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Protective effects of Sm-p80 in the presence of resiquimod as an adjuvant against challenge infection with *Schistosoma mansoni* in mice

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SUMMARY

Objectives: To determine the prophylactic efficacy of an Sm-p80-based vaccine formulation against challenge infection with *Schistosoma mansoni* in mice using an approach comprising of initial priming with DNA and boosting with recombinant protein in the presence of resiquimod (R848) as an adjuvant.
Methods: In the first experiment (prime–boost approach), mice were primed with Sm-p80–pcDNA3 (week 0) and boosted at weeks 4 and 8 with recombinant Sm-p80 formulated in resiquimod (R848). Each mouse in the control group first received only pcDNA3 and was boosted with R848. In the second set of experiments (recombinant protein approach), mice were immunized (week 0) and boosted (weeks 4 and 8) with rSm-p80 formulated in R848. Animals of the control group in this series of experiments received only R848 at 0, 4, and 8 weeks. All of the animals from both the 'prime–boost' and 'recombinant protein' groups were challenged with cercariae of *S. mansoni*, 4 weeks after the last immunization. The mice were sacrificed 6 weeks post-challenge and the reductions in worm burden and egg production were determined. Sm-p80-specific antibody titers were estimated in the mice sera by ELISA. Cytokine mRNA and protein production by proliferating splenocytes in response to in vitro stimulation with Sm-p80, were estimated via RT-PCR and ELISA, respectively.
Results: Vaccination with Sm-p80 (prime–boost approach) showed 49% reduction in worm burden; with the recombinant protein approach the protection was found to be 50%. The protection levels were correlated with antibody production. Upon antigenic stimulation with recombinant Sm-p80, splenocytes secreted significant levels of interferon (IFN)-γ and interleukin (IL)-2, indicating that the immune responses were Th1-biased and this was further supported in terms of distribution of antibody isotypes and mRNA expression of cytokines.
Conclusions: In conclusion the present study clearly demonstrates that Sm-p80 consistently maintained its protective nature, and resiquimod as an immunopotentiating agent slightly boosted the protective effects of Sm-p80 in both 'DNA prime–protein boost' and 'recombinant protein' immunization approaches in a murine model.

1. Introduction

Schistosomiasis affects over 200 million people, with 20 million seriously suffering from severe anemia, chronic diarrhea, internal bleeding, and organ damage (e.g., intestine, liver and spleen) caused by worms, and especially their eggs, and the immune system reaction that the eggs provoke leading to the formation of granulomas.[1] Despite ongoing control efforts, schistosomiasis remains a major source of morbidity in 76 countries, with 280 000 deaths per annum attributed to this disease in sub-Saharan Africa alone.[2,3]

Currently schistosomiasis control is mainly based on the treatment of infected individuals with praziquantel.[4] The large extension of endemic areas and constant reinfection of drug-treated individuals, combined with the poor sanitary conditions in tropical and subtropical countries, makes it necessary to look for other control strategies in addition to drug treatment. To this effect the advent of a prophylactic vaccine would be of great benefit in the sustainable control of schistosomiasis. A potent vaccine against schistosomiasis is expected to enhance protective immune responses in at-risk populations and thereby lead to reduced worm burden and decreased egg production.[5–7]

Due to its significant protective and antifecundity effects in both rodent and nonhuman primate models, Sm-p80 is now considered a leading putative vaccine candidate antigen for the development of a schistosomiasis vaccine.[4] In the present study, in

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order to investigate the feasibility of further improving the immunoprotective efficacy of the Sm-p80 vaccine candidate, we used Sm-p80 in combination with resiquimod (R848), a low molecular weight imidazoquinolinamine compound with immune response modifier properties, in 'DNA prime–protein boost' and 'recombinant protein–resiquimod' approaches.

2. Methods

2.1. Animals

Laboratory inbred female C57BL/6 mice were purchased from Charles River Laboratories International Inc. (Wilmington, MA, USA). At the outset of the immunization study the mice were 3–4 weeks old and weighed 10–12 g.

2.2. Parasites

Schistosoma mansoni (Puerto Rican strain)-infected *Biomphalaria glabrata* snails were obtained from the National Institute of Allergy and Infectious Diseases Schistosomiasis Resource Center (Biomedical Research Center, Rockville, MD, USA). The cercariae were collected from these snails. The viability of the larvae was determined under an optical microscope.

2.3. Vaccination schedules and challenge infection

For the entire immunization study, a total of 60 mice were divided into the four major groups, each consisting of 15 animals. Each major group of mice was subdivided into two subgroups comprising seven and eight mice and each of the subgroups processed as independent experiments to obtain two repeats. Animals in experimental group I (prime–boost experimental group) were inoculated intramuscularly with 100 μg Sm-p80–pcDNA3 and boosted with 25 μg recombinant Sm-p80 protein containing 10 μg resiquimod (R848) at week 4 and week 8. The animals in the control group for this experiment (control group I or prime–boost control group) were immunized with 100 μg naked pcDNA3 and boosted with 10 μg R848 at the same time intervals as described above. Animals in experimental group II (recombinant protein experimental group) were immunized with 25 μg rSm-p80 protein containing 10 μg R848 and boosted at week 4 and week 8 with the same vaccine formulation. The animals for the control group (control group II or recombinant protein control group) of this experiment received 10 μg R848 at 0, 4, and 8 weeks. Blood samples were collected prior to the immunization and biweekly thereafter. Four weeks after the second boost, all of the animals were challenged with 150 *S. mansoni* cercariae via tail exposure method.

2.4. Necropsy and estimation of worm and egg burdens in the animal tissue

Forty-six days after the challenge infection, all of the animals were sacrificed. The worms were recovered from the portal system by perfusion and also manually removed from the mesenteric veins. The worm burden reduction rate was calculated as described previously.[8] The liver and intestine from individual animals in all the four major groups (eight subgroups) were removed at necropsy and digested overnight at 37 °C in 4% KOH; the numbers of eggs were counted by two independent individuals before calculating the egg burdens.

2.5. Estimation of antibody responses

An enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of IgG (and its isotypes), IgA, and IgM antibodies. Details of the ELISA protocols have been described previously.[8–10] The antibody responses in the pooled sera were determined in all four groups (eight subgroups) of animals involved in the present study. The results are expressed as mean end-point titers ± standard error (SE).

2.6. Estimation of key Th1- and Th2-type cytokines by ELISA

For the estimation of various cytokines in the culture supernatants, the single cell suspensions of pooled splenocytes from each group of animals were prepared and cultured/stimulated in vitro with Sm-p80 for 48 h, as described previously.[11] The concentrations of interleukin (IL)-2, IL-4, IL-10, and interferon (IFN)-γ were measured by ELISA using the eBioscience ELISA Ready-Set-Go Kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

2.7. Estimation of expression of cytokine mRNA by RT-PCR in vaccinated and control groups

Splenocytes of C57BL/6 mice were cultured in the presence or absence of rSm-p80 protein for 48 h. The details of culture media and the cell culture method have been described previously.[11] Total RNA was extracted by TRIzol reagent as per the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA). Details of the reverse transcription reactions for the first strand cDNA synthesis are described elsewhere.[12] Expression levels of the following cytokines: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (= MIP-2), IL-9, IL-10, IL-11, IL-12α, IL-12β, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, tumor necrosis factor (TNF)-α, IFN-γ, transforming growth factor (TGF)-β1, and TGF-β2 and the expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined by RT-PCR. The protocol details have been described previously.[13–18]

2.8. Statistical analysis

The computer program SPSS (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. One-way analysis of variance (ANOVA) was used to calculate the significance between the two groups. The paired *t*-test was employed to determine in-group significance. In order to avoid reaching a false conclusion, Bonferroni adjustments were included and the results were considered statistically significant if the *p*-value was <0.05.

3. Results

3.1. Appraisal of the protective efficacy of vaccination

As shown in Table 1, both 'DNA prime–protein boost' and 'recombinant protein' immunization approaches in combination with the immunomodulatory agent resiquimod (R848) significantly reduced the worm burden in the vaccinated animals as compared with the respective control groups, which received either plasmid DNA with R848 or R848 alone. Resiquimod boosted the protective nature of candidate vaccine Sm-p80 to 49% in 'prime–boost' experiments and to 50% in 'recombinant protein' experiments.

3.2. Reduction in eggs entrapped in the tissues of the vaccinated animals

Since the vaccine effect on egg output is one of the parameters for an effective anti-morbidity vaccine, we measured the response of the two vaccine regimens on egg production by determining the entrapped eggs in liver and intestine of the vaccinated animals. In

Table 1
Anti-worm effects in C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Immunization group	Mean ± SE worm burden/mouse (*n*)		Total for two experiments (*n*)	% reduction in worm burden
	Experiment 1	Experiment 2		
Control prime–boost (pcDNA3–R848)	28.42 ± 4.95 (7)	31.28 ± 5.34 (7)	29.85 ± 5.15 (14)	-
Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	13.16 ± 3.04 (6)	17.00 ± 4.15 (7)	15.08 ± 3.59 (13)	49.49
Control protein vaccine (R848)	25.28 ± 4.29 (7)	28.50 ± 3.45 (8)	26.89 ± 3.87 (15)	-
Experimental protein vaccine (rSm-p80–R848)	15.33 ± 2.88 (6)	11.28 ± 2.04 (7)	13.30 ± 2.46 (13)	50.53

Table 2
Anti-egg effects in C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Immunization group	Mean ± SE egg burden/mouse (*n*)		Total for two experiments (*n*)	% reduction in egg burden
	Experiment 1	Experiment 2		
Control prime–boost (pcDNA3–R848)	256.78 ± 268.41 (7)	839.81 ± 442.99(7)	683.30 ± 155.70 (14)	-
Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	229.21 ± 229.21 (6)	719.49 ± 566.54(7)	474.30 ± 397.87 (13)	30.57
Control protein vaccine (R848)	536.86 ± 291.89 (7)	645.13 ± 270.73 (8)	590.99 ± 281.31 (15)	-
Experimental protein vaccine (rSm-p80–R848)	452.72 ± 270.66 (6)	538.65 ± 316.95(7)	495.68 ± 293.80 (13)	16.12

the case of the DNA immunization and protein boost, an approximate 30% reduction in egg count was recorded in the tissues of vaccinated animals. In animals vaccinated via 'recombinant protein' approach, the reduction in egg production was found to be 16% (Table 2).

3.3. Titers of anti-Sm-p80 antibody responses

Sera from the control and vaccinated C57BL/6 mice were examined in order to determine the titers of total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA using ELISA. The end-point titers for all these antibodies are shown in Tables 3–9. Briefly, as shown in Table 3, in the case of the DNA prime–protein–R848 boost group of animals, the total IgG titer started rising 6 weeks after initial immunization, and at 12 weeks post-vaccination the antibody titer in this group was 1:204800. In the case of protein–R848 vaccinated animals, the titer of IgG rose quickly just 2 weeks after initial immunization and remained high at 12 weeks post-vaccination (end-point titer = 1:204 800). In contrast, sera from the control animals immunized either with pcDNA or immunomodulatory agent R848 did not react with rSm-p80 (Table 3).

IgG1 antibody levels were similar in both the vaccine regimens, with 1:6400 titers at 12 weeks post-immunization (Table 4). Both IgG2a and IgG2b started rising early in the case of the recombinant Sm-p80 protein–R848 vaccinated group at 2 weeks post-immunization, but the end-point titers for both the 'DNA prime–protein boost' and 'recombinant protein' vaccinated animals were the same at the 1:25 600 level (Tables 5 and 6).

A very high titer of IgG3 antibody was observed in both vaccine regimen groups of mice, as can be seen in Table 7. The IgG3 antibody titer for both vaccination formulations was 1:51 200 at 12 weeks post-immunization, although the initial rise in the IgG3 level was slow for the 'DNA prime–protein boost' vaccine group (Table 7). As expected, the IgM titer initially rose for both

Table 3
End-point titers of anti-Sm-p80 total IgG in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 13.18	100 ± 4.35	100 ± 3.23	100 ± 12.69
2	100 ± 10.49	100 ± 5.04	100 ± 7.32	800 ± 44.29
4	100 ± 12.06	100 ± 14.18	100 ± 9.45	6400 ± 1459.65
6	100 ± 14.00	12 800 ± 5443.68	100 ± 9.76	6400 ± 829.63
8	100 ± 2.56	204 800 ± 2872.60	100 ± 4.12	204 800 ± 5639.20
10	100 ± 0.93	204 800 ± 1230.58	100 ± 2.56	204 800 ± 2377.43
12	100 ± 1.91	204 800 ± 2844.44	100 ± 14.53	204 800 ± 4850.26

Table 4
End-point titers of anti-Sm-p80 IgG1 in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 4.05	100 ± 1.43	100 ± 2.82	100 ± 9.33
2	100 ± 4.00	100 ± 2.70	100 ± 4.11	100 ± 2.63
4	100 ± 0.00	100 ± 7.69	100 ± 0.00	100 ± 1.54
6	100 ± 4.21	400 ± 6.30	100 ± 2.63	1600 ± 112.56
8	100 ± 1.64	800 ± 0.00	100 ± 1.64	1600 ± 73.85
10	100 ± 3.28	6400 ± 93.66	100 ± 4.76	6400 ± 124.68
12	100 ± 2.94	6400 ± 43.54	100 ± 0.00	6400 ± 562.64

Table 5
End-point titers of anti-Sm-p80 IgG2a in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 10.71	100 ± 1.89	100 ± 2.86	100 ± 6.60
2	100 ± 3.81	100 ± 1.04	100 ± 1.00	200 ± 4.76
4	100 ± 13.64	100 ± 0.00	100 ± 2.97	800 ± 11.85
6	100 ± 6.56	1600 ± 21.77	100 ± 5.65	6400 ± 693.98
8	100 ± 10.68	6400 ± 35.96	100 ± 1.02	12 800 ± 874.53
10	100 ± 5.00	25 600 ± 3020.22	100 ± 8.77	25 600 ± 1855.07
12	100 ± 7.22	25 600 ± 1404.88	100 ± 23.33	25 600 ± 1338.56

Table 6
End-point titers of anti-Sm-p80 IgG2b in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 5.09	100 ± 7.18	100 ± 0.43	100 ± 9.50
2	100 ± 0.92	400 ± 13.12	100 ± 4.55	800 ± 48.39
4	100 ± 4.37	800 ± 26.23	100 ± 0.44	3200 ± 359.69
6	100 ± 3.83	6400 ± 176.38	100 ± 6.07	12 800 ± 294.25
8	100 ± 7.80	25 600 ± 1808.18	100 ± 5.69	25 600 ± 1786.05
10	100 ± 5.29	25 600 ± 1983.76	100 ± 5.88	25 600 ± 973.38
12	100 ± 7.59	25 600 ± 2822.81	100 ± 4.67	25 600 ± 1835.13

Table 7
End-point titers of anti-Sm-p80 IgG3 in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 12.69	100 ± 1.52	100 ± 2.80	100 ± 8.20
2	100 ± 7.62	100 ± 0.91	100 ± 0.87	400 ± 9.76
4	100 ± 0.00	100 ± 2.31	100 ± 9.79	3200 ± 246.15
6	100 ± 4.80	3200 ± 285.71	100 ± 0.76	12 800 ± 696.23
8	100 ± 1.48	25 600 ± 3131.92	100 ± 1.56	25 600 ± 1629.09
10	100 ± 3.60	51 200 ± 4536.71	100 ± 2.78	51 200 ± 3324.68
12	100 ± 5.07	51 200 ± 2426.54	100 ± 8.11	51 200 ± 4633.48

Table 8
End-point titers of anti-Sm-p80 IgM in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 0.69	100 ± 4.07	100 ± 2.86	100 ± 0.67
2	100 ± 8.09	100 ± 0.77	100 ± 9.63	200 ± 4.30
4	100 ± 7.38	100 ± 1.65	100 ± 2.59	400 ± 17.61
6	100 ± 8.39	1600 ± 8.70	100 ± 2.21	6400 ± 479
8	100 ± 6.00	3200 ± 82.47	100 ± 1.28	6400 ± 172
10	100 ± 6.67	12 800 ± 163	100 ± 12.26	12 800 ± 136
12	100 ± 9.74	6400 ± 795.27	100 ± 16.18	6400 ± 533

Table 9
End-point titers of anti-Sm-p80 IgA in the sera of C57BL/6 mice following immunization either with prime–boost regimen ((Sm-p80–pcDNA3)–(rSm-p80–R848)) or only with the recombinant protein (rSm-p80–R848)

Week	Control prime–boost (pcDNA–R848)	Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	Control protein vaccine (R848)	Experimental protein vaccine (rSm-p80–R848)
0	100 ± 11.45	100 ± 6.21	100 ± 5.15	100 ± 18.84
2	100 ± 4.72	100 ± 2.10	100 ± 5.11	800 ± 9.70
4	100 ± 3.23	100 ± 14.29	100 ± 9.24	3200 ± 120.43
6	100 ± 5.69	6400 ± 34.97	100 ± 0.91	12 800 ± 940.11
8	100 ± 4.05	12 800 ± 104.92	100 ± 17.05	25 600 ± 8290.91
10	100 ± 20.21	25 600 ± 5493.12	100 ± 12.40	25 600 ± 1055.67
12	100 ± 4.05	25 600 ± 2203.28	100 ± 17.05	25 600 ± 5222.4

Table 10
Levels of cytokine production by splenocytes after 48 h stimulation with recombinant Sm-p80 in vitro[a]

Immunization group	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	IFN-γ (pg/ml)
Control prime–boost (pcDNA–R848)	32.32 ± 0.48	39.23 ± 0.00	165.82 ± 13.72	46.32 ± 8.03
Experimental prime–boost ((Sm-p80–pcDNA3)–(rSm-p80–R848))	164.00 ± 4.85[b]	41.41 ± 9.96	88.47 ± 4.16	144.64 ± 21.47[b]
Control protein vaccine (R848)	32.10 ± 2.55	40.88 ± 10.64	123.68 ± 4.23	48.09 ± 8.61
Experimental protein vaccine (rSm-p80–R848)	1691.89 ± 42.01[b]	39.74 ± 7.95	204.45 ± 105.32	408.74 ± 35.61[b]

IL, interleukin; IFN, interferon.
[a] The values in the table represent mean ± SD.
[b] $p \leq 0.05$ vs. corresponding control group stimulated by recombinant Sm-p80 using the independent sample *t*-test.

'DNA prime–protein boost' and 'recombinant protein–R848' vaccinated group of animals, reaching a peak of 1:12 800 at 10 weeks post-immunization, gradually declining thereafter (Table 8). The titer of IgA antibody was also monitored in the present study and a high level of IgA antibody could be detected in both of the vaccination regimens (Table 9). The end-point titer for IgA antibody stood at 1:25 600 at 12 weeks post-immunization.

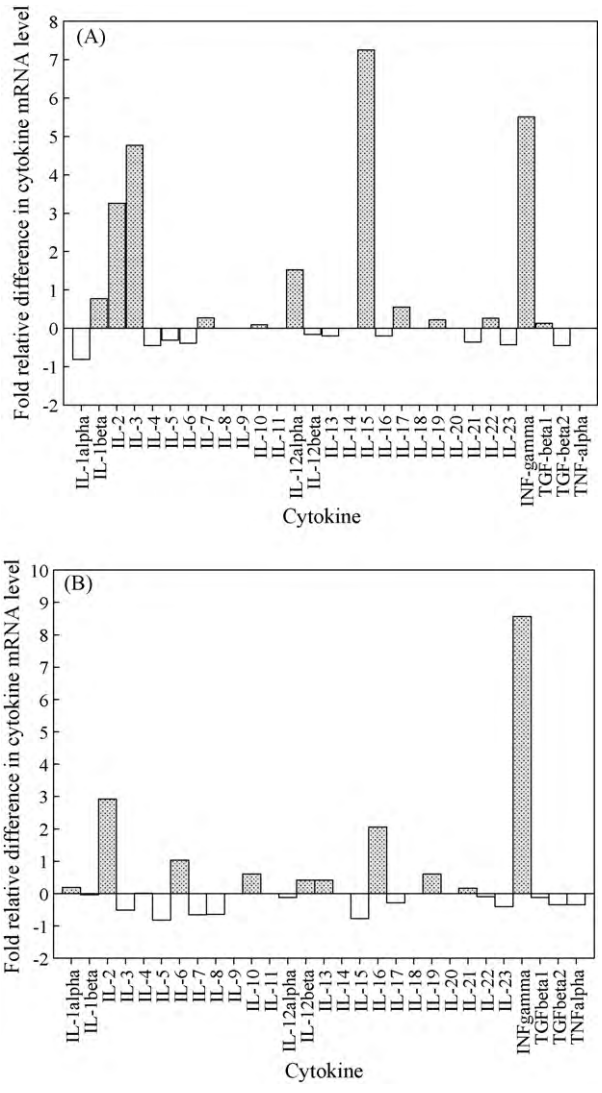

Figure 1. Relative cytokine mRNA expression levels by splenocytes after 48 h stimulation with recombinant Sm-p80 in vitro. The relative cytokine mRNA expression was calculated by comparing the differences in the message levels of the control group with the respective experimental group after standardization using glyceraldehyde 3-phosphate dehydrogenase (GAPDH); (A) 'DNA prime–protein boost' group; (B) 'recombinant protein–R848' group..

3.4. Cytokine production

High levels of IL-2 and IFN-γ were detected in the supernatant of cultured splenocytes 48 h after culture, as detected by ELISA in both the 'DNA prime–protein boost' and 'recombinant protein–R848' vaccinated group as compared with their respective control groups (Table 10). However no significant increase in the level of IL-4 or IL-10 was observed for either vaccination regimen group.

3.5. mRNA expression profiles of cytokines as detected by RT-PCR

Using RT-PCR, the mRNA expression profiles of a number of cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (= MIP-2), IL-9, IL-10, IL-11, IL-12α, IL-12β, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, TNF-α, IFN-γ, TGF-β1, TGF-β2) and GAPDH were determined and the results thus obtained are shown in Figure 1. Relative gene expression was calibrated to the expression of the GAPDH gene and reported as fold relative difference levels in the normalization of normal control samples. In the 'DNA prime–protein boost' group (Figure 1A), higher levels of IL-2, IL-3, IL-12α, IL-15 and IFN-γ genes were expressed, while in the case of the 'recombinant protein–R848' group, besides high level expression of IL-2 and IFN-γ genes, several additional cytokines genes, for example, IL-6 and IL-16, were also up-regulated (Figure 1B).

4. Discussion

In immunization approaches with the aim of stimulating protective immune responses with the potential to facilitate the host in rejecting the initial establishment and subsequent sexual maturation of the parasite, the choice of immune-enhancer is of significant importance. Although Sm-p80 has been shown to be exposed at the host–parasite interface of larval and adult parasites and is naturally immunogenic, this natural immunogenicity of the molecule does not stimulate the immune responses that could protect the host from infection.[19–23] We have previously demonstrated that Sm-p80 can protect murine as well as nonhuman primate animals significantly against experimental schistosomiasis if presented in such a way that it induces a Th1-skewed protective immune response.[3,8–11,24–26] For example, recently[11] using oligodeoxynucleotide (ODN) 10104 (Coley Pharmaceutical Group, Wellesley, MA, USA) as an adjuvant we could enhance the immunoprotective efficacy of Sm-p80 leading to a reduction in worm burden of 70%, a protection level previously reported only with irradiated cercarial vaccine.[27–29]

In our continual efforts to improve the efficacy of Sm-p80 by enhancing the Th1-type protective immune response, we studied the adjuvant effect of a TLR 7/8 agonist imidazoquinolinamine compound – resiquimod. Resiquimod has demonstrated potency as an inducer of the Th1 response enhancer cytokines both in vitro and in vivo[30] and also stimulates dendritic cells to secrete cytokines, up-regulate co-stimulatory molecule expression, and enhance antigen presentation to T cells. Additionally, R848 has

demonstrated vaccine adjuvant properties in a number of animal models.[31]

In the present study we designed two experiments, expecting Sm-p80 delivered in combinations with R848 either in a 'DNA prime–protein boost' approach or 'recombinant Sm-p80 protein–R848' approach to augment its protective efficacy. As expected, resiquimod did indeed enhance the antigen-specific total IgG responses (1:204 800 titer) and also shifted the response towards the Th1-type, as determined by higher levels of production of IgG2a following immunization with the 'DNA prime–protein boost' vaccination strategy. However the elevation of IgG2a did not lead to higher protection levels in terms of worm reduction and reduction in egg production, as previously was observed using ODN 10104 as an adjuvant.[11] Besides this, the proliferating splenocytes secreted significantly higher levels of IL-2 and IFN-γ in the culture supernatants. Traditionally it has been reported that IFN-γ plays a significant role in vaccine-mediated protection against schistosome challenge infection in animal model studies.[8,9,11,24,26,32–35] The up-regulation in expression of IL-12 may be the reason for a very high titer of IgG2a antibodies. Previously it has been reported that IL-12 induces a switch in immunoglobulin isotypes by acting on B cells both directly and indirectly via T-cell-derived IFN-γ, resulting in enhanced production of IgG2a antibodies and inhibition of IgE synthesis.[36] Additionally, in the present vaccination regimen in mice, as well as the known Th1 response enhancing cytokines, for example IL-2, IFN-γ and IL-12α, up-regulation of IL-3, IL-6, IL-15 and IL-16 was also observed. IL-5 which has structural similarity to IL-2 is involved in the induction and proliferation of natural killer cells and its regulation has previously been implicated in enhancing resistance to microbes.[37] Up-regulation of IL-16 in the present study is very interesting, since IL-16 is a multi-functional cytokine that uses CD4 as a receptor to signal diverse biological activities by target cells, including T-lymphocytes, monocytes, and eosinophils.[38]

In conclusion the present study clearly demonstrates that Sm-p80 consistently maintained its protective nature and that resiquimod as an immunopotentiating agent slightly boosted the protective effect of Sm-p80 both in 'DNA prime–protein boost' and 'recombinant protein' immunization approaches in a murine animal model.

Conflict of interest

No conflict of interest to declare.

Ethical approval

All of the studies were performed after approval was obtained from the Institutional Animal Care and Use Committee of the Texas Tech University Health Sciences Center.

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