

BRIEF REPORT

# The parasitic worm product ES-62 up-regulates IL-22 production by $\gamma\delta$ T cells in the murine model of collagen-induced arthritis

Margaret M. Harnett<sup>1</sup>, William Harnett<sup>2</sup>, Miguel A. Pineda<sup>1</sup>

<sup>1</sup>Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow G12 8TA, UK

<sup>2</sup>Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G4 0NR, UK

Correspondence: Miguel Pineda

E-mail: Miguel.Pineda@glasgow.ac.uk

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ES-62 is a phosphorylcholine (PC)-containing glycoprotein secreted by the filarial nematode *Acanthocheilonema viteae* that acts to modulate the host immune response to promote the establishment of chronic helminth infection. Reflecting its anti-inflammatory actions, we have previously reported that ES-62 protects mice from developing Collagen-Induced Arthritis (CIA); thus, as this helminth-derived product may exhibit therapeutic potential in Rheumatoid Arthritis (RA), it is important to understand the protective immunoregulatory mechanisms triggered by ES-62 in this model *in vivo*. We have established to date that ES-62 acts by down-regulating pathogenic Th17/IL-17-mediated responses and up-regulating IL-10. In addition, our studies have identified that IL-22, another member of the IL-10 family of cytokines, exerts dual pathogenic and protective roles in this model of RA with ES-62 harnessing the cytokine's inflammation-resolving and tissue repair properties in the joint during the established phase of disease. Here, we discuss the counter-regulatory roles of IL-22 in the murine model of CIA and present additional novel data showing that ES-62 selectively induces  $\gamma\delta$  T cells with the capacity to produce IL-22 and that  $\gamma\delta$  T cells with the capacity to produce IL-22, but not IL-17, induced during CIA can be identified by their expression of TLR4. Moreover, we also show that treatment of mice undergoing CIA with the active PC moiety of ES-62, in the form of PC conjugated to BSA, is not only sufficient to mimic the ES-62-dependent suppression of pathogenic IL-17 responses shown previously but also that of the IL-22 and IL-10 up-regulation observed with the parasitic worm product during CIA. These findings not only reinforce the potential of IL-22, firstly described as a Th17-related pro-inflammatory cytokine, as a protective factor in arthritis but also suggest that drugs based on the PC moiety found in ES-62 may be able to harness the joint-protecting activities of IL-22 therapeutically.

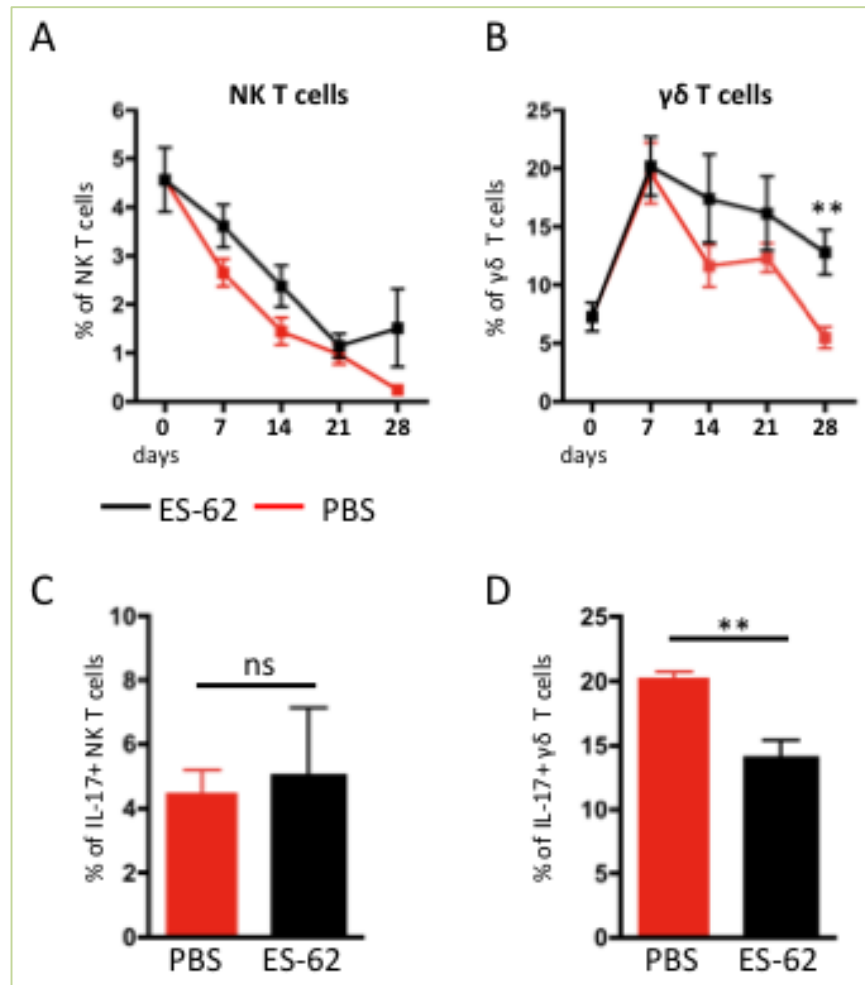
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## Introduction

ES-62 is a phosphorylcholine (PC)-containing

glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae* and a homologue of excretory-

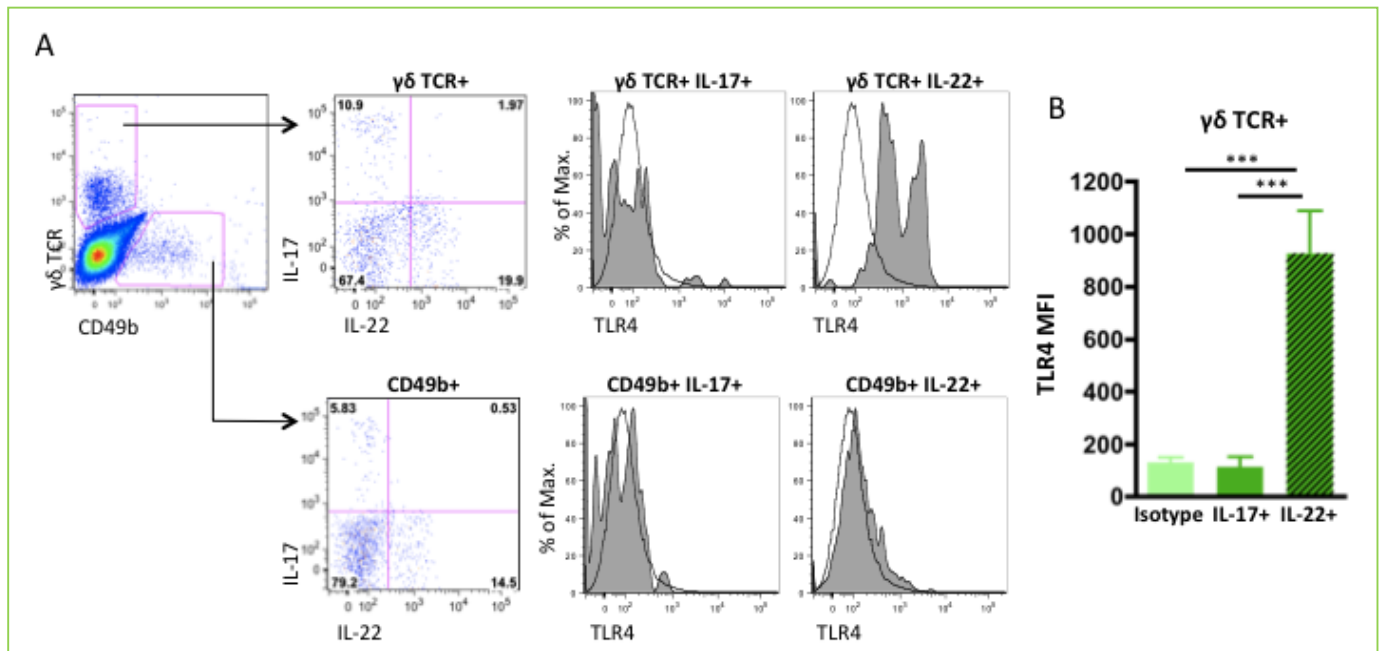


**Figure 1. Kinetic analysis of IL-22-producing NK and  $\gamma\delta$  T cells in DLN of mice with CIA.** ES-62- and PBS-treated mice undergoing CIA were culled every 7 days (n=4 for each treatment group at each time-point) and IL-22 expression by DLN cells analysed by intracellular flow cytometry. Proportions of spontaneously IL-22-producing, CD49b<sup>+</sup> NK T cells (A) and  $\gamma\delta$  TCR<sup>+</sup> T cells (B) from individual mice were evaluated as described in the Material and Methods. The proportions of IL-17-producing NK (C) and  $\gamma\delta$  T cells (D) were also analysed at d28. Red symbols represent CIA mice treated with PBS whilst black symbols represent mice treated with ES-62. Values are the mean  $\pm$  SEM of 4 individual mice at each time-point. \*p<0.05, \*\*p<0.01.

secretory products that can be detected in the bloodstream of humans infected with *Brugia malayi* and *Onchocerca volvulus* [1]. Our early *in vitro* studies revealed that this parasite-derived molecule exhibits a broad range of immunomodulatory effects, for example the dampening of the pro-inflammatory responses of macrophages and dendritic cells<sup>[2, 3]</sup>, that likely promote persistence of the parasite in the host without severely immunocompromising it. Although its mechanism of action is not yet fully understood, TLR4 is a major target of ES-62 [4] and most of its anti-inflammatory properties depend on its unusual post-translational addition of PC: thus, when PC is chemically linked to an irrelevant protein such as BSA or ovalbumin, as with ES-62, it inhibits full activation of macrophages and DCs following TLR stimulation in a TLR4-dependent

manner<sup>[5]</sup>.

In accordance with the Hygiene Hypothesis [6] that has led to the proposal that the recent dramatic increase in allergy and autoimmune diseases in industrialized countries is related to the eradication of parasitic infections, a wide range of studies have shown infection with helminths to be protective against such disorders in experimental models, suggesting that such parasites or their immunomodulatory products could be exploited therapeutically. Reflecting this, we have shown that treatment with ES-62, either prophylactically or therapeutically, reduces disease severity and incidence of Collagen-Induced Arthritis (CIA), a murine model of human Rheumatoid Arthritis (RA)<sup>[7]</sup> and PC-BSA is able to protect mice in a similar

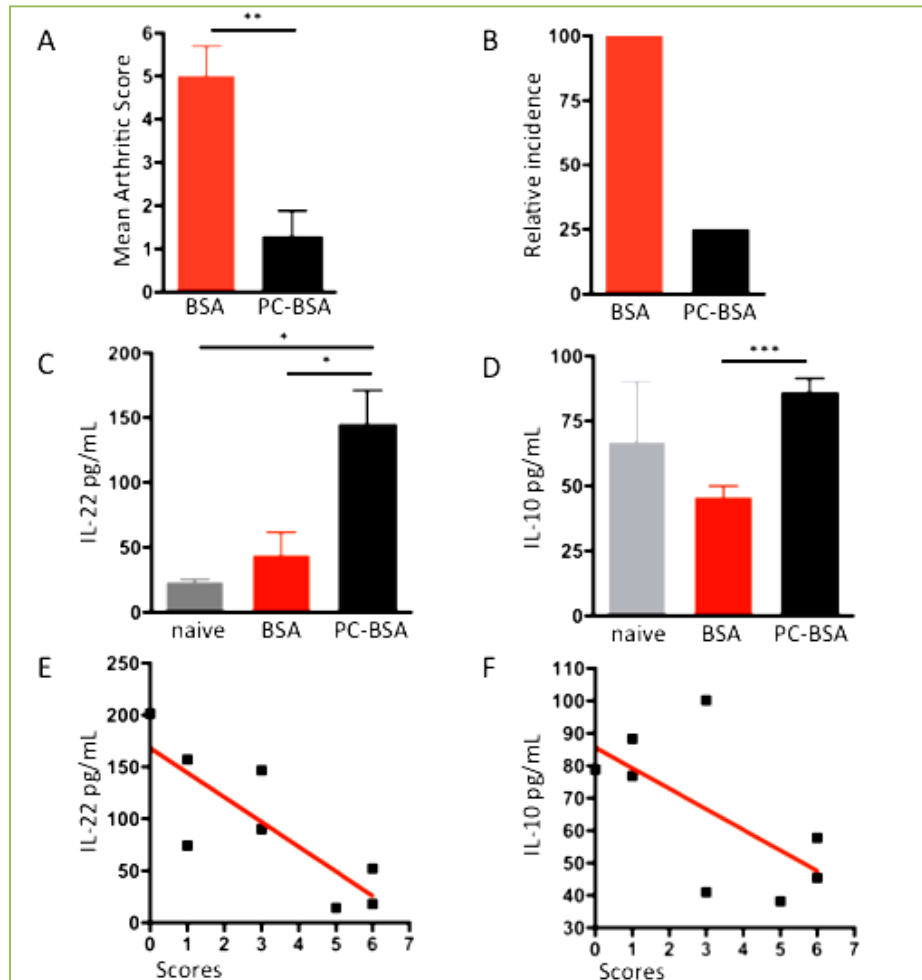


**Figure 2. TLR4 expression by  $\gamma\delta$  T cells is associated with their production of IL-22 in mice with Collagen-Induced Arthritis.** Representative plots of the gating strategy for analysis of intracellular IL-17 and IL-22 expression by  $\gamma\delta$  TCR<sup>+</sup> and CD49b<sup>+</sup> NK T cells in the DLN from a single CIA mouse following *ex vivo* stimulation with PMA/ionomycin are shown (A). Expression of TLR4 (tinted grey histograms) was determined according to appropriate isotype controls in  $\gamma\delta$  T cells and CD49b<sup>+</sup>NK T cells respectively. Data represent Mean Fluorescence Intensity (MFI)  $\pm$  SD of DLN cells (B) from 5 individual mice (\*\**p*<0.001) undergoing CIA from a single experiment representative of two independent experiments.

fashion to that of ES-62 [8, 9]. An increasing number of studies have shown that helminths and their secreted products exert their anti-inflammatory effects by actively modulating host immune effector mechanisms, for example, by reducing aberrant Th1 or Th2 responses and promoting IL-10-dependent regulatory pathways [10-12]. Indeed, some of these actions have been associated with the mechanism of action underlying ES-62-mediated protection in CIA. Thus, protection was first associated with suppressed Th1 responses, reduced levels of collagen-specific IgG2a antibodies and increased IL-10 production [7, 13]. However, ES-62 failed to protect against other Th1-driven models of disease [14, 15], suggesting that alternative pathways might be targeted in CIA. Indeed, ES-62-mediated protection was subsequently shown to be associated with down regulation of IL-17 production, a finding consistent with increasing evidence that this cytokine is a major pathogenic driver in RA as IL-17 induces hyperplasia and proliferation of synoviocytes, production of pro-inflammatory mediators and promotion of TLR-dependent responses and recruitment of immune system cells including T cells, B cells and neutrophils [16-18]. Interestingly, ES-62 down-regulates IL-17 production by a multi-pronged mechanism, inhibiting Th17 polarization both directly and indirectly, the latter via the suppression of IL-6 and IL-23 release by DCs, key cytokines in DC-priming of Th17 differentiation. ES-62

also targets DCs to modulate the cross-talk between  $\gamma\delta$  T cells and DCs, resulting in reduced levels of IL-17 production by the innate lymphocytes. Collectively, these actions result in down-regulated expression of IL-17 in the joints of mice with CIA and reduced synovial inflammation [19]. Importantly, as biologics targeting IL-17 have resulted in some opportunistic fungal infections under clinical trial, IL-17-producing NK cells which have been shown to be protective in such infections remain unaffected in ES-62-treated mice undergoing CIA [20]. Thus ES-62 attenuates IL-17-mediated responses without fully inhibiting them, as a result suppressing pathogenic, but not infection-combating sources of IL-17 and these findings may go some way to explaining why helminth infections or ES-62-based treatments do not substantially immunocompromise the host.

More recently, we have investigated the effect of ES-62 on another component of the IL-23/IL-17 inflammatory axis in autoimmune arthritis, namely IL-22 which had also been reported to be a pro-inflammatory cytokine in RA [21, 22]. Consistent with this, we showed that neutralisation of IL-22 prior to onset of disease blocks joint pathology. However, we have also shown that IL-22 is required for ES-62's anti-arthritis effects, via a mechanism in which IL-22-signalling desensitizes synovial fibroblast (SF) responses to pro-inflammatory factors such as IL-17 in the joint [23]. As a result, SFs produce less IL-6 and the number of



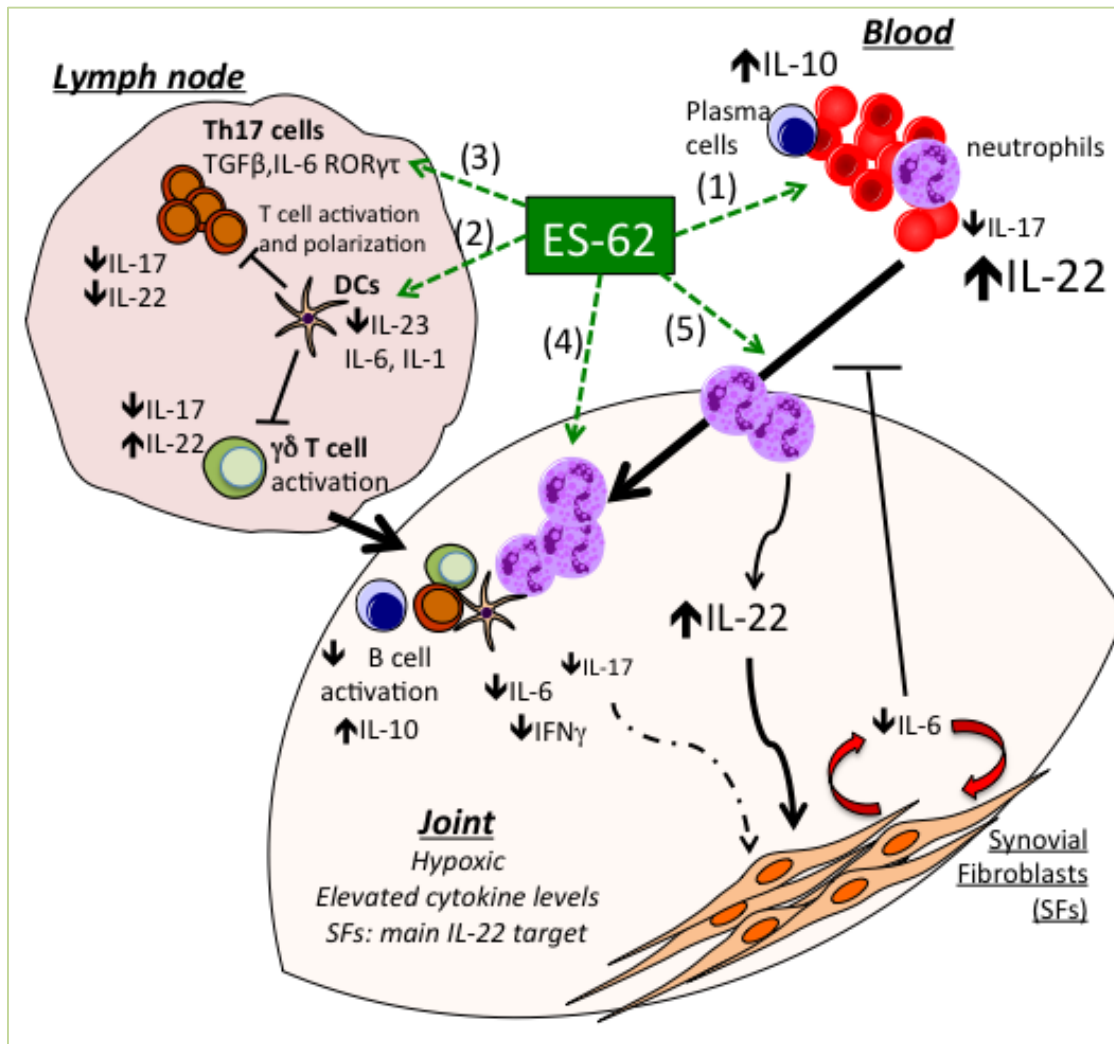
**Figure 3. PC-BSA protection against CIA is associated with up-regulation of serum IL-22 and IL-10.** Mice undergoing CIA were treated with BSA (n=4) or PC-BSA (n=4). Arthritis scores (A) at day 32 after initial collagen injections are shown, expressed as mean scores  $\pm$  SEM for BSA- or PC-BSA-treatment groups. Disease incidence is also shown (B), indicated by the % of mice developing a severity score  $\geq 2$ . \*\* =  $p < 0.01$ . IL-22 (C&E) and IL-10 (D & F) levels were evaluated by ELISA in serum of naïve (n=3), BSA- (n=4) and PC-BSA-treated (n=4) individual mice and plotted as mean values of triplicate analyses (C & D). \* $p < 0.05$  and \*\*\* $p < 0.001$ . The correlations between IL-22 (E; n = 8,  $r = 0.6726$ ,  $p = 0.0127$ ) and IL-10 (F; n = 8,  $r = 0.406$ ,  $p = 0.088$ ) serum concentrations for all mice undergoing CIA (BSA- and PC-BSA-treated groups) and clinical scores are shown (E & F).

neutrophils infiltrating the joints is significantly reduced. These findings are consistent with increasing evidence suggesting that IL-22 can play dual pathogenic and, reflecting its wound healing properties [24], protective roles in RA. It is therefore essential to fully understand the role(s) of IL-22 in the pathogenesis of arthritis and the resolution of inflammation and protection against joint damage induced by the helminth product in order to translate these potentially therapeutic actions to the clinic. The aim of this report is therefore to provide further insight into the sources of IL-22 modulated by ES-62 in mice with CIA and in particular we show that a subset of  $\gamma\delta$  T cells that express TLR4 *in vivo* is a major potential producer of such protective IL-22. In addition, we also report that PC-BSA

mimics the ability of ES-62 to up-regulate IL-22 production during protection from CIA: thus as we have recently provided proof of principle that small drug-like PC-based compounds can be protective in mouse models of arthritis and asthma<sup>[9, 25]</sup>, this latter finding highlights the novel potential of parasite-derived products to harness the therapeutic properties of IL-22 in RA.

### Material and Methods

**CIA.** Animals were maintained in the Biological Services Units in accordance with the Home Office UK Licences PPL60/3580, PPL60/3119 and PIL60/12183 and the Ethics Review Boards of the Universities of Glasgow



**Figure 4. Protective effects of ES-62 observed in the CIA model.** ES-62 targets a complex cell network to regulate cytokine production and effector responses in the joint of CIA mice. In serum, ES-62/PC-BSA up-regulates IL-10 and IL-22, but strongly down-regulates IL-17 (1). In draining lymph nodes, IL-17 production by  $\gamma\delta$  T cells and conventional CD4 T cells, is down-regulated by ES-62 via modulation of DC responses (2). This is reflected *in vitro* by ES-62 targeting DCs to down-regulate TLR-mediated production of IL-23, IL-6 and IL-1, resulting in reduced Th17 priming and inhibited induction of IL-17-producing  $\gamma\delta$  T cells. In addition, ES-62 directly inhibits IL-1-dependent differentiation of Th17 *in vitro* by downregulation of MyD88 (3). Analysis of joint cells by flow cytometry and immunohistochemistry (4) revealed that at the site of inflammation, ES-62 down-regulates the levels of antibody producing B cells and restores the levels of IL-10 producing B cells. In addition, the IL-17/IL-22 balance is reset with protective IL-22 responses desensitising synovial fibroblasts to IL-17, further suppressing IL-17-dependent inflammatory pathways, including the IL-6 production by synoviocytes and the subsequent recruitment of pro-inflammatory cells like neutrophils (5). Furthermore, the residual neutrophils infiltrating the joint exhibit a modulated phenotype and produce higher amounts of IL-22 (and lower levels of IL-17/IFN $\gamma$  and IL-6), to perpetuate the protective mechanisms of ES-62 in the joint.

and Strathclyde. CIA was induced in male DBA/1 mice (8-10 weeks old; Harlan Olac; Bicester, UK) by intradermal immunization with bovine type II collagen (CII, MD Biosciences) in complete Freund's adjuvant (FCA) and mice were treated with purified endotoxin-free ES-62 from *A. viteae* or PC-BSA (2  $\mu$ g/dose) or PBS subcutaneously on days -2, 0 and 21<sup>77, 81</sup>. Mice were monitored for clinical symptoms of arthritis: severity scores represent 0=normal;

1=erythema; 2=erythema plus swelling; 3=extension of swelling; 4=loss of function, and the disease score is the sum for the four limbs.

**Ex vivo analysis.** DLN cells (10<sup>6</sup>/ml) were stimulated in the presence and absence of PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 1 h before being incubated with 10  $\mu$ g/ml Brefeldin A (Sigma-Aldrich, UK) for 5 h at 37°C with 5% CO<sub>2</sub>. Live cells were discriminated by the

LIVE/DEAD fixable aqua dye (BioLegend, UK) and phenotypic markers labelled using FITC-conjugated anti-CD49b (BioLegend, UK), APC-conjugated anti- $\gamma\delta$  TCR (eBioscience, UK) and biotin-conjugated anti-TLR4 antibodies before the cells were fixed and permeabilized according to BioLegend protocols. Pacific Blue-conjugated streptavidin was used for detection of biotinylated antibodies. Cytokines were stained using PerCP-Cy5.5-conjugated anti-IL-17A (BioLegend, UK) or PE-conjugated anti-IL-22 antibodies (R&D Systems, UK) for 30 minutes prior to analysis by flow cytometry, gating according to appropriate isotype controls<sup>[19, 23]</sup>.

**Cytokine analysis.** Serum levels of IL-22 and IL-10 were determined by ELISA, according to the manufacturers' recommendations (R&D Systems; Biolegend).

**Statistical analysis.** Data were analyzed by Student's unpaired 1-tailed t-test. Articular scores were analysed by the Mann-Whitney test. P values  $* < 0.05$  were considered significant.

## Results and Discussion

**ES-62 differentially regulates IL-22 production by  $\gamma\delta$  and NK T cells in DLN from mice undergoing CIA.** Protection by ES-62 in the CIA model has been associated with counter-regulation of IL-17 and IL-22 levels in serum and joints during the established phase of pathology, with exposure to the parasite product elevating IL-22 but reducing IL-17 levels<sup>[19, 23]</sup>. Similarly, exposure to ES-62 reduces IL-17, but not IL-22 production by DLN and joint cells *ex vivo* and analysis revealed these cytokines to be predominantly produced by distinct populations of cells at both sites<sup>[19, 23]</sup>. Moreover, whilst ES-62 inhibited the levels of both spontaneous and PMA/ionomycin-stimulated IL-17 production by CD4<sup>+</sup> DLN cells, it only reduced the proportion of unstimulated CD4<sup>+</sup> DLN cells expressing IL-22<sup>[23]</sup>. However, although we have shown the parasite product to additionally suppress the levels of IL-17<sup>+</sup>  $\gamma\delta$  T cells whilst leaving the levels of IL-17-producing NK cells unaffected<sup>[26]</sup>, the effects of ES-62 on IL-22 production by these subsets of innate lymphocytes was not investigated. We therefore decided to evaluate this in order to potentially identify a source of protective IL-22 production in the DLN during CIA progression (Fig.1). Consistent with our previous observations that exposure to ES-62 appeared to have little effect on the levels of IL-22-producing DLN cells<sup>[23]</sup>, whilst kinetic analysis suggested that the proportion of both NK and  $\gamma\delta$  T cells expressing IL-22 was elevated in those DLN from ES-62 exposed mice undergoing CIA following challenge (day 21; Fig. 1A&B), this only reached significance for  $\gamma\delta$  T cells (at d28; Fig. 1B). By contrast, the proportion of  $\gamma\delta$  IL-17<sup>+</sup> but not NK IL-17<sup>+</sup> T cells was reduced at this time point (Fig. 1C&D).

These data suggest that  $\gamma\delta$  T cells, which by bridging innate and adaptive immunity play important immunoregulatory roles in RA<sup>[27]</sup>, are selectively targeted by ES-62 to modulate the IL-17/IL-22 balance in the CIA model to mediate its protective effects. Interestingly, this kinetic analysis also revealed, consistent with IL-22 playing a pathogenic role during the initiation phase of disease that the levels of IL-22-producing  $\gamma\delta$  T cells in the DLN peaked within about 7 days and then generally declined with disease progression in PBS-treated mice undergoing CIA. By contrast, and supporting their proposed protective role, exposure to ES-62 helps maintain the levels of IL-22<sup>+</sup>  $\gamma\delta$  T cells at time points where pathology is normally established in the joint. Although we do not know whether these cells can migrate to the joint and hence are representative of the IL-22-expressing lymphocyte-like cells previously shown to be elevated in ES-62-treated mice at this stage<sup>[23]</sup>, these results could suggest that the observed increase of IL-22 in serum and joints of ES-62-treated mice might be a result of  $\gamma\delta$  T cell modulation rather than effects on conventional Th22 cells and may correlate with a report that CD4<sup>+</sup> T cells isolated from synovial samples from RA patients were not able to produce IL-22<sup>[22]</sup>.

**IL-22-producing  $\gamma\delta$  T cells, but not IL-17-producing  $\gamma\delta$  T cells, express Toll-like receptor 4 (TLR4).** It is not clear how ES-62 selectively targets  $\gamma\delta$  but not NK T cells, to render them respectively sensitive or resistant to modulation of IL-17/IL-22 production as both these cell types express pattern recognition receptors (PRR) important in CIA pathogenesis<sup>[28]</sup>, such as TLR2<sup>[29]</sup> and ES-62 has been shown to subvert TLR signalling by down regulation of MyD88 in a variety of cell types<sup>[4, 19, 30-33]</sup>. However, TLR4 is required for ES-62 action and although this PRR is not generally considered to be expressed by either  $\gamma\delta$  or NK T cells, TLR4<sup>+</sup>  $\gamma\delta$  T cells have been described following burn injury<sup>[34]</sup> and we have recently reported that a population of these cells, but not NK T cells can be found in the DLN of mice undergoing CIA<sup>[20]</sup>. Thus, as TLR4 signalling has been reported to promote IL-17 production in  $\gamma\delta$  T cells isolated from lungs and lymphoid organs<sup>[35]</sup>, we hypothesised that differential expression of this PRR may be involved in the selective regulation of IL-17/IL-22 production by  $\gamma\delta$  T cells and NK T cells in the CIA model. Although analysis of DLN cells from mice with CIA confirmed that TLR4<sup>+</sup>  $\gamma\delta$  T cells did not express IL-17<sup>[20, 28]</sup>, perhaps surprisingly, but consistent with our previous findings that IL-17 and IL-22 were predominantly expressed by distinct populations of DLN cells, we observed a positive correlation between IL-22 expression and TLR4 on  $\gamma\delta$ , but not NK, T cells (Fig. 2A & B). This is the first observation of IL-22<sup>+</sup>TLR4<sup>+</sup>  $\gamma\delta$  T cells in the CIA model, and this subset may be analogous to the population of IL-22<sup>+</sup>IL-17<sup>-</sup>  $\gamma\delta$  T cells that has been shown to be protective in models of colon inflammation<sup>[36]</sup> and lung

fibrosis [37]. A similar association between other cell surface markers and cytokine profile expression in  $\gamma\delta$  T cells has previously been reported in that IL-17<sup>+</sup>  $\gamma\delta$  T cells and IFN $\gamma$ <sup>+</sup>  $\gamma\delta$  T cells differentially express the markers CD25 and CD122 respectively [38, 39], suggesting that expression of particular cell sensors can dictate functional  $\gamma\delta$  T cell responses and thus, phenotyping may allow the possibility of distinguishing potentially protective IL-22<sup>+</sup>  $\gamma\delta$  T cells from pathogenic IL-17<sup>+</sup>  $\gamma\delta$  T cells. Whether ES-62 can induce "protective" IL-22<sup>+</sup>  $\gamma\delta$  T cells via TLR4 signalling and/or differentially and temporally modulate IL-17 and IL-22 production by distinct subsets of  $\gamma\delta$  T cells is currently under investigation in our laboratory. Thus, although whether ES-62 acts via TLR4 to modulate IL-22 expression by  $\gamma\delta$  T cells during CIA is still unclear, it is an attractive hypothesis in terms of allowing selective modulation of the immune response given that we have previously shown that ES-62-mediated down regulation of IL-17 production by  $\gamma\delta$  T cells reflects inhibition of their activation by DCs [23] and that NK T cells, which do not express TLR4, are not affected by ES-62 in terms of either IL-17 or IL-22 production [20] (Fig. 1).

**Phosphorylcholine (PC) mimics the ability of ES-62 to increase IL-22 levels in the bloodstream of CIA animals.** Collectively, our data indicate that ES-62 can selectively modify critical regulatory IL-17/IL-22 pathways in the CIA model. Of note, therapies based on its mode of action promoting resetting of the protective: pathogenic T cell effector balance of this inflammatory axis that focus on the differential suppression of IL-17/IL-22-producing innate cell populations are perhaps less likely to leave the host open to opportunistic fungal infections, than current strategies involving novel biological agents like monoclonal IL-17 neutralizing antibodies. This new generation of immunomodulators is in phase II clinical trials (secukinumab [40], ixekizumab [41] and brodalumab [42]), but already it seems clear that more studies are needed to establish their long-term safety as some reports showed increased rates of such infection concomitant with treatment [43-45]. ES-62 thus presents certain advantages as a potential anti-arthritic treatment: reduced risk of immunosuppression since IL-17-driven responses are limited but not abrogated, regulation of a complex network of cells rather than a key regulatory molecule and the additional ability to trigger damage repair mechanisms, such as IL-22-mediated pathways of inflammation resolution and wound healing. However, as a large potentially immunogenic protein, ES-62 still presents important limitations preventing its translation into the clinic. Nevertheless, new ES-62-based interventional approaches can still be developed as the bioactive PC moiety, when conjugated to an "inert" carrier protein such as BSA, can also protect mice against CIA (Fig. 3A-B). Indeed, studies involving PC-BSA were the starting point

for our development of small molecular analogues (SMAs) that mimic the anti-inflammatory ability of ES-62 in targeting pathogenic IL-17 responses in mouse models of arthritis and asthma [9, 25] and provide proof of concept for helminth-based drugs in inflammatory disease.

Although protection by PC-BSA against CIA also reflects down-regulation of pathogenic IL-17-dependent responses [9], our earlier studies suggested that PC does not necessarily reproduce all of the potentially protective effects of ES-62. For example, whilst PC conjugated to ovalbumin (PC-OVA) also ameliorated joint pathology in CIA models, it did not reduce the levels of potentially pathogenic anti-collagen IgG2a antibodies that are additionally decreased in ES-62-treated mice [8]. Therefore, we decided to investigate whether PC-BSA-mediated protection against CIA also reflected the up-regulation of IL-22 required for protection by ES-62 [23]. Interestingly therefore, whilst as we have shown previously, CIA mice presented elevated levels of IL-22 in serum compared to naïve mice [23], it was found that such PC-BSA-treated mice exhibit significantly higher levels of IL-22 (3.5-fold increase, Fig. 3C) and these serum levels of IL-22 inversely correlate with the clinical scores of the mice with CIA (Fig. 3E). Taken together, these findings corroborate our previous results observed with ES-62 and further support our hypothesis that the high levels of IL-22 observed in the serum of such mice during established disease, reflect the ability of this cytokine to resolve inflammation and joint damage properties of this cytokine in the CIA model [23].

**Increased levels of IL-22 are also associated with elevated levels of IL-10 in serum obtained from PC-BSA-treated CIA mice.** We have previously shown that protection against CIA in mice afforded by ES-62 is associated not only with inflammation-resolving IL-22 responses, but also with elevated splenic IL-10 production [7] which may reflect restoration of the levels of B cells with potential (IL-10-dependent) regulatory capacity [13]. Interestingly, therefore, a recent study has suggested that IL-22-mediated amelioration of CIA severity is associated with increased levels of IL-10 [46]. Moreover, IL-22 has also recently been reported to suppress experimental autoimmune uveitis via generation of IL-10-producing regulatory CD11b<sup>+</sup> antigen presenting cells [47]. Therefore, we decided to evaluate the levels of IL-10 in serum of PC-BSA-treated mice, to determine whether there was concomitant up-regulation of IL-22 and IL-10 levels, as observed with ES-62. Indeed, treatment with PC-BSA was found to restore the levels of serum IL-10 which had tended to be down-regulated in CIA mice to those observed in naïve mice (Fig. 3D): moreover, and consistent with a protective role for this cytokine, such IL-10 levels also inversely correlated with CIA articular disease scores (Fig. 3F). These results suggest that PC-BSA is able to reproduce the ability of ES-62 to promote IL-10 production in the CIA

model<sup>[8]</sup>: although the cell type responsible for such IL-10 production is still unknown, it is possible that PC-BSA promotes IL-10-producing regulatory B cells, rather than regulatory T cells, as with ES-62 in CIA mice <sup>[13]</sup>. Mechanistically, since neutralizing anti-IL-10 antibodies were shown to abrogate the protective effect of IL-22 in CIA <sup>[46]</sup>, such restoration of IL-10 levels by PC-BSA might impact on DC-dependent priming of Th17 and IL-17-producing  $\gamma\delta$ T cells, consistent with the reduced levels of IL-17 observed in serum of PC-BSA-treated CIA mice <sup>[9]</sup>. Thus understanding the mechanisms responsible for controlling cytokine networks such as those resulting in the ES-62-mediated counter-regulation of IL-10/IL-22/IL-17 responses (Summarized in Fig. 4), rather than the analysis of the individual cytokines, may clarify the contradictory data relating to, for example, whether IL-22 is a pro- or anti-inflammatory mediator in RA.

### Concluding Remarks

IL-22 was originally described as a pro-inflammatory mediator in RA. This reflects findings that mice deficient in this cytokine were resistant to developing CIA<sup>[48]</sup> as well as reports that IL-22 levels and Th22 cells are elevated in the serum of RA patients <sup>[21]</sup> and also that IL-22 has been shown to induce proliferation of human SF and promote RANKL production and osteoclastogenesis *in vitro*<sup>[49]</sup>. However, whilst IL-22 levels are up-regulated in RA patients and IL-22 has been shown to be involved in bone damage and RANKL production, certain studies report that such IL-22 up regulation does not contribute to the induction of synovial inflammation <sup>[21, 49-51]</sup>. Moreover, supporting our proposal of dual pathogenic and protective roles for IL-22 in RA based on the protective action of ES-62 in CIA <sup>[23]</sup>, recent studies have also shown that IL-22 can play protective roles in CIA, via enhancing IL-10-mediated immunoregulation <sup>[46]</sup> and, as with ES-62, desensitizing SFs to IL-17 <sup>[52]</sup>. Collectively, these data suggest that the precise role of IL-22 in RA - protective versus pathogenic -probably depends on the inflammatory context. This is likely to especially be the case with respect to its differential effects on SFs, which in RA are subject to a complex pro-inflammatory cocktail involving cytokines, chemokines and alarmins generated by the hypoxic conditions pertaining in the arthritic joint. These results therefore question the hypothesis that serum IL-22 levels provide a pathogenic biomarker in established RA and may even suggest that IL-22 is up-regulated in a homeostatic mechanism to limit and resolve joint inflammation and promote tissue repair. In any case, they dictate that therapies targeting IL-22 should be reconsidered while the role of the cytokine in the initiation and progression of synovial inflammation in RA awaits full understanding.

### Conflicting interests

The authors have declared that no competing interests exist.

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