Published in final edited form as:
Parasite Immunol. 2009 March ; 31(3): 156–161. doi:10.1111/j.1365-3024.2008.01091.x.

Sm-p80-based DNA vaccine made in a human use approved vector VR1020 protects against challenge infection with *Schistosoma mansoni* in mouse

Weidong Zhang[a], **Gul Ahmad**[a], **Workineh Torben**[a], and **Afzal A. Siddiqui**[a,b]

[a]Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, TX 79430

[b]Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

SUMMARY

Although there is an effective drug (praziquantel) available for the treatment of schistosomiasis, yet the disease is still spreading unabated and is rampant in 76 countries. Control via praziquantel treatment has so far been insufficient in reducing the disease transmission. Therefore a vaccine in addition to other strategies, for example, improving sanitation and introduction of new drugs are essential to successfully control and eventually eradicate schistosomiasis. To this effect we have targeted a functionally important antigen, Sm-p80 as a vaccine candidate. In the present study, full length cDNA of Sm-p80 was cloned in VR1020, a FDA approved vector for human use. The protective efficacy of this vaccine formulation was tested in a murine model. Sm-p80-VR1020 vaccine formulation was able to induce 47% reduction in worm burden. Serology on samples obtained from vaccinated animals revealed a strong antibody response which included IgG and all of its subtypes, IgM and IgA. Proliferating splenocytes in response to recombinant Sm-p80 produced a wide spectrum of cytokines representing Th1, Th2 and Th17 types, as ascertained via RT-PCR analysis. These findings further strengthen the importance of Sm-p80 molecule as a vaccine candidate for intestinal schistosomiasis.

Keywords

Sm-p80; calpain; *Schistosoma mansoni*; vaccine; DNA vaccine

INTRODUCTION

The overall health problems related to schistosomiasis seems greater than ever; according to recent estimates up to 210 million people are infected with the disease in more than 76 countries (1-2), and an additional 779 million people are at risk of acquiring this infection (3). This is primarily because of the suboptimal success rates of most conventional control approaches especially as it relates to the developing world. Thus, the emphasis has been placed to develop new approaches involving a vaccine as an addition to the existing methods to control morbidity and mortality of the disease.

Corresponding author Afzal A. Siddiqui Department of Microbiology and Immunology, 3601 4th Street, Mail Stop 6591, Texas Tech University Health Sciences Center, Lubbock, TX79430, USA Tel: (806) 743-2639; Fax (806) 743-2334 Afzal.Siddiqui@ttuhsc.edu.

Disclosures: None

To this effect, a key schistosome protein (Sm-p80) has been identified; this antigen plays an important role in the escape of parasite from immune killing by the host and thus is a viable vaccine target (4-13). The protein was originally identified to be involved in the surface membrane biogenesis (14-15). The vaccine potential of Sm-p80 has been described both for *S. mansoni* (16) and *S. japonicum* (17). Different vaccine formulations of Sm-p80 antigen have been explored in terms of its protective efficacy, both in the mouse and in nonhuman primate models (8-9,18-19). In our ongoing efforts to improve and refine the efficacy of Sm-p80-based vaccine, in this study, we have examined the prophylactic effects of a Sm-p80 based DNA vaccine formulation in human use approved vector, VR1020, against *Schistosoma mansoni* in the murine model

MATERIALS AND METHODS

Animals and Parasites

Female C57BL/6 mice were purchased from Charles River Laboratories International Inc. (Wilmington, MA). The cercariae of *Schistosoma mansoni* were collected from infected *Biomphalaria globrata* snails which were obtained from the Schistosomiasis Resource Center (Biomedical Research Institute, Rockville, MD).

DNA Vaccine Construct

The large subunit of *S. mansoni* calpain (Sm-p80) was subcloned into BamHI/BglII sites of VR1020 (Vical Incorporated, San Diego, CA). The construct thus obtained was named as Smp80-VR1020. Transient transfection of CHO and COS-7 cells were used to ascertain the expression of Sm-p80-VR1020 (5,8-9,18-19). Plasmid DNA was prepared via conventional alkaline lysis method.

Immunization Schedules and Challenge Infection

Thirty mice were divided into two groups. Each of the 15 animals in group 1 (experimental group) were immunized with 100μg Sm-p80-VR1020 plasmid DNA on 0, 4, 8 and 12 weeks. The animals in group 2 (control group) were immunized with the same amount of VR1020 plasmid DNA. Blood samples were collected prior to the immunization and thereafter at 2 weeks intervals. Four weeks after the third boost all of the animals were challenged with 150 *S. mansoni* cercariae via tail exposure method. All of the animals were sacrificed 6 weeks post challenge and the worms were recovered by perfusion from the hepatic portal system and also individually removed from the mesenteric veins. The number of worms recovered from each animal was recorded and percent reduction in worm burdens in vaccinated verses control animals was calculated (5).

Measurement of Antibody Responses

Antibody responses against recombinant Sm-p80 protein were assessed in the sera of vaccinated and control mice by ELISA as described previously (5,8). Briefly after coating each well of a microtiter plate with 1.2 μg Sm-p80 overnight, wells were washed three times in PBST and blocked for one hour at 37 °C with 2 % bovine serum albumin in PBST. Sera were titrated using doubling dilutions from 1:100 to 1:12800. Plates were incubated as mentioned above, washed and then reacted with optimally diluted anti-mouse antibody conjugated with HRP. The absorbance was measured calorimetrically in an automated plate reader (Biotek Instruments, Inc, Vermont, USA).

Cell proliferation and Cytokine production Assay

Single cell suspensions were prepared from the spleens of control and experimental animals as described elsewhere (9). For determination of cell proliferation and cytokine production,

5×10^6 splenocytes/ml were cultured in a final volume of 200 μl in 96-well flat-bottom plates in the presence of either 1.2 μg recombinant Sm-p80 protein or 0.5 μg Con A. One hundred μl culture supernatants were collected after 48 h incubation to estimate IL-2, IL-4, IL-10 and INF-γ production. All of these cytokines were measured by using a murine cytokine Th1/Th2 ELISA panel kit (eBiosciences Inc., San Diego, CA), following the procedure provided by the manufacturer. The remainders of the cell cultures were processed for cell proliferation assays as described previously (9).

RNA Extraction, cDNA synthesis and RT-PCR

Total RNA was extracted from splenocytes of C57BL/6 mice before and after stimulation of the cells with rSm-p80. TRIzol reagent was used for the extractions of RNA as per the manufacturer's instructions (Invitrogen, Carlsbad, CA). Reverse transcription reactions for first strand cDNA synthesis was carried out as described earlier (20). Expression levels of GAPDH and various cytokines [IL-1α, IL-1β, IL-2, IL-3, IL4, IL-5, IL6, IL-7, IL-8 (=MIP-2), IL-9, IL-10, IL-11, IL-12α, IL-12β, IL-13,IL-14, IL-15, IL16, IL-17,IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, TNF-α, IFN-γ, TGF-β1, TGF-β2] were analyzed using RT-PCR. The details of the protocols used have previously described elsewhere (21-28).

Statistical analysis

To calculate the total sample size of experimental animals required per immunization Fisher's exact test (2 sided) was used, assuming the minimum detectable difference in response proportions P_E and P_C with 80% power and type I error rate of 0.05. If $P_C = 0$ (no response in control animals) and $P_E = 0.5$ (any measurable immune response, e.g., antibody production as determined by Western analysis or ELISA, T cell stimulation, cytokine production, protection from challenge infection, in 50% of the experimental animals) the number of animals needed per group is 15.

Statistical analysis of the data was performed using SPSS computer program. Significance between two groups was calculated by one-way ANOVA and in group significance by paired t-test. Boferroni adjustments were included to reduce the risk of reaching false conclusions. Results were considered statistically significant when the analysis returned a $p < 0.0\ 5$.

RESULTS

Assessment of protective activity of naked DNA vaccination

Using DNA vaccination strategy, the protective potential of Sm-p80 was determined. As shown in Table 1, mice inoculated with Sm-p80-VR1020 showed a statistically significant worm reduction of 46.87% ($p<0.001$).

Antibody responses

To determine end point titers of anti-Sm-p80 specific antibodies, pooled sera from the control and vaccinated mice were tested by ELISA. Significant titers of specific IgG antibodies were observed in vaccinated mice (Figure1). It was observed that the IgG titer started rising 2 weeks after inoculation and continued to rise thereafter with every booster. The end point IgG titer for vaccinated group was 1:6400 at 16 weeks post immunization at the time animals were challenged (Figure 1A).

To determine the IgG isotype profile induced by vaccination, specific IgG1, IgG2a and IgG2b to Sm-p80 was determined. It was observed that the titer of IgG1 began to rise 4 weeks after the first immunization and continued to rise and reached a peak of 1: 3200 at 16 weeks just before the challenge infection (Figure 1B). Similarly the titer of IgG2a started

increasing at week 4 after the first inoculation and the titer was 1: 3200 at 16 week of the vaccination. The end point titer for IgG2b at 16 week was 1: 6400 which was double of IgG2a (Figure 1C &D). The titers of IgG3 antibody was much lower which at its peak stood at 1:800 (Figure 1E). As expected the IgM titer started rising 8 week after the initial immunization and peaked at 12 week which gradually fell to the background level by 16 week post immunization (Figure 1F) just before the challenge infection. We also determined the IgA levels in this study and observed only a small level of this antibody in the vaccinated group of animals. The end point titer for IgA was 1:800 (Figure 1G).

Assessment of splenocytes proliferation assay—The splenocytes from the Sm-p80-VR1020 vaccinated group showed a significant increase in proliferation compared with the control group (which received only VR1020) after stimulation by recombinant Sm-p80 protein *in vitro* (Table 2). Further as expected, Sm-p80 stimulated proliferation of splenocytes was much lower when compared with the stimulation index of cells stimulated by Con A (Table 2).

Assessment of Th1 and Th2 cytokine production by splenocytes of vaccinated and control animals as detected by Capture ELISA—The effect of Sm-p80-VR1020 vaccination on the production of different cytokines was investigated *in vitro* in spelenocytes culture. As shown in Figure 2, IL-2 and INF-γ production was significantly higher in vaccinated animals as compared with the control group. It was observed that there is no statistically significant difference in the IL-4 and IL-10 production when compared with the control group.

Assessment of Cytokine mRNA profile—A large repertoire of cytokines [IL-1α, IL-1β, IL-2, IL-3, IL4, IL-5, IL6, IL-7, IL-8 (=MIP-2), IL-9, IL-10, IL-11, IL-12α, IL-12β, IL-13,IL-14, IL-15, IL16, IL-17,IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, TNF-α, , IFN-γ, TGF-β1, TGF-β2] were studied via RT-PCR. Specifically, in addition to the Th1 type cytokines, IL-2 and INF-γ, a number of other cytokines including IL-1β, IL-3, IL-5, IL-6, IL-8,IL-11, IL-12, IL-15-18, IL-21, TGF–β, and TNF α were up regulated as compared with the control group (Figure 3 and 4).

DISCUSSION

A vaccine for schistosomiasis that can be used in endemic areas would definitely complement drug treatment and other control strategies and will help immensely in reducing the levels of transmission of this disease. Several groups around the world have been working to develop a subunit vaccine for schistosomiasis but thus far none of them have achieved consistent and independently reproducible levels of protection which is acceptable for human use (27-30). In the present investigation we report high levels of protection with Sm-p80 DNA vaccine constructed in VR1020 plasmid which is an FDA approved vector for human use. Previously, we have shown that Sm-p80 has significant promise as a vaccine candidate based on the data obtained from both murine as well as nonhuman primate models (5-6,8-9,18-19,31).

In the present study Sm-p80-VR1020 DNA vaccination induced high titers of anti-Sm-p80 IgG, just 2 weeks after the initial immunization while the titers of IgG1 and IgG2a were considerably low even 6 weeks following the first vaccination. Conversely, the titers of IgG2b were found to be much higher compared to IgG1 and IgG2a. These findings further support our previous reports (5-6,8-9,18-19,31) and of others (32) and suggest some bias towards the Th1 type of immune response after vaccination with DNA vaccine formulations. This is further supported by cytokine analysis in which Sm-p80-VR1020 immunization resulted in a Th1-skewed type of immune response as ascertained by high level of IFN-γ and

IL-2 and significantly low levels of IL-4 and IL-10 production by splenocytes following *in vitro* stimulation with recombinant Sm-p80. Further, high message levels of IL-18 were observed in vaccinated animals which are again indicative of a Th1-bias, because IL-18 is known to induce IFN-γ production and other Th1 cytokines thus promoting a Th1 development and NK activity (33). Upregulation of IL-15 in vaccinated animals is interesting; IL-15 possesses structural similarity to IL-2 and induces cell proliferation of natural killer cells. Overproduction of IL-15 has been shown to improve resistance to pathogens in other systems (34).

Additionally, induction of a Th17 type of immune response was also observed in these studies as characterized by the up regulation of TGF-βand IL-17 cytokines. Upregulation of IL-6 and TGF–β1 perhaps expanded Th17 immune responses leading to significant worm burden reduction. Recently, Tallima *et al*. (35) have reported a significant increase in plasma levels of TGF-β and IL-17 cytokines in C57BL/6 mice experimentally infected with *Schistosoma mansoni* and attributed the worm burden reduction to the increased levels of these cytokines. Additionally, the down regulation of IL-4 cytokine, observed in the present study, is in agreement with the published literatures that induction of IL-4 molecules suppresses the growth and maturation of Th1 and Th17 cells (36-38). It has been postulated that Th17 responses are likely to constitute an early immune response to several pathogens which could not be tackled properly by Th1 or Th2 type of immune response alone (37). Lastly, the present findings further strengthen our previous reports that Sm-p80 is an efficacious vaccine candidate and should be further developed and optimized for human trials.

Acknowledgments

This work is supported by a grant from the National Institute of Allergy and Infectious Diseases, NIH (R01AI071223) to Afzal A. Siddiqui.

REFERENCES

1. Berriman M, Haas BJ, LoVerde PT, et al. The genome of the blood fluke *Schistosoma mansoni*. Nature. 2009; 460:352–358. [PubMed: 19606141]
2. Hotez PJ, Molyneux DH, Fenwick A, et al. Control of neglected tropical diseases. N Engl J Med. 2007; 357:1018–1027. [PubMed: 17804846]
3. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis. 2006; 6:411–425. [PubMed: 16790382]
4. Young BW, Podesta RB. Complement and 5-HT increase phosphatidylcholine incorporation into the outer bilayers of Schistosoma mansoni. J Parasitol. 1986; 72:802–803. [PubMed: 3806337]
5. Ahmad G, Torben W, Zang W, Matt W, Siddiqui AA. Sm-p80 based DNA vaccine formulation induces potent protective immunity against Schistosoma mansoni,. Parasite Immunol. 2008; 31:156–161. [PubMed: 19222788]
6. Hota-Mitchell S, Siddiqui AA, Dekaban GA, Smith J, Tognon C, Podesta RB. Protection against Schistosoma mansoni infection with a recombinant baculovirus-expressed subunit of calpain. Vaccine. 1997; 15:1631–1640. [PubMed: 9364694]
7. Hota-Mitchell S, Clarke MW, Podesta RB, Dekaban GA. Recombinant vaccinia viruses and gene gun vectors expressing the large subunit of Schistosoma mansoni calpain used in a murine immunization-challenge model. Vaccine. 1999; 17:1338–1354. [PubMed: 10195769]
8. Siddiqui AA, Phillips T, Charest H, et al. Enhancement of Sm-p80 (large subunit of calpain) induced protective immunity against Schistosoma mansoni through co-delivery of interleukin-2 and interleukin-12 in a DNA vaccine formulation. Vaccine. 2003; 20:2882–2889. [PubMed: 12798631]

9. Siddiqui AA, Pinkston JR, Quinlin ML, et al. Characterization of protective immunity induced against Schistosoma mansoni via DNA priming with the large subunit of calpain (Sm-p80) in the presence of genetic adjuvants. Parasite. 2005; 12:3–8. [PubMed: 15828575]
10. Ridi RE, Tallima H. Schistosoma mansoni ex vivo lung-stage larvae excretory-secretory antigens as vaccine candidates against schistosomiasis. Vaccine. 2009; 29:666–673. [PubMed: 19056448]
11. Jankovic D, Aslund L, Oswald IP, et al. Calpain is the target antigen of a Th1 clone that transfers protective immunity against Schistosoma mansoni. J Immunol. 1996; 157:806–814. [PubMed: 8752932]
12. Ohta N, Kumagai T, Maruyama H, Yoshida A, He Y, Zhang R. Research on calpain of Schistosoma japonicum as a vaccine candidate. Parasitol Int. 2004; 53:175–81. [PubMed: 15081949]
13. Zhang R, Yoshida A, Kumagai T, et al. Vaccination with calpain induces a Th1-biased protective immune response against Schistosoma japonicum. Infect Immun. 2001; 69:386–391. [PubMed: 11119528]
14. Siddiqui AA, Zhou Y, Podesta RB, et al. Characterization of Ca(2+)-dependent neutral protease (calpain) from human blood flukes, Schistosoma mansoni. Biochim Biophys Acta. 1993; 24:37–44. [PubMed: 8457603]
15. Karcz SR, Podesta RB, Siddiqui AA, Dekaban GA, Strejan GH, Clarke MW. Molecular c loning and sequence analysis of a calcium-activated neutral protease (calpain) from Schistosoma mansoni. Mol Biochem Parasitol. 1991; 49:333–336. [PubMed: 1775175]
16. Siddiqui AA, Ahmad G, Damian RT, Kennedy RC. Experimental vaccines in animal models for schistosomiasis. Parasitol Res. 2008; 102:825–833. [PubMed: 18259777]
17. Ohta N, Kumagai T, Maruyama H, Yoshida A, He Y, Zhang R. Research on calpain of Schistosoma japonicum as a vaccine candidate. Parasitol Int. 2004; 53:175–181. [PubMed: 15081949]
18. Siddiqui AA, Phillips T, Charest H, et al. Induction of protective immunity against Schistosoma mansoni via DNA priming and boosting with the large subunit of calpain (Sm-p80): adjuvant effects of granulocyte-macrophage colony-stimulating factor and interleukin-4. Infect Immun. 2003; 71:3844–51. [PubMed: 12819068]
19. Siddiqui AA, Pinkston JR, Quinlin ML, et al. Characterization of the immune response to DNAvaccination strategies for schistosomiasis candidate antigen, Sm-p80 in the baboon. Vaccine. 2005; 23:1451–1456. 10. [PubMed: 15670880]
20. Smith JK, Siddiqui AA, Modica LA, et al. Interferon-alpha upregulates gene expression of aquaporin-5 in human parotid glands. J. Interferon Cytokine Res. 1999; 19:929–935. [PubMed: 10476940]
21. Brandt K, Bulfone-Paus S, Foster DC, et al. Interlukin-21 inhibits dendritic cell activation and maturation. Blood. 2003; 102:4090–4098. [PubMed: 12893770]
22. Godinez I, Haneda T, Raffatellu M, et al. T cells helpto amplify inflammatory responses induced by Salmonella enterica serotype Typhimurium in the intestinal mucosa. Infect Immun. 2008; 76:2008–2017. [PubMed: 18347048]
23. Lauwerys BR, Garot N, Renauld JC, et al. Cytokine production and killer activity of NK/TNK cells derived with IL-2, IL-15, or a combination of IL-12and Il-18. J Immunol. 2000; 165:1847–1853. [PubMed: 10925263]
24. Montgomery RA, Dallman MJ. Analysis of cytokine gene expression during fetal thymic ontogeny using the polymerase chain reaction. 1991; 147:554–560.
25. Ren F, Xhan X, Martens G, et al. Pro-IL-16 regulation in activated murine CD4+ lymphocytes. J Immunol. 2005; 174:2738–2745. [PubMed: 15728482]
26. Shen L, Zhang C, Wang T, et al. Development of autoimmunity in IL-14 alpha-transgenic mice. J Immunol. 2006; 177:5676–5686. [PubMed: 17015757]
27. McManus DP, Loukas A. Current status for schistosomiasis. Clinical Microbiol Rev. 2008:225–242. [PubMed: 18202444]
28. Capron A, Riveau G, Capron M, Trottein F. Schistosomes:the road from host-parasitic interactions to vaccines in clinical trials. Trends Parasitol. 2005; 21:143–149. [PubMed: 15734662]

29. Lebens M, Sun JB, Czerkinsky C, Holmgren J. Current status and future prospects for a vaccine against schistosomiasis. Expert Rev Vaccins. 2004; 3:315–328.

30. Pearce EJ. Progress towards a vaccine for schistosomiasis. Acta Trop. 2003; 86:309–313. [PubMed: 12745147]

31. Ahmad G, Zhang W, Torben W, Damian RT, et al. Protective and antifecundity effects of Sm-p80-based DNA vaccine formulation against Schistosoma mansoni in a nonhuman primate model. Vaccine. 2009; 27:2830–2837. [PubMed: 19366570]

32. Cardoso FC, Macedo GC, Gava E, et al. Schistosoma mansoni tegument protein Sm29 is able to induce a Th1 type of immune response and protection against parasite infection. PLoS Negl Trop Dis. 2008; 2(10):e308. Doi:10.1371/journal.pntd.0000308. [PubMed: 18827884]

33. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev. 2008; 223:20–38. [PubMed: 18613828]

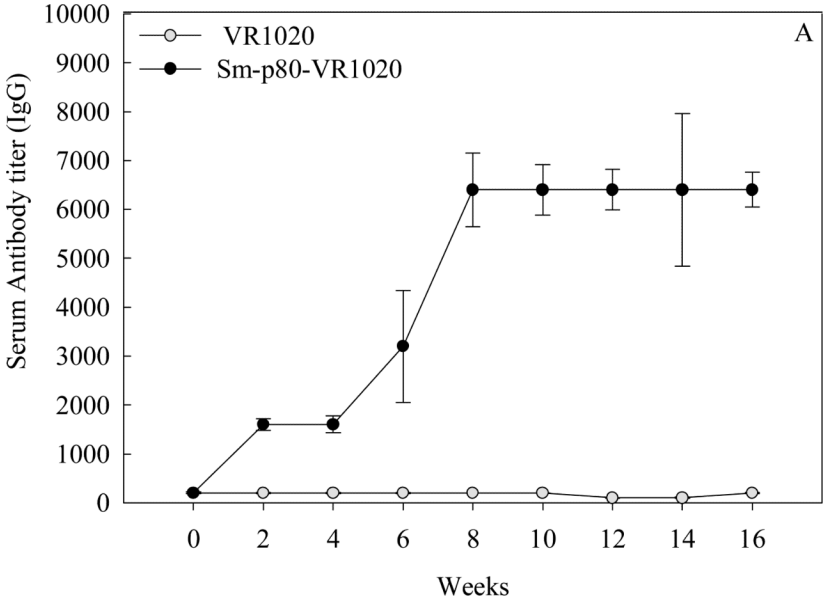
34. Ward PA. The curiosity of IL-15. Nature Medicine. 2007; 13:903–904.

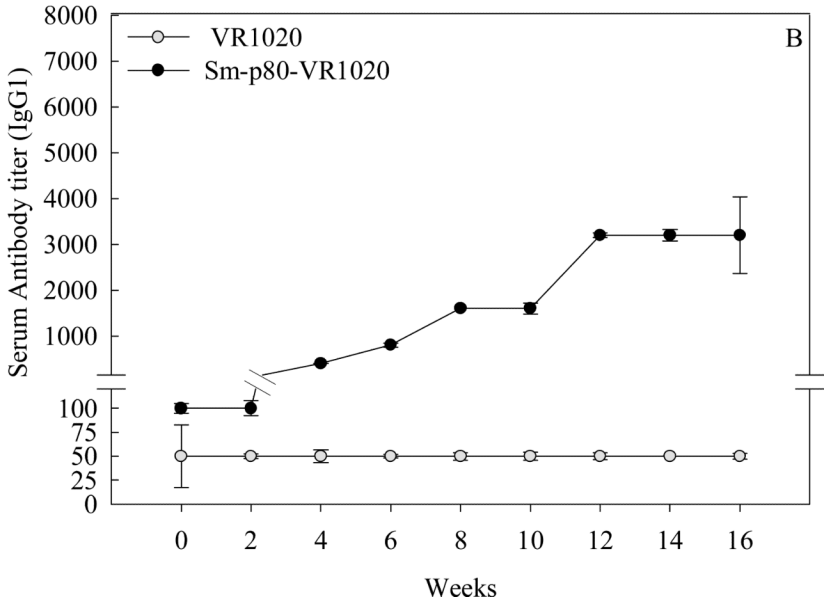
35. Tallima H, Salah M, Guirguis FR, El Ridi R. Transforming growth factor-b and Th17 responses in resistance to primary murine schistosomiasis mansoni. Cytokine. 2009 doi:10.1016/i.cyto.2009.07.581.

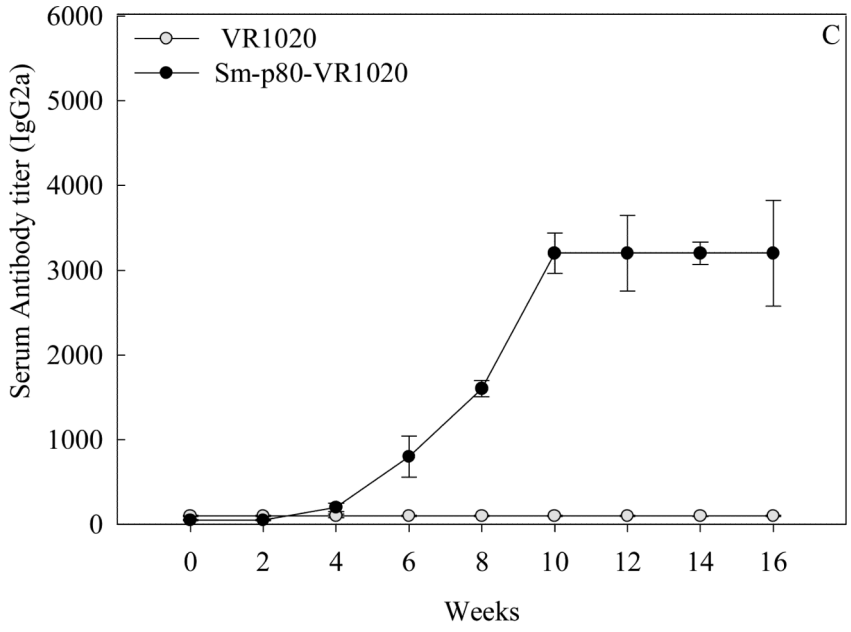
36. Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T (H) 17 cells. Nature. 2008; 453:1051–1057. [PubMed: 18563156]

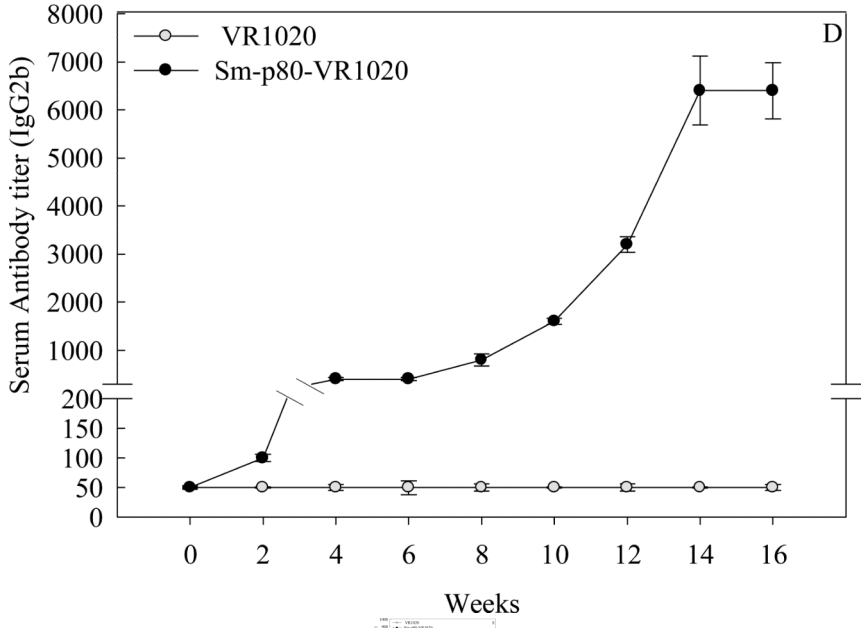
37. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity. 2008; 28:445–453. [PubMed: 18400187]

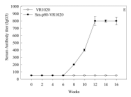
38. Dong C. Th17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol. 2008; 8:337–348. [PubMed: 18408735]

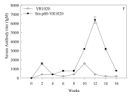












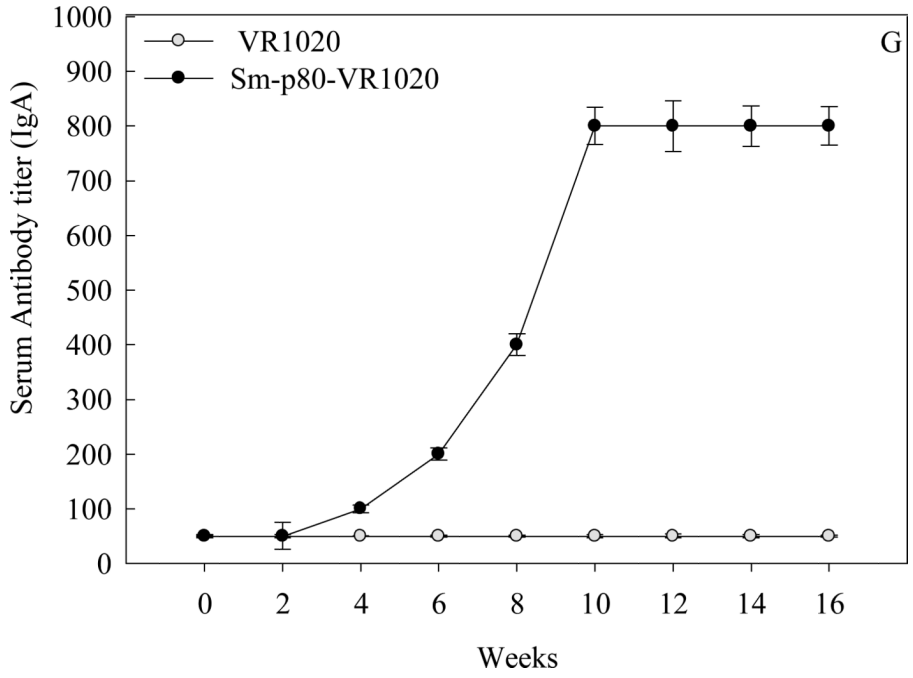


Figure 1.

Titers of anti-Sm-p80 antibodies in immunized mice. ELISA was performed with a pool of sera obtained by mixing equal volumes of serum collected from each mouse(biweekly) in their respective groups (VR1020 and Sm-p80-VR1020). IgG (A), IgG1 (B) IgG2a (C), IgG2b (D) IgG3 (E) IgM(F) and IgA (G).The value represent the mean of three experiments ± standard error. Statistic significance ($P<0.05$) are indicated by (*) compared with VR1020 control group.

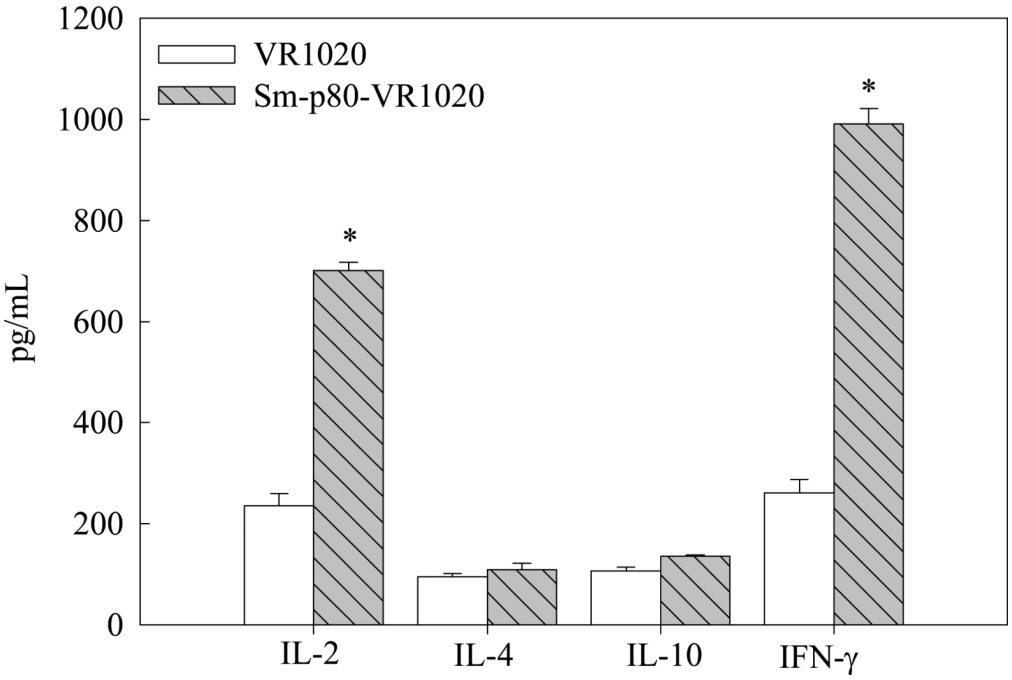


Figure 2.

Levels of cytokine production by splenocytes after 48 hr stimulation with recombinant Sm-p80 *in vitro*. Groups of mice were inoculated with VR1020 and Sm-p80-VR1020. Data are shown as mean ± S.D. Statistical significance ($P<0.05$) are indicated by (*) compared with VR1020 control group using independent sample t- test.

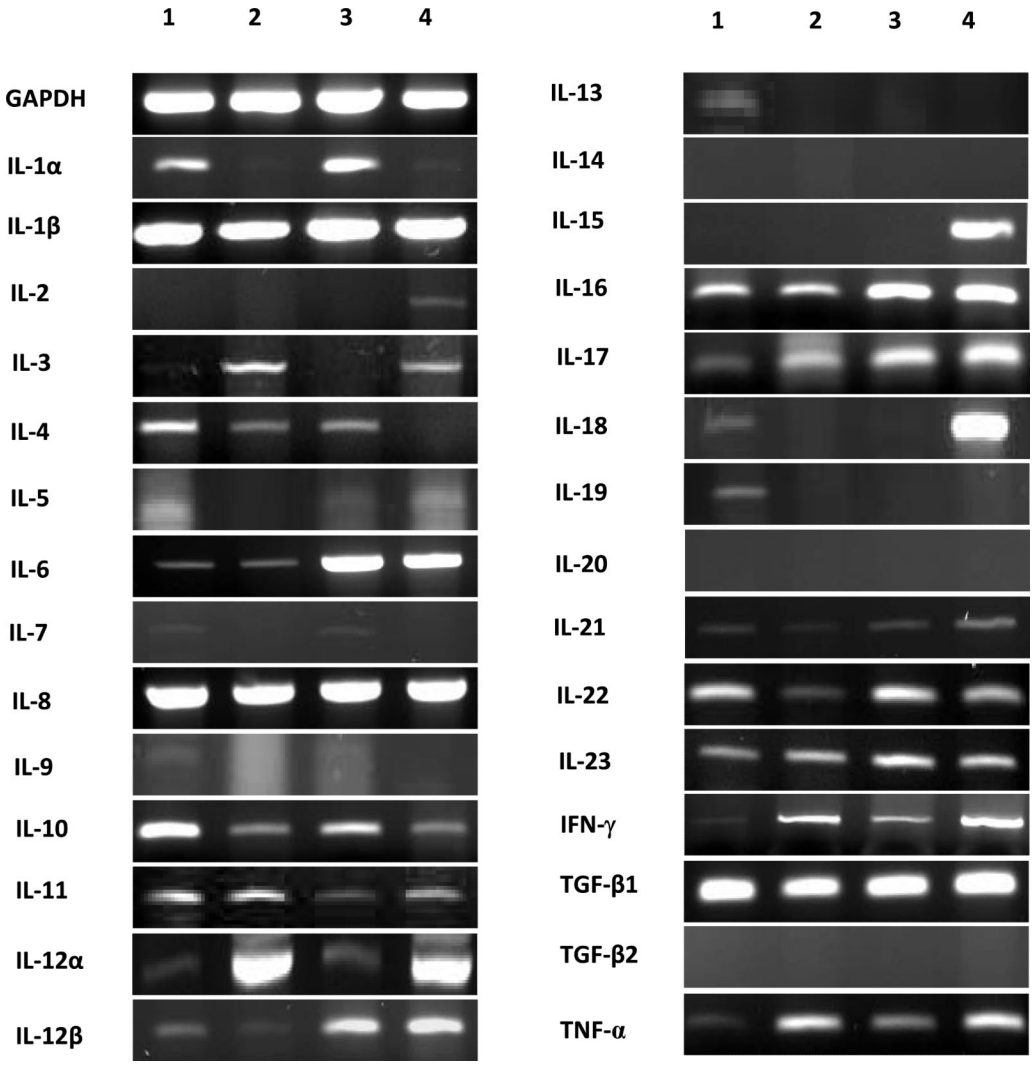


Figure 3.

RT-PCR for cytokine expression by splenocytes after 24 hr stimulation with recombinant Sm-p80 *in vitro*. Panels 1, and 3, control groups inoculated with VR1020; Panels 2, 4, experimental groups inoculated with Sm-p80-VR1020. 1, 2, medium control; 3,4, splenocytes stimulated with recombinant Sm-p80.

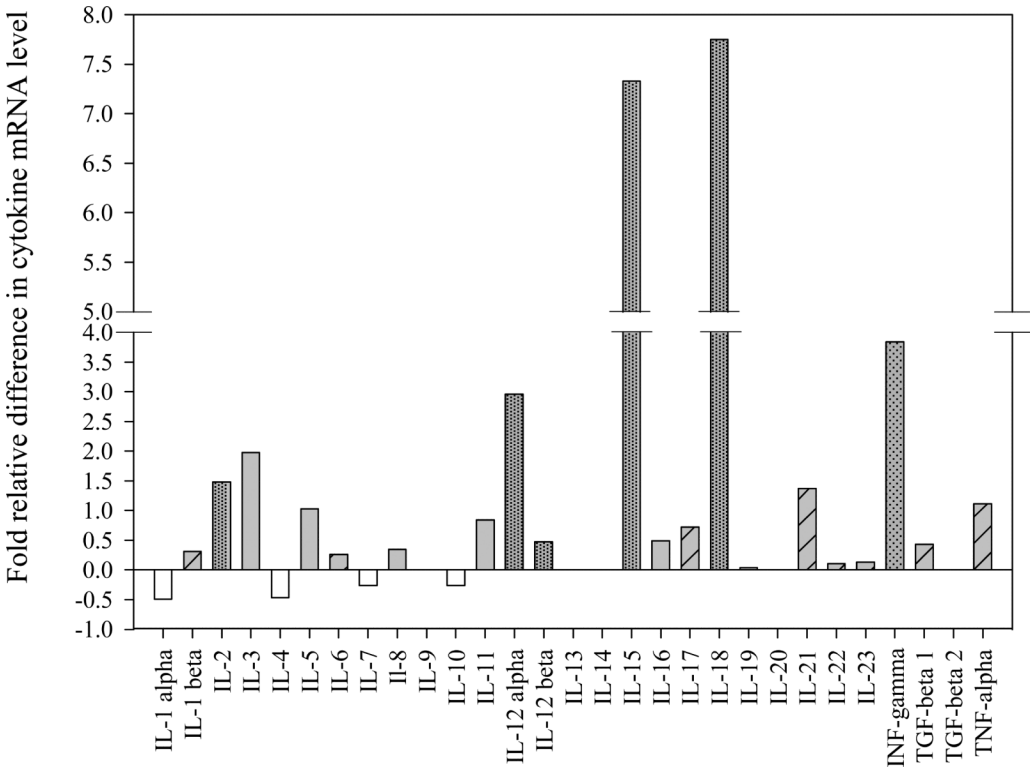


Figure 4.

Fold relative difference of cytokine mRNA level by splenocytes after 24 hr stimulation with recombinant Sm-p80 *in vitro*. The fold difference was calculated by comparing the differences in the message levels of the control group (VR1020) with the experimental group (Sm-p80-VR1020) after standardization using respective GAPDH.

Table 1

Anti-worm effect in C57BL/6 mice following immunization with Sm-p80-VR1020

Immunization Groups	n	Worm burden (Mean ± S.E.)	% Reduction in worm burden ($P < 0.001$)
VR1020	15	40.53±2.53	-
Sm-p80-VR1020	15	21.53±2.65	46.87%

Table 2

Splenocyte proliferation induced by recombinant Sm-p80 after 48hrs of culturing *in vitro*.

Groups	Stimulation index(SI)	
	ConA	Sm-p80
VR1020	3.42±0.36	2.26±0.83
Sm-p80-VR1020	5.15±0.57*	3.96±1.33*

The values in the table represent mean ± S.D.

*$P \leq 0.047$ vs. VR1020 group stimulated by recombinant Sm-p80 respectively using independent samples test