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Autor: Harn, A.
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Department of Medicine, Robert B. Brigham Hospital, Boston, Massachusetts, USA

Immunization with schistosome membrane antigens

A. Harn

In schistosomiasis, animals harboring experimental, natural infections or those immunized with irradiated cercariae, develop significant levels of resistance to challenge infection [1–4]. The surface membranes of cercariae and early schistosomula have been considered to be the major targets of protective host mechanisms responsible for resistance to challenge infection. This view has been based on many in vitro observations which demonstrated that unlike lung or adult worms, young schistosomula are susceptible to a wide variety of immune effector mechanisms [5, 6].

Therefore, studies on the detection and characterization of surface membrane antigens present on cercariae and developing schistosomula were initiated by many different groups. To characterize these antigens, our laboratory and several others, have produced and selected monoclonal antibodies which bind to the surface membranes of cercariae and/or developing schistosomula, and which also are able to passively transfer resistance to cercarial challenge in naive animals [7–15].

Protective monoclonal antibodies have been produced with both rat and mouse lymphocytes. The level of protection in passive transfer experiments has ranged from 30–45% in mice and as high as 60% in rats. These monoclonal antibodies have been produced from animals harboring natural infectons [7–9, 13], immunized with membrane enriched extracts of cercariae or schistosomula [11, 12, 14, 15], or immunized with eggs or attenuated larvae [10, 15].

To date, monoclonal antibodies which protect in vivo have described surface membrae antigens at the following molecular weights: 38 kD [8]; 160 and 130 kD [9, 10]; 155 kD [16]; 28 kD [11]; 22 kD [12]; 30, 45 kD [13]; 32 kD and 17–18 kD [15].

Additionally, it should be noted that a number of surface membrane antigens have been detected by monoclonal antibodies which may be effective in vitro but do not passively transfer immunity in vivo [17–22].

The purification of putatively protective antigens has been accomplished

Correspondence: Dr. Donald A. Harn, Department of Medicine, Robert B. Brigham Hospital, The Seeley G. Mudd Building, 250 Longwood Avenue, Boston, MA 02115, USA

using the monoclonal antibodies in an affinity column, or by excising the antigen from a polyacrylamide gel after SDS-PAGE [23] separation of a parasite extract. In the latter method, it is then possible to immunize with the antigen in the polyacrylamide, or it may be eluted from the gel. The purity of the antigens and the relative yield may be estimated by SDS-PAGE.

Using an immunoaffinity column with protective monoclonal antibodies as ligands, Smith and Clegg [16] first reported on the isolation of membrane antigens from both adult worms and schistosomulae. The antigens had molecular weights of 155 kD and 53 kD as determined by Western blot analysis [24]. Trial vaccination studies with the purified antigens were administered intramuscularly in alhydrogel, and the animals were challenged with cercariae two weeks after the boost. Immunized mice had a statistically significant reduction in adult worms, 26%, when compared to sham immunized controls. The levels of protection ranged from 0.0% to 64%. In the only experiment with monkeys, the three which were immunized with the 155 kD antigen had adult worm burdens reduced by 30% when compared to three sham immunized controls.

In the only other published report which I am aware of, Hazdai et al. [13] immunized mice with purified antigen from cercariae. The antigen was initially administered with complete Freunds adjuvant, however no adjuvant was used in the boost. In this experiment mice were challenged with cercariae and the immunized group showed a significant level of protection which was apparently dose dependent. Animals which received 5 μg of antigen per injection had a 34% reduction in adult worm burden while mice which recieved only 1.5 μg of antigen had a 17% reduction in adult worms.

Our laboratory has been involved with the purification of three distinct membrane antigens from schistosomula. The molecular weights as determined by Western blots and immunoprecipitations with Bolton-Hunter [25] radiolabeled samples are: 22 kD, 28 kD, and 160 kD. Both the 22 kD and the 28 kD antigens can be purified from a membrane enriched deoxycholate extract of schistosomula [10] by immunoaffinty chromatography. However, the relative yields of antigen obtained in this manner are low. We estimate, based on silver staining, that we are able to obtain 5 μg of pure 22 kD and 8 μg of pure 28 kD per 100,000 schistosomula extracted. Thus we initiated trial vaccinatons with the 22 kD antigen using small quantities of antigen.

We immunized naive mice intradermally with approximately 50 ng of pure antigen mixed with BCG as described by James et al. [26]. Additionally, we used maalox at a 1:1 volume for volume ratio. Mice were boosted 4 times at three week intervals. Four weeks after the last boost, mice were challenged with cercariae by belly penetration. The immunized mice had adult worm burdens reduce 27% $p < 0.01$ compared to sham immunized controls. In subsequent experiments where fewer boosts were given, the protection level dropped to 10–17%. However, the antigen did appear to invoke a memory response as the animals were challenged 4 weeks after the last boost.

We next immunized mice with purified 28 kD antigen. For these experiments we immunized mice with immune complexes. The complexes consisted of protein A-sepharose beads to which we bound our anti-28 kD antibody. Antigen was next bound to the beads, and the beads were injected subcutaneously. The amount of antigen injected was estimated at 5 μg per mouse. The mice were boosted 4 weeks after the prime. We waited 4 weeks after the boost before we challenged the vaccinated mice with cercariae. Six weeks post-infection, adult worms were harvested. The mice vaccinated with the 28 kD antigen had adult worm burdens reduced 38% compared to sham immunized controls.

The last antigen which we are working with is the antigendetected by our protective anti-egg monoclonal antibody [10]. The amount of antigen in the schistosomula extracts is small compared to what we can get from soluble egg antigen, so we have performed these experimetns with cross-reactive egg antigen. We primed and boosted naive mice with approximately 50–100 ng of pure antigen. The antigen was mixed with BCG and maalox as described earlier. Mice were immunized intradermally and intramuscularly. Four weeks after the boost the mice were challenged with cercariae. The vaccinated mice had adult worm burdens reduced 37% when compared to sham immunized controls.

Although these are preliminary results, they are encouraging. When taken together with the two published studies, they clearly demonstrate that defined antigens can be used to vaccinate naive mice. Our studies also indicate that it is possible to induce a memory response.

Because these preliminary studies have been successful, we are optimistic that future research concerning optimal antigen doses, the different types of adjuvants, and even combinations of defined antigens will give rise to far superior vaccines. When considering studies where multiple antigens may be employed, it should be noted that all of the studies other than with the 22 kD antigen which I have mentioned are early stage antigens. Thus it is likely a vaccine which would combine epitopes from early and late stages may achieve protection levels as high as 80–90%.

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