5-15-2005

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Eric James
*Medical University of South Carolina*

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**Eric James**

Department of Ophthalmology, Medical University of South Carolina, Charleston

**(See the article by Leiriao et al., on pages 1576–81.)**

Of the >2 billion people who live in areas where malaria occurs, >500 million are estimated to become infected each year, and as many as 1.2 million die as a direct result, the majority of whom are children <5 years old. Additionally, some 10,000 travelers from developed nations return home with malaria annually. Resistance, both in *Plasmodium* species to the first-line chemotherapeutic agents and in the *Anopheles* mosquito vectors to insecticides, is widespread. The potential value of an antimalaria vaccine has long been recognized.

The malaria life cycle presents 3 sets of targets for vaccine intervention, associated sequentially with invasion, erythrocyte infection, and sexual reproduction in the mosquito. However, malaria infection, clinical disease, and transmission all would be prevented by a vaccine targeting only the exoerythrocytic (EEC) sporozoite and liver stages. In the quest for a malaria vaccine, the first significant benchmark was set in 1967, when Ruth Nussenzweig and colleagues [1] reported that the injection of radiation-attenuated sporozoites of the rodent malaria parasite, *Plasmodium berghei,* was capable of inducing protection against challenge in >90% of immunized mice. Subsequent studies, in Nussenzweig's lab and in others, of rodent, monkey, and human forms of malaria have established radiation-attenuated sporozoites as the "gold standard" for malaria vaccination.

In a natural infection, sporozoites injected into the skin by an infected mosquito travel through the bloodstream to the liver sinusoids, where they actively migrate through Kupffer cells and into hepatocytes [2]. Inside a hepatocyte, the sporozoite is sequestered within a parasitophorous vacuole, where it metamorphoses, feeds, and divides asexually into numerous merozoites to form a maturing liver schizont. Optimally irradiated sporozoites also are able to invade hepatocytes but fail to undergo nuclear division and development into schizonts.

A series of parasite proteins, characteristic initially of sporozoites and latterly of developing liver and erythrocytic stages, is expressed in the liver. Antibodies to proteins expressed by the sporozoites and early liver-stage parasites, together with $CD8^+$ T cells and natural killer cells, form the core of the host immune response to the EEC infection [3].

Until now, it had been assumed that the protection induced by radiation-attenuated malaria parasites was derived from an extended period of expression of the EEC-stage antigens by the intracellular hepatocytic parasites. However, an additional, alternative explanation was recently suggested [4]: presentation of the parasite antigens in the context of host cell apoptosis. A host cell that is invaded by a pathogen shows a natural proclivity for undergoing apoptosis in an attempt to prevent further development of the invader and dissemination of the infection to new host cells. Intracellular pathogens—viruses, bacteria, and protozoa—have evolved a remarkable array of strategies for circumventing or neutralizing this host cell response. Attenuation induced by radiation, it was reasoned, might be capable of abrogating the ability of a parasite to suppress the host cell's inclination to undergo apoptosis. Thus, host cells infected with irradiated parasites would die by apoptosis and be phagocytosed and processed by dendritic cells.

In this issue of the *Journal of Infectious Diseases,* Leiriao et al. [5] present data that support this hypothesis. Using the mouse malaria parasite, *P. yoelii,* these authors show that, after invasion of hepatocytes in vivo by irradiated sporozoites, the infected hepatocytes subsequently undergo apoptosis. This observation fits with the notion that, with the inhibitors of host cell apoptosis disabled through irradiation of the parasite, the infected hepatocyte dies as it is programmed to do after invasion.

It seems likely that the apoptotic malaria parasite–infected hepatocytes observed by Leiriao et al. died through activation of the intrinsic pathway of apoptosis rather than the extrinsic death receptor–mediated or granzyme/perforin pathways, but this remains to be confirmed. The intrinsic

Received 21 December 2004; accepted 21 December 2004; electronically published 4 April 2005.

Reprints or correspondence: Dr. Eric James, Dept. of Ophthalmology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425 (jameser@musc.edu).

**The Journal of Infectious Diseases 2005; 191:1573–5**

0022-1899/2005/19110-0001$15.00

pathway of apoptosis operates through disruption of the mitochondrial outer membrane and activation of caspase-9, which then activates the executioner caspases, caspase-3, -6, and -7. The extrinsic pathway is usually initiated through activation of caspase-8 upstream of caspase-3. Leiriao et al. demonstrate strong activity of caspase-3 in the malaria-infected apoptotic hepatocytes. Other studies have reported that the immune response to liver-stage parasites is not abrogated in perforin-deficient or Fas-deficient mice [6], suggesting that it is unlikely that these pathways are involved. The decline in the numbers of irradiated-parasite–infected hepatocytes in Leiriao et al.'s study began within a few hours of sporozoite inoculation, and parasitized host cell numbers had already fallen to ~20% of initial numbers by 48 h after inoculation. Hepatocyte apoptosis thus precedes the appearance of parasite-reactive $CD8^+$ T cells, which become detectable 48–72 h after inoculation [7], further suggesting that the observed hepatocyte apoptosis is unlikely to be mediated via the granzyme/perforin or extrinsic pathways triggered by activated T cells. Under the assumption that the apoptosis seen in Leiriao et al.'s mouse hepatocytes does follow the intrinsic pathway, this would be the first evidence that intracellular parasites attenuated by irradiation do fail to prevent the inherent drive of the host cell to undergo apoptosis.

Irradiation is a powerful inducer of the intrinsic pathway of apoptosis that operates through DNA damage and up-regulated expression of p53, leading to mitochondrial disruption. This, at least, is the pathway in mammalian cells. A process very similar to apoptosis has been observed in protozoa [8, 9]; however, since the molecular machinery and the apoptotic transduction pathway(s) are currently unknown for protozoa, it is not yet possible to determine whether an apoptotic death is also the ultimate demise of irradiated sporozoites. Even if it did not die as a result of radiation-induced apoptosis, the intracellular parasite might also be disassembled coincidently with the host cell contents by the host apoptotic machinery. After invasion of a hepatocyte by an irradiated sporozoite, both parasite and host cell, or just the host cell, could die by apoptosis. Either way, the apoptotic hepatocyte would be flagged for phagocytosis; however, if the parasite died without the concomitant apoptotic death of its host hepatocyte, this antigen-presentation pathway would not operate.

The work of Leiriao et al. additionally provides evidence that liver dendritic cells are recruited to phagocytose the hepatocyte corpses. This observation is also significant, since data from other systems have indicated that dendritic cells are important for the phagocytosis and processing of apoptotic cell debris and cross-presentation of antigens for priming $CD8^+$ T cells [10, 11], and $CD8^+$ T cells are critical to the development of immunity against an irradiated-sporozoite infection. The impetus for recruitment of dendritic cells, as well as for their maturation, could be provided by inflammatory mediators released by Kupffer cells and hepatocytes wounded during the migration of sporozoites within the liver sinusoids before the hepatocyte of choice is selected [2], as Leiriao et al. suggest, as well as by the apoptotic hepatocytes themselves.

Cells that have undergone apoptosis as a consequence of normal tissue growth or development do not release "danger signals," and the phagocytosis of these cells does not usually lead to the induction of immunity [12]. However, "stressed" apoptotic cells, particularly those expressing heat shock proteins, are very effective stimulators of dendritic cells and activators of T cells [13, 14]. It might be reasonable to suspect that hepatocytes infected with radiation-attenuated sporozoites are stressed. However, even if this is not the case, the parasite itself could be a source of stress proteins and other danger signals. Coincidentally, Leiriao et al. used *Plasmodium* Hsp70 to monitor host cell infection and dendritic cell phagocytosis. Danger signals of microbial or host cell origin can function as adjuvant molecules; therefore, if certain molecules originating from the irradiated sporozoites do assume the role of danger signals, the vaccine organisms could essentially provide their own adjuvants, as can occur with immunization with bacteria. Alternatively, the danger signals could come from those wounded Kupffer cells and hepatocytes traversed by the sporozoites during invasion.

An issue related to vaccination using irradiated sporozoites that remains controversial is whether the induction of protective immunity occurs centrally, through antigen processing in the spleen and lymph nodes, or peripherally, at the sites where sporozoite invasion of host cells occurs. The data presented by Leiriao et al. provide evidence that, in the liver, the latter scenario is at least plausible. Others may disagree, since it is thought that only a minority of irradiated sporozoites actually reaches the liver. Local induction of immunity to parasite antigens through processing of apoptotic infected host cells by dendritic cells could, thus, be just one component of several diverse mechanisms of antigen processing and presentation that are activated after inoculation with irradiated sporozoites. However, since hepatocyte invasion is unique to the live attenuated vaccine approach and immunization with apoptotically derived antigens is increasingly being recognized in several systems as a potent method of inducing protective immunity, this mechanism, involving hepatocyte apoptosis and dendritic cells, could be what accounts for the "gold standard" status of the radiation-attenuated–sporozoite vaccine.

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