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Everts, B.

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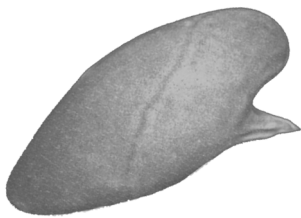
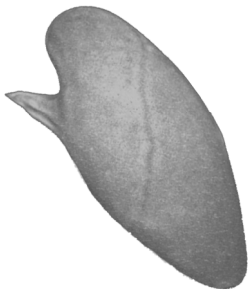
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Chapter

4



Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses



Bart Everts, Georgia Perona-Wright,
Hermelijn H. Smits, Cornelis H. Hokke,
Alwin J. van der Ham, Colin M. Fitzsimmons,
Michael J. Doenhoff, Jürgen van der Bosch,
Katja Mohrs, Helmut Haas, Markus Mohrs,
Maria Yazdanbakhsh and Gabriele Schramm

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Abstract

Soluble egg antigens of the parasitic helminth *Schistosoma mansoni* (SEA) induce strong Th2 responses both *in vitro* and *in vivo*. However, the specific molecules that prime the development of Th2 responses have not been identified. Here we report that omega-1, a glycoprotein secreted from *S. mansoni* eggs and present in SEA, is capable of conditioning human monocyte-derived DCs *in vitro* to drive Th2 polarization with similar characteristics as whole SEA. Furthermore, using IL-4 dual reporter mice we show that both natural and recombinant omega-1 alone are sufficient to generate Th2 responses *in vivo* even in the absence of IL-4R signaling. Finally, omega-1-depleted SEA displays an impaired capacity for Th2 priming *in vitro*, but not *in vivo*, suggesting the existence of additional factors within SEA that can compensate for the omega-1-mediated effects. Taken together, we identify omega-1, a single component of SEA, as a potent inducer of Th2 responses.

Introduction

Helminth parasites are the most potent natural inducers of T helper 2 (Th2) cell-polarized responses. Infection with *Schistosoma mansoni* elicits strong Th2 responses, in humans and in experimental animal models. The development of this Th2 polarization coincides with the onset of egg production by adult worms [1]. The ability of *S. mansoni* eggs to induce Th2 differentiation during infection is underscored by the observation that schistosome eggs alone, or *S. mansoni* soluble egg antigen (SEA) are sufficient to drive Th2 polarization in naïve mice even in the absence of infection [2;3].

Various cells of the innate immune system are thought to contribute to the activation of Th2 responses after infection with *S. mansoni*. Granulocytes like basophils, eosinophils and mast cells, have been shown to represent potential innate sources of Th2-associated cytokines like IL-4, during infection, which can contribute to the polarization, sustenance and amplification of Th2 responses [4;5]. Although these cells may well support Th2 development, professional antigen presenting cells (APCs), in particular dendritic cells (DCs), are thought to play a dominant role in the initiation of these T cell responses [6]. DCs have been shown to efficiently sense, capture and process antigens derived from *S. mansoni* eggs [7;8], resulting in the capacity of these DCs to prime for strong Th2 polarization both *in vitro* and *in vivo* [9;10].

While the ability of *S. mansoni* eggs and their soluble antigens to promote potent Th2 responses has been well documented, the specific components responsible for this activity are only beginning to be characterized. In this respect, glycans on proteins from *S. mansoni* eggs have been shown to contribute to the Th2 polarizing properties of SEA [11;12]. In addition, IPSE/alpha-1 and peroxiredoxin, both glycoproteins secreted by the eggs, have recently been shown to trigger basophils to produce IL-4 [13;14] and to induce the development of alternatively activated macrophages [15], respectively, both of which can contribute to Th2 polarization following exposure to egg antigens. However, the specific molecules responsible for the initiation of Th2 differentiation have remained elusive.

Here we show that omega-1, a glycoprotein present in both SEA [16] as well as excretory/secretory products (ESP) from live eggs [17], potently instructs human DCs to prime highly Th2-polarized responses from naïve human CD4⁺ T cells *in vitro*. In addition, we demonstrate that injection of omega-1 alone into IL-4 dual reporter mice is sufficient to prime Th2 responses *in vivo*, even in the absence of the IL-4R α chain. Together, these findings demonstrate that omega-1 is a potent initiator rather than amplifier of Th2 responses.

Results and Discussion

Excretory/secretory products from *S. mansoni* eggs condition human DCs for Th2 priming

DCs are known to play a pivotal role in the initiation and polarization of T cell responses and *S. mansoni* egg preparations have been shown to prime Th2 cells via the functional modulation of DCs [9;10]. To study and identify the components from *S. mansoni* egg preparations that instruct Th2 development, we used a well-established co-culture system of human monocyte-derived DC and naïve CD4⁺ T cells, which is generally thought to mimic in vivo DC-mediated T helper cell polarization [6]. It stands to reason that excretory/secretory products (ESP) from live eggs [17] are the first egg-derived molecules to interact with cells of the innate immune system, including DCs. Therefore, we initially tested ESP for their capacity to condition DCs to prime Th2 development from naïve CD4⁺ T cells. Similar to SEA, exposure of DCs to ESP resulted in a robust Th2 skewing irrespective of the presence or absence of LPS as a neutral maturation factor (Fig 1A). In a recent report Williams and colleagues [17] identified omega-1 and IPSE/alpha-1 as the most abundant proteins within ESP from *S. mansoni* eggs. Separation of ESP preparations by SDS-PAGE (Fig 1B) followed by Western blotting with specific monoclonal antibodies revealed prominent bands representing IPSE/alpha-1 and omega-1 (Fig 1C), which was confirmed by mass spectrometry (data not shown). Both, omega-1 and IPSE/alpha-1, are glycoproteins which are specifically expressed in and secreted from *S. mansoni* eggs. Omega-1 has been demonstrated to display RNase activity and hepatotoxic effects [16;18] whereas IPSE/alpha-1 has previously been shown to trigger IL-4 production by human and murine basophils [13;14].

Natural omega-1 modulates human DC maturation and cytokine production in vitro

The observation that ESP can instruct human DCs to drive highly polarized Th2 responses prompted the question whether omega-1 and IPSE/alpha-1 as prominent ESP components are responsible for this activity. While some immunological properties of IPSE/alpha-1 have been described [13;14], the effects of omega-1 and IPSE/alpha-1 on DC-driven T helper cell polarization have not been investigated. To this end, natural omega-1 and IPSE/alpha-1 were purified from SEA (Fig 2) and used for the conditioning of human DCs in comparison with whole SEA. The concentrations of omega-1 and IPSE/alpha-1 used in these assays were equivalent to those in the unfractionated SEA preparations. As described before [8;19], stimulation with SEA did not lead to classical maturation of DCs, based on surface marker expression (Suppl Fig 1). Likewise, omega-1 and IPSE/alpha-1 did not induce the expression of these markers on DCs (Suppl Fig 1). Apart from the failure to induce the maturation of DCs, SEA is also

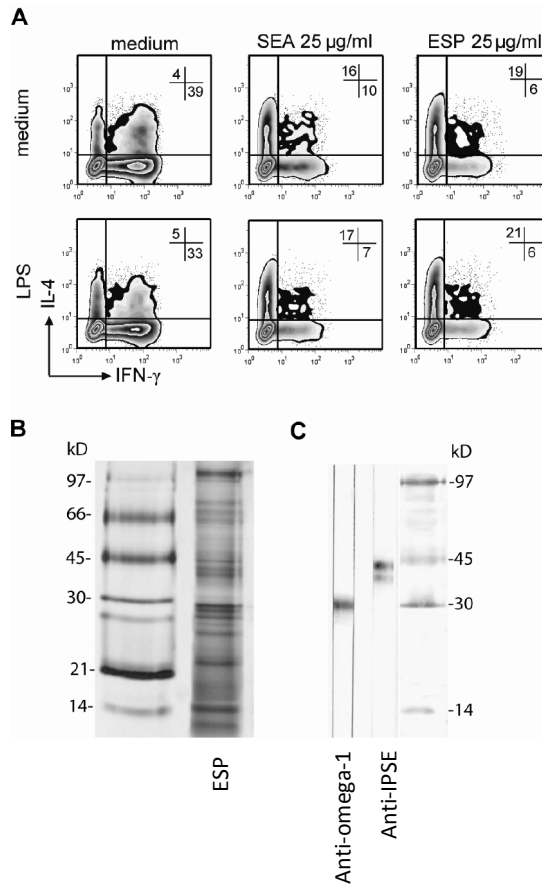


Figure 1. Immunological and biochemical characterization of excretory/secretory products (ESP) from *S. mansoni* eggs. (A) Monocyte-derived DCs pulsed for 48 h with the different antigen preparations in the absence (upper panel) or presence (lower panel) of LPS (100 ng/ml) as a maturation factor were co-cultured with allogeneic naive CD4⁺ T cells for 12 days in the presence of staphylococcal enterotoxin B and IL-2. Intracellular cytokine production was assayed by FACS 6 h after the stimulation of primed T cells with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The frequencies of each population are indicated as percentages in the plot. One representative result from 3 independent experiments is shown. (B) ESP (5 µg/cm) were separated under non-reducing conditions by SDS-PAGE and silver stained. The presence of omega-1 and IPSE/alpha-1 was confirmed on Western blots (C) by staining with specific anti-IPSE/alpha-1 and anti-omega-1 monoclonal antibodies.

known to interfere with TLR-mediated DC activation [8;19]. Indeed, when DCs were matured with the TLR4 ligand LPS, a non-polarizing maturation factor for human DCs, the presence of SEA significantly impaired the LPS-induced up-regulation of CD83 and CD86 surface expression (Fig 3A). Strikingly, omega-1 alone was sufficient to suppress the induction of these molecules on LPS-stimulated DCs to a similar extent, whereas IPSE/alpha-1 had no effect (Fig 3A).

DCs exposed to parasitic helminth-derived antigens, including SEA, are distinguished by their low production of IL-12, which is thought to be a prerequisite for their Th2-inducing capacity [20]. We analyzed the cytokine production of conditioned DCs following re-stimulation with a CD40-L expressing cell line mimicking the interaction with T cells. DCs stimulated with LPS in the presence of SEA displayed a potent reduction in the production of IL-12p70 (Fig 3B). Importantly, omega-1 alone was sufficient to inhibit the release of IL-12 (Fig 3B). Of note, the impact of 500 ng/ml omega-1 on IL-12 production was equal to that of 25 µg/ml SEA (Fig 3B). In contrast, IPSE/alpha-1 did not significantly affect IL-12 production (Fig 3B). Taken together, these data demonstrate that omega-1, but not IPSE/alpha-1, down-modulates DC maturation and cytokine production to a similar extent as SEA.

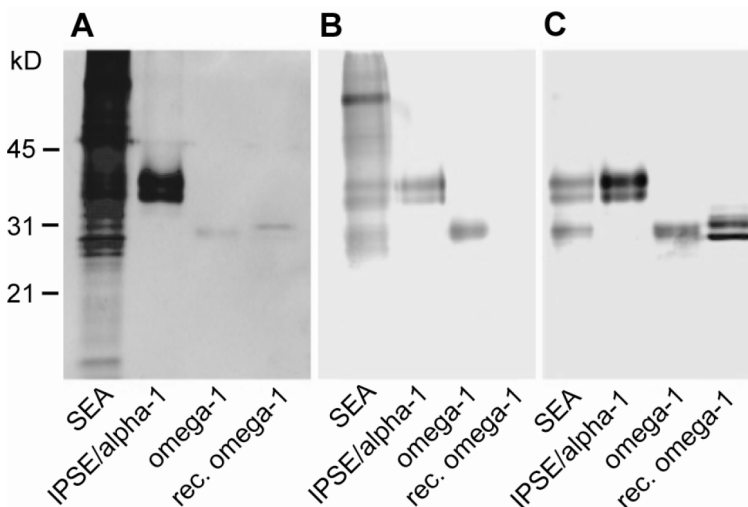


Figure 2. SDS-PAGE of SEA, natural omega-1 and natural IPSE/alpha-1 as well as recombinant omega-1 (silver staining and Western blotting). (A-C) SEA (5 µg/cm), omega-1 and IPSE/alpha-1 (each 0.3 µg/cm) purified from SEA, were separated by SDS-PAGE and silver stained or blotted onto nitrocellulose membrane. Silver staining (A) revealed a weak banding intensity of both natural and recombinant omega-1 compared to IPSE/alpha-1, although the purified proteins were applied to the gel at the same amounts (0.3 µg/cm). The two bands stained by anti-IPSE/alpha-1 represent posttranslational variants of the same protein [13]. On Western blots alkaline phosphatase-labelled *Aleuria aurantia* agglutinin (B) or a mixture of specific anti-IPSE/alpha-1 and anti-omega-1 monoclonal antibodies followed by alkaline phosphatase-labelled anti-mouse IgG second antibody (C) were used for detection. (B) While *Aleuria aurantia* agglutinin clearly binds to omega-1 and IPSE/alpha-1 as well as to a variety of other fucosylated components present in SEA, it does not bind to recombinant omega-1 whose glycans are lacking fucose residues. (C) On the other hand, all purified proteins but no irrelevant SEA components are detected by the mixture of specific monoclonal antibodies.

Natural omega-1 primes human DCs to induce a Th2 response *in vitro*

To evaluate the capacity of DCs exposed to these schistosome egg-derived antigens to direct T helper polarization, human DCs were pulsed for 40 h with the different egg antigens in the presence of LPS as a neutral maturation factor and then co-cultured with naïve CD4⁺ T cells. Two weeks later cytokine production by the CD4⁺ T cells was determined by intracellular cytokine staining. In contrast to IFN- γ -stimulated DCs that were used as a Th1-polarizing control, SEA-stimulated DCs potently skewed the response towards a Th2 cytokine profile (Fig 3C + D). Omega-1 alone displayed the same Th2-inducing potency as SEA, even at a 50-fold lower protein concentration (500 ng/ml versus 25 μ g/ml) (Fig 3C + D). Moreover, the robust Th2 priming by omega-1-conditioned DCs was also observed in the absence of LPS as a neutral maturation factor (Suppl Fig 2). IPSE/alpha-1-treated DCs, on the other hand, did not drive significant Th2 polarization, which is in keeping with its inability to suppress the production of IL-12 by these DCs (Fig 2B-D).

Recombinant omega-1, like natural omega-1, conditions human DCs for Th2 polarization *in vitro*

To further establish that omega-1 alone is sufficient to prime Th2 polarization through the functional modulation of DCs, we tested recombinant omega-1 expressed by human embryonic kidney (HEK) cells (Fig 2). As described for natural omega-1 [18], recombinant omega-1 displayed RNase activity (Fig 4A), proving its biological activity (Fig 4A). In contrast to natural omega-1, the recombinant protein was not bound by the fucose-specific lectin *Aleuria aurantia* agglutinin revealing differences in the glycosylation pattern (Fig 2B). Importantly, recombinant omega-1 significantly reduced IL-12 production by DCs (Fig 4B) and conditioned DCs to prime Th2 responses (Fig 4C), albeit with reduced potency compared to natural omega-1 (Fig 3B + C).

These data presented so far demonstrate that omega-1 alone, in contrast to IPSE/alpha-1, can initiate Th2 polarization via the modulation of human DCs with similar characteristics as unfractionated SEA.

Omega-1 primes a Th2 response *in vivo*

To investigate whether omega-1 has the capacity to prime Th2 responses *in vivo*, we administered omega-1 to 4get/KN2 IL-4 dual-reporter mice [21]. In these mice IL-4-competent cells are GFP⁺ and IL-4-producing cells additionally express huCD2, allowing the direct visualization of Th2 differentiation and IL-4 production. Following the s.c. injection of SEA, omega-1 or IPSE/alpha-1 into the footpad, the draining popliteal lymph nodes were harvested on day 7 and CD4⁺CD44^{high} effector T cells were analyzed for the expression of GFP and huCD2 directly *ex vivo*. Injection of SEA resulted in a

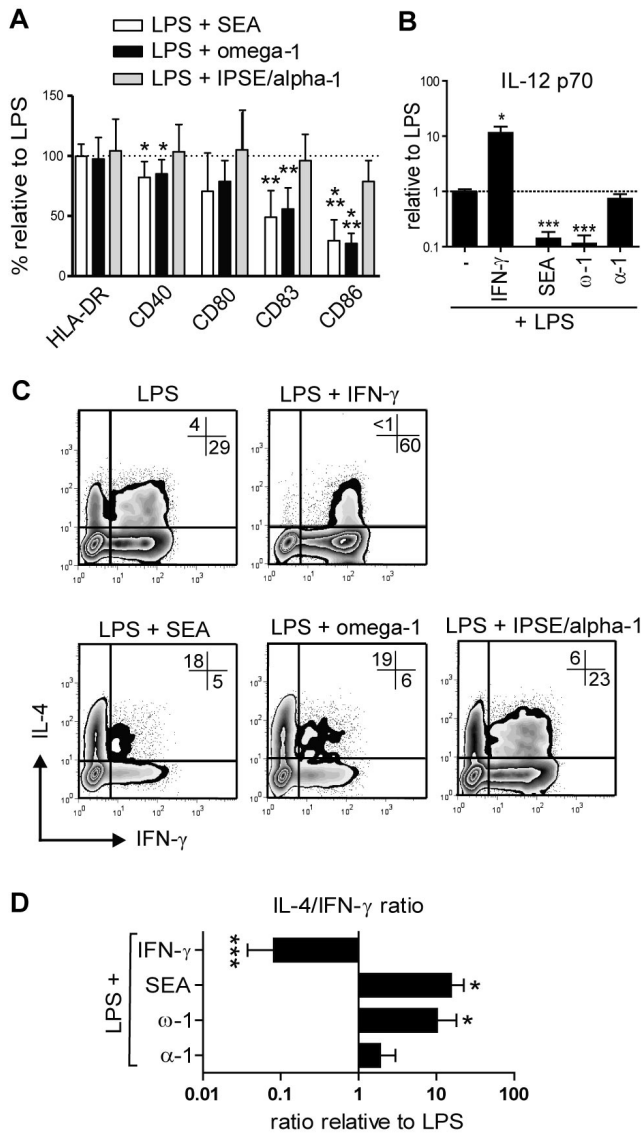


Figure 3. Omega-1 modulates human DC maturation, cytokine production and T cell-polarizing capacity with similar characteristics as SEA. (A) DCs were pulsed for 48 h with SEA (25 μ g/ml), omega-1 (500 ng/ml) or IPSE/alpha-1 (500 ng/ml) in combination with LPS (100 ng/ml) as a maturation factor and surface expression of maturation markers was determined by FACS analysis. The expression levels, based on geometric mean fluorescence, of different maturation markers are shown relative to the DCs stimulated with LPS alone, which is set to 100% for each marker (dashed line). (B) DCs were co-cultured for 24 h with a CD40-L expressing cell line, to mimic the interaction with T cells. IL-12p70 cytokine expression levels are shown relative to the DCs stimulated with LPS alone, which is set to 1 (dashed line). (C and D) T cell-polarizing capacity of the conditioned DCs was evaluated as described in legend of Fig. 1. (C) Representative plots out of at least 4 independent experiments are shown. (D) Based on intracellular cytokine staining, the ratio of T cells single-positive for either IL-4 or IFN- γ was calculated relative to the control condition. (A, B, D) Bars represent mean \pm SD of at least 4 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for values significantly different from the LPS control, based on paired analysis (one-sided paired t-test). ω -1, omega-1; α -1, IPSE/alpha-1.

significant increase of GFP⁺ and huCD2⁺ cells, a result reflecting the induction of Th2 differentiation and acute IL-4 production in vivo (Fig 5A). Importantly, omega-1 alone also induced a marked Th2 response and the production of IL-4, whereas IPSE/alpha-1 did not (Fig 5A). The Th2-inducing capacity of omega-1 was further substantiated by the observation that immunization with recombinant omega-1 led to the induction of a Th2 response and the production of IL-4 in these mice, although to a lesser degree than natural omega-1 (Fig 5B).

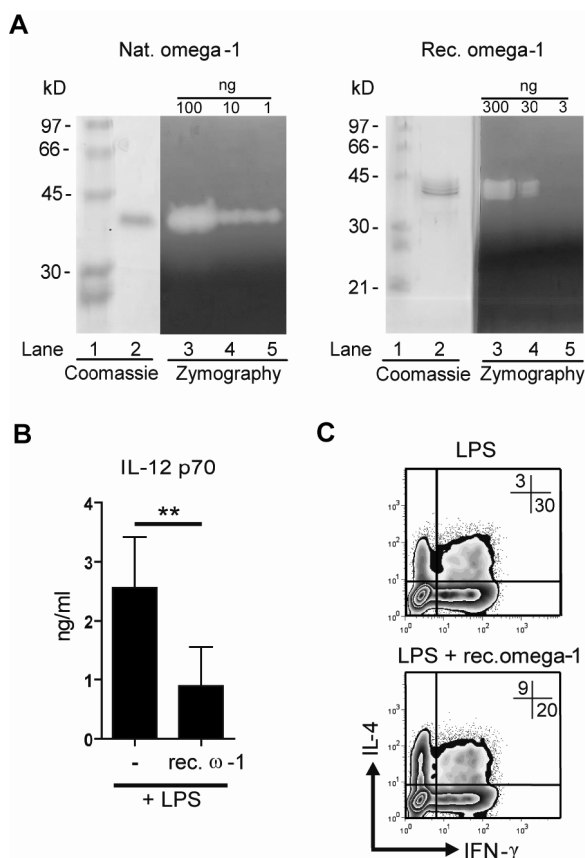


Figure 4. Recombinant omega-1 has RNase and Th2-polarizing activity similar to natural omega-1. (A) Recombinant omega-1 is a functional RNase as determined by negative-staining RNase zymography. Samples containing the indicated amount of protein, were run under non-denaturing conditions on 11% SDS polyacrylamide gels containing 2 mg/ml yeast RNA. Protein bands were detected by Coomassie blue staining (lane 2) or SDS was removed and RNase activity was detected by toluidine blue (lanes 3-5). Lane 1 contains molecular weight standards. (B) Monocyte-derived DCs were treated as described in the legend of Fig. 2. IL-12 p70 concentrations were determined by ELISA. Bars represent mean \pm SD of 4 independent experiments. (C) T cell polarizing capacity of the conditioned DCs was evaluated as described in legend of Fig. 1. One representative result from 4 independent experiments is shown. ** $p < 0.01$ (one-sided paired t -test). ω -1, omega-1; α -1, IPSE/alpha-1.

Although IL-4 has been shown to play an important role in both the differentiation and amplification of Th2 responses, there is clear evidence that initial Th2 priming can occur in the absence of IL-4 signaling, as has been shown for SEA [20;22;23]. To establish whether omega-1 can induce Th2 polarization in the absence of IL-4R signaling, we immunized 4get/KN2 IL-4 dual-reporter mice on the IL-4R $\alpha^{-/-}$ background [24]. Injection of omega-1 as well as SEA resulted in an increased frequency of GFP⁺ and huCD2⁺ cells in IL-4R $\alpha^{-/-}$ mice (Fig 5C) albeit with reduced magnitude, as previously reported for SEA [22]. The observation that IL-4R signaling is dispensable for the *in vivo* priming of Th2 responses by omega-1 further supports that omega-1 itself can provide the initial triggers driving Th2 differentiation, rather than simply amplifying the process.

Omega-1 is a major factor in SEA that conditions DCs for Th2 priming but not the only Th2-inducing component of SEA

Given the potency of omega-1 to condition DCs for Th2 priming *in vitro* and to drive Th2 polarization *in vivo* with similar characteristics as SEA, we depleted omega-1 from SEA (Suppl Fig 3) to address the extent to which the Th2-polarizing capacity of SEA can be attributed to omega-1. Depletion of omega-1 almost completely abrogated the inhibitory effect of SEA on LPS-induced *in vitro* maturation (Fig 6A) and IL-12 cytokine production (Fig 6B) by DCs. Consistent with this observation, the Th2-polarizing capacity of omega-1-depleted SEA was also significantly reduced compared to whole SEA in the presence (Fig 6C) or absence of LPS (Suppl Fig 4). This suggests that omega-1 is a principal factor in SEA mediating the conditioning of DCs for Th2 priming *in vitro*. In contrast, omega-1-depleted SEA was not impaired in its capacity to prime Th2 responses *in vivo* (Fig 6D). Thus, additional components in SEA are able to compensate for omega-1 with respect to Th2 priming *in vivo*. An interesting candidate could be the glycoprotein peroxiredoxin present in SEA, as this molecule has recently been shown to induce the development of alternatively activated macrophages [15], which may render it capable of initiating a Th2 response [25].

In the present study we identify omega-1, a glycoprotein secreted by *S. mansoni* eggs, as a strong inducer of Th2 responses *in vitro* and *in vivo*. Although omega-1 was known to be secreted by live *S. mansoni* eggs and to be one of the most abundant molecules present in SEA [16;26], its immunological properties have remained elusive. This study shows that omega-1 alone is sufficient to drive Th2 responses both *in vitro* and *in vivo*. Using a well-established *in vitro* model to study the T helper polarization of naïve human T cell by DCs, we demonstrate that omega-1 can elicit Th2 responses via the conditioning of DCs. Nonetheless, our observations do not exclude the possibility, that *in vivo* omega-1-driven Th2 responses are also supported

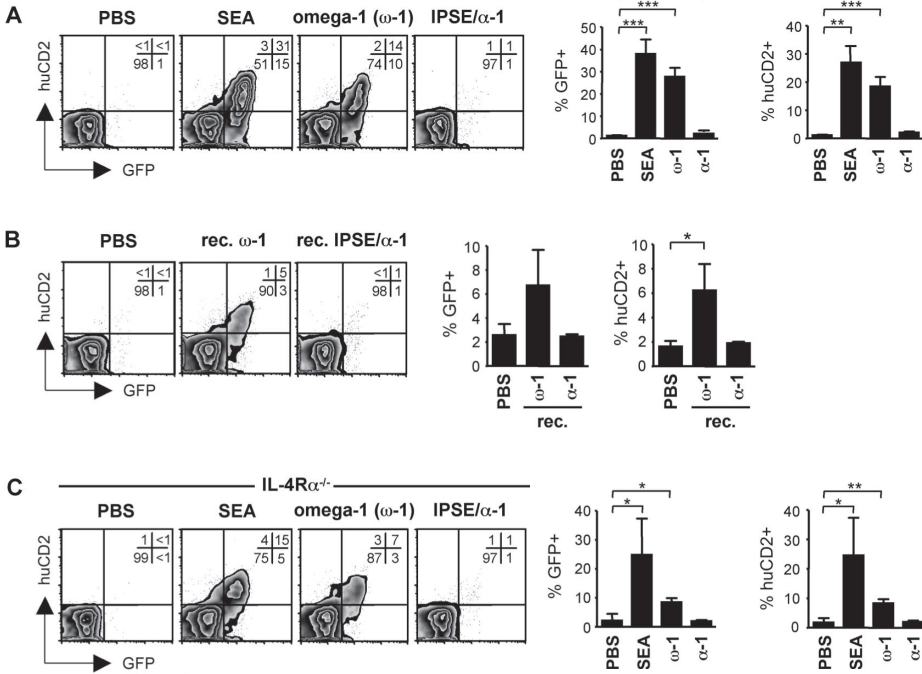


Figure 5. Omega-1 is sufficient to drive Th2 polarization in vivo, independently of IL-4R signaling. (A) 4get/KN2 IL-4 dual reporter mice were injected s.c. with SEA (20 μg), omega-1 (2 μg) or IPSE (2 μg) into the footpad. After 7 days the frequency of GFP⁺ and huCD2⁺ within the CD4⁺CD44^{high} effector T cell population was determined by flow cytometry in the draining popliteal lymph nodes. Depicted are representative plots with percentages indicated and the combined data of three individual mice per group. (B) 4get/KN2 mice were injected with recombinant proteins and analyzed as in (A). (C) IL-4Rα^{-/-} 4get/KN2 mice were treated and analyzed as in (A). The frequencies of each population are indicated as percentages in the plots. One of 2 independent experiments with n=5 is shown. Bars represent mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001 for values significantly different from the PBS control (two-sided paired t-test).

by cell types other than DCs, like for instance basophils [5]. However, our *in vivo* studies with IL-4Rα^{-/-} mice demonstrated that IL-4R signaling is dispensable for the *in vivo* priming of Th2 responses by omega-1. Given that DCs have the unique capacity to initiate Th2 responses independently of IL-4 signaling *in vivo* [27], these data support a role for DCs in omega-1-driven Th2 priming. Our findings are corroborated by an independent study by Jankovic and coworkers [28] showing that omega-1-conditioned bone marrow-derived mouse DCs prime Th2 responses in vitro and upon transfer into naive mice also *in vivo*. Collectively these studies support a role for omega-1 in driving Th2 responses via the functional modulation of DCs.

The molecular basis underlying the immunomodulatory property of omega-1 still remains to be determined. Carbohydrates present in SEA have been found to contribute to the Th2-polarizing properties of this antigen preparation [11;12]. Since

omega-1 is a glycosylated protein, the glycosylation pattern of omega-1 might play a role in its Th2 priming activity. Furthermore, its RNase activity could provide an alternative or additional mechanism through which omega-1 drives Th2 polarization, since several RNases have been implicated in Th2 responses [29;30]. Whether the reduced Th2-inducing activity of recombinant compared to natural omega-1 *in vitro* (Fig 4C versus Fig 3C) and *in vivo* (Fig 5B versus Fig 5A) is due to differences in the glycosylation pattern or reduced RNase activity, remains to be determined. The specific modification of the glycosylation and/or the RNase activity of omega-1 will define their respective roles and will help to identify the molecular pathways through which omega-1 conditions DCs to initiate Th2 polarization.

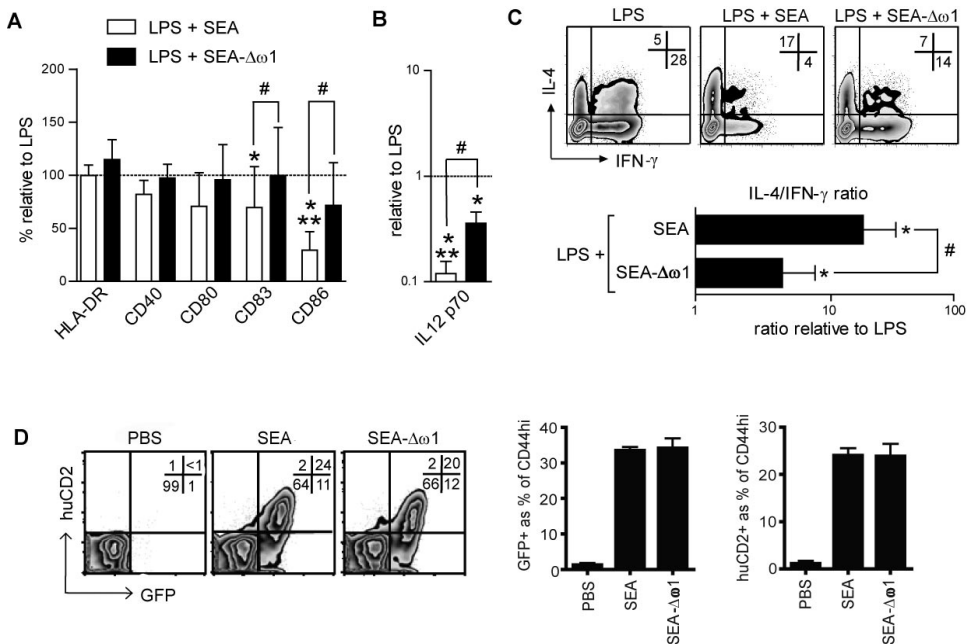


Figure 6. Omega-1 is a major factor in SEA that conditions DCs for Th2 priming but not the only Th2-inducing component present in SEA. (A and B) Monocyte-derived DCs were pulsed for 48 h with SEA (25 μ g/ml) or omega-1-depleted SEA (25 μ g/ml) in combination with LPS (100 ng/ml) and analysed for surface expression of maturation markers and IL-12 production as described in legend of Fig. 2. Bars represent mean \pm SD of 4 independent experiments. (C) T cell polarizing capacity of the conditioned DCs was evaluated as described in legend of Fig. 1. Depicted are representative plots with percentages indicated. Based on intracellular cytokine staining, the ratio of T cells single-positive for either IL-4 or IFN- γ was calculated relative to the control condition. Bars represent mean \pm SD of 5 independent experiments. (D) 4get/KN2 mice were injected with recombinant proteins and analyzed as in Fig. 4. Depicted are representative plots with percentages indicated and the combined data of three individual mice per group. *,# $p < 0.05$, **,### $p < 0.01$, ***,### $p < 0.001$ for values significantly different from the controls (*) or SEA (#) based on paired analysis (one-sided paired t-test). ω -1, omega-1.

While the immunological processes resulting in Th1 polarization have been extensively characterized, it is still poorly understood how exactly Th2 responses are initiated. SEA has often been used as a model antigen mixture to study the immunological mechanisms underlying the induction of Th2 responses [3;20;31]. Now, two groups using different but complementary models have independently identified omega-1 as a single glycoprotein in SEA with potent Th2-polarizing properties. These findings will pave the way for the use of a defined molecule, omega-1, to further delineate the cellular mechanisms and molecular signals that drive Th2 differentiation.

Materials and Methods

Preparation and purification of *S. mansoni* egg-derived antigens

Freshly isolated *S. mansoni* eggs from trypsinized livers from infected hamsters were washed in RPMI medium with 300 U/ml penicillin, 300 µg/ml streptomycin and 500 µg/ml fungizone. To obtain ESP, 3×10^5 eggs/ml were incubated in the same medium for 48 h at 37°C in a humidified incubator. Supernatant containing ESP was harvested and centrifuged to remove residual eggs. SEA was prepared as described previously [10]. Omega-1 and IPSE/alpha-1 were purified from SEA via cation exchange chromatography as described earlier [13;16]. Omega-1 was then separated from IPSE/alpha-1 by affinity chromatography using specific anti-IPSE/alpha-1 monoclonal antibodies coupled to a NHS-HiTrap Sepharose column according to the manufacturer's instructions (GE Healthcare). Purified components were concentrated and dialysed. Omega-1-depleted SEA was prepared by adding back purified IPSE/alpha-1 to the remaining SEA fraction left from the cation exchange chromatography. The purity of the preparations was controlled by SDS-PAGE and silverstaining. In parallel, Western Blotting was performed both with specific anti-omega-1 (140-3E11) and anti-IPSE/alpha-1 (74-1G2) monoclonal antibodies followed by alkaline phosphatase-labelled anti-mouse IgG (Dianova) detection antibody and with alkaline phosphatase-labelled *Aleuria aurantia* agglutinin, which binds specifically to fucose residues. Protein concentrations were tested using the Bradford or BCA procedure.

Production of recombinant omega-1

Recombinant omega-1 was purified from human 293 HEK cells transfected with the expression vector pSecTag2-omega-1. The pSecTag2 plasmide was from Invitrogen. Secreted recombinant omega-1 was sequentially purified from the culture medium by immobilized metal affinity chromatography and size exclusion chromatography.

Zymography

Ribonuclease activity was determined as described previously [18].

Human DC culture, stimulation and analysis

Monocytes were isolated from venous blood of healthy volunteers using Institutional Review Board-approved protocols by density centrifugation on ficoll followed by a Percoll gradient as described [10] and were cultured in RPMI medium supplemented with 10% FCS, human rGM-CSF (500 units/ml, a gift from Schering-Plough, Uden, The Netherlands) and human rIL-4 (250 units/ml) (R&D Systems). On day 3, culture medium including the supplements was replaced and on day 6 immature DCs were stimulated with the indicated reagents in the presence of ultrapure LPS (100 ng/ml) (E. coli O111 B4 strain, InvivoGen). As a Th1 control DCs were also pulsed with IFN- γ (1000 U/ml). After 48 h, DCs were harvested for co-culture with naïve T cells. In addition, 1×10^4 matured DCs were co-cultured with 1×10^4 CD40L-expressing J558 cells for 24 h to determine cytokine production by the DCs following activation by CD40L. IL-12p70 concentrations were determined by ELISA using mouse anti-human IL-12, clone 20C2 as capture antibody and biotinylated mouse-anti-human IL-12, clone C8.6 as detection antibody (both Becton Dickinson). The expression of maturation markers on the pulsed DCs was determined by FACS (FACS caliber) through staining with CD83-PE (Immunotech), HLA-DR-PerCP, CD40-APC, CD80-FITC and CD86-PE (all Becton Dickinson).

Human T cell culture and determination of T cell polarization

To determine T cell polarization, 5×10^3 48 h-pulsed DCs were co-cultured with 2×10^4 naïve T cells that were purified using a human CD4⁺/CD45RO⁻ column kit (R&D, Minneapolis, MN) in the presence of staphylococcal enterotoxin B (100 μ g/ml; Sigma) in 96-well flat-bottom plates (Costar). On day 5, rhIL-2 (10 U/ml, Cetus Corp., Emeryville, CA) was added and the cultures were expanded for another 7 days. For intracellular cytokine production, the primed CD4⁺ T cells were restimulated with 50 ng/ml phorbol 12-myristate 13-acetate plus 2 μ g/ml ionomycin for 6 h. 10 μ g/ml brefeldin A was added during the last 2 h (all Sigma). The cells were stained with a combination of IL-4-PE and IFN- γ -FITC antibodies (BD).

***In vivo* experiments**

WT [21] and IL-4R α ^{-/-} [24] 4get/KN2 mice were bred and housed in the animal facility of the Trudeau Institute and used at 8-12 weeks of age. All experimental procedures were approved by the Institutional Animal Care and Use Committee. Mice were immunized s.c. into one hind footpad with SEA (20 μ g), omega-1 (2 μ g), or IPSE (2 μ g) in a volume of 30 μ l and the draining popliteal lymph nodes were analyzed one week later.

Statistical analysis

Data were analyzed for statistical significance using a one-sided paired *t*-test. All *p*-values < 0.05 were considered significant.

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Supplemental figures

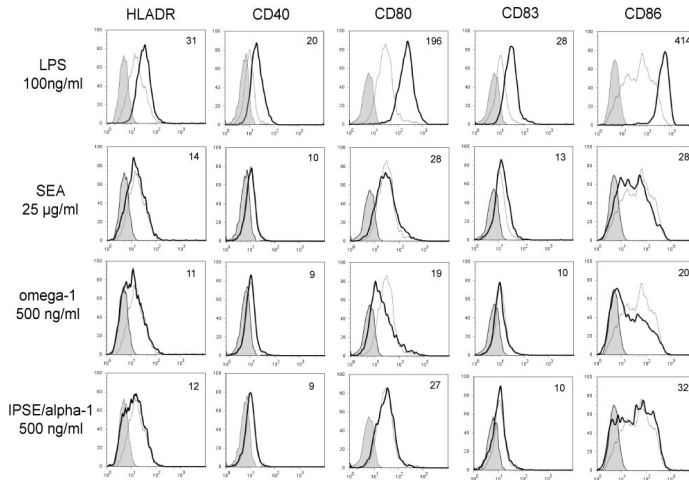


Figure S1. SEA, omega-1 and IPSE/alpha-1 do not induce expression of DC maturation markers. Monocyte-derived DCs were pulsed for 48 h with LPS, SEA, omega-1 or IPSE/alpha-1 and surface expression of maturation markers was determined by FACS analysis. Filled histograms represent unstained cells, while the thin black line represents unstimulated DCs. The thick black line represents the DCs pulsed with the different antigen preparations. One representative result from at least 3 independent experiments is shown. The geometric mean of the fluorescence intensity of the different surface markers is depicted in the upper right corner of each plot.

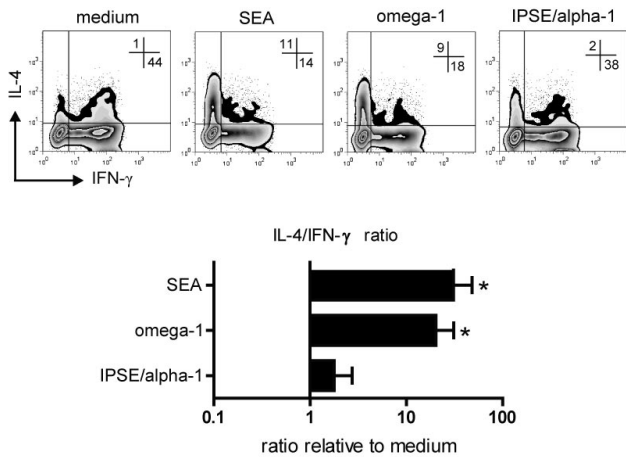


Figure S2. Omega-1 drives Th2 polarization also in the absence of LPS. 48 h-stimulated dendritic cells were co-cultured with allogeneic naive CD4⁺ T cells for 2 weeks in the presence of staphylococcal enterotoxin B and IL-2. Intracellular cytokine production was assayed as described in the legend of Fig. 1. Representative plots out of 4 of independent experiments are shown. Based on intracellular cytokine staining, the ratio of T cells single-positive for either IL-4 or IFN-γ was calculated relative to the control condition. Bars represent mean ± SD of 4 independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 for values significantly different from medium control, based on paired analysis (one-sided paired t-test). ω-1, omega-1.

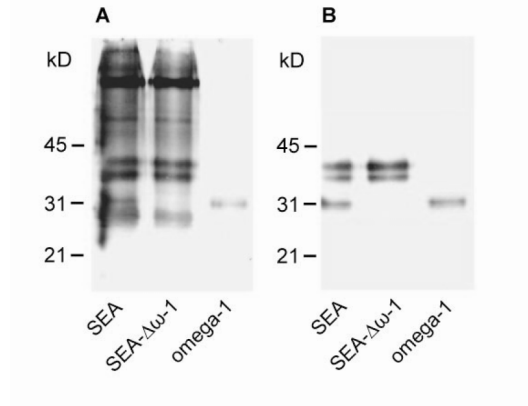


Figure S3. SDS-PAGE of SEA and omega-1-depleted SEA (Western blotting). (A and B) SEA (5 $\mu\text{g}/\text{cm}$), omega-1 (each 0.3 $\mu\text{g}/\text{cm}$) purified from SEA, and SEA depleted for omega-1 (5 $\mu\text{g}/\text{cm}$) were separated by SDS-PAGE and blotted onto nitrocellulose membrane. On Western blots alkaline phosphatase-labelled *Aleuria aurantia* agglutinin (A) or a mixture of specific anti-IPSE/alpha-1 and anti-omega-1 monoclonal antibodies followed by alkaline phosphatase-labelled anti-mouse IgG second antibody (B) were used for detection. ω -1, omega-1.

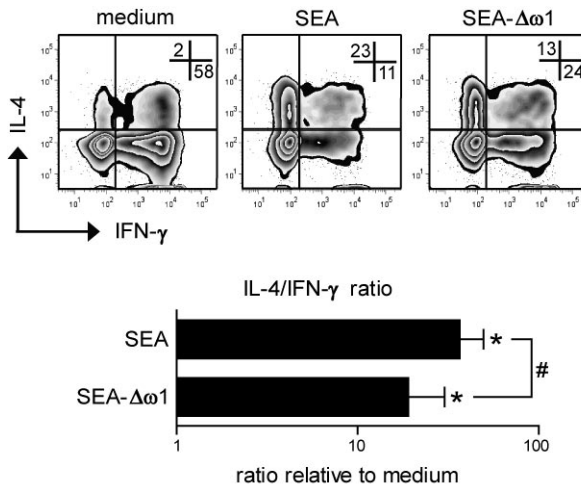


Figure S4. Reduced Th2 polarization via DCs by SEA depleted of omega-1 in the absence of LPS. 48 h-stimulated dendritic cells were co-cultured with allogeneic naive CD4^+ T cells for 2 weeks in the presence of staphylococcal enterotoxin B and IL-2. Intracellular cytokine production was assayed as described in the legend of Fig. 1. Representative plots out of 3 independent experiments are shown. Based on intracellular cytokine staining, the ratio of T cells single-positive for either IL-4 or IFN- γ was calculated relative to the control condition. Bars represent mean \pm SD of 3 independent experiments. *,# $p < 0.05$ for values significantly different from the controls (*) or SEA (#) based on paired analysis (one-sided paired t -test). ω -1, omega-1.