REVIEW

Why the radiation-attenuated cercarial immunization studies failed to guide the road for an effective schistosomiasis vaccine: A review

Rashika El Ridi *, Hatem Tallima

Zoology Department, Faculty of Science, Cairo University, Cairo 12613, Egypt

GRAPHICAL ABSTRACT

Schistosomula- and adult worms-derived antigens induce predominant Th1 immune responses. The radiation-attenuated cercariae vaccine efficacy is dependent on induction of Th1 and Th2 immune responses. Accordingly, schistosomula- and adult worms-derived antigens used for effective vaccination must be combined with Th2 immune responses-inducing cytokines or molecules as adjuvant.

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 5 October 2014

ABSTRACT

Schistosomiasis is a debilitating parasitic disease caused by platyhelminthes of the genus Schistosoma, notably Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum. Pioneer researchers used radiation-attenuated (RA) schistosome larvae to immunize laboratory rodents and non-human primate hosts. Significant and reproducible reduction in challenge worm
Introduction

Schistosomiasis is a severe parasitic disease caused by members of the genus Schistosoma, notably *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*. More than 200 million persons are infected and up to 800 million, mostly children, are at risk. These statistics may well be underestimated because of the difficulty in assessing the true prevalence. The infective schistosome stage, the cercariae, are commonly used for inducing resistance to challenge infection following radiation attenuation (RA) [4]. Mechanically transformed schistosomula (tailless cercariae) attenuated by X- or gamma irradiation and injected intramuscularly (im) successfully protected mice and cynomolgus monkeys against challenge *S. mansoni* infection [5,6]. However, percutaneously applied RA cercariae were more effective in stimulating resistance (60%) than irradiated, im-administered, schistosomula (40%) [7]. Approximately 500 RA (30 krad of gamma irradiation) 6-day-old lung *S. mansoni* schistosomula, injected im, intraperitoneally (ip), or intravenously (iv) into NIH/Nmri CV and C57BL/6J mice, were also capable of inducing significant (P < 0.001) levels of challenge worm reduction (36–56%) that were not very different from approximately 850 RA cercariae as immunizing agents. These findings were construed to indicate that the extravascular stages of development within the skin are not required for the induction of resistance [8]. Conversely, iv-injected RA lung-stage schistosomula derived from optimally RA cercariae failed to confer protection in C57BL/6 mice, suggesting that successful vaccination is not dependent on systemic (vascular), antigen presentation [9,10]. Additionally, irradiated day 21 (#105) and day 28 (#58) worms induced much less resistance (reduction in challenge worm burden of 15–27%) than RA cercariae [8].
Parameters of immunization of mice with $^{60}\text{Co}$ Cobalt-irradiated *Schistosoma mansoni* cercariae were first described by Minard et al. [4] and related to protection against subsequent challenge infection. Optimal protection was found to be dependent on dose of irradiation, number of immunizing cercariae, and number and time course of immunizations. Low levels of resistance were obtained with low irradiation doses. In general, resistance increased with increasing irradiation doses, up to approximately 48–56 krad. Maximal resistance (70–80% reduction in challenge worm burden) was elicited by a single exposure to 250–500 cercariae, irradiated at a dose rate of 2 krad/min to a total dose of 56 krad. In C57BL/6 mice, *S. mansoni* cercariae RA with $^{60}\text{Co}$ 15 krad induced higher levels of protection than 50 krad, and protection was maximal following 4× immunizations with moderately or highly RA cercariae [11]. Cobalt-60 RA cercariae and schistosomula vaccine was widely used in mice [3,4,7,12] and baboons [3,13] for protection against *S. mansoni*, in calves for protection against *Schistosoma bovis* [14], and in cattle and buffaloes for protection against homologous *Schistosoma japonicum* infection [15]. In parallel comparison studies, Cesium-137-attenuated cercariae afforded better protection than the $^{60}\text{Co}$ RA vaccine. The optimal total radiation with $^{137}\text{Cs}$ was between 45 and 50 krad [16]. Cercariae of *S. mansoni* attenuated by exposure to 30–60 krad gamma radiation from a $^{137}\text{Cs}$ source induced >50% protection in baboons against homologous, but not *S. haematobium*, infection challenge [17], and in the vervet monkey, where a protection ceiling of 48% was achieved following 3 vaccinations [18].

X-irradiated *S. mansoni* cercariae were also effective in protecting mice against homologous challenge infection, provided using the optimum number of immunizing cercariae (500), dose of X-irradiation (48 krad), the number of immunizations (5), the time interval between immunization and challenge (up to 1 year), and the size of the challenging dose (up to 500 cercariae) [19,20]. X-irradiated *S. japonicum* tailless cercariae were employed for protecting rhesus monkeys [21] and cattle [22] against schistosomiasis japonicum, with reduction in challenge worm burden varying between 42% and 96%.

The expenses and inconvenience of gamma and X-ray irradiation promoted studies using ultraviolet (UV) irradiated vaccine, which is cost-effective, and only requires simple devices [23]. Dean et al. demonstrated that single immunization of mice with UV-attenuated *S. mansoni* cercariae, using a small, portable S-68 Mineralight Lamp adjusted to deliver 330–440 μwatts/cm², conferred similar levels of resistance to infection (50–70%) as with 50 krad gamma-RA cercariae [24]. Ultra-violet-irradiated *S. mansoni* cercariae were capable of leading to reduction in challenge infection in guinea pigs (approximately 40%), but not Mongolian gerbils [25]. Of note, Mongolian gerbils were also not protected against *S. mansoni* challenge infection when vaccinated with 20 krad gamma-irradiated cercariae [26]. Likewise, UV-attenuated cercarial vaccine was highly effective with *S. japonicum* in protecting mice, water buffaloes, and pigs against homologous schistosome infection [27–31], but induced low, unstable level of protection in some inbred mice, notably C57BL/6 [32].

Studies using tissue mincing and incubation, histopathology, and autoradiographic tracking techniques revealed that similarly to normal larvae, RA cercariae are able to penetrate the epidermis of the host and henceforth to the dermis en route to the dermal blood or lymph capillaries, with only a slight difference in timing of skin exit, whereby attenuated larvae persist in the skin much longer than normal parasites [33–35]. A significant number of immunizing RA larvae were located in lymph nodes draining the skin site of exposure [34]. Migrating schistosomula derived from RA *S. mansoni* cercariae (approximately 50% of penetrants) attain the lung in 6 or 7 days, and differently from their intact counterparts linger, not to leave this site, and die therein. Indeed, schistosomula are detected in the lung for up to 3 weeks following infection with *RA cercariae*, and a proportion therefrom are located extravascularly within the alveoli [33–39]. Schistosomula transforming from cercariae attenuated with low doses of irradiation may make their route to the liver, but usually fail to copulate and lay eggs [35,39]. Accordingly, RA schistosome larvae confer high levels of protection without causing pathological symptoms [3].

The failure of schistosomula derived from RA cercariae to migrate beyond the lung stage was attributed to the impact of irradiation on the parasite neuromuscular function with consequent lower mobility, slow alternating body extensions and contractions, and limited maximum body elongation and extension [40]. In support, microarray examination of the gene expression in cultured schistosomula derived from normal and RA cercariae revealed down-regulation of transcripts encoding G-protein-coupled and neuro receptors, resulting into diminished parasite response to external stimuli and giving an explanation to the extended transit through skin-draining lymph nodes and the lung [41]. Radiation attenuation of *S. mansoni* larvae was reported to lead to profound inhibition of protein and glycoprotein synthesis and radiosialysis of surface carbohydrates that likely enhance the immunogenicity of the larval antigens and/or stimulate exposure of cryptic epitopes [42–45]. No studies are, however, available to delineate whether the death of RA schistosomula in the lungs is a result of the radiation insult and/or to the host immune effector responses. This question might be resolved by tracking the fate of RA cercariae in thymectomized or anti-thymocyte serum-treated mice [46].

**Effects on challenge worm burden and fecundity**

Immunization of mice with $^{60}\text{Co}$-attenuated (46–96 krad) larvae of *S. mansoni*, once or twice, resulted in a 70% reduction in challenge worm burden administered 3 and up to 15 weeks after immunization [4,7]. Treatment with immunosuppressive drugs or excision of sites of infection following immunization revealed that RA larvae need to persist in the host for between 1 and 2 weeks to stimulate optimum protection. Antigens released during protracted stay in the skin and lung likely induce the effector immune responses mediating the resistance to challenge infection [34,35,47,48]. Elucidating the challenge parasites major attrition site was a subject of controversy. Thus, in inbred CBA/Ca mice exposed to 400 *S. mansoni* cercariae attenuated
with 20 krad of $^{60}$Co irradiation, challenge parasites were found to be killed within the first 4 days after challenge, i.e., at the skin stage [12,49–51]. Conversely, in mice immunized by exposure to $S.\ mansoni$ RA cercariae (50 krad, 2 krad/min of $^{60}$Co radiation), mincing and incubation [52] as well as autoradiographic studies of challenge infection with approximately 200 L-($^{75}$Se) selenomethionine-labeled but otherwise normal cercariae indicated that worm elimination occurs after the skin stage, essentially in the lungs [12,33,34,39,53–56]. Challenge schistosomula were found to reach the liver in reduced numbers or are killed or cleared extravasally in the liver in greater number in immunized mice, suggesting that the liver is a site of challenge worm attrition in mice immunized with RA larvae [53] or previously infected mice as well [57]. In guinea pigs vaccinated with $^{60}$Co-RA (20 krad) $S.\ mansoni$ cercariae, and challenged 4–5 weeks after immunization with normal cercariae, lung-stage or 2–6 week-old parasites, the liver appeared to be an important attrition site [58]. Combined microautoradiographic and histopathological studies revealed that immune elimination of challenge larvae does not result from a cytolytic hit, but is essentially due to extravascular exit during migration. Schistosomula surrounded by leukocytic foci in alveoli or in the vasculature did not show any attached leukocyte and appeared entirely free of structural damage [59].

Immune protection was found to be schistosome species-specific as mice exposed to 20 krad-irradiated $S.\ mansoni$ cercariae showed 53–67% reduction in homologous challenge worm burden, while heterologous vaccination with $S.\ bovis$, $S.\ haematobium$, or $S.\ japonicum$ conferred only 5–12% protection [60]. The RA vaccine cross-protection in mice was limited to species of the $S.\ haematobium$, but not $S.\ mansoni$, group [61]. In inbred mice immunized with UV-irradiated cercariae of $S.\ mansoni$ or $S.\ haematobium$, homologous protection ranged from 56% to 69% for $S.\ mansoni$ and 88% to 99% for $S.\ haematobium$. Significant heterologous protection was consistently induced against $S.\ haematobium$ by immunization with $S.\ mansoni$, but not against $S.\ mansoni$ by immunization with $S.\ haematobium$ [62]. Moreover, induction of resistance with RA cercariae of $S.\ mansoni$ varied with mouse strain, with C57BL/6 showing the highest and P/N the lowest level of reduction in challenge worm burden [63–65].

The RA schistosome vaccine induced a high level of protective immunity in experimental rodent hosts and importantly was also efficacious in baboons, whereby 9000 cercariae attenuated by exposure to 30–60 krad of gamma radiation induced $>$50% protection to a challenge with normal larvae [17]. Significant protection, with 64–89% reductions in worm burden and parallel reductions in egg production, was achieved in baboons immunized with gamma-irradiated $S.\ haematobium$ cercariae [66]. Cynomolgus monkeys im-injected with $^{60}$Co (50 krad at 4 krad/min)-RA $S.\ mansoni$ tailless cercariae had 52% fewer challenge worm, and at 7 weeks post-challenge excreted 80% fewer eggs than did the control animals [6].

The data together gave strong evidence that protective immunity could be induced against schistosome infection. The RA vaccine-mediated protection was invariably partial, with surviving worms able to copulate, and daily deposit hundreds of eggs [67]. Moreover, the RA vaccine did not result in significant decrease in challenge worm fecundity in CBA and C57BL/6 mice immunized once or more with gamma-irradiated $S.\ mansoni$ larvae [7,11]. Inbred and outbred mice receiving one exposure to UV RA $S.\ mansoni$ cercariae, and challenged five weeks later with approximately 100 normal cercariae were assessed for worm burden and worm egg counts in liver and intestine at 5, 6, 7 and 8 weeks after infection. Reduction in worm burden varied between 27 and 65% (8 experiments). Decrease in egg counts and female fecundity was highly significant in vaccinated versus control mice at 5, 6, and 7 weeks after challenge. At 8 weeks after challenge, the egg count/mouse and per female worm was similar in immunized and control mice suggesting that the RA vaccine-mediated decrease in worm egg load is only transient [68]. In studies complete regarding egg sampling, significant reduction in fecundity of challenge worms was not observed in baboons immunized with $S.\ haematobium$ [66], or $S.\ mansoni$ [69] RA cryopreserved schistosomula.

**RA vaccine-induced immune responses**

**Skin**

Vaccination of CBA or C57BL mice with RA cercariae induces localized skin inflammatory foci comprising 50% macrophages and 50% eosinophils at the site of immunization that appeared to be responsible for attrition of challenge parasite within few days of entry [51,70]. In support, ip injection of a monoclonal antibody (mAb) specific to neutrophils, but apparently also effective against macrophages and eosinophils, on the day of challenge, greatly reduced (67% mean reduction) the RA-induced resistance [71]. Moreover, passive transfer of serum from RA vaccine-protected mice was able to transfer resistance against challenge infection in mice via induction of subdermal inflammatory reactions, comprising 60% mononuclear cells and 40% eosinophils [72]. Whole body irradiation of RA cercariae-immunized CBA mice 3 days prior to challenge infection revealed that eosinophils, rather than macrophages, are central to the RA vaccine-induced protection [73].

The importance of the skin-draining lymph nodes (LN) for the RA vaccine-mediated immunity was shown in mice percutaneously immunized once with 500 $S.\ mansoni$ cercariae attenuated with 20 krad $^{60}$Co radiation, LN draining the vaccination site removed five days prior, or 5, 10, 15, or 20 days after vaccination, and challenged 35 days post-immunization with 200 normal cercariae. Highly significant reduction in resistance to challenge infection was observed in the lympho-adenectomized as compared to intact mice. The results were construed to suggest that for induction of immune protection, presentation of antigens to leukocytes in the draining LN during the first days of RA larvae skin residence is more important than antigen presentation to the spleen cells (SC) during larval intravascular migration [74]. This assumption was supported by finding marked increase in T-, and to a greater extent of B-lymphocytes in skin- and lung-draining LN, but not in spleen of C57Bl/6 mice on days 2–4 post 1x vaccination with $S.\ mansoni$ cercariae attenuated with 20 krad from a $^{60}$Co source [75]. Localized hyperemia (increased blood flow) appeared to explain the accumulations of lymphocytes in draining LN [76]. This finding suggests that leukocytes in draining LN may well be stimulated by larval antigens released intravascularly and not uniquely by antigens released extravasally, in the dermis or lung parenchyma [77]. The draining LN leukocytes of RA cercariae-vaccinated mice were shown to be essentially of the CD4+ type and responded to parasite
antigens by production of T helper (Th) dominant immune responses, notably increased production of interferon-gamma (IFN-\(\gamma\)) and interleukin (IL)-12 [78–82]. Yet, these LN cells released significant amounts of IL-4 and did not generate an anamnestic Th1 response to parasite antigens after challenge infection whereby IFN-\(\gamma\) production was profoundly down-regulated and large amounts of IL-4 were generated [83].

The results together certainly indicate that RA S. mansoni vaccine-induced protection of mice to challenge infection is dependent on site of vaccination-draining LN build-up of Th1 and Th2-immune responses.

Lung
Schistosomula must negotiate the thin-walled and convoluted pulmonary capillaries before attaining the liver sinusoids and then the portal vein. The migration is obligatorily intravascular, but during the strenuous journey in the lung, many larvae are detected in the alveolar spaces, destined to disintegrate and die [59,84,85]. The larval-derived antigens stimulate intense immune responses characterized by accumulation of lymphocytes and macrophages in dense foci. Similar events occur in RA cercariae-vaccinated rodents with a larger proportion of migrating schistosomula ending into the alveolar spaces and surrounded by larger leukocytic foci [85–91]. These inflammatory foci are generated in response to antigens derived from larvae destined to die, and there is no proof they are the agents responsible for parasite attrition in normal or RA cercariae-immunized mice. Indeed, in spite of the inflammation, no direct lethal cytolytic hit to the schistosomula was observed [59,85,87,92,93]. Intravascular healthy larvae release extremely minute amounts of molecules, the excretory–secretory products (ESP), the scent, and attract no or minute foci [59,92,93]. Intravascular dying or dead larvae, especially in RA vaccine-administered mice, stimulate more or less intense inflammatory foci characterized by the presence of large numbers of eosinophils [92,93]. Some histopathological studies showed the intravascular leukocytic loci destroy the blood-air barrier, thus facilitating larval exit and subsequent death, but also blood spill in the alveoli, a phenomenon rarely, if never, observed [85]. Conversely, it was reported that pulmonary intravascular foci around larvae are rather small [59,92,93]. The results together do not provide conclusive evidence that the inflammatory foci in the lung parenchyma are the agents responsible for parasite deflection in the alveoli.

The dogma stipulating that immune responses to challenge schistosome infection following RA cercariae vaccination must be Th1 polarized to achieve protection has its foundation in several studies that measured C57BL/6 mice bronchialalveolar lavage leukocytes (BAL) immune responses to parasite antigens. As stated above, BAL are situated in lung parenchyma and alveolar tissue and are stimulated by antigens released by extravasated dying larvae. Schistosome larval antigens predominantly induce Th1-related responses [78,94–98]. Accordingly, it is expected that BAL release Th1-related cytokines upon culture in \emph{vitro} in the absence or presence of larval antigens [80,95]. Yet, there is no proof that the BAL-mediated Th1 immune responses are major players in extravasation of challenge intravascularly migrating worms.

Spleen
Schistosomes are obligatory intravascular residents. Like other blood-born antigens, ESP released by healthy parasites and molecules derived from intravascularly dying, dead and degenerated worms reach the spleen, are trapped by residents macrophages and dendritic cells (DC), and stimulate T and B lymphocytes that circulate thereafter in tissue and blood [88]. Leukocytes in blood, rather than in tissue-draining LN, are the ones that interact with developing larvae and might mediate their extravasation and potential attrition. Yet, SC immune responses in the RA vaccine model were seldom looked at. C57BL/6 mice were percutaneously vaccinated with S. mansoni cercariae attenuated with 20 krad of gamma irradiation from a \(^{60}\)Co source, SC and LN cells obtained at 3 day interval for 24 days post-immunization, and tested for proliferation and cytokine release in response to soluble schistosomal (18 h-old larvae) antigens. Similarly to the axillary, inguinal and mediastinal LN, SC cultures released significant amounts of IFN-\(\gamma\) that reached a peak at day 18 post-vaccination; no information was shown related to SC IL-4 production [80]. Following challenge with 200 normal cercariae, SC differed from BAL in displaying vigorous proliferation but production of low levels of IFN-\(\gamma\) in response to \emph{in vitro} stimulation with schistosomal antigens [95]. In our laboratory, SC obtained from C57BL/6 mice 1–6 weeks following secondary immunization with RA (25 krad of gamma irradiation from a \(^{60}\)Co source, or 330 \(\mu\)W/cm\(^2\) UV radiation) were found to consistently release IL-2, IFN-\(\gamma\), and IL-4 in response to \emph{in vitro} stimulation with electroseparated soluble schistosomal or adult worm antigens [99,100].

T cell mediated or humoral immunity?
The association between leukocytic accumulations in the lung parenchyma of RA larvae-vaccinated and challenge cercariae-infected mice and high protection levels led to the assumption that resistance in vaccinated mice may be T cell rather antibody-mediated [84,85]. In RA cercariae once vaccinated mice, results were compatible with that hypothesis and further stressed that the mechanism of immunity depends on T lymphocytes-macrophages interaction triggered by antigens released from lung larvae, leading to focal cell-mediated effector immune responses that block onward challenge larvae migration and cause their deflection in the alveoli and attrition [84–93,101]. The results together suggested that challenge larvae are predominantly eliminated through delayed-type hypersensitivity (DTH) reactions [79,90]. In support, mice of the P/N strain that are characterized as deficient in their ability to mount DTH and macrophage activity, and mice of the 129 strain with disruption of the gene encoding the tumor necrosis factor receptor consistently failed to display resistance to challenge infection following once vaccination [65,102]. In contrast, nitric oxide produced by leukocytes accumulations in the lung tissue of RA cercariae vaccinated mice was shown to be not essential for challenge parasite elimination [103]. Additionally, one-third of B cell-deficient C57BL/6 mice vaccinated once with RA cercariae failed to display resistance to challenge infection [104].
findings were supported in mice made deficient in T or B lymphocytes [105]. A strong evidence for the importance of antibodies came from studies of Mangold and Dean [106] who conclusively showed that passive iv transfer of serum obtained from C57BL/6 mice 3 weeks following last (of 2–3) immunization with RA (50 krad from a 60Co source) S. mansoni cercariae into syngeneic naive mice elicited reductions in challenge worm burdens of 20–50%. The highest level of protection was achieved when immune serum was administered at a time coincident with larval migration in the pulmonary vasculature. The antibody-mediated protection levels were never as high as in the donor mice, implying that other immune effector arms, likely cell-mediated immunity, are required for optimal resistance [106] and Table 1. Highly significant protection was also achieved in C57BL/6 mice upon passive transfer of serum from RA S. mansoni cercariae vaccinated rabbits [107]. The serum fraction responsible for resistance transfer was conclusively shown to be antibodies of the IgG class [106,107]. Similar results were obtained in BALB/c mice passively transferred with RA S. mansoni vaccine immune serum from syngeneic mice or rabbits [108] and were entirely confirmed in the RA S. japonicum vaccine model [109]. Furthermore, protective immunity displayed by baboons vaccinated with RA S. mansoni cercariae was suggested to essentially be antibody-dependent [110]. In mice, the titer of antibodies following RA cercariae immunization appeared of critical importance for the development of resistance to challenge infection [111].

**Th1 versus Th2?**

Treatment of RA cercariae once vaccinated-mice with neutralizing mAb to mouse IL-4, IL-5, or IFN-γ, on day 14 or 7, and day 1 before and again at weekly intervals after challenge infection indicated a preponderant role for IFN-γ-dependent cell-mediated effector mechanisms in the elicited protection, while IL-4, IL-5, and cosinophils are of negligible importance [112]. Yet, mice with disrupted IFN-γ receptor gene displayed an impaired, yet not abrogated, resistance to challenge infection following vaccination with RA S. mansoni cercariae; of note, the reduction in worm burdens in wild type was in the range of a modest 50% [113]. The results, thus, suggest that IFN-γ-independent mechanisms are necessary for optimal protection in the RA vaccine model. Additionally, all cytokine measurements concentrated on BAL and/or total lung tissue [113,114] while it must be reiterated that S. mansoni strive inside the blood vasculature in lungs and elsewhere. In contrast to conclusions reported using mice treated with a mAb targeting inducible nitric oxide synthase [103], nitric oxide direct effector functions and its role in activation of macrophages and endothelial cells for killing migrating larvae were advocated as key elements in the acquisition of protection in the murine RA vaccine model [114,115]. The debate over the effector functions of nitric oxide in protection against schistosome infection is not as yet settled [116,117]. On the other hand, lung tissue or SC production of IL-4, IL-13, IL-10 and other Th2-related cytokine responses appeared to be responsible for the overall limited protection in high [115] and low [118] responder mice.

Different results were attained with 50 krad RA (from a 137Cs source) S. mansoni cercariae once or thrice vaccination of B cell-deficient mice, whereby challenge worm burden reductions were only 33–43%, considerably less than wild type mouse. Additionally, the decrease in protection in IFN-γ knockout mice was not striking compared to wild type counterparts vaccinated in parallel with RA S. mansoni cercariae once (46% versus 63%) or thrice (64% versus 80%) [119]. Moreover, signaling via IL-4 receptor alpha chain was absolutely required for significant RA cercariae vaccination-mediated reduction in BALB/c mice [120]. Finally, several studies using knockout mice closed the controversy by conclusively demonstrating that optimal protection in the RA vaccine model is dependent on the induction of both type-1 and type-2-associated immune responses [121–123].

**Molecules recognized by antibodies and lymphocytes of RA-immunized hosts**

Antibodies of C57BL/6 mice exposed twice via tail immersion to approximately 500 S. mansoni RA (50 krad) cercariae selectively bound to several schistosomular molecules, notably a 38 kDa glycoprotein of *in vitro* cultured 5 day-old schistosoma, seven adult worm antigens among which a 94–97 kDa glycoprotein, as well as, an antigen of 200 kDa present in schistosomular and adult worm soluble extracts [124–127]. A DNA encoding a 62 kDa portion of the 200 kDa molecule was cloned and sequenced and found to share homology with myosins of other species; subcutaneous or ip immunization of C57BL/6 mice with the expressed recombinant protein, designated rIrV-5, elicited 75% protection against challenge worm burden [127]. Similar studies led to identification of SmIrV1, which showed homology to calnexin and calreticulin [128,129]. Additionally, studies with SC of mice vaccinated with RA S. mansoni cercariae used to produce mAb against newly transformed schistosomular surface antigen resulted into selection of a larval surface membrane 18 kDa polypeptide. Polyclonal antibodies generated against the 18 kDa molecule isolated recombinant clones from an adult worm cDNA library constructed in λgt11 [130]. The target molecule was found to be of exactly 23 kDa, designated Sm23, and identified as worm...
integral surface transmembrane antigen and glycosyl inositol phosphatidyl-anchored as well [131]. Furthermore, antibodies of RA S. mansoni cercariae-vaccinated CBA mice were found to specifically recognize schistosomulum surface antigens of >200, 38, 32, 20, and 15 kDa. The >200 and 15 kDa molecules were also recognized by CBA mice immunized with RA S. hae-matothbium cercariae; conversely, the molecules of the 20–38 kDa range showed species-specificity [132,133], thus indicating that some, but not all schistosome molecules confer cross-protection. Most importantly, when vaccinated mice of the C57BL/6 and CBA strain were compared, both strains recognized Sm23, glutathione-S-transferase (GST) and cathepsin B, thus suggesting that these molecules may be used for vaccination of different mouse strains, in contrast to Sm32 and paramyosin that were recognized only by CBA, and heat shock protein 70 exclusively by C57BL/6 mice [134].

Since T cells mediate cellular immunity and control antibody production, it was of importance to identify the schistosome antigens recognized by T cells as well as humoral antibodies of mice vaccinated with RA S. mansoni cercariae. Axillary LN cells of C57BL/6 and CBA mice vaccinated once with cercariae attenuated with 15 or 50 kGy gamma irradiation were in vitro stimulated with adult worm antigens fractionated by isoelectric focusing. The LN cells proliferative and lymphokine responses and humoral antibody binding revealed that Sm23, paramyosin, heat shock protein 70, triose phosphate isomerase (TPI), and GST appeared to be the molecules that stimulate the most intense immune responses in the murine RA vaccine model [135,136]. We have used the T cell western and western blotting assays to identify the schistosomulum and adult worm antigens recognized by LN and spleen T cells and serum antibody of outbred and inbred mice immunized twice with gamma or UV-radiation-attenuated S. mansoni cercariae [99,100,137]. The molecules most consistently recognized, and presumably of importance in inducing resistance against challenge infection in this model, were selected and identified as S. mansoni enolase, and S. mansoni calreticulin [99,100,138,139].

Some of the molecules putatively responsible for the induction of protection against challenge infection following RA cercariae vaccination, notably Irv5, Sm23, paramyosin, GST, TPI-derived peptides in a multiple antigen construct (MAP), probably emulsified in Freund’s or alum were used in controlled vaccination and protection studies in C57BL/6 and BALB/c mice. None succeeded in inducing protection higher than the 40% benchmark sent by the World Health Organization for progression of schistosome vaccine antigens into pre- and clinical trials [140,141].

The outcome of the missed lessons

The majority of the murine RA vaccine model studies concentrated on the C57BL/6 strain because it proved to be the highest responder. BALB/c and CBA mice showed moderate response, A/J mice marginal resistance, while other strains, notably RF/J, and P/N appeared to display negligible protection following immunization with RA larvae [63-65]. These findings suggest that vaccination results using schistosome subunit antigens in preferred 2 or 3 inbred mouse strains may not be readily confirmed in other laboratories using different mouse strains, or extrapolated to the outbred humans. Nevertheless, the majority of studies related to development of a schistosomiasis vaccine disregarded this limitation, over-relied on the C57BL/6 strain, and neglected the use of outbred mice. Fortunately, several schistosome vaccine studies were performed in baboons, despite the challenges of the costs and experimental settings [110,142–148].

In every histopathological or mincing/incubation study regarding the RA vaccine model, no evidence was ever obtained for tight adherence of leukocytes to the lung-stage schistosomula surface, direct cytolytic hit, or structural damage presumably mediated by antibody-dependent cell-mediated cytotoxicity [39,52,56,59,67,84–87,89–93]. These results were in entire accord with the plethora of articles documenting the inaccessibility of healthy schistosome surface membrane antigens to antibody binding and the insusceptibility of developing larvae to antibody-dependent attrition mechanisms [9, reviewed in 149,150]. These well-established, confirmed, and reproducible findings imply that parasite surface membrane or tegumental antigens may not mediate access of effector immune responses to challenge infection parasites whether in the dermis or during intravascular migration and residence. Nevertheless, the great majority of articles focused on schistosome surface membrane or tegumental molecules as vaccine candidates, notwithstanding the fact that if surface membrane molecules were at any time accessible to the host effector immune responses, the parasite would not survive days, not to mention decades, in the host blood stream. The outcome of these lessons neglect is obtention of protection against challenge infection of limited significance (P < 0.05–<0.01) and reduction percentages of 30–40% that are not reproduced from experiment to experiment, leading to damping of these molecules out of the vaccine candidate list [reviewed in 149,150]. An outstanding example was the S. mansoni glucose transporter SGTP4, a molecule at the host-parasite interface of critical importance for the parasite survival [151]. Vaccination of outbred and inbred mice with the molecule extracellular domains in recombinant or synthetic peptide constructs and emulsified in Freund’s adjuvant induced considerable cellular and humoral immune responses but entirely failed to provide protection against challenge S. mansoni infection [152]. Fortunately, however, several antigens readily released from invading worms and potential inducers of protection in the RA vaccine model were used as vaccine candidates among which calpain [143–148], GST [142], which has now moved to phase 1 clinical trials [153], and paramyosin, whereby recombinant full-length S. japonicum paramyosin, rS97 was produced and assessed for efficacy and safety in rodents and large-animal models [154].

One of the salient lessons gained from the extensive studies concerning the RA vaccine model is that protection elicited essentially depends on both Th1 and Th2-associated immune responses [3]. Since schistosome candidate vaccine molecules are documented to stimulate polarized Th1-related immune reactivity, it was of importance to look for and use an adjuvant that would skew the immunogen-induced polarized Th1 toward the Th2 immunity axis. That did not happen. On the contrary, many candidate vaccines, including calpain, were used as DNA constructs known to predominantly elicit Th1-related responses [143–145 and reviewed in 150,155]. We have used the candidate vaccine antigen and larval ESP, S. mansoni glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) in a recombinant (r), linear peptide or MAP form, emulsified in Freund’s or other Th1 adjuvants for immunization of outbred and inbred mice and only obtained occasional,
and barely significant (P < 0.05) reduction in challenge worm burden and egg load of less than 35% [156–159]. We have used other larval ESP, notably S. mansoni 14-3-3 and p18 protein in a recombinant form, and aldolase, calpain, and thioredoxin peroxidase (TPX) = 2 cys peroxiredoxin-derived peptides in MAP constructs emulsified in Freund’s adjuvant or aluminum hydroxide for immunization of C57BL/6 and BALB/c mice. While the molecules were strongly immunogenic, eliciting biased Th1-related immune responses whether administered in conjunction with Freund’s adjuvant or alum, the protection levels were suboptimal and rather erratic [160]. Not very different results were attained with the numerous trials using S. mansoni or S. japonicum tegumental and surface membrane associated molecules in conjunction with Th1-biased adjuvants for immunization of inbred mice [reviewed in 149,150,161]. The outcome is up of today, the schistosomiasis vaccine still remains an unmet clinical need [123,149].

The outcome of the well-learned lessons

We have learned our lessons and focused on the use of larval ESP, such as SG3PDH and TPX, relied on outbred mice, and most importantly performed extensive studies to find an adjuvant that would skew these molecules-mediated Th1 responses toward the Th2 axis. We found that alum [160], polynosinic-polycytidylic acid and peptidoglycan [162] drive C57BL/6 and BALB/c to respond to S. mansoni larval ESP by production of IFN-γ and IL-17. Conversely, thymic stromal lymphopoietin (TSLP), the master regulator of type 2 responses, succeeded in directing the larval ESP-mediated immune responses toward a Th2-biased profile in prototypical Th1 and Th2 mice [162]. We thus understood that the type 2 cytokines, notably TSLP, IL-25, and IL-33, which stimulate the group 2 innate lymphoid cells [163–165] and type-2 cytokines-inducing molecules such as the cysteine peptidase, papain [166,167], are the immunomodulatory adjuvants needed to drive larval ESP-mediated vaccination toward generation of type 2-associated immune responses. Challenge infection larvae are, thus, met by both Th1- and Th2 cell-dependent immunity, as studies of the RA vaccine model recommended. Administration of outbred mice with rSG3PDH and TPX MAP in conjunction with papain, TSLP, IL-25, or IL-33 consistently and reproducibly elicited Th1- and Th2-associated cytokines and antibodies, and significant (P < 0.0001) reductions of a minimum of 50% and up to 78% in challenge worm burden and worm egg counts [168]. Since schistosome cysteine peptidases are both ESP and potential type-2 cytokines-inducers, it was reasonable to assess their protective potential in outbred mice alone or as adjuvants to the larval ESP, rSG3PDH and TPX MAP. The considerable and highly significant (P < 0.0001) reduction of 50–83% in worm burdens and worm egg load in each of 7 consecutive experiments, each involving 4–8 animal groups, led us to devise a formula for the schistosomiasis vaccine, notably rSG3PDH + S. mansoni cathepsin B + S. mansoni cathepsin L. The latter peptidase was required for its potential role in worm reproduction and impact on eliminating the Th2 cytokine-associated transient increase in challenge worm fecundity [169,170]. Benefiting from another lesson of the RA vaccine model, notably that S. mansoni molecules may protect hosts against S. haematobium infection [62], we have vaccinated outbred mice and hamsters with the S. mansoni antigens mentioned in the formula and obtained consistent, reproducible, and highly significant (P < 0.0001) reductions of 70% in challenge worm burden and worm egg counts [171].

Accordingly, we recommend retesting the various available schistosome candidate vaccine antigens, notably calpain, GST, TPI, enolase, paramyosin, and Sm14 in conjunction with cathepsin B and cathepsin L for their protective potential in laboratory outbred rodents and baboons against challenge S. mansoni, S. haematobium, and S. japonicum infection. Evidence regarding the longevity of the generated protection must be established in an aim of achieving the highly coveted goal of a sterilizing schistosomiasis vaccine.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Acknowledgment

The authors acknowledge funding of The Science and Technology Development Fund (STDF), Egypt, Grant No. 2073 to R. El Ridi.

References

[1] World Health Organization. Schistosomiasis: population requiring preventive chemotherapy and number of people treated in 2010 [pdf 873kb]. Weekly Epidemiol Rec 2012;87(4):37–44.

[2] Murray CI, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. Lancet 2012;380:2197–223.

[3] Coulson PS. The radiation-attenuated vaccine against schistosomes in animal models: paradigm for a human vaccine? Adv Parasitol 1997;39:271–336.

[4] Minard P, Dean DA, Jacobson RH, Vannier WE, Murrell KD. Immunization of mice with covalt-60 irradiated Schistosoma mansoni cercariae. Am J Trop Med Hyg 1978;27(1 Pt 1):76–86.

[5] Eveland LK, Morse SI. Schistosoma mansoni: infectivity and immunizing effects of in vitro derived schistosomula attenuated by X irradiation. Exp Parasitol 1978;45(1):19–25.

[6] Murrell KD, Clark SS, Dean DA, Vannier WE. Schistosoma mansoni: immunization of cynomolgus monkeys by injection of irradiated schistosomula. Exp Parasitol 1979;48(3):415–20.

[7] Bickle QD, Taylor MG, Doenhoff MJ, Nelson GS. Immunization of mice with gamma-irradiated intramuscularly injected schistosomula of Schistosoma mansoni. Parasitology 1979;79(2):209–22.

[8] Dean DA, Cioli D, Bukowski MA. Resistance induced by normal and irradiated Schistosoma mansoni: ability of various worm stages to serve as inducers and targets in mice. Am J Trop Med Hyg 1981;30(5):1026–32.

[9] Sher A, Benno D. Decreasing immunogenicity of developing schistosome larvae. Parasite Immunol 1982;4(2):101–7.
Re-evaluation of the RA cercariae vaccine lessons

[10] Coulson PS, Mountford AP. Fate of attenuated schistosomula administered to mice by different routes, relative to the immunity induced against *Schistosoma mansoni*. Parasitology 1989;99(Pt 1):39–45.

[11] Reynolds SR, Harn DA. Comparison of irradiated-cercariae schistosome vaccine models that use 15- and 50-kilorder doses: the 15-kilorder dose gives greater protection, smaller liver sizes, and higher gamma interferon levels after challenge. Infect Immun 1992;60(1):90–4.

[12] Miller KL, Smithers SR. *Schistosoma mansoni*: the attrition of a challenge infection in mice immunized with highly irradiated live cercariae. Exp Parasitol 1980;50(2):212–21.

[13] Stek Jr M, Minard P, Dean DA, Hall JE. Immunization of baboons with *Schistosoma mansoni* cercariae attenuated by gamma irradiation. Science 1981;212(4502):1518–20.

[14] Bushara HO, Hussein MF, Saad AM, Taylor MG, Dargie JD, Marshall TF, et al. Immunization of calves against *Schistosoma bovis* using irradiated cercariae or schistosomula of *S. bovis*. Parasitology 1978;77(Pt 3):303–11.

[15] Hsu SY, Xu ST, He YX, Shi FH, Shen W, Hsu HF, et al. Vaccination of bovines against *schistosomiasis japonica* with highly irradiated schistosomula in China. Am J Trop Med Hyg 1984;33(5):891–8.

[16] Stek Jr M, Minard P, Cruess DF. Murine immunization by *Schistosoma mansoni* irradiated cercariae. J Helminthol 1978;52(4):303–4.

[17] Yole DS, Pemberton R, Reid GD, Wilson RA. Protective immunity induced in the olive baboon *Papio anubis* by the irradiated cercaria vaccine. Parasitology 1996;112(Pt 1):23–6.

[18] Yole DS, Pemberton R, Reid GD, Wilson RA. Protective immunity to *Schistosoma mansoni* cercariae dependent on the size of the challenging dose? J Parasitol 1984;70(3):398–402.

[19] Yole DS, Pemberton R, Reid GD, Wilson RA. Immunization of bovines against *Schistosoma mansoni* in immunosuppressed mice. Jpn J Parasitol 1983;32(4):790–3.

[20] Wales A, Fukumoto SI, Otieno MF, Kusel JR. Effects of gamma irradiation on the viability of *Schistosoma mansoni* cercariae. Parasitology 1983;86(Pt 3):429–38.

[21] Wales A, Fukumoto SI, Otieno MF, Kusel JR. Effects of gamma irradiation on the viability of *Schistosoma mansoni* cercariae. Parasitology 1983;86(Pt 3):429–38.

[22] Ireland AG, Murrell KD. The migration and survival of *Schistosoma mansoni* cercariae in male gerbils, *Meriones unguiculatus*, vaccinated with gamma-irradiated cercariae of *Schistosoma mansoni* against a homologous challenge infection. J Parasitol 1993;79(4):616–20.

[23] Yole DS, Reid GD, Wilson RA. Protective immunity induced in the vervet monkey *Cercopithecus aethiops* by the *Schistosoma mansoni* cercaria vaccine. Parasitology 1983;83(2):367–70.

[24] Ruppel A, Shi YE, Moloney NA. *Schistosoma mansoni* and *S. japonicum*: comparison of levels of ultraviolet irradiation for vaccination of mice with cercariae. Parasitology 1990;101(Pt 1):23–6.

[25] Shi YE, Jiang CF, Han JJ, Li YL, Ruppel A. *Schistosoma japonicum*: an ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes. Exp Parasitol 1990;71(1):100–6.

[26] Shi YE, Jiang CF, Han JJ, Li YL, Ruppel A. Immunization of pigs against infection with *Schistosoma japonicum* using ultraviolet-attenuated cercariae. Parasitology 1993;106(Pt 5):459–62.

[27] Tian F, Lin D, Wu J, Gao Y, Zhang D, Ji M, et al. Immunization of calves against *Schistosoma japonicum* infection as compared to single vaccination. Parasit Vectors 2011;4:103.

[28] Zhang M, Tian F, Gao Y, Ji M, Wu G. Ultraviolet-attenuated cercariae of *Schistosoma japonicum* fail to effectively induce a Th1 response in spite of up-regulating expression of cytotoxicity-related genes in C57BL/6 mice. J Biomed Res 2010;24(4):277–84.

[29] Minard P, Dean DA, Vannier WE, Murrell KD. Effect of immunization on migration of *Schistosoma mansoni* larvae through lungs. Am J Trop Med Hyg 1978;27(1):87–93.

[30] Wang GL, Dean DA. The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the induction of resistance in mice. Parasitology 1984;88(Pt 2):249–65.

[31] Mountford AP, Coulson PS, Wilson RA. Antigen localization and associated immune responses in *Schistosoma japonicum* using highly X-irradiated cercariae. J Immun 1992;60(1):90–4.

[32] Wilson RA. The saga of schistosome migration and attrition. Parasitology 2009;136(12):1581–92.

[33] Harrop R, Wilson RA. Irradiation of *Schistosoma mansoni* cercariae impairs neuromuscular function in developing schistosomes. J Parasitol 1993;79(2):286–9.

[34] Hilton GP, Feltwell T, Skelton J, Coulson PS, Wilson RA, Ivens AC. Altered patterns of gene expression underlying the enhanced immunogenicity of radiation-attenuated schistosomes. PLoS Negl Trop Dis 2008;2(5):e240.

[35] Procaccio V, Skelton J, 注意: 这段文本中的格式会导致混乱，建议删除或重新组织。
Bickle QD. Studies on the relationship between the survival of Schistosoma mansoni larvae in mice and the degree of resistance produced. Parasitology 1982;84(1):111–22.

Bickle QD. Radiation-attenuated schistosome vaccination—a brief historical perspective. Parasitology 2009;136(12):1621–32.

Smithers SR, Miller KL. Protective immunity in murine Schistosomiasis mansoni: evidence for two distinct mechanisms. Am J Trop Med Hyg 1980;29(5):832–41.

Miller KL, Smithers SR, Sher A. The response of mice immune to Schistosoma mansoni to a challenge infection which bypasses the skin: evidence for two mechanisms of immunity. Parasite Immunol 1981;3(1):25–31.

Miller KL, Smithers SR. Localized skin changes at the site of immunization with highly irradiated cercariae of Schistosoma mansoni are associated with enhanced resistance to a challenge infection. Parasitology 1982;85(2 Pt 2):305–14.

Stek Jr M, Dean DA, Clark SS. Attrition of schistosomes in an irradiation-attenuated cercarial immunization model of Schistosoma mansoni. Am J Trop Med Hyg 1981;30(5):1033–8.

Dean DA, Mangold BL, Georgi JR, Jacobson RH. Comparison of Schistosoma mansoni migration patterns in normal and irradiated cercaria-immunized mice by means of autoradiographic analysis. Evidence that worm elimination occurs after the skin phase in immunized mice. Am J Trop Med Hyg 1984;33(1):89–96.

Mangold BL, Dean DA, Coulson PS, Wilson RA. Site requirements and kinetics of immune-dependent elimination of intravascularly administered lung stage schistosomula in mice immunized with highly irradiated cercariae of Schistosoma mansoni. Am J Trop Med Hyg 1986;35(2):332–44.

Dean DA, Mangold BL, Kassim OO, Von Lichtenberg F. Sites and mechanisms of schistosome elimination. Mem Inst Oswaldo Cruz 1987;82(Suppl.4):31–7.

Dean DA, Mangold BL, Lewis FA. Comparison of two strains of Schistosoma mansoni with respect to the sites and kinetics of immune elimination in cercaria-immunized mice. J Parasitol 1995;81(1):43–7.

Dean DA, Mangold BL. Autoradiographic analysis of resistance to re-infection with Schistosoma mansoni in mice. Evidence that the liver is a major site of worm elimination. Am J Trop Med Hyg 1984;33(1):97–103.

McLaren DJ, Rogers MV. Schistosoma mansoni: liver phase challenge attrition is a stage-dependent phenomenon in guinea-pigs vaccinated with highly irradiated cercariae. Parasite Immunol 1986;8(4):307–18.

Kassim OO, Dean DA, Mangold BL, Von Lichtenberg F. Combined microautoradiographic and histopathologic analysis of the fate of challenge Schistosoma mansoni schistosomula in mice immunized with irradiated cercariae. Am J Trop Med Hyg 1992;47(2):231–7.

Bickle QD, Andrews BJ, Doenhoff MJ, Ford MJ, Taylor MG. Resistance against Schistosoma mansoni induced by highly irradiated infections: studies on species specificity of immunization and attempts to transfer resistance. Parasitology 1985;90(Pt 2):301–12.

Navarrete SI, Rollinson D, Agnew AM. Cross-protection between species of the Schistosoma haematobium group induced by vaccination with irradiated parasites. Parasite Immunol 1994;16(1):19–25.

Dean DA, Mangold BL, Harrison RA, Ricciardone MD. Homologous and heterologous protective immunity to Egyptian strains of Schistosoma mansoni and S. haematobium induced by ultraviolet-irradiated cercariae. Parasite Immunol 1996;18(8):403–10.

Murrell KD, Clark S, Dean DA, Vannier WE. Influence of mouse strain on induction of resistance with irradiated Schistosoma mansoni cercariae. J Parasitol 1979;65(5):829–31.

James SL, Labine M, Sher A. Mechanisms of protective immunity against Schistosoma mansoni infection in mice vaccinated with irradiated cercariae. I. Analysis of antibody and T-lymphocyte responses in mouse strains developing differing levels of immunity. Cell Immunol 1981;65(1):75–83.

James SL, Sher A. Mechanisms of protective immunity against Schistosoma mansoni infection in mice vaccinated with irradiated cercariae. II. Identification of a mouse strain, P/N, that fails to respond to vaccination. Parasite Immunol 1983;5(6):567–75.

Webbe G, Sturrock RF, James ER. Schistosoma haematobium in the baboon (Papio anubis): effect of vaccination with irradiated larvae on the subsequent infection with percutaneously applied cercariae. Trans R Soc Trop Med Hyg 1982;76(3):354–61.

Dean DA. Schistosoma and related genera: acquired resistance in mice. Exp Parasitol 1983;55(1):1–10.

El Ridi R, Ozaki T, Sato H, Inaba T, Kamiya H. Immunization of mice with ultraviolet-attenuated cercariae of Schistosoma mansoni transiently reduces the fecundity of challenge worms. Int J Parasitol 1997;27(5):581–6.

Damián RT, Powel MR, Roberts ML, Clark JD, Stirewalt MA, Lewis FA. Schistosoma mansoni: parasitology and immunology of baboons vaccinated with irradiated cryopreserved schistosomula. Int J Parasitol 1985;15(3):333–44.

Ward RE, McLaren DJ. Schistosoma mansoni: evidence that eosinophils and/or macrophages contribute to skin-phase challenge attrition in vaccinated CBA/Ca mice. Parasitology 1988;96(1 Pt 1):63–84.

McLaren DJ, Strath M, Smithers SR. Schistosoma mansoni: evidence that immunity in vaccinated and chronically infected CBA/Ca mice is sensitive to treatment with a monoclonal antibody that depletes cutaneous effector cells. Parasite Immunol 1987;9(6):667–82.

McLaren DJ, Smithers SR. Serum from CBA/Ca mice vaccinated with irradiated cercariae of Schistosoma mansoni protects naive recipients through the recruitment of cutaneous effector cells. Parasitology 1988;97(2 Pt 2):287–302.

Delgado VS, McLaren DJ. Evidence that radio-sensitive cells are central to skin-phase protective immunity in CBA/Ca mice vaccinated with radiation-attenuated cercariae of Schistosoma mansoni as well as in naive mice protected with vaccine serum. Parasitology 1990;100(1 Pt 1):45–56.

Mountford AP, Wilson RA. Schistosoma mansoni: the effect of regional lymphadenectomy on the level of protection induced in mice by radiation-attenuated cercariae. Exp Parasitol 1990;71(4):463–9.

Constant SL, Mountford AP, Wilson RA. Phenotypic analysis of the cellular responses in regional lymphoid organs of mice vaccinated against Schistosoma mansoni. Parasitology 1990;101(1 Pt 1):15–22.

Constant SL, Wilson RA. In vivo lymphocyte responses in the draining lymph nodes of mice exposed to Schistosomamansoni: preferential proliferation of T cells is central to the induction of protective immunity. Cell Immunol 1992;139(1):145–61.

Riengrojpitak S, Anderson S, Wilson RA. Induction of immunity to Schistosoma mansoni: interaction of schistosomula with accessory leukocytes in murine skin and draining lymph nodes. Parasitology 1998;117(4 Pt 4):301–9.

Vignali DA, Crocker P, Bickle QD, Cobbold S, Waldmann H, Taylor MG. A role for CD4+ but not CD8+ T cells in immunity to Schistosoma mansoni induced by 20 krad-irradiated and Ro 11-3126-terminated infections. Immunology 1989;67(4):466–72.

Ratcliffe EC, Wilson RA. The magnitude and kinetics of delayed-type hypersensitivity responses in mice vaccinated with...
irradiated cercariae of *Schistosoma mansoni*. Parasitology 1991;103(Pt 1):65–75.

- [80] Pemberton RM, Smythies LE, Mountford AP, Wilson RA. Patterns of cytokine production and proliferation by T lymphocytes differ in mice vaccinated or infected with *Schistosoma mansoni*. Immunology 1991;73(3):327–33.

- [81] Mountford AP, Coulson PS, Pemberton RM, Smythies LE, Wilson RA. The generation of interferon-gamma-producing T lymphocytes in skin-draining lymph nodes, and their recruitment to the lungs, is associated with protective immunity to *Schistosoma mansoni*. Immunology 1992;75(2):250–6.

- [82] Hogg KG, Kumkate S, Anderson S, Mountford AP. Interleukin-12 p40 secretion by cutaneous CD11c+ and F4/80+ cells is a major feature of the innate immune response in mice that develop Th1-mediated protective immunity to *Schistosoma mansoni*. Infect Immun 2003;71(6):3563–71.

- [83] Pemberton RM, Wilson RA. T-helper type-1-dominated lymph node responses induced in C57BL/6 mice by optimally irradiated cercariae of *Schistosoma mansoni* are down-regulated after challenge infection. Immunology 1995;84(2):310–6.

- [84] Crabtree JE, Wilson RA. *Schistosoma mansoni*: an ultrastructural examination of pulmonary migration. Parasitology 1986;92(Pt 2):243–54.

- [85] Crabtree JE, Wilson RA. The role of pulmonary cellular reactions in the resistance of vaccinated mice to *Schistosoma mansoni*. Parasite Immunol 1986;8(3):265–85.

- [86] Aitken R, Coulson PS, Wilson RA. Pulmonary leukocytic responses are linked to the acquired immunity of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. J Immunol 1988;140(10):3573–9.

- [87] Coulson PS, Wilson RA. Examination of the mechanisms of pulmonary phase resistance to *Schistosoma mansoni* in vaccinated mice. Am J Trop Med Hyg 1988;38(3):529–39.

- [88] Munson EN, Coulsen PS, Wilson RA. *Schistosoma mansoni*: circulating and pulmonary leukocyte responses related to the induction of protective immunity in mice by irradiated parasites. Parasitology 1989;98(Pt 1):43–55.

- [89] Vignali DA, Klaus SN, Bickle QD, Taylor MG. Histological examination of the cellular reactions around schistosomula of *Schistosoma mansoni* in the lungs of sublethally irradiated and unirradiated, immune and control rats. Parasitology 1989;98(Pt 1):57–65.

- [90] Kambara T, Wilson RA. In situ pulmonary responses of T cell and macrophage subpopulations to a challenge infection in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. J Parasitol 1990;76(3):365–72.

- [91] Ratcliffe EC, Wilson RA. The role of mononuclear-cell recruitment to the lungs in the development and expression of immunity to *Schistosoma mansoni*. Parasitology 1992;104(Pt 2):299–307.

- [92] von Lichtenberg F, Sher A, McIntyre S. A lung model of schistosome immunity in mice. Am J Pathol 1977;87(1):105–23.

- [93] Von Lichtenberg F, Correa-Oliveira R, Sher A. The fate of challenge schistosomula in the murine anti-schistosome vaccine model. Am J Trop Med Hyg 1985;34(1):96–106.

- [94] Mountford AP, Harrop R, Wilson RA. Antigens derived from lung-stage larvae of *Schistosoma mansoni* are efficient stimulators of proliferation and gamma interferon secretion by lymphocytes from mice vaccinated with attenuated larvae. Infect Immun 1995;63(5):1980–6.

- [95] Smythies LE, Pemberton RM, Coulson PS, Mountford AP, Wilson RA. T cell-derived cytokines associated with pulmonary immune mechanisms in mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. J Immunol 1992;148(5):1512–8.

- [96] Mountford AP, Harrop R. Vaccination against Schistosomiasis: the case for lung-stage antigens. Parasitol Today 1998;14(3):109–14.

- [97] Harrop R, Jennings N, Mountford AP, Coulson PS, Wilson RA. Characterization, cloning and immunogenicity of antigens released by transforming cercariae of *Schistosoma mansoni*. Parasitology 2000;121(Pt 4):385–94.

- [98] El Ridi R, Tallima H, Mahana N, Dalton JP. Innate immunogenicity and in vitro protective potential of *Schistosoma mansoni* lung schistosomula excretory–secretory candidate vaccine antigens. Microbes Infect 2010;12(10):700–9.

- [99] Osman A, El Ridi R, Guirguis N, Dean DA. Identification of *Schistosoma mansoni* antigens recognized by T cells of C57BL/6 mice immunized with gamma-irradiated cercariae. J Parasitol 1994;80(3):421–31.

- [100] Osman A, El Ridi R, Guirguis N, Dean DA. Identification of *Schistosoma mansoni* antigens recognized by spleen cells of C57BL/6 mice immunized with ultraviolet-irradiated cercariae. Int J Parasitol 1994;24(7):943–50.

- [101] Coulson PS, Wilson RA. Recruitment of lymphocytes to the lung through vaccination enhances the immunity of mice exposed to irradiated schistosomes. Infect Immun 1997;65(1):42–8.

- [102] Street M, Coulson PS, Sadler C, Warnock LJ, McLaughlin D, Blumenthm H, et al. TNF is essential for the cell-mediated protective immunity induced by the radiation-attenuated schistosome vaccine. J Immunol 1999;163(8):4489–94.

- [103] Coulson PS, Smythies LE, Betts C, Mabbott NA, Sternberg JM, Wei XG, et al. Nitric oxide produced in the lungs of mice immunized with the radiation-attenuated schistosome vaccine is not the major agent causing challenge parasite elimination. Immunology 1998;93(1):55–63.

- [104] Anderson S, Coulson PS, Ljubojevic S, Mountford AP, Wilson RA. The radiation-attenuated schistosome vaccine induces high levels of protective immunity in the absence of B cells. Immunology 1999;96(1):22–8.

- [105] Sher A, Hiens S, James SL, Asosky R. Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T lymphocytes, B lymphocytes, or complement. J Immunol 1982;128(4):1880–4.

- [106] Mangold BL, Dean DA. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against *Schistosoma mansoni* in mice. J Immunol 1986;136(7):2644–8.

- [107] Mangold BL, Dean DA. The role of IgG antibodies from irradiated cercaria-immunized rabbits in the passive transfer of immunity to *Schistosoma mansoni*-infected mice. Am J Trop Med Hyg 1992;47(6):821–9.

- [108] Luo J, LoVerde PT. The ability of fractionated sera from animals vaccinated with irradiated cercariae of *Schistosoma mansoni* to transfer immunity to mice. J Parasitol 1989;75(2):252–60.

- [109] Dunne DW, Jones FM, Cook L, Moloney NA. Passively transferable protection against *Schistosoma japonicum* induced in the mouse by multiple vaccination with attenuated larvae: the development of immunity, antibody isotype responses and antigen recognition. Parasite Immunol 1994;16(12):655–68.

- [110] Soisson LA, Reid GD, Farah IO, Nyindo M, Strand M, Strand M. Protective immunity in baboons vaccinated with a recombinant antigen or radiation-attenuated cercariae of *Schistosoma mansoni* is antibody-dependent. J Immunol 1993;151(9):4782–9.

- [111] Vignali DA, Devey ME, Bickle QD, Taylor MG. The role of antibody affinity and titre in immunity to *Schistosoma mansoni* following vaccination with highly irradiated cercariae. Immunology 1999;100(2):195–201.

- [112] Sher A, Coffman RL, Hiens S, Cheever AW. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. J Immunol 1990;145(11):3911–6.
266 R. El Ridi and H. Tallima

[113] Wilson RA, Coulson PS, Betts C, Dowling MA, Smythies LE. Impaired immunity and altered pulmonary responses in mice with a disrupted interferon-gamma receptor gene exposed to the irradiated *Schistosoma mansoni* vaccine. Immunology 1996;87(2):275–82.

[114] Wynn TA, Oswald IP, Eltoum IA, Caspar P, Lowenstein CJ, Lewis FA, et al. Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni*. J Immunol 1994;153(1):5200–9.

[115] James SL, Cheever AW, Caspar P, Wynn TA. Inducible nitric oxide synthase-deficient mice develop enhanced type 1 cytokine-associated cellular and humoral immune responses after vaccination with attenuated *Schistosoma mansoni* cercariae but display partially reduced resistance. Infect Immun 1998;66(8):3510–8.

[116] Ahmed SF, Oswald IP, Caspar P, Hiency S, Keefe L, Sher A, et al. Developmental differences determine larval susceptibility to nitric oxide-mediated killing in a murine model of vaccination against *Schistosoma mansoni*. Infect Immun 1997;65(1):219–26.

[117] Ascenzi P, Fasano M, Gradoni L. Do hemoglobin and hemocyanin impair schistosoma killing by no? IUBMB Life 2002;53(6):287–8.

[118] Oswald IP, Caspar P, Wynn TA, Scharton-Kersten T, Williams ME, Hiency S, et al. Failure of *P* strain mice to respond to vaccination against schistosomiasis correlates with impaired production of IL-12 and up-regulation of Th2 cytokines that inhibit macrophage activation. Eur J Immunol 1998;28(6):1762–72.

[119] Jankovic D, Wynn TA, Kullberg MC, Hiency S, Caspar P, James S, et al. Optimal vaccination against *Schistosoma mansoni* requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. J Immunol 1999;162(1):345–51.

[120] Mountford AP, Hogg KG, Coulson PS, Brombacher F. Signaling via interleukin-4 receptor alpha chain is required for successful vaccination against schistosomiasis in BALB/c mice. Infect Immun 2001;69(1):228–36.

[121] Anderson S, Shires VL, Wilson RA, Mountford AP. In the absence of IL-12, the induction of Th1-mediated protective immunity by the attenuated schistosome vaccine is impaired, revealing an alternative pathway with Th2-type characteristics. Eur J Immunol 1999;28(9):2827–38.

[122] Hoffmann KF, James SL, Cheever AW, Wynn TA. Studies with double cytokine-deficient mice reveal that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccine-induced immunity to *Schistosoma mansoni*. J Immunol 1999;163(2):327–38.

[123] Wynn TA, Hoffmann KF. Defining a schistosomiasis vaccination strategy – is it really Th1 versus Th2? Parasitol Today 2000;16(11):497–501.

[124] Dalton JP, Strand M, Mangold BL, Dean DA. Identification of *Schistosoma mansoni* glycoproteins recognized by protective antibodies from mice immunized with irradiated cercariae. J Immunol 1986;136(12):4689–94.

[125] Dalton JP, Strand M. *Schistosoma mansoni* polypeptides immunogenic in mice vaccinated with radiatation-attenuated cercariae. J Immunol 1987;139(7):2474–81.

[126] Pearce EJ, James SL, Dalton J, Barrall A, Ramos C, Strand M, et al. Immunochemical characterization and purification of Sm-97, a *Schistosoma mansoni* antigen monospecifically recognized by antibodies from mice protectively immunized with a nonliving vaccine. J Immunol 1986;137(11):3593–600.

[127] Soisson LM, Masterson CP, Tom TD, McNally MT, Lowell GH, Strand M. Induction of protective immunity in mice using a 62-kDa recombinant fragment of a *Schistosoma mansoni* surface antigen. J Immunol 1992;149(11):3612–20.

[128] Hawn TR, Tom TD, Strand M. Molecular cloning and expression of SmIrV1, a *Schistosoma mansoni* antigen with similarity to calnexin, calreticulin, and OvRall1. J Biol Chem 1993;268(11):7692–8.

[129] Hawn TR, Strand M. Developmentally regulated localization and phosphorylation of SmIrV1, a *Schistosoma mansoni* antigen with similarity to calnexin. J Biol Chem 1994;269(21):19883–9.

[130] Dalton JP, Tom TD, Strand M. Cloning of a cDNA encoding a surface antigen of *Schistosoma mansoni* schistosomula recognized by sera of vaccinated mice. Proc Natl Acad Sci USA 1987;84(12):4268–72.

[131] Kö ster B, Strand M. *Schistosoma mansoni*: Sm23 is a transmembrane protein that also contains a glycosyl phosphatidyl inositol anchor. Arch Biochem Biophys 1994;310(1):108–17.

[132] Simpson AJ, Hackett F, Walker T, de Rossi R, Smithers SR. Antibody response against schistosomulum surface antigens and protective immunity following immunization with highly irradiated cercariae of *Schistosoma mansoni*. Parasite Immunol 1986;7(2):133–52.

[133] Omer Ali P, Hagan P, Hackett F, Smithers SR, Simpson AJ. Variable species and stage specificity of schistosomulum surface epitopes recognized by mice vaccinated with highly irradiated cercariae. Parasite Immunol 1989;11(3):257–67.

[134] Richter D, Harn DA. Candidate vaccine antigens identified by antibodies from mice vaccinated with 15- or 50-kilorad-irradiated cercariae of *Schistosoma mansoni*. Infect Immun 1993;61(1):146–54.

[135] Richter D, Reynolds SR, Harn DA. Candidate vaccine antigens that stimulate the cellular immune response of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. J Immunol 1999;163(1):256–65.

[136] Richter D, Harn DA, Matuschka FR. The irradiated cercariae vaccine model: looking on the bright side of radiation. Parasitol Today 1995;11(8):288–93.

[137] El Ridi R, Abdel Tawab N, Guirguis N. *Schistosoma mansoni*: identification and protective immunity of adult worm antigens recognized by T lymphocytes of outbred Swiss mice immunized with irradiated cercariae. Exp Parasitol 1993;76(3):265–77.

[138] Abdel Tawab N. Identification and molecular characterization of protective antigens against murine *Schistosomiasis mansoni*. Ph.D. Thesis, Faculty of Science, Cairo University; 1994. 280 p.

[139] El Gengehi N, El Ridi R, Tawab NA, El Demellawy M, Mangold BL. A *Schistosoma mansoni* 62-kDa band is identified as an irradiated vaccine T-cell antigen and characterized as calreticulin. J Parasitol 2000;86(5):993–1000.

[140] Bergquist NR, Colley DG. *Schistosomiasis mansoni* vaccine: research to development. Parasitol Today 1999;15(3):99–104.

[141] Todd CW, Colley DG. Practical and ethical issues in the development of a vaccine against *Schistosomiasis mansoni*. Am J Trop Med Hyg 2002;66(4):348–58.

[142] Boulanger D, Reid GD, Sturrock RF, Wolowczuk I, Balloul JM, Grezel D, et al. Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. Parasite Immunol 1994;16(3):133–5.

[143] Siddiqui AA, Pinkston JR, Quinlin ML, Saeed Q, White GL, Shearer MH, et al. Characterization of the immune response to DNA vaccination strategies for schistosomiasis candidate antigen, Sm-p80 in the baboon. Vaccine 2005;23(12):1451–6.

[144] Ahmad G, Zhang W, Torben W, Damian RT, Wolf RF, White GL, et al. Protective and antifecondity effects of Sm-p80-based DNA vaccine formulation against *Schistosoma mansoni* in a nonhuman primate model. Vaccine 2009;27(21):2830–7.

[145] Zhang W, Ahmad G, Torben W, Noor Z, Le L, Damian RT, et al. Sm-p80-based DNA vaccine provides baboons with levels of protection against *Schistosoma mansoni* infection
comparable to those achieved by the irradiated cercarial vaccine. J Infect Dis 2010;201(7):1105–12.

[146] Torben W, Ahmad G, Zhang W, Siddiqui AA. Role of antibodies in Sm-p80-mediated protection against Schistosoma mansoni challenge infection in murine and nonhuman primate models. Vaccine 2011;29(12):2262–71.

[147] Ahmad G, Zhang W, Torben W, Ahgorev A, Damian RT, Wolf RF, et al. Preclinical prophylactic efficacy testing of Sm-p80-based vaccine in a nonhuman primate model of Schistosoma mansoni infection and immunoglobulin G and E responses to Sm-p80 in human serum samples from an area where schistosomiasis is endemic. J Infect Dis 2011;204(9):1437–49.

[148] Karmakar S, Zhang W, Ahmad G, Zhang W, Siddiqui AA. Role of Schistosoma mansoni paramyosin, a vaccine candidate for schistosomiasis japonica. Parasitol Today 1998;14(10):436.

[149] El Ridi R, Tallima H, Veprek P, Velek J, Jezek J, El Ridi R. Differences in immunogenicity and vaccine potential of peptides from Schistosoma mansoni glyceraldehyde 3-phosphate dehydrogenase. Vaccine 2003;21(23):3290–300.

[150] El Ridi R, Montash M, Tallima H, Immunogenicity and vaccine potential of dipeptic multiple antigen peptides from Schistosoma mansoni glyceraldehyde 3-phosphate dehydrogenase. Scand J Immunol 2004;60(4):392–402.

[151] El Ridi R, Montash M, Tallima H, Veprek P, Jezek J, Velek J, Tallima H, Montash M, El Ridi R. Peptides and multiple antigen peptides from Schistosoma mansoni glyceraldehyde 3-phosphate dehydrogenase: preparation, immunogenicity and immunoprotective capacity in C57BL/6 mice. J Pept Sci 2004;10(6):350–62.

[152] El Ridi R, Tallima H. Schistosoma mansoni ex vivo lung-stage larvae excretory-secretory antigens as vaccine candidates against schistosomiasis. Vaccine 2009;27(5):666–73.

[153] El Ridi RAF, Tallima HA-M. Novel therapeutic and prevention approaches for schistosomiasis. J Adv Res 2013;4(5):467–78.

[154] El Ridi R, Tallima H. Adjuvant selection for vaccination against murine schistosomiasis. Scand J Immunol 2012;76(6):552–8.

[155] Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. J Immunol 2008;180(4):2443–9.

[156] Monticelli LA, Sonnenberg GF, Artis D. Innate lymphoid cells: critical regulators of allergic inflammation and tissue repair in the lung. Curr Opin Immunol 2012;24(3):284–9.

[157] Saenz SA, Siracusa MC, Monticelli LA, Ziegler CG, Kim BS, Brestoff JR, et al. IL-25 simultaneously elicits distinct populations of innate lymphoid cells and multipotent progenitor type 2 (MPPtype2) cells. J Exp Med 2013;210(9):1823–37.

[158] Sokol CL, Medzhitov R. Role of basophils in the initiation of Th2 responses. Curr Opin Immunol 2010;22(1):73–7.

[159] Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya Hl, Kundu K, et al. The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. Nat Immunol 2010;11(7):608–17.

[160] El Ridi R, Tallima H. Vaccine-induced protection against murine schistosomiasis mansoni with larval excretory-secretory antigens and papain or type-2 cytokines. J Parasitol 2013;99(2):194–202.

[161] El Ridi R, Tallima H, Selim S, Donnelly S, Cotton S, Santana B Gonzales, et al. Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. PLoS One 2014;9(1):e85401.

[162] El Ridi R, Tallima H, Dalton JP, Donnelly S. Induction of protective immune responses against schistosomiasis using functionally active cysteine peptidases. Front Genet 2014;5:119.

[163] Tallima H, El Ridi R, Dalton JP. Induction of protective immune responses against schistosomiasis haematobium in hamsters and mice using cysteine peptidase-based vaccine. Front Immunol 2015. http://dx.doi.org/10.3389/immu.2015.00130.