



Review

Antigen Receptor Signaling Is Subverted by an Immunomodulatory Product Secreted by a Filarial Nematode

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Abstract. ES-62 is a phosphorylcholine (PC)-containing glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae* which is able to inhibit antigen receptor-stimulated proliferation of B and T lymphocytes *in vitro* and *in vivo*. The active component of ES-62 appears to be PC, as the results obtained with ES-62 are broadly mimicked by PC conjugated to bovine serum albumin or PC alone. Such desensitization of lymphocyte responsiveness appears to reflect an uncoupling of the antigen receptors from key intracellular proliferative signaling events, such as the phosphoinositide-3-kinase, protein kinase C and Ras mitogen-activating protein kinase pathways. ES-62 mediates such immunomodulatory effects at concentrations equivalent to those found for PC-containing molecules in the bloodstream of parasitized humans and, thus, ES-62 provides a model system for dissecting the mechanisms of immune evasion induced by related PC-containing glycoproteins expressed by human filarial nematodes.

Key words: filarial nematode; immune evasion; lymphocyte; phosphorylcholine; signal transduction.

Introduction

Filarial nematodes, particularly *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*, are a major cause of morbidity in the tropics⁴⁹. It is currently estimated that about 150 million people are infected with one or more of these worms and a significant proportion of these individuals suffer major health problems, including severe skin lesions, elephantiasis and eye damage leading to blindness. Infection with filarial nematodes is essentially a life-long condition⁴⁷ and parasite longevity suggests that immune responses to the parasite appear to be suppressed or at least modulated in the majority of infected individuals (reviewed

in^{26, 38}). Although the mechanisms underlying such immunomodulation have not been fully elucidated, “excretory-secretory products” (ES) released by the worms have been found in serum obtained from humans and animals infected with these parasites, and these molecules appear to have immunomodulatory properties. Certainly, we²² and others¹⁵ have demonstrated that these molecules can inhibit lymphocyte proliferation *in vitro*. In addition, a recent study on humans infected with *W. bancrofti* has demonstrated the existence of an inverse relationship between levels of ES and levels of total antibody in the bloodstream³⁰.

We²² have focused on the effect of ES-62, a phosphorylcholine (PC)-containing glycoprotein which is

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a major ES of the rodent filarial parasite *Acanthocheilonomema viteae*²¹, on the proliferation of B cells following crosslinking of the antigen receptors (BCR/sIg). At concentrations comparable to those of PC-containing ES molecules found in the bloodstream of infected humans (0.2–2 µg/ml²⁹), ES-62 was able to substantially inhibit (by up to 60%) the proliferation of B cells resulting from activation of the BCR. That the PC-moiety was almost certainly responsible for these inhibitory effects of ES-62 was demonstrated by the finding that it could be mimicked by PC conjugated to bovine serum albumin (BSA) or even PC alone. Moreover, we have found that the levels of the BCR-stimulated proliferation of B cells derived from mice exposed to PC *in vivo* are suppressed compared with those of B cells from normal animals²⁰.

In order to understand the molecular mechanisms underlying such ES-62-mediated suppression of BCR-stimulated B cell proliferation, we investigated the ef-

fect of the parasite product on some of the early signaling events underlying sIg-mediated B cell activation^{10, 11, 22}. The BCR comprises a clonotypic antigen-binding component, sIg and its accessory immunoreceptor tyrosine-based activation motif (ITAM)-containing transducing molecules, Ig-α and Ig-β. Ligation of BCR induces protein tyrosine kinase (PTK) activity^{5, 41} and resultant tyrosine phosphorylation of the ITAMs. Such tyrosine phosphorylation induces recruitment of Syk kinase and the reorientation, enhanced binding and activation of ITAM-associated *src*-family PTKs, Blk, Fyn, Lck and Lyn leading to BCR-association of a number of key signal transducers (Fig. 1), implicated in cellular activation and proliferation, such as phospholipase C (PLC)-γ, phosphoinositide-3-kinase (PI-3-K), protein kinase C (PKC) and the components of the Ras/MAPK (mitogen-activating protein kinase) signaling cascades^{5, 41}. We have found that the ES-62-mediated inhibition of B cell activation reflects selective effects on BCR sig-

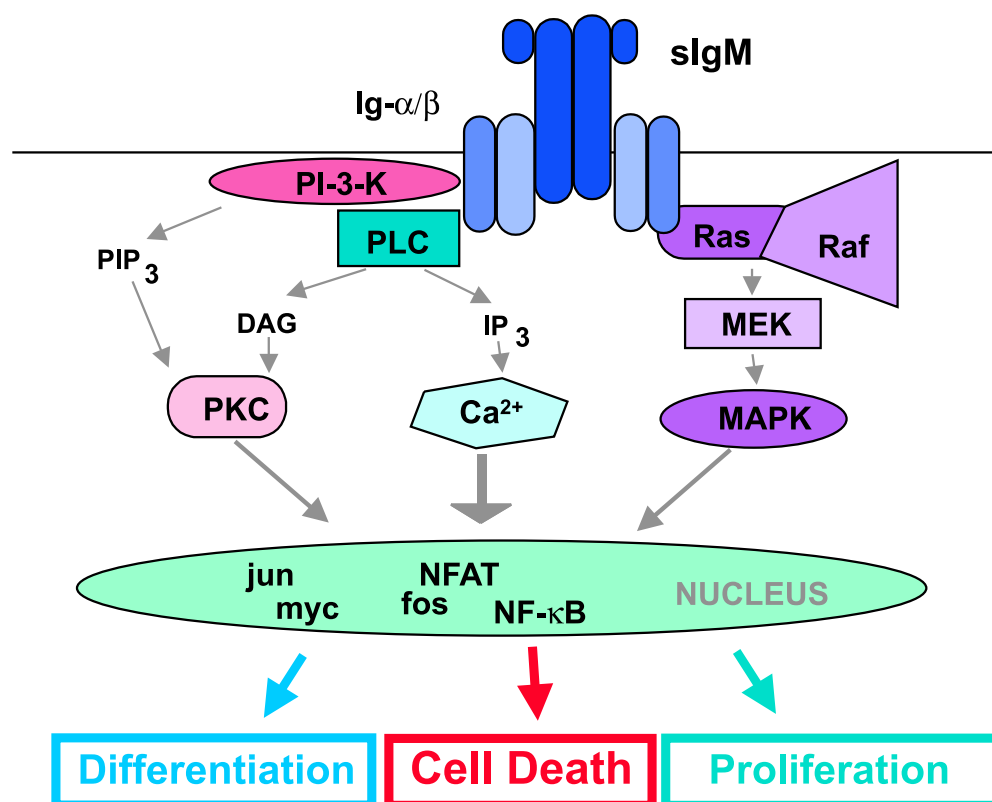


Fig. 1. BCR signaling. Although the events leading to B cell proliferation have not been fully elucidated, it is clear that ligation of the B cell antigen receptor complex, which comprises a clonotypic antigen-binding component, sIg and its accessory immunoreceptor tyrosine-based activation motif (ITAM)-containing transducing molecules, Ig-α and Ig-β, induces protein tyrosine kinase (PTK) activity. Such tyrosine phosphorylation induces recruitment of Syk kinase and the reorientation, enhanced binding and activation of ITAM-associated *src*-family PTKs, Blk, Fyn, Lck and Lyn, leading to BCR-association of a number of key signal transducers implicated in cellular activation, transcription and proliferation, such as phospholipase C γ (PLC-γ), phosphoinositide-3-kinase (PI-3-K) and the components of the Ras/MAPK (mitogen-activating protein kinase) signaling cascades

naling: for example, ES-62 does not target the early sIg-coupled PLC- γ -mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdInsP₂), which generates the second messengers, inositol trisphosphate (InsP₃) and diacylglycerol (DAG)²², but rather appears to downregulate total cellular levels of PKC expression and activity²² in resting and sIg-stimulated B cells^{11, 22}. Moreover, we have also found that the parasite molecule can modulate the activation status of key sIg-coupled proliferative pathways, such as the PI-3-K and Ras/MAPK signaling cascades. In this review, therefore, we discuss how these findings provide a molecular mechanism to explain how ES-62 could modulate B cell responsiveness during filarial infections.

ES-62 Differentially Modulates Expression and Activation of PKC Isoforms in B Lymphocytes

PKC comprises a superfamily of phospholipid-dependent serine/threonine kinases of which the isoforms α , β , δ , ϵ , γ , η , ι/λ (ι and λ are the same protein, originally identified in distinct species), μ , θ , and ζ have now been cloned (reviewed in^{12, 25}). Alternatively spliced variants of β and ϵ have also been shown to exist. These PKC isoforms have been shown to have distinct enzymological properties and varied cellular distributions, and have now thus been subclassified into 3 major groups according to similarities in their structure and function^{12, 25}. For example, the "conventional" PKC subfamily, cPKC, comprises the α , β , γ isoforms, which are calcium-, DAG- and phospholipid-dependent enzymes and have, hence, been postulated as being the downstream targets of PtdInsP₂-derived second messengers. In contrast, whilst the "novel" PKCs (nPKC; δ , ϵ , η , θ) are DAG, but not calcium, dependent enzymes, the "atypical" PKCs (aPKC; ζ , ι/λ) are not activated by DAG or the pharmacological activator of most PKCs, phorbol 12-myristate 13-acetate (PMA), but have been shown to transduce PI-3-K-mediated signals. A recent addition to the PKC superfamily, PKC μ or PKD, which appears to have the most homology with the nPKC family, may in fact represent the identification of a new PKC family, because this isoform has an additional N-terminal region which contains a putative transmembrane domain, suggesting that this PKC may be an intrinsic receptor ser/thr kinase^{12, 25}. Since several of these PKC isoforms (α , β , δ , ϵ , ζ , η and μ) have previously been shown to be expressed in B cells, we decided to investigate whether the anti-proliferative action of ES-62 was reflected by differential modulation

of PKC isoform expression and activation. We found that, whilst ES-62 selectively downregulates expression of the α , β , ζ , δ or ι/λ isoforms of PKC, it upregulates expression of PKC- γ and $-\epsilon$ in murine splenic B cells. Little (γ , ϵ) or no (θ , μ) expression of certain isoforms of PKC could be detected in resting B cells, and PKC- θ or $-\mu$ were not induced by culture with ES-62. Inhibitor and kinetics studies showed that ES-62 appears to promote PKC downregulation, which is maximal following 4 h of culture with ES-62 (2.5 μ g/ml), predominantly by stimulating proteolytic degradation as this downregulation can be blocked by protease inhibitors such as leupeptin¹¹. Interestingly, the distinct PKC isoforms exhibited differential sensitivity to ES-62-mediated downregulation and this may reflect their distinct subcellular localisation and/or functional properties^{12, 25}. Moreover, although ES-62-mediated downregulation of PKC was found to be predominantly mediated by proteolysis rather than changes in PKC synthesis, either at the mRNA or protein levels, ES-62-mediated downregulation of certain PKC isoforms could also be prevented by treatment with actinomycin D (PKC- δ , $-\iota$, $-\zeta$, and to a lesser extent, $-\alpha$) or cycloheximide (PKC- β , $-\iota$ and $-\zeta$). These results therefore suggested that ES-62 may also have the capacity to differentially modulate the transcriptional (including mRNA stabilisation)/translational regulatory mechanisms associated with the expression of PKC isoforms.

Given that prolonged stimulation of B cells via the antigen or IL-4 receptors leads to an overall upregulation of PKC activity^{3, 19, 22} and promotes B cell proliferation, ES-62-mediated downregulation of overall PKC activity provides a signaling mechanism to explain the observed suppression of sIg-driven B cell proliferation¹⁹. Moreover, as we found that ES-62 may exert its anti-proliferative effects, at least in part, by downregulating PKC- α , $-\beta$ and $-\iota/\lambda$, our data are consistent with proposals that activation-induced upregulation of PKC- α , $-\epsilon$, $-\iota/\lambda$ (anti-Ig³) and $-\beta$ (anti-Ig and PMA³) plays a role in B cell activation. Further support for these proposals is provided by the finding that not only can IL-4 overcome the downregulatory effects of ES-62 on PKC- α and $-\iota/\lambda$ isoform expression in B cells¹¹, but also that IL-4 can provide a co-mitogenic signal for ES-62 in B cells²⁰.

The molecular mechanisms underlying the transduction of proliferative signals by PKC isoforms are as yet poorly understood, but a number of recent studies have reported that overexpression of several PKC isoforms is not only associated with an enhanced expression of the early-response genes and/or transcription factors, but also that overexpression of PKC- α or $-\beta$

has been shown to result in cell transformation (reviewed in^{1, 25}). In addition, a number of recent findings are consistent with the hypothesis that certain isoforms may exert their proliferative effects by translocating to the nucleus and modulating DNA synthesis via phosphorylation of targets involved in the regulation of DNA synthesis. The relevance of such studies to B cell activation is underlined by reports that the nuclear protein lamin B is phosphorylated by a PKC-like activity in B cells and that PKC activation is required for NF- κ B, Fos, Egr-1 and Myc induction following mitogenic stimulation via the antigen receptors^{16, 24, 27, 35, 43}. Interestingly, therefore, in addition to showing that ES-62 can modulate PKC expression, we have found that ES-62 can also modulate PKC signaling by interfering with the normal activation and nuclear translocation patterns of the α and ι/λ isoforms of PKC resulting from antigen receptor-driven activation of B cells¹¹. Thus, in the light of the recent developments outlined above, our results may suggest that sIg-mediated nuclear translocation of PKC- α , - β and - ι/λ , and subsequent phosphorylation of elements associated with the regulation of

DNA synthesis, may play an important role in the transduction of proliferative signals via the antigen receptors in B cells.

ES-62 Disrupts B Lymphocyte Activation by Targeting the RasMAPK Pathway

In addition to its effects on PKC signaling, we have also found that ES-62 can induce activation of the PTKs Lyn, Syk and Blk and ErkMAPK in B cells. Furthermore, pre-exposure to the parasite product selectively inhibits sIg-mediated tyrosine phosphorylation and resultant coupling to key sIg-coupled proliferative pathways, such as PI-3-K and the RasMAPK cascades¹⁰. 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, like anti-Ig, can induce activation of the PTKs Lyn, Syk and Blk (upstream regulators of Ras and PI-3-K) and ErkMAPK (downstream effector of Ras)¹⁰. These data therefore suggested either that activation of these key

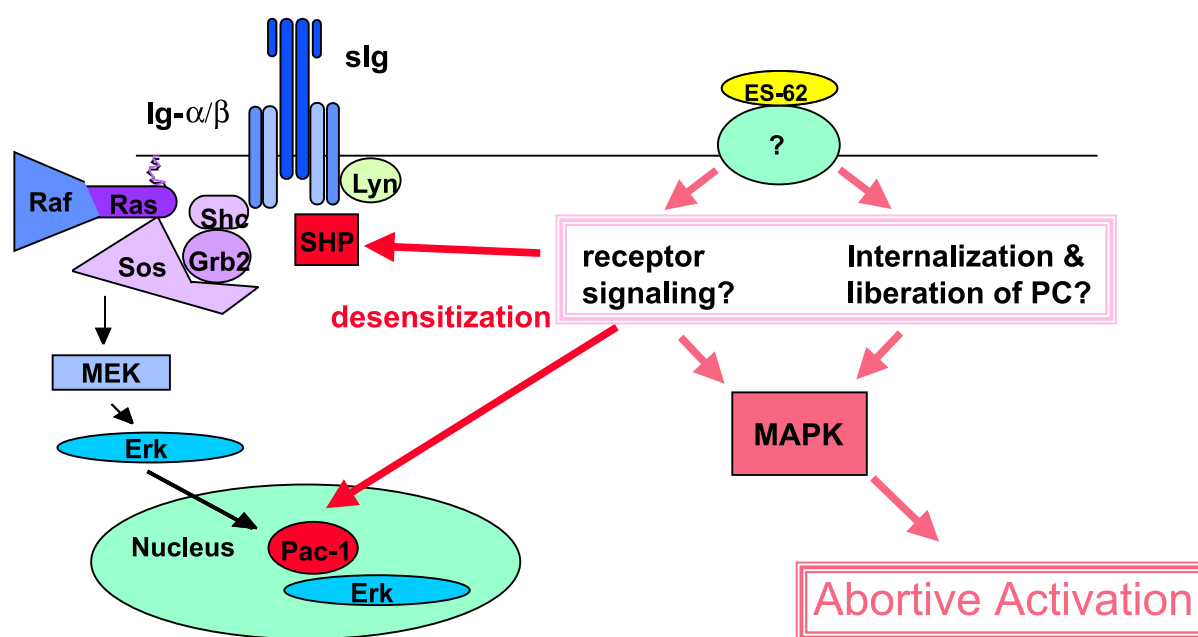


Fig. 2. ES-62 uncouples the BCR from the RasErkMAPK cascade. Following ligation of the BCR, the PTK, Lyn, tyrosine phosphorylates the ITAMs on the accessory transducing molecules Ig- α and Ig- β . The Ras adaptor protein, Shc, binds to the phosphorylated ITAMs and, in turn, is phosphorylated, leading to the recruitment of the Grb2Sos complexes required for activation of Ras. Active Ras initiates the ErkMAPK cascade by binding and activating Raf, leading to stimulation of MEK and consequent activation and nuclear translocation of Erk. ES-62/PC, either by subversion of immune receptor signalling and/or internalisation, appears to target two major negative regulatory sites in the control of BCR-coupling to the Ras MAPK cascade. First, ES-62 induces the activation of SHP tyrosine phosphatase to prevent initiation of BCR signaling by maintaining the ITAMs in a resting, dephosphorylated state and, hence, prevents recruitment of the ShcGrb2Sos complexes required to activate Ras. Second, ES-62 recruits the nuclear MAPK dual phosphatase, Pac-1 to terminate any ongoing Erk signals. This dual-pronged mechanism results in a rapid and profound desensitization of BCR-coupling to the RasErkMAPK cascade

BCR-associated PTKs is not sufficient for Ras or PI-3-K activation or, alternatively, that ES-62 recruited additional undefined signals which acted to negatively modulate Ras and PI-3-K activity. The latter hypothesis turned out to be the case, as we found that ES-62 does not mediate its uncoupling of the BCR from the PI-3-K or RasMAPK cascades by targeting activation of Syk, Lyn or Blk, but rather induces the tyrosine phosphatase SHP-1, which serves to prevent activation of the BCR complex by dephosphorylating the ITAMs and, hence, preventing recruitment of the RasMAPK 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 or Erk activity, respectively. This dual-pronged mechanism provides for a rapid and profound desensitization of BCR-stimulated proliferative signaling (Fig. 2).

Of course, the finding that ES-62 alone can selectively stimulate Blk, Syk and Lyn and MAPK activation, whilst it does not appear to modulate either Ras or PI-3-K activity, also suggests that MAPK can be activated in B cells via alternative PTK-dependent pathways. This proposal is consistent with the increasing evidence for Ras-independent pathways of MAPK activation involving lipid second messengers and PKC⁴. We have not, however, found any evidence to support the proposal that ES-62 or sIg stimulates MAPK activity via lipid second messengers derived from phosphatidylcholine-specific phospholipase C (PtdCho-PLC), PtdCho-phospholipase D (PLD) or sphingomyelinase-dependent pathways, but PKC- α -mediated activation of MAPK via Raf and MEKK pathways^{2, 40} could provide a rationale for our earlier finding that, whilst ES-62 initially upregulates PKC- α expression, prolonged pretreatment with ES-62 acts to reduce this PKC activity^{11, 22}. Indeed, it is tempting to speculate that ES-62 may exert its suppressive effects on B cell proliferation by inhibiting sIg-activation of this key signaling cascade and downstream regulators of gene transcription via Ras, PTK- and PKC-mediated pathways. Recent findings that are consistent with this hypothesis of convergent signaling are that the product of PI-3-K, PtdInsP₃ reportedly activates PKC- ζ and the atypical PKCs- δ , - ϵ , and - η *in vitro*^{36, 45}. Since PKC- δ and - ζ are expressed in B cells^{11, 34}, ES-62-mediated abrogation of sIg-coupled PI-3-K activity could contribute to the suppression of PKC activity which results from exposure to the parasite product^{11, 22}. Moreover, given that activation of PKC- ζ leads to phosphorylation and inactivation of I κ B and, hence, activation of the transcription factor NF- κ B^{13, 14}, and that PI-3-K has also been

implicated in activation of S6 kinases^{6, 48}, ES-62-mediated disruption of PTK, PKC- and Ras-mediated activation of MAPK and such downstream pathways would be likely to have profound inhibitory effects on B cell activation.

ES-62 Disrupts Antigen Receptor Signalling in T Cells

Similarly¹⁸, we have found that ES-62 (2 μ g/ml) can also render the human T cell line Jurkat anergic to cellular signaling via the T cell antigen receptor (TCR; for review see⁴⁴). ES-62-mediated desensitization of TCR signaling is associated with disruption of TCR coupling to PLD, PKC, PI-3-K and Ras-MAPK signaling but, as with murine B cells, not the PLC-mediated generation of inositol phosphates. Again, as for the disruption of BCR signaling, PC appears to be the active moiety, as culture with PC or PC-BSA has similar effects to ES-62 on the coupling of the TCR to PTK activation (ZAP-70, Lck and Fyn) and the PLC, Ras and MAPK signaling cascades¹⁸. These findings are therefore consistent with an earlier report showing that PC-containing molecules of another filarial nematode, *B. malayi*, inhibit the response of human T cells to mitogens²⁸.

Mechanism of Action of ES-62?

PC therefore appears to target key elements in the transduction of transcriptional and proliferative signals associated with ligation of antigen receptors both in B and T cells. How is it able to do this? Examination of the literature reveals that PC is considered to be a putative second messenger which may play a role in the regulation of cell growth. It is known, for example, that most, if not all, human tumors contain elevated levels of PC^{9, 33} and that *ras*-transformed cell lines produce elevated levels of PC, which are necessary for cell proliferation^{23, 42}. Thus, since we have shown for B cells that ES-62 does not itself induce the generation of cellular PC¹⁰, it is conceivable that the PC component of ES-62 could result in a partial activation of B cells which renders them desensitized to subsequent activation via the antigen receptor. Certainly, it has been shown that PC added to culture medium enhances DNA synthesis in cells such as NIH 3T3 fibroblasts, but at much higher concentrations of the molecule than employed in our study^{7, 46}. Abortive activation by the PC component of ES-62 would require the internalization of the protein, but it is possible that this could

occur via the platelet-activating factor (PAF) receptor that is expressed on B and T cells³². Certainly, *Streptococcus pneumoniae*, which expresses PC on its surface, has been shown to enter human cells by this mechanism⁸. Alternatively, PC on ES-62 could simply desensitize the cells to subsequent challenge to antigen by abortively signaling via immune receptors such as the PAF receptors^{17, 31}. This latter proposal may be particularly pertinent as not only does signaling via the PAF receptor augment certain B cell activation responses such as antibody secretion, but also there is no heterologous desensitization of IP₃-sensitive calcium mobilisation between the antigen and PAF receptors³², a situation reminiscent of the PLC-independent suppression of B cell activation induced by ES-62²².

Conclusions and Perspectives

In summary, we have shown that a filarial nematode PC-ES renders B and T cells anergic to stimulation via the antigen receptors by selectively uncoupling sIg from key proliferative pathways such as the PKC, PI-3-K and RasMAPK cascades. Although anti-parasite responses can generally be detected in filarial infections, a number of studies suggest that, in some patients at least, these may be impaired^{30, 37, 39}. We propose that the results we have obtained in this study provide a molecular mechanism which could contribute to impaired lymphocyte responses during filarial infection. Moreover, our approach, by elucidating the nature of lymphocyte defects at the molecular level, may ultimately lead to the design of strategies to overcome such immune dysfunction.

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