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# Attenuated parasites and the development of an erythrocytic-stage vaccine

By W. M. C. ELING\*

Department of Medical Microbiology, Catholic University of Nijmegen,  
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Asexual-stage vaccine candidates may be identified by recognition of antibodies to them in the sera of naturally immune individuals. Once identified, recombinant molecules expressing the relevant antigen can be produced, although such recombinant molecules only seem to be effective at inducing immunity if mixed with adjuvant. More information is needed about the role of antibodies in immunity to malaria. In an animal model, that of *Plasmodium berghei* in mice, a fairly effective immune response can be produced by inoculating mice with live, proliferating parasites and then keeping the infections subclinical, by drug treatment, for 5 weeks. However, the resultant 'immune' individuals carry small numbers of parasites despite the presence of antibody (premunition). Repeated challenge infections are limited by the hosts' immune responses but sterile immunity is not observed. The immunity that is established only fades in the absence of the parasite. In contrast, inoculation with preparations of disrupted parasites, which do not seem to contain all the antigenic information of the living parasites, do not lead to an immune response which limits parasitaemias. These observations may be the result of antigenic variation. Immunity induced in mice by live, attenuated parasites (but not by extracts) protects against challenge with virulent parasites. Immunity against the attenuated but not the virulent parasite can be transferred by inoculating naïve mice with spleen cells from the immunized mice. The mice given spleen cells can then be protected against the virulent parasite by challenge with proliferating, attenuated parasites (but not with extracts). Immune B cells from the immune donor and non-immune CD4 cells in the naïve recipient are essential for transfer of immunity.

A strategy for the development of a vaccine against the erythrocytic stages of the malarial parasite is to look in the serum of a protected individual for antibody reactivity against a molecule on the surface of the parasite or of a parasitized erythrocyte. Such molecules may be identified, isolated, expressed in pro- and eu-karyotic systems and then used for immunization. Although, usually, the recombinant molecules which have been prepared in this way only produce a useful immune response if inoculated with complete Freund's adjuvant (CFA), it is believed that this strategy will eventually result in the development of a molecular or epitope vaccine. To resolve possible problems with antigenic variation or diversity, a vaccine cocktail is envisaged. Whether or not such an antibody-based vaccine is compatible with the characteristics and mechanisms of

malarial immunity remains unclear. In fact, the mechanisms of malarial immunity are still not fully understood.

Although there are differences between experimental and human malaria, there are parallels too. In humans, immunity develops after repeated infection and takes years to become effective. In animal models, repeated infections, controlled by chemotherapy, are also effective. An easy way to immunize mice against *Plasmodium berghei*, for example, is to infect them with the parasite and then keep the infections at subclinical levels by adding sulphonamide to the rodents' drinking water for 5 weeks (Eling and Jerusalem, 1977). However, live, proliferating parasites must be present (at about one parasite/ $10^5$ – $10^7$  erythrocytes) during the whole of the 5-week period for effective immunity to develop (Eling, 1978a). Repeated injection of disrupted parasites, which supposedly contain

\* E-mail: w.eling@mmb.azn.nl; fax: +31 24 3540216.

all the molecules/epitopes of the living parasites and which are regularly used to identify antibody reactivity (e.g. by immunoprecipitation), is, in comparison, a poor way to immunize mice in the absence of CFA (Jerusalem and Eling, 1969). Why are live parasites better for immunization than disrupted parasites? Antigenic diversity in the parasite populations used or the generation of antigenic variants during their proliferation *in vivo* could explain this puzzle.

Premunition occurs in murine malaria as in human malaria, and so mice 'immune' to *P. berghei* still carry the parasite. Challenge with as many as  $10^9$  parasites is rapidly suppressed to subpatent levels by these mice, and repeated high-dose challenges are controlled, but the mice eventually become and remain carriers of subpatent numbers of the parasite (Eling, 1978a, c). This premunition cannot be boosted to sterile immunity. Why do parasites in low numbers survive despite a fully effective immune system? If immunity is antibody mediated, why are not all parasites taken out? Is antigenic variation the explanation? When the parasites present in immune mice are given to naïve mice, a normal, virulent infection develops. The parasites present in 'immune' mice therefore appear to be as virulent as the original, wild-type parasites used for immunization.

Premunition or carrier-immunity is functional; as long as the parasites are present there is resistance to re-infection (Eling, 1978a, b, c, 1980; Eling *et al.*, 1991). If the parasites disappear spontaneously or are eliminated by radical cure (chloroquine treatment), immunity fades gradually within months, both in human and experimental malaria. The longer the parasites are absent, the more severe the course of a challenge infection and the more difficult it becomes to control. If mice are left long enough without *P. berghei*, the challenge eventually resembles an infection in untreated controls and all challenged mice die. This fading of immunity is prevented if subclinical numbers of proliferating parasites are present or if such parasites are inoculated at regular intervals (e.g. monthly). In the latter case, the inoculated parasites

must be viable and proliferating; repeated injection of high numbers ( $10^8$ ) of freeze-thawed parasites or of live parasites under chloroquine treatment (to prevent proliferation) fails to prevent the immunity fading (Eling, 1978c, Eling *et al.*, 1991). Thus, proliferating parasites are important for both the development and the maintenance of immunity. We must learn to understand why proliferating parasites rather than freeze-thawed preparations of them are important.

Why are proliferating parasites needed for a period of 5 weeks to develop immunity to *P. berghei*? Infected mice given anti-T-cell treatment during the first 2–3 weeks still develop immunity, but if such treatment is given later in the 5-week infection it does limit development of the immunity, increasing in effectiveness the later it is given (Eling, 1979). T-cell reactivity, which does not, therefore, appear to be an important factor in the first 3 weeks of the immunization period, was further analysed by Celluzzi (1994) and Celluzzi *et al.* (1995). At varying time points during the 5-week immunization period, as well as after the challenge, when the mice had developed immunity to the challenge infection, spleen cells were transferred to naïve recipients. All of the recipients developed infections and died, indicating that there were parasites among the transferred spleen cells and that the proliferation of these parasites could not be controlled by the recipients. Even transfer of all the spleen cells from one immune mouse with an established immunity to one recipient failed to control the subsequent infection. Repeated boosting infections before the transfer were ineffective. However, after adding sulphonamide to the drinking water of the recipients of spleen cells to limit their infections, it became apparent that transfer of spleen cells from mice on or near day 28 of the 5-week immunization period induced immunity in the recipients in 3 weeks. Surprisingly, transfer of peripheral blood from these 'day-28' mice also resulted in immunity in sulphonamide-treated recipients in 3 weeks. It finally became clear that it was the parasites in the spleen-cell preparations and in the peripheral blood, not the spleen cells themselves,



that were responsible for the immunization of the recipients in a 3-week period. The parasites from day-28 mice were therefore isolated, cloned, subinoculated by syringe passage into splenectomized mice and frozen in liquid N<sub>2</sub>. These parasites had an attenuated character. Whereas the virulent parasites induced cerebral malaria, infections with the attenuated parasites developed slowly and, irrespective of the number inoculated, never induced cerebral malaria. When <1000 of these attenuated parasites were inoculated, parasitaemias either never came up (if <10 parasites were used) or remained at low levels for 3 weeks and then, in some of the mice, disappeared. Such mice also resisted a subsequent challenge with the virulent parasite. (To indicate how surprising this was, naïve mice given a single virulent parasite each and left untreated all develop intense parasitaemias and die.) Infection with high numbers of the attenuated parasite, obtained from passage animals and controlled by sulphonamide treatment given in the drinking water of the recipients, induced immunity in 3 weeks, a feature never observed with the virulent parasite.

Recipients of immune spleen cells could not control the virulent parasite, whether the virulent parasites were present in the transferred spleen-cell preparations or were removed (by chloroquine treatment) from the immune donor before collection of the spleen cells and given to the recipients after the transfer. When immune spleen cells free of virulent parasites were transferred, recipients were immune against infection with the attenuated, day-28 parasite. Recipients of immune spleen cells that were infected with the attenuated parasite were also immune to the virulent parasite when given 1 week after the attenuated parasites. A week-long, subclinical infection with proliferating, attenuated parasites was necessary to develop immunity to the virulent parasite; inoculations with freeze-thawed attenuated parasites for a week, even when given every other day, failed to induce this immunity. Thus, in mice with established immunity the virulent parasites persist and are controlled, but immune spleen cells only transfer immunity to the attenuated parasites

and the attenuated parasites induce immunity to the virulent parasites within 1 week. These conclusions provided a basic model for further transfer studies (Celluzzi, 1994; Celluzzi *et al.*, 1995). Mice with established immunity to the virulent parasite received chloroquine treatment to remove all parasites. Spleen cells from them were then transferred to recipients, which were immediately infected with the attenuated parasite and 7 days later challenged with the virulent parasite. Macrophage-depletion experiments showed that macrophages, from the donor or the recipient, were not necessary for successful immunization by transfer of immune spleen cells. However, B-cells had to be present among the transferred immune spleen cells for development of immunity in the recipients. CD4 cells also had to be present but, surprisingly, not necessarily in the transferred immune spleen cells. When CD8 cells were depleted in the recipients (CD4 cells present), transferred immune spleen cells depleted of CD4 cells provided immunity to the attenuated and (7 days later) to the virulent parasites. In mice with established immunity to the virulent parasite, CD4 depletion resulted in loss of immunity to the virulent parasite.

What about fading of immunity and the capacity to transfer immunity using spleen cells? When mice with established immunity against both attenuated and the virulent parasites were radically cured by chloroquine treatment, they lost immunity to the virulent parasite after 3–4 months. After 6 months, however, they were still immune to the attenuated parasite and immunity to the virulent parasites developed when the virulent parasites were given 7 days after the attenuated parasite. Immune donors lost their capacity to transfer immunity via their spleen cells after being parasite-free for approximately 2 months. When recipients received parasite-free immune spleen cells only and infection, with the attenuated parasites followed, 7 days later, by the virulent parasite, was delayed, their capacity to develop immunity was lost after a delay of 4 months. Could this mean that immunity to the attenuated parasite is dependent on the presence of immune

B cells and that (non-immune) CD4 cells are necessary for the development of immunity against the virulent parasite? What is the difference between the attenuated and the virulent parasite, and why do we need live, proliferating parasites for development of the immune reactions? Do attenuated and virulent parasites differ in their repertoires of antigenic variants. Does the attenuated parasite simply

lack an antigen that induces pathology? Further study of the attenuated parasite should be useful in the development of a malaria vaccine. We need to know whether similar attenuated parasites can develop in *P. falciparum*-human interactions and whether the mechanisms observed in the mouse model also operate in human immunity.

## REFERENCES

- CELLUZZI, C. (1994). *Attenuated immunogenic parasites: essential stimulators of Plasmodium berghei immunity*. Thesis, University of Nijmegen, The Netherlands.
- CELLUZZI, C. M., LIEM, P. L., VAN DE WIEL, T. & ELING, W. M. C. (1995). Attenuated immunogenic parasites are essential in the transfer of immunity to virulent *Plasmodium berghei*. *Immunology*, **85**, 509-515.
- ELING, W. (1978a). Malaria immunity and premunition in a *Plasmodium berghei*-mouse model. *Israel Journal of Medical Science*, **14**, 542-553.
- ELING, W. (1978b). Fading of malaria immunity in mice. *Tropenmedizin und Parasitologie*, **29**, 77-84.
- ELING, W. (1978c). Survival of parasites in mice immunized against *Plasmodium berghei*. *Tropenmedizin und Parasitologie*, **29**, 204-209.
- ELING, W. (1979). The effect of ATS on induction of malaria immunity in a *Plasmodium berghei*-mouse model. *Experimental Parasitology*, **47**, 403-409.
- ELING, W. (1980). *Plasmodium berghei*: premunition, sterile immunity and loss of immunity in mice. *Experimental Parasitology*, **49**, 80-96.
- ELING, W. & JERUSALEM, C. (1977). Active immunization against the malaria parasite *Plasmodium berghei*. Sulfathiazole treatment of a *P. berghei* infection and induction of immunity. *Tropenmedizin und Parasitologie*, **28**, 158-174.
- ELING, W. M. C., CELLUZZI, C., HERMSEN, C. C., VAN DE WIEL, T., CURFS, J. & LIEM, P. L. (1991). The need for live parasites for longterm immunity in malaria. *Acta Leidensia*, **60**, 167-175.
- JERUSALEM, C. & ELING, W. (1969). Active immunization against *Plasmodium berghei* malaria in mice, using different preparations of plasmodial antigen and different pathways of administration. *Bulletin of the World Health Organization*, **40**, 807-818.