage and DC signalling

Induction of cytokine production by macrophages and DCs in response to many pathogen products occurs

We have recently shown that ES-62 achieves its modulation of macrophage and DC activation in a TLR4-dependent manner.³⁹ Low-level cytokine induction by ES-62 alone was abolished in macrophages/DCs from TLR4 knockout mice and also MyD88 knockout mice. Similarly ES-62-mediated suppression of cytokine induction by TLR ligands (BLP and CpG) was dependent on the presence of TLR4. In contrast, ES-62 effects were TLR2 and TLR-6 independent. Modulation of surface expression of major histocompatibility complex class II and costimulatory molecules (CD40, CD80, CD86) was also suppressed in DCs from TLR4 knockout mice, although these latter effects were only partially dependent on the presence of MyD88. Interestingly, macrophages and DCs from C3H/HeJ mice, which are unresponsive to LPS because of their production of a defective form of TLR4 resulting from a point mutation in the intracellular TIR domain, remain responsive to ES-62 as evidenced by the modulation of cytokine production and costimulatory molecule expression. Thus it appears that TLR4 must be present but not necessarily fully functional for ES-62 responsiveness. This may be because of 'non-classical' coupling to downstream signal transduction pathways from TLR4 or the recruitment of a signalling coreceptor.

It is not currently clear whether TLR4 is required for the direct recognition of ES-62. Preliminary evidence suggests that the PC moiety of ES-62 is at least in part required for its recognition (unpublished data) and hence it is tempting to speculate that TLR4 may recognize this molecular pattern, especially because other PC-containing molecules have been reported to act via TLR4.^{61–63}

Conclusions and future prospects

In summary, our studies over the past decade have demonstrated extensively how a single parasite glycoprotein can cause profound modulation of the host immune response to enable coexistence of host and parasite, via co-ordinated targeting of multiple cells of the immune system. Importantly, ES-62 is not unique to *A. viteae*; homologues of ES-62 are found in other filarial nematodes, including the human parasites *B. malayi* and *O. volvulus*.^{14,15,64} For example, *A. viteae* ES-62 shares 77% homology with an ES-62 cDNA of *B. malayi* (see Fig. 4). In addition, PC-containing molecules are produced by a diverse range of pathogens (reviewed in ref. 22); hence PC represents an important molecular pattern in the detection of and response to pathogens.

Of additional interest, dissection of the mechanisms of immune modulation by ES-62 ultimately provides information that can be utilized in the development of novel strategies not only for the treatment of filariasis and other infections, but also for the treatment of pathological conditions caused by excessive or inappropriate immune responses resulting from immune dysfunction. Indeed, we

recently demonstrated that exposure to ES-62 prevented the initiation of arthritis in a murine collagen-induced arthritis model of rheumatoid arthritis and also suppressed the progression of established disease.⁶⁵ These effects correlated with the inhibition of collagen-specific pro-inflammatory/Th1 cytokine production (TNF- α , IL-6 and IFN- γ). In human studies, ES-62 was also able to suppress the *in vitro* release of pro-inflammatory cytokines by synovial cells derived from patients with rheumatoid arthritis. Thus, ES-62 constitutes a pathogen-derived immunomodulator with significant therapeutic potential.

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