Original Article

Using poly-N-acetyl glucosamine gel matrix to deliver IL-12 with anti-schistosomasis vaccination

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Abstract

Background: Interleukin (IL)-12 is a potential adjuvant in a variety of diseases including schistosomiasis. The clinical use of IL-12, however, is limited by the toxicity associated with its systemic administration. We have developed a novel delivery system (designated F2 gel matrix) composed of poly-N-acetyl glucosamine that has the dual properties of sustaining the release of proteins (e.g. interleukins) and adjuvant effects. The main aim of this study was to use a mouse model to test whether IL-12 released from F2 gel can induce adjuvant effects in the schistosomiasis setting as compared to those obtained after systemic delivery of IL-12.
Methodology: First, we compared the toxicity induced by paracrine (delivered by F2 gel) and systemic IL-12. Second, we compared the induction of cytokines induced by paracrine and systemic IL-12. Third, we compared the adjuvant effects of paracrine and systemic IL-12-based prophylactic vaccination against schistosomiasis using soluble worm antigen preparation (SWAP).
Results: IL-12 released from F2 gel did not induce significant toxicity measured by alanine aminotransferase (ALT). We found similar serum levels of IFN-γ, TNF-α and IL-2 after paracrine and systemic IL-12 treatments. We also found that vaccination with F2 gel/SWAP/IL-12 induced higher anti-schistosomal effects than IL-12/SWAP as evidenced by 1) the decrease in the total liver egg counts; 2) the reduction in the granuloma size and fibrotic reaction in the liver; and 3) the amelioration of the liver functions.
Conclusion: Collectively, these results indicate that IL-12-F2 gel delivery approach could be considered as a potential strategy for the treatment of schistosomiasis.

Key words: cytokines, IL-12, Paracrine treatment, Schistosoma mansoni, SWAP, systemic treatment, vaccination

Introduction

Schistosomiasis is a disease caused by the trematode *Schistosoma mansoni* after infection with cercariae. The adult worms of *S. mansoni* live in the blood vessels around the intestine and bladder of the human host [1]. The deposition of thousands of eggs by the adult worm in the liver is the hallmark of the disease. The disease-dependent egg deposition induces a granulomatous reaction in the liver, leading to abnormalities in the liver function due to the induced fibrosis [2]. With the progress of infection, the liver can be blocked by the deposited eggs, causing portal hypertension, hepatomegaly and death due to haematenesis [3].

It is estimated that about 200 million people in the world are currently affected by schistosomiasis, and an additional 600 million people are at risk of acquiring this infection [4]. Schistosomiasis control is essentially based on the use of molluscicides to kill the snail intermediate host, and drugs to cure patients [5]. However, acquisition of tolerance to these drugs, particularly praziquantel, has been reported [6]. Therefore, defining an alternative approach that can induce worm killing and modulate the infection-related complications is of a great significance. One potential approach is the use of a vaccine against schistosomiasis to increase protection from the *S. mansoni* worm so that the numbers of deposited eggs can be decreased in the liver and intestine. Eventually, these effects could decrease the hepatic and intestinal fibrosis, which is the hallmark complication of schistosome infection [7]. Several studies have developed different approaches of vaccination against schistosomiasis [8]. It appears, however, that the nature of the adjuvant incorporated

with the vaccine is a critical factor. Cytokines, in particular Th1 type cytokines such as IL-12, are potential candidate adjuvants.

Since its discovery in the mid-1980s, the critical role of endogenous IL-12 in host resistance to infectious disease has been established [9]. Exogenous administration of IL-12 during immunization with *S. mansoni*-egg antigen, irradiated cercariae or soluble worm antigen preparation (SWAP) also can reduce subsequent egg-induced lung pathology [10] and modulation of Th1/Th2 responses [11], suggesting that this cytokine may be useful for promoting both humoral and cell-mediated protective responses against schistosomiasis [12,13]. In addition to the use of IL-12 protein, IL-12 plasmid DNA has been used as an adjuvant [14], where it elicited significant levels of protective immunity against S. japonica challenge infection [15]. Thus IL-12 appears to significantly benefit the immuno-prophylactic treatment of schistosomiasis. The clinical application of IL-12, however, has been limited with its associated toxicity [16]. Therefore, defining approaches that can lower its toxicity and enhance its adjuvant effects would improve its application.

Through our collaboration with Marine Polymer Technologies, Inc., (Boston, USA) we have developed a highly purified polysaccharide (Poly-N-acetyl glucosamine) that can be formulated into a stable gel matrix (designated F2 gel matrix). F2 gel matrix has been certified to be fully biocompatible, biodegradable, and nontoxic. A membrane patch formulation of the F2 gel precursor has been FDA approved and is in commercial use as a topical haemostatic agent [17-19]. Formation of an emulsion stable for vaccination does not require vigorous sonication, and labile proteins are efficiently incorporated without denaturation [20, 21]. F2 gel can be formulated into a stable matrix in combination with antigenic peptides and adjuvant cytokines, creating a vaccine delivery system capable of providing the sustained release of the incorporated protein in vivo, establishing a potent micro-environment for antigen presentation [20-23]. With regard to IL-12, we have recently reported that F2 gel matrix can release low but functional levels of IL-12 [21,24-27]. Therefore, the aim of the present study was to test whether these low levels of IL-12 delivered from F2 gel can induce beneficial effects to protect against *S. mansoni* infection.

Materials and methods

Mice

Adult male albino mice Mus musculus (CD1 strain), C57BL/6 and C3He/J mice weighing between 16 and 22 g were purchased from Helwan Research Animal Center (Cairo, Egypt). The mice were maintained in a quiet room at 28°C. The mice received laboratory chow and water *ad libitum* and were allowed a period of 10 days, prior to the initiation of experiments, to acclimatize to the laboratory conditions. The mice were housed at the animal facility center of the Zoology Department, Faculty of Science, Tanta University, in accordance with the institutional guidelines at Tanta University.

Materials and reagents

Soluble worm antigen preparation (SWAP) was purchased from Theodor Bilharz Institute (Giza, Egypt), dissolved in PBS to a concentration of 100 μg/100 μl, and chill frozen at -20°C until use. Recombinant murine IL-12 (rmIL-12) was purchased from R&D Systems (Minneapolis, MN) and reconstituted in 0.1% BSA (Sigma-Aldrich). Highly purified poly-*N*-acetyl glucosamine (*p*-GlcNAc) in a gel formulation, referred to as F2 gel matrix, was obtained as a gift from Marine Polymer Technologies Inc., Boston, USA.

Preparation of IL-12-based vaccination system

The F2 gel matrix was prepared by chemical deacetylation of *p*-GlcNAc natural nano-fibers to 70% with conversion of the polymer to a lactate salt [19-22]. A final 5% polymer concentration in gel form was achieved by hydration. Next 50 μl of this gel matrix was mixed with 50 μl of PBS, IL-12 alone, and SWAP/IL-12 to form F2 gel/PBS, F2 gel/IL-12, and F2 gel/SWAP/IL-12, respectively. Mixing F2 gel with the reagents was performed using two 1-ml syringes connected by a four-way stopcock (Medex; Hilliard, Ohio, USA). The mixing process was performed on ice to prevent denaturation of IL-12 and SWAP. Mice were treated with subcutaneous (s.c.) injection of 100 μl of the formulation at the base of the tail.

Measurement of ALT

C3H mice (n = 4/group) were treated s.c. with IL-12, F2 gel/PBS, or F2 gel/IL-12. Mice were bled seven days after treatment and sera were separated from non-heparinized peripheral blood by centrifugation at 1500 rpm for 15 minutes at room temperature. Alanine aminotransferase (ALT)

enzymatic activities were determined using Biotron kits (Biotron diagnostic Inc., CA, USA) as previously described [28].

Measurement of cytokines in serum

B6 mice (n = 4/group) were treated with s.c. injection in their flanks with 2 µg/mouse rmIL-12 (systemic IL-12), F2 gel/IL-12 (paracrine IL-12), or F2 gel matrix alone. Treated mice were bled at serial time points (15 minutes, 1 hour, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours and 7 days) and the sera were collected for measurement of IFN-γ, TNF-α, IL-2, and IL-5 by ELISA (Pharmingen, USA) as we previously described [29].

Vaccination and infection with S. mansoni

To test the efficacy of IL-12-based vaccination on *S. mansoni* infection, five groups each of 10 mice were used. Mice were immunized with s.c. injection of SAWP alone (100 µg), IL-12 alone (2 µg), SWAP (100 µg) mixed with IL-12 (2 µg), F2 gel alone (100 µL), or F2 gel (100 µL) loaded with SWAP (100 µg) and IL-12 (2 µg). Mice were vaccinated with the vaccine formulation two times, 2 weeks apart. One group of naïve (non-vaccinated) and the all vaccinated mice were infected with 100 cercariae of *S. mansoni* by the tail immersion method as previously described [30]. Then, the infected mice were sacrificed after eight weeks for the analysis below.

Evaluation of worm burden

The livers of mice were perfused for worm recovery as previously described [30]. All worms (male, female, or couples) from perfusion fluid (mesentery and liver) were counted. The percentage worm reduction (% protection) was calculated according to the following equation: % protection = (C − I) / C x 100; where (C) is the mean worm recovery of the non-immunized group, and (I) is the mean worm recovery of the immunized group.

Evaluation of egg deposition (oogram)

Samples of liver tissue weighing about 0.1 g were transferred to clean slides. Slides were then covered with glass slips and pressed to spread the liver tissue homogeneously. Triplicate samples of each liver were prepared and the average number of eggs was expressed as number of eggs/g tissue. All viable and dead eggs were counted and classified according to the criteria previously described [31].

Evaluation of liver fibrosis

For histological examination, liver specimens from different lobes of each mouse of each group were fixed in 10% neutral buffer formalin. After dehydration, paraffin sections of 6 µm thick were prepared and stained with conventional haematoxylin and eosin method. For morphometric measurements, granuloma dimensions per liver section were measured using an ocular micrometer as previously described [32] based on the following equation: granuloma size (mm3) = 4/3 π r3, where r is the radius of granuloma.

Statistical analysis

Student's *t*-test was used to compare the significance of the observed differences between the mean values of the experimental and control groups with the level of significance set as $P < 0.05$ and $P < 0.01$.

Results

### Paracrine delivery of IL-12 from F2 gel matrix induces less toxicity

A major concern with the adjuvant effects of systemic administration of IL-12 is the associated toxicity [33]. We have recently reported releases of low levels of IL-12 from the novel delivery system designated F2 gel matrix. These low levels of paracrine IL-12 induced higher adjuvant effects than those obtained after the systemic administration of IL-12 as evidenced by the enhanced post vaccination anti-tumor responses of CD8^{+} T cells [21]. In the present study, we performed experiments to compare the toxicity obtained after systemic and paracrine treatment with IL-12. We used the C3H/HeJ mouse strain because these mice are susceptible to IL-12 treatment [34]. Thus, female C3H mice (n = 3/group) were treated with s.c. injection of 1) 100 µl PBS; 2) 100 µl F2 gel matrix; 3) 100 µl F2 gel matrix containing 2µg/mouse rmIL-12 (i.e. paracrine IL-12); or 4) 100 µL PBS containing 6µg/mouse rmIL-12 (i.e. systemic IL-12). Mice were bled on day 7 after treatment and the activity of ALT enzyme was assessed as the indication for the liver toxicity. As shown in Figure 1, we found that treatment of mice with F2 gel alone had no effect on the ALT activity as compared to PBS-treated mice. Systemic treatment of mice with IL-12, however, resulted in an increase in the activity of ALT. Interestingly, when IL-12 was delivered from F2 gel matrix its toxicity was significantly lower than those induced by systemic IL-12 treatment. Similar results were obtained with

Figure 1. ALT activity in sera after paracrine and systemic IL-12 treatment.
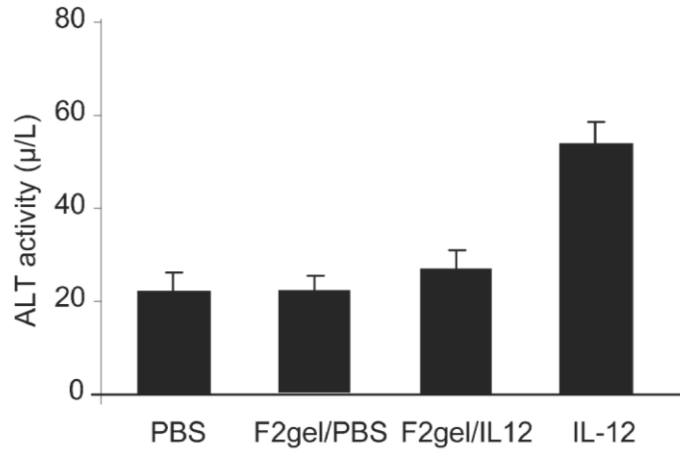
C3H mice (n = 3/group) were treated with subcutaneous injection in their flanks with 100 µl PBS, 100 µl F2 gel/PBS, 100 µl F2 gel mixed with 2 µg rmIL-12 (paracrine IL-12), or 100 µl PBS containing 2 µg IL-12 (systemic IL-12). Treated mice were bled after 7 days of treatment and sera were collected for ALT using the commercial kits.

Figure 2. Induction of inflammatory cytokines in serum.
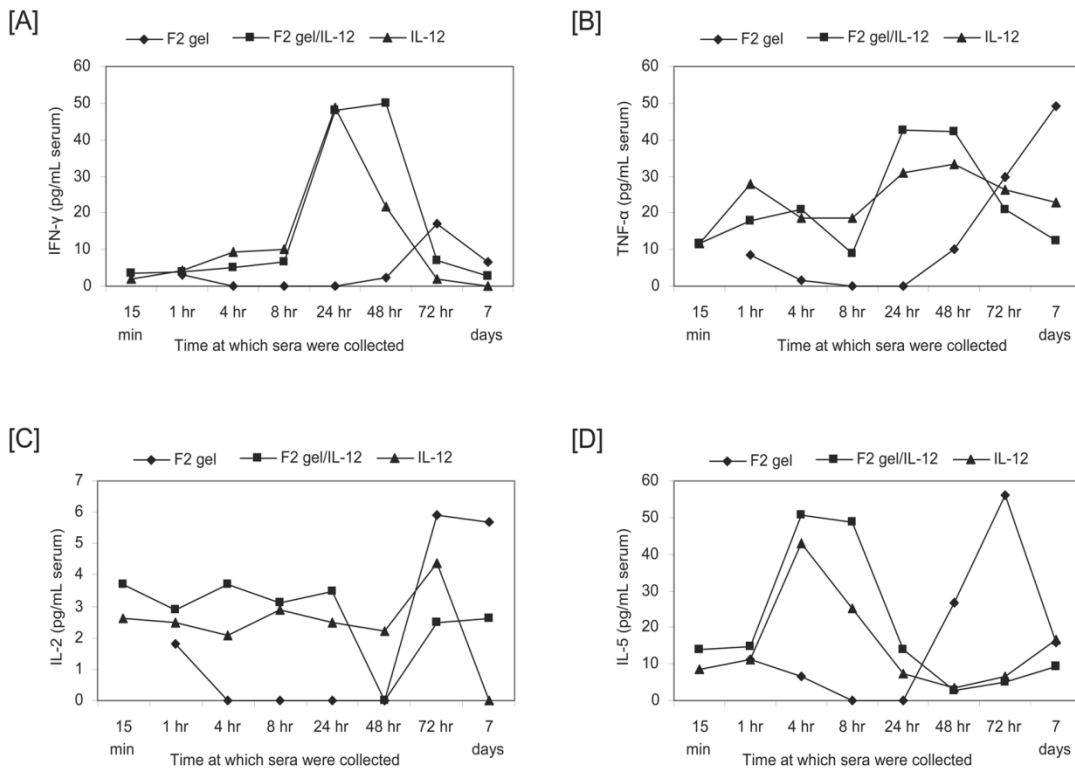
B6 mice (n = 4/group) were treated with s.c. injection in their flanks with 2µg/mouse rmIL-12 (systemic IL-12), F2 gel loaded with 2 µg IL-12 (F2 gel/IL-12; paracrine IL-12), or F2 gel matrix alone. Treated mice were bled at the indicated time points and the sera were collected for measurement of IFN-□ (A), TNF-α (B), IL-2 (C), and IL-5 (D) by ELISA (Pharmingen, USA).

another marker for liver functions, AST (data not shown). Taken together, these data indicate that F2 gel matrix loaded with IL-12 can deliver low functional levels of IL-12 with no significant toxicity.

Low levels of paracrine IL-12 induce inflammatory cytokines in serum

Given that low levels of IL-12 delivered from F2 gel did not induce significant toxicity as shown in Figure 1 above, the next question was whether these levels of IL-12 can induce comparable levels of inflammatory cytokines to those induced by systemic IL-12 treatment. It has been established that IL-12 treatment induces IFN-γ and TNF-α, which are the typical Th1 cytokines [35,36]. Therefore, we analyzed the levels of these cytokines in addition to IL-2 (another Th1 cytokine) and IL-5, a typical Th2 cytokine. Thus, B6 mice (n = 4/group) were treated with s.c. injection in their flanks with 2μg/mouse rmIL-12 (systemic IL-12), F2 gel/IL-12 (paracrine IL-12), or F2 gel matrix alone. Treated mice were bled at the indicated time points and the sera were collected for measurement of IFN-γ, TNF-α, IL-2, and IL-5. As shown in Figure 2, similar levels of the Th1 cytokines IFN-γ and TNF-α were induced in the sera of mice treated with paracrine or systemic IL-12. The levels of these cytokines peaked from 24 to 48 hours after treatment and then declined. The levels of IL-2 induced by IL-12 were very low. With regard to IL-5, its levels were also induced after treatment with either paracrine or systemic IL-12. The peak of the induction of this Th2 cytokine, however, appeared earlier than those of IFN-γ and TNF-α. Of note, treatment with F2 gel alone also induced the tested cytokines but generally at a lower level with significantly delayed kinetics than with IL-12 treatment, either alone or via F2 gel.

Effect of IL-12-based vaccination system on worm burden

Mice infected with *S. mansoni* cercariae (100/mouse) with no vaccination showed a total worm recovery of 47 ± 8.8 worms/mouse. Vaccination with any of the vaccine formulations did not show significant changes in the total worm recovery. As shown in Figure 3, infected mice vaccinated with SWAP alone, IL-12 alone, SWAP/IL-12, F2 gel alone, and F2 gel/SWAP/IL-12 showed a total worm recovery of 45 ± 6.9, 43.0 ± 8.6, 40.0 ± 9.2, 45.0 ± 11.8, and 43.0 ± 9.2, respectively. The numbers of males and females were then analyzed. The numbers of male worms were 30 ± 5.7

in infected mice with no vaccination, and 23 ± 3.1, 24 ± 6.5, 24 ± 4.3, 24 ± 7.1, and 24 ± 4.3, respectively, in infected mice vaccinated with SWAP alone, IL-12 alone, SWAP/IL-12, F2 gel alone, and F2 gel/SWAP/IL-12. Although these data show that each of the vaccine components can slightly reduce the numbers of male worms, the changes were not statistically significant. The numbers of female worms were 17 ± 5.1 in infected mice with no vaccination, and 22 ± 5.7, 19 ± 5.3, 16 ± 5.9, 21 ± 5.3, and 19 ± 4.9, respectively, in infected mice vaccinated with SWAP alone, IL-12 alone, SWAP/IL-12, F2 gel alone, and F2 gel/SWAP/IL-12. Based on the numbers of total worms, the percent of protection was not significant with any of the treatments.

*Effect of IL-12-based vaccination system on egg burden*

The numbers of total, viable, and dead eggs in the liver from infected mice with or without vaccination are shown in Figure 4. The total liver egg count of infected mice with no vaccination was 914 ± 242 eggs/g liver tissue, of which 58% were live and 42% were dead. Vaccination with SWAP alone slightly, but not significantly, increased the number of eggs deposited in the liver of infected mice. Vaccination with IL-12 or IL-12/SWAP showed insignificant changes in the total numbers of eggs. Treatment with F2 gel alone or F2 gel loaded with SWAP/IL-12 induced significant decreases in the total number of eggs; the effect of F2 gel alone was higher (*P* < 0.05; *P* < 0.01). Interestingly, treatment with vaccine formulations, except with SWAP/IL-12, resulted in significant decreases in the number of viable eggs. Treatment with only SWAP/IL-12 or F2 gel induced decreases in the number of dead eggs.

Effect of IL-12-based vaccination system on granuloma size

Granuloma size of infected mice was 0.022 mm^3. Vaccination of infected mice with SWAP and IL-12 did not cause significant changes in the size of granuloma. Although vaccination with SWAP/IL-12 increased the granuloma size, F2 gel alone or F2 gel loaded with SWAP/IL-12 caused a significant reduction in the size of the granulomas (*P* < 0.01) as compared to the non-vaccinated infected mice, with granuloma sizes of 0.016 mm^3 and 0.012 mm^3, respectively (Figure 5).

Effect of IL-12-based vaccination on liver pathology

Figure 3. Effect of IL-12- based vaccination on worm burden (worm/mouse) of mice infected with *S. mansoni*.
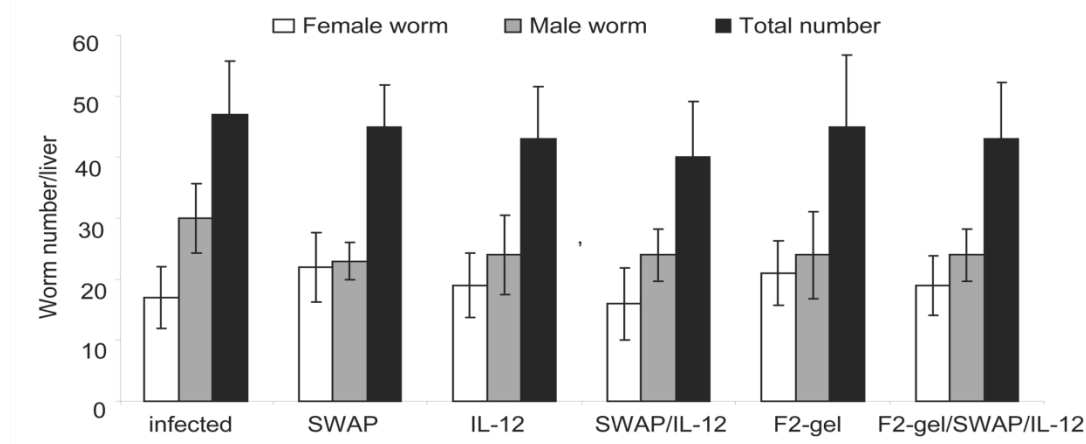
Mice (n = 10/group) were immunized with subcutaneous (s.c.) injection of SAWP alone (100 µg), IL-12 alone (2 µg), SWAP (100 µg) and IL-12 (2 µg), F2 gel alone (100 µL), or F2 gel (100 µL) loaded with SWAP (100 µg) and IL-12 (2 µg). Mice were vaccinated with the vaccine formulation two times, 2 weeks apart. One group of naïve (non-vaccinated) and the all vaccinated mice were infected with 100 cercariae of S. mansoni by tail immersion. Then, the infected mice were sacrificed after 8 weeks.

Figure 4. Effect of IL-12- based vaccination on oogram (ova/100 mg of liver tissue) of mice infected with *S. mansoni*.
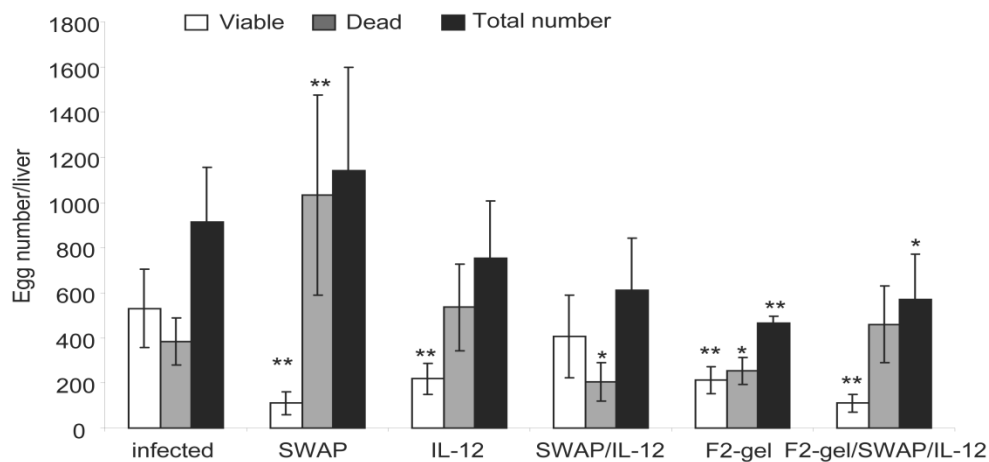
Mice (n = 10/group) were immunized with subcutaneous (s.c.) injection of SAWP alone (100 µg), IL-12 alone (2 µg), SWAP (100 µg) and IL-12 (2 µg), F2 gel alone (100 µL), or F2 gel (100 µL) loaded with SWAP (100 µg) and IL-12 (2 µg). Mice were vaccinated with the vaccine formulation two times, 2 weeks apart. One group of naïve (non-vaccinated) and the all vaccinated mice were infected with 100 cercariae of *S. mansoni* by tail immersion. Then, the infected mice were sacrificed after 8 weeks. (*$P < 0.05$; **$P < 0.01$)

Histological examination of the liver sections of *S. mansoni*-infected mice with no vaccination showed numerous granulomatous reactions containing viable eggs. In some sections, more than one egg containing live miracidium can be identified. These livers also showed pronounced inflammatory cellular infiltrations around the deposited egg (Figure 6a). Although vaccination of infected mice with SWAP (Figure 6b) or IL-12 (Figure 6c) alone did not significantly impact the granulomatous reactions, vaccination with SWAP/IL-12 (Figure 6d) showed an increase in the granulomatous reactions as indicated by the increase in the contents of the inflammatory cellular infiltration. Interestingly, vaccination with either F2 gel alone (Figure 6e) or F2 gel/SWAB/IL-12 (Figure 6f) showed marked decreases in the magnitude of the inflammatory cellular infiltration and reduction in the granuloma size as compared to the liver sections from the infected mice with no vaccination.

**Figure 5.** Effect of IL-12- based vaccination on granuloma size (mm^3) of mice infected with *S. mansoni*.
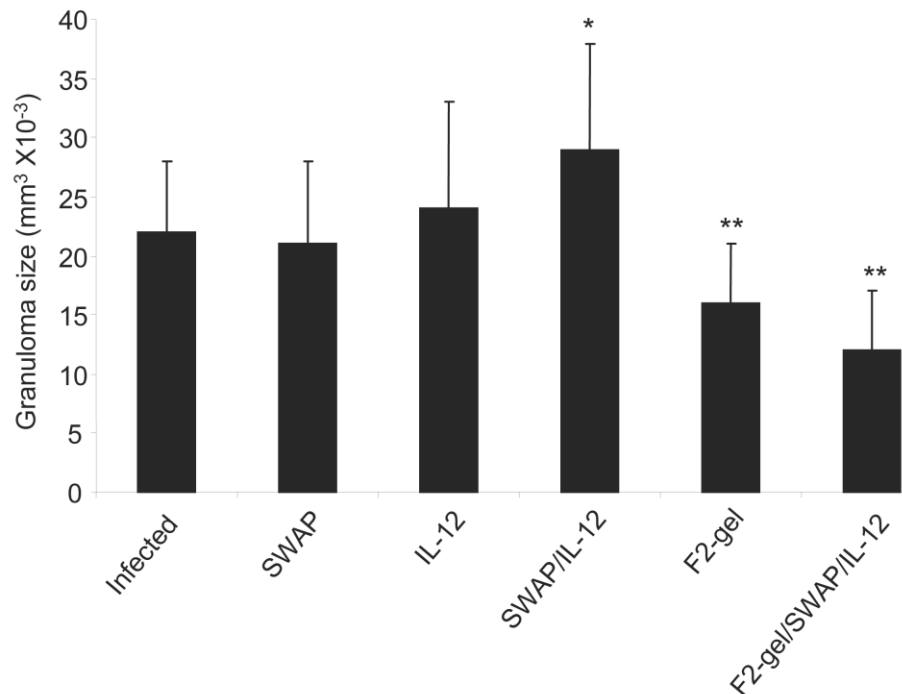
Mice (n = 10/group) were immunized with subcutaneous (s.c.) injection of SAWP alone (100 µg), IL-12 alone (2 µg), SWAP (100 µg) mixed with IL-12 (2 µg), F2 gel alone (100 µL), or F2 gel (100 µL) loaded with SWAP (100 µg) and IL-12 (2 µg). Mice were vaccinated with the vaccine formulation two times, 2 weeks apart. One group of naïve (non-vaccinated) and the all vaccinated mice were infected with 100 cercariae of *S. mansoni* by tail immersion. Then, the infected mice were sacrificed after 8 weeks. (*P <0.05; **P <0.01).

Discussion

Although systemic administration of IL-12 is known to be a potent T cell adjuvant [37-39], it is associated with dose- and schedule-dependent toxicity in humans [40-42]. Our group has established that the F2 gel matrix can release low but effective functional levels of IL-12, as evidenced by the enhanced post-vaccination responses of CD8$^+$ T cells and anti-tumor responses [21]. The main aim of the present study was to test whether IL-12 released from F2 gel can induce comparable adjuvant effects to those obtained after systemic delivery of IL-12 without toxicity in the schistosomiasis setting. The present study demonstrated that 1) IL-12 released from F2 gel did not induce significant toxicity as compared with systemic IL-12; 2) there were similar levels of IFN-γ, TNF-α and IL-2 after paracrine and systemic IL-12 treatments; 3) vaccination with F2 gel/SWAP/IL-12 induced a higher anti-schistosomal activity as compared with systemic IL-12 as evidenced by the decrease in the total liver egg counts and the reduction in fibrotic reaction and granuloma size. Collectively, these results indicate the beneficial effects of F2 gel/IL-12 adjuvant system

against schistosomiasis-induced hepatic fibrosis without induction of toxicity. Therefore, this IL-12/F2 gel delivery approach would be a potential strategy for the treatment of schistosomiasis in the clinical setting.

Previous studies revealed that the predominant immune response to larval and adult schistosome worm antigens appears to be skewed toward the production of Th1 cytokines [43,44]. Although CD4$^+$ T cells have been shown to be crucial for the development of egg-induced granulomas [45,46], there have been conflicting observations on the roles of Th1 and Th2 cells and the cytokines they secrete in the development of the granulomatous response following egg deposition [47]. This could explain why Th1 immune response with increased IFN-γ predominates in early infection and Th2 type predominates as egg production and tissue reaction begins. Furthermore, the decrease in INF-γ as infection progresses is accompanied by an increase in granuloma formation [48]. Thus, an enhanced Th2 cytokine synthesis was observed during the formation of granulomas in infected mice [49,50] or in animals injected with schistosome eggs [51,52]. In contrast,

Figure 6. Effect of IL-12- based vaccination on liver pathology of mice infected with *S. mansoni*.
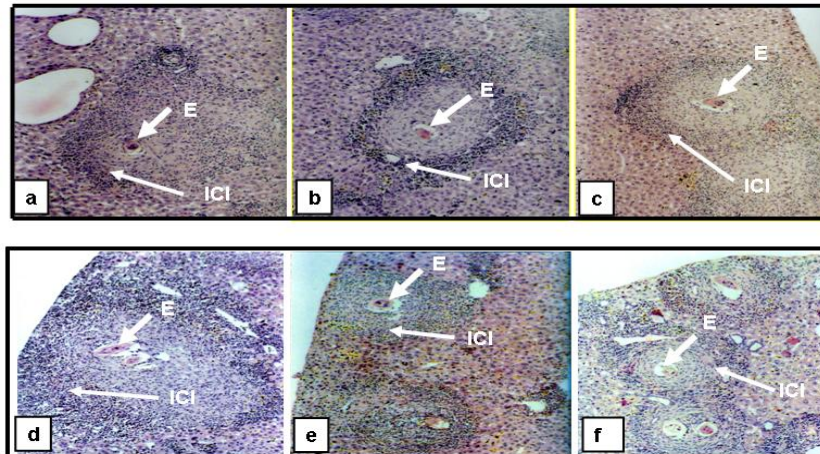
Mice were infected with 100 cercariae of *S. mansoni*, sacrificed 8 weeks after infection, and liver samples were harvested for histological staining. (A) Shows stained liver section from infected mice with no vaccination. Mice were vaccinated twice, 2 weeks apart s.c. with 100 µg SAWP alone (B), 2 µg IL-12 (C), 100 µg SWAP mixed with 2 µg IL-12 (D), 100 µl F2 gel (E), or 100 µl F2 gel loaded with 100 µg SWAP mixed with 2 µg IL-12 (F). The red arrows indicate the inflammatory cellular infiltration around the deposited egg in the formed granuloma. The blue arrows indicate the deposited eggs. Sections were stained with haematoxylin and eosin, X 100.

the inhibition of egg pathology was observed after in vivo depletion of either IL-4 or IL-2 [52], two cytokines required for the generation of Th2 responses [53]. This dichotomy in the nature of anti-schistosomiasis Th1/Th2 responses is consistent with in vitro studies in human intestinal schistosomiasis. In these studies, when inflammatory cells collected at various stages of infection were stimulated by egg or adult worm antigens, antigen-specific Th1 cytokine responses predominated in the acute infection stage [54]. In line with these studies, copious amount sof the Th1 inflammatory cytokines IFN-γ and TNF-α were rapidly induced upon injection of F2 gel/IL-12 or IL-12. These high levels of IFN-γ and TNF-α could skew the immune response to Th1 type, and thus decrease the Th2-mediated granulomatous reaction. This could explain the decrease in granuloma size after IL-12-based vaccination which was consistent with the reduction in the numbers of viable eggs.

The results of the present study showing the induction of IFN-γ by F2 gel/IL-12 are consistent with the previous studies which show that IL-12 has potent IFN-γ stimulating activities on NK cells as well as on CD4^{+} and DC8^{+} T lymphocytes [55,56]. In addition, IL-12 has been demonstrated in vitro to play a major role in directing the differentiation of Th1 effector CD4^{+} [57, 58]. The results indicate that IL-12 and INF-γ are important immunomodulators of granulomatous inflammation in the liver. Therefore, we suggested that it could be possible to stimulate IFN-γ expression and thus simultaneously inhibit

granuloma formation by treatment with exogenous IL-12. This suggestion is consistent with the observation that exogenous IL-12 caused a near complete inhibition of both primary and secondary granuloma formation, while suppressing *S. mansoni* egg-induced Th2 response [59].

In this report we have shown the beneficial effects of the F2 gel/IL-12 adjuvant system toward schistosomiasis-induced liver fibrosis. The added beneficial effect of F2 gel to the adjuvant effects of IL-12 could be explained because F2 gel provides a danger signal via the innate immune system and at the same time serves as a sustained release delivery vehicle for SWAP antigen and IL-12 at the vaccine site [20,21]. This creates a beneficial microenvironment in which SWAP antigen is presented in association with a danger signal, F2 gel per se plus IL-12 enhanced IFN-γ and TNF-α production, inducing protective cell-mediated immune responses. These anti-schistosomiasis adjuvant effects of the low levels of IL-12 released from F2 gel are comparable to those obtained after systemic IL-12 treatment, which often induces toxicity. These results show also that F2 gel can induce anti-fibrotic effects. Therefore, F2 gel/IL-12 adjuvant system could be used as an "anti-pathology" vaccine for preventing granulomatous disease. Further studies, however, are required to confirm and expand the beneficial effects of the F2 gel/IL-12 adjuvant system and to evaluate its effects on different toxicity-related parameters.

Acknowledgements

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