LETTERS to the EDITOR

Protection against malaria induced by irradiated sporozoites

SIR,—Protection against a virulent sporozoite challenge has been achieved by intravenous (iv) injection of γ-irradiated sporozoites of *Plasmodium yoelii* or *P berghei* in mice, and *P falciparum* or *P vivax* in man.[1] Protection has also been obtained in mice by iv injection of non-irradiated sporozoites in animals treated by chloroquine to prevent erythrocyte infection.[2,3] In contrast, limited or no protection was induced either by irradiated sporozoites injected by intramuscular or subcutaneous routes, or by dead sporozoites, sporozoite extracts, or molecules from the sporozoite surface irrespective of route.[1] Since it has been shown in mice by in-vitro[4] and in-vivo (Londono A, et al, unpublished) methods that γ-irradiated sporozoites are viable organisms able to penetrate hepatocytes and transform into uninucleate liver trophozoites, we believe these experiments indicated that the transformation of sporozoite to liver stage (LS) was required, at least in mice, to achieve protection.[5] We investigated this hypothesis in man. Volunteers vaccinated with *P falciparum* sporozoites exposed to a higher dose of irradiation (20–23 krad) than that used in previous studies (12–15 krad) did not seem to be protected on challenge with non-irradiated sporozoites, despite appropriate antibody response to sporozoite antigens.[6] We therefore decided to investigate in vitro the fate of *P falciparum* sporozoites exposed to the same doses. This was done in the human hepatocyte in-vitro model with the procedure described previously[7] and the same parasites (NF 54 strain) used in the in-vivo vaccine experiment.

The results (table) clearly demonstrate that the rate of penetration in human hepatocytes is inversely related to the dose of irradiation. At 14 krad, the dose that induced protection in earlier experiments, about half the number of LS found without irradiation was obtained. At 21 krad, the dose which did not confer protection in man, the number of LS obtained compared with controls was under 10%. The same assay was done with *P yoelii* (17X-NL strain) sporozoites with Balb C mouse hepatocytes.[8] In three sets of experiments much the same results were obtained. Penetration and transformation to young LS in vitro was reduced compared with controls with 10 krad-exposed sporozoites, and was close to zero when sporozoites were exposed to 20 krads.

MEAN NUMBER OF LS (LIVER STAGE TROPHOZOITES) PER WELL (DUPLICATES) IN HEPATOCYTES INFECTED WITH *P FALCIPARUM* OR *P YOELLI* SPOROZOITES (48 h CULTURES)

—	Irradiation dose (krad)		
	0	14	21
P falciparum No of LS in human hepatocytes	29	13 (45%)	2 (7%)
	0	10	20
P yoelii No of LS in mouse hepatocytes	75	19 (25%)	0·5 (0·7%)

% of transformation of sporozoites to LS compared with controls (non-irradiated sporozoites) in parentheses.

In further experiments three groups of 6 Balb C mice were immunised twice at 15 day intervals by iv injection of *P yoelii* sporozoites (7×10^5 and 3×10^5, respectively), irradiated at 10, 20, or 30 krad. They were challenged 15 days later by 1000 non-irradiated sporozoites. In the group receiving 10 krad sporozoites 6 of 6 were protected, whereas in all 12 receiving 20 and 30 krad sporozoites blood infection developed.

From these in-vivo and in-vitro data it seems that there is in both man and mice a correlation between the ability of sporozoites to induce protection against an infectious challenge and their viability in respect of transformation to LS. These findings strongly suggest that the presence of infected liver cells is necessary to achieve protection. In turn this may indicate that antigens expressed at the LS level and not those harboured by sporozoites are responsible for triggering the species-specific protection achieved. Alternatively the blocked liver trophozoites could act as an antigen depot which increases the immunogenicity of sporozoite, or sporozoite and LS shared, antigens. However, the protection achieved in mice by the use of non-irradiated sporozoites in animals treated with chloroquine, in which maturation of LS occurs,[2,3] argues against this hypothesis and strengthens the case for LS-specific antigens.

These findings open novel approaches to the design of a pre-erythrocytic stage malaria vaccine, in which the analysis of the immunological responses to various *P falciparum* LS antigens in individuals protected by means of 14 krad irradiated sporozoite is an essential part.

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1. Cochrane AH, Nussenzweig RS, Nardin EH. Immunization against sporozoites. In: Kreier, ed. Malaria, vol 3. New York: Academic Press, 1980: 163–202.
2. Beaudoin R-L, Strome CPA, Mitchell F, Tubergen TA. *Plasmodium berghei*: immunization of mice against the Anka strain using the unaltered sporozoite as an antigen. *Exp Parasitol* 1977; 42: 1–5.
3. Onih AU, Cochrane AH, Nussenzweig RS. Comparative studies on the immunogenicity of infective and attenuated sporozoites of *Plasmodium berghei*. *Trans R Soc Trop Med Hyg* 1982; 76: 57–61.
4. Ramsey JM, Beaudoin RL, Hollingdale MR. Infection of tissue-culture cells with Co gamma-irradiated malaria sporozoites. In: Nuclear techniques in the study of parasitic infections. Vienna: International Atomic Energy Agency, 1982: 19–25.
5. Druilhe P, Marchand C. From sporozoite to liver stage: the saga of the irradiated sporozoite vaccine. In: McAdam, ed. New strategies in parasitology. Edinburgh: Churchill Livingstone, 1989.
6. Herrington D, Clyde D, Davis J, et al. Clinicals trial of sporozoite vaccine at the University of Maryland. In: Pre-erythrocytic stage malaria vaccine development: current status and future prospects. (Bethesda, 12–15 April, 1989, WRAIR AID WHO meeting). *WHO Bull* (in press).
7. Mellouk S, Mazier D, Druilhe P, Berbiguier N, Danis M. In vitro and in vivo results suggesting that anti-sporozoite antibodies do not totally block *Plasmodium falciparum* sporozoite infectivity. *N Engl J Med* 1986; 315: 648.
8. Mellouk S, Berbiguier N, Druilhe P, et al. Inhibitory effect of antibodies to sporozoites on the pre-erythrocytic stages of malaria parasites. In: Pre-erythrocytic stage malaria vaccine development: current status and future prospects. (Bethesda, 12–15 April, 1989 WRAIR AID/WHO meeting). *WHO Bull* (in press).