In summary, cell clone E9 dominated the anti-DNP response while the clone was actively proliferating and producing antibody. This exclusive production of E9 anti-DNP, with its characteristic isoelectric spectrum, persisted for up to 100 days within individual mice, and through several serial spleen cell transfers. The implication is that immature anti-DNP precursor cells are not able to proliferate or differentiate in the presence of clone E9 forming large amounts of antibody. Clonal dominance was complete as long as E9 production was in excess of 50 μg anti-DNP per ml. serum.

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Sporozoite and Normal Salivary Gland Induced Immunity in Malaria

STUDIES using sporozoites as antigenic material to induce protection against malaria have been relatively scarce compared with those employing antigens of the blood stages. Many workers[1–5] have reported considerable protection against challenging homologous sporozoites of *Plasmodium gallinaceum*. Richards[4], using three injections of sporozoites exposed to ultraviolet, dried or treated with formalin, found at least eighteen birds out of twenty were protected, but with freeze-thawed sporozoite material he found only fifteen out of twenty protected. By using six to seven injections of X-irradiated sporozoites of *Plasmodium berghei*, Nussenzweig *et al.*[6,7] obtained 90–100% protection when the animals were challenged with 2,000 sporozoites two weeks after the last injection.

In the experiments described here we used 3–4 week old A/J inbred female mice which were randomly grouped according to weight. All animals were vaccinated intraperitoneally with *Plasmodium berghei* NK65 and challenged intraperitoneally with 2,000 sporozoites. Those which had positive blood films died.

In the first experiment, seven injections of 25,000 sporozoites, heat inactivated at 40°–42° C for 45 min, were given at intervals of fourteen days. After challenge, seven out of eight normal control mice became infected while four out of eight control mice injected with salivary gland protein and nine out of ten sporozoite injected mice showed no infection.

In the second experiment, six injections of 450,000 sporozoites, heat inactivated at 40°–42° C for 60 min, were given as in the first experiment. After challenge, all ten normal control mice became infected, while seven out of ten salivary gland control mice and seven out of nine sporozoite injected mice remained uninfected.

In the third experiment, six injections, each of 400–500 μg protein of normal salivary gland material heated at 40°–42° C for 60 min, were given at intervals of fourteen days. Following challenge at nineteen weeks, all the ten untreated control mice and six out of nine normal salivary gland mice became infected.

In the fourth experiment, six injections of 450,000 sporozoites, inactivated by freezing and thawing three times, were injected at fourteen day intervals. After challenge, none of the ten normal control mice remained uninfected, but seven out of ten salivary gland injected and nine out of ten sporozoite injected animals remained negative. Thus freezing and thawing do not seem to inhibit the activity of the antigen.

The most striking result in each of the experiments is that controls injected with tissue from normal mosquitoes showed unexpectedly good protection although always at a somewhat lower level than sporozoite injected mice. Nevertheless some protection appears to last as long as nineteen weeks, which is equivalent to about a fifth of the mouse life span. The simplest explanation for this protection seems to be non-specific macrophage type immunity, although Rivera[8] reported that mouse non-specific spleen activity usually returns to normal fourteen days after similar injections. Our results suggest that the protection with normal salivary gland material is not of a non-specific macrophage type.

Alternative explanations for this protection may possibly be antigenic mimicry as suggested by Damian[9] or the combination of antibody with mosquito antigen carried on the surface of the sporozoite which, with the aid of complement, may destroy the parasite. Alternatively there may be cross-reaction between mosquito and parasite antigens, or possibly antigens of contaminating bacteria or viruses cross-react with the parasite.

Neither heat treating nor freeze-thawing seems to interfere with the induction of protection by either sporozoite or normal salivary gland antigens.

Although the possibility of protective immunization with normal mosquito tissue is exciting because the various stages of the parasite itself are so difficult to procure, the mechanism of this immunity remains obscure. Fractionation of both sporozoite and normal mosquito tissue may be a first step toward understanding this phenomenon.

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