Zeitschrift: Acta Tropica

Herausgeber: Schweizerisches Tropeninstitut (Basel)

Band: 44 (1987)

Heft: (12): Prospects for immunological intervention in human schistosomiasis

Artikel: Induction of cell-mediated immunity as a strategy for vaccination against "Schistosoma mansoni"

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DOI: https://doi.org/10.5169/seals-313841

Acta Tropica *44*, Suppl. 12, 50–54 (1987)

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Induction of cell-mediated immunity as a strategy for vaccination against *Schistosoma mansoni*

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Although not widely considered a viable alternative strategy for vaccine production in the past, induction of T cell-mediated anti-schistosome immunity has more recently been shown to possess strong protective potential. As determined decades ago by Mackaness and others working on microbial resistance [1], cell-mediated immunity (CMI) is antigen specific in the induction phase, but can operate nonspecifically in the effector phase. That is, in contrast to antibody-dependent processes, the cytotoxic reactivity of lymphokine-activated macrophage effector cells need not be directed toward specific surface epitopes on the target. Rather, macrophages distinguish and selectively damage abnormal or non-host targets in an antigen nonspecific manner [2]. In terms of schistosome immunity, this mechanism of resistance would offer the distinct advantage over humoral immunity of overcoming the parasite's immune evasion strategies of surface antigen masking or shedding and antibody cleavage [3]. A further advantage for vaccine production is that virtually any schistosome antigen, whether surface, internal or secreted, against which the host is immunized in a way that specifically sensitizes the correct T cell subset and to which the host will again be exposed upon challenge infection could theoretically have protective value. A final advantage of an antigen specific CMI vaccination protocol is that the protective reaction is compartmentalized in the area of secondary antigen recognition by sensitized T cells, which produce macrophage chemotactic and activating lymphokines locally [2]. That is, if the host were immunized against schistosome antigens, CMI would be expressed upon challenge infection only where immune T cells become re-exposed to the sensitizing antigens – generally, in the vicinity of challenging parasites. Thus, such a vac-

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cine would be unlikely to provoke significant systemic pathology. In this manuscript we document evidence that such a vaccine, based on induction of CMI, is feasible.

The parasite is susceptible to killing by lymphokine-activated macrophages. Macrophages that have been activated by lymphokine exposure in vitro or in vivo can kill 3 h schistosomula and 2 to 3 week old juvenile worms [4]. Thus, the timing of parasite susceptibility to macrophage effector cells in vitro is consistent with the early and late phases of resistance described in experimental models of immunity in vivo [3, 5]. With respect to kinetics and morphology of damage, as well as mechanism and genetic control of activation, macrophage-mediated cytotoxicity against schistosomes appears similar to that against tumor cell targets [6]. The observation that macrophages activated through CMI mechanisms can kill the parasite in vitro establishes the possibility that such mechanisms could also be effective in vivo.

Macrophages are activated by T cell-dependent mechanisms in experimental models of immunity to S. mansoni. Mice immunized against *S. mansoni* by prior exposure to living infective or radiation-attenuated larvae demonstrate antigen-specific CMI upon secondary exposure. Lymphocytes from immunized mice respond to in vitro culture with schistosomula or soluble extracts from adult worms by proliferation as well as production of macrophage chemotactic and activating lymphokines, IL-2 and IL-3 [6, 7]. In vivo, specific antigen challenge elicits delayed hypersensitivity and recruits activated (larvicidal and tumoricidal) macrophages to the site [7]. Thus, the existence of potentially protective CMI has been established in immunized mice.

Levels of resistance and macrophage activity correlate in several models of protective immunity. Several models have now been developed for testing the relationship between CMI and protection. For example, inbred strains of mice with characterized defects in macrophage activation and delayed hypersensitivity, such as P and A strains, fail to become resistant to *S. mansoni* infection as a result of immunization [6, 8]. Genetic studies following the inheritance of resistance and macrophage larvicidal activity in the irradiated cercariae vaccine model show that in P × C57BL/6 F2 and backcross progeny the P-associated defects in these traits are each controlled by single major loci. There is a highly significant correlation between the levels of macrophage function and resistance, suggesting that these loci may be identical or closely linked [8]. Evidence for association of these defects in C57BL/6 × P crosses is consistent with a cause and effect relationship.

A correlation was also observed between CMI and resistance in the cryopreserved irradiated vaccine model, in which schistosomula with the least freezing-associated damage were most capable of inducing protective immunity

as well as CMI, whereas animals immunized with cryodamaged parasites showed minimal CMI and no resistance [9].

Thus, in every known model of immunization against *S. mansoni* using living parasites (early concomitant immunity, radiation-attenuated vaccine and cryopreserved vaccine), an association has been shown between macrophage activity and resistance.

Induction of protective immunity by a nonliving vaccine based on CMI. An immunization protocol using nonliving antigens that elicits antigen specific CMI was designed to test the hypothesis that macrophage activation is associated with resistance. Intradermal injection of schistosome antigens in conjunction with bacterial adjuvant (*Mycobacterium bovis* strain BCG or *Bordetella pertussis* vaccine) sensitizes for CMI, including antigen specific delayed hypersensitivity, lymphokine production and macrophage activation in C57BL/6 mice [10–12]. In addition, this method of immunization induces up to 70% resistance to challenge *S. mansoni* infection [10]. Immunization with the same antigens via an intravenous route fails to induce either resistance or CMI [10, 12]. The latter observation again suggests a correlation between the two properties, and indicates that the route of antigen presentation may be critical to vaccine production. That resistance is based on CMI in this model is further supported by the inability to protect the macrophage-defective P strain [13]. One other nonresponder strain, BALB/c, has been identified and was also found to be defective in CMI response under these conditions [13]. In contrast, outbred and many other inbred strains of mice, including those with inherent defects in immediate hypersensitivity, endotoxin responsiveness and IgM production as well as mice rendered B cell deficient by μ suppression, develop strong resistance as a result of intradermal immunization [13].

Characterization of the protective immunogens in the intradermal vaccine. The protective antigen(s) in this model are apparently not stage specific, being present in cercariae, 3 h schistosomula and adult worms [11]. Preliminary characterization studies suggest that the immunogenic material is a soluble protein which is not heavily glycosylated [14]. Mice immunized with crude schistosome extracts plus BCG develop minimal humoral response to parasite surface antigens but produce anitbodies against a single internal protein of M_r 97,000 (Sm 97) [11]. Upon gel filtration, the immunogenic activity in soluble worm extracts separated in a high molecular weight fraction containing Sm 97 [14].

While it appears likely that the effector mechanism of resistance in this model involves CMI, the remarkable specificity of the antibody response suggests that Sm 97 may be uniquely recognized by T_H cells and may thus also reflect the repertoire of cellular responsiveness. We have therefore initially focused on determining the immunogenicity of this molecule. Affinity purified Sm 97 elicits delayed hypersensitivity in mice immunized with crude schisto-

some antigens plus BCG, and sensitizes for delayed hypersensitivity when injected with BCG – indicating that this molecule is indeed immunogenic for T cells. Experiments assessing the ability of Sm 97 to induce lymphokine production by T cells from immunized mice are now in progress.

Two cDNA clones expressing products reactive with monoclonal anti-Sm 97 have been identified and one of these has been partially sequenced. The corresponding amino sequence indicates that Sm 97 has a coiled coil alpha-helical structure typical of a myosin-like molecule. Because its apparent molecular weight is much lower than myosin itself, we postulate that Sm 97 is paramyosin, a molecule found exclusively in invertebrates which forms a core stabilizing the structure of myosin in the muscle. The explanation for the unique immunogenicity of this protein when administered intradermally remains to be determined. Vaccine trials employing affinity purified Sm 97 as well as the recombinant DNA expression product are currently underway. It remains to be determined whether molecules other than Sm 97 present in crude antigenic preparations elicit CMI or are protective in this model.

Conclusion

The data reviewed above indicate that CMI can protect against schistosome infection through a mechanism involving, minimally, T cell recognition of parasite antigens resulting in LK production and activation of macrophage effector cells. The concept of a vaccine that does not require antibody recognition of parasite surface antigens is a radical departure from common thinking, and opens up new possibilities for production of purified protective immunogens. The key element of vaccine production may in fact be the method of antigen presentation and the type of immune responses thus elicited rather than identification of any one protective antigen. It is possible that different antigens can be protective in the context of different immune mechanisms, humoral or cellular, and that a successful vaccine will involve a combined approach.

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