

The Role of Natural Killer Cells in Immunity Against Malaria

Natural killer (NK) cells are non-adherent, non-phagocytic mononuclear cells, the surface of which bear receptors for the Fc portion of IgG.¹ NK cells share a number of features with T cells and with macrophages. Thus, about half of the human NK cells express receptors for the red blood cells of sheep² and the cells grow in response to T-cell growth factor (TCGF).³ Enriched populations of human NK cells undergo blastogenic transformation in the presence of T-cell mitogens, phytohaemagglutinin and concanavalin A.⁴ NK cells, however, are not thymic-dependent, since high NK activity could be detected in athymic nude or neonatally thymectomized mice and rats.^{5,6} The properties of human NK cells that are shared with macrophages include reaction of the NK cell surface marker with OKM1 monoclonal antibody.⁷ Morphologically, NK cells are large granular lymphocytes which comprise about 5 per cent of the peripheral blood leukocytes.⁸

NK cells can exert cytolytic activity against a wide variety of syngeneic, allogeneic and xenogeneic cells.⁹ Cells susceptible to NK cytotoxicity include malignant cells, fetal cells and virus-infected cells. NK activities are enhanced by interferon.¹ Stimulated NK cells also produce mediators, one of which is interferon.¹⁰ Accumulated evidence gathered in recent years shows that

NK cells play an important role in host defence against tumours, virus-infected cells, bacteria and parasites. This lytic activity is independent of T cells. For example, neonatal thymectomized mice do not have a particularly high incidence of spontaneous or carcinogen-induced tumours and they are also resistant to some microbial agents.¹¹⁻¹³ In addition, the resistance in vivo to the growth of implanted allogeneic tumour cell lines has been shown to be correlated with the level of NK activity in recipient animals, i.e. poorer tumour cell growth in recipients with higher NK activity and vice versa.^{14,15} NK cells also play a role against syngeneic autologous primary tumour cells as demonstrated by NK cell accumulation at the site of small spontaneous mammary carcinomas in mice as well as in small primary mouse tumours in mice that have been induced by murine sarcoma virus.¹⁶

NK cells may also be involved in surveillance against primary tumours as supported by the following findings: (1) A high incidence of lymphoproliferative diseases in patients with Chediak-Higashi syndrome, who have markedly reduced NK activity;¹⁷ (2) A high incidence of lymphoma in beige mice with selective deficit of NK activity; and (3) Depressed NK activity and high risk of tumour development among recipients of kidney transplants who have been treated with immuno-

suppressive drugs.¹⁸

Evidence has been accumulated that indicates a possible role for NK cells in virus infections; the genetic resistance of mice to severe herpes virus type I infections and Marek diseases has been shown to be associated with NK activity.^{19,20}

The contention that NK cells might participate in the body's defence against malaria was initiated by the findings of Eugui and Allison²¹ that shows the apparent association between genetic susceptibility to *P. chabaudi* and NK activity. Thus, strain-A mice with low NK activity have been shown to be susceptible while C57B1 and CBA mice with high NK activity have been shown to be resistant to *P. chabaudi* infection. Strain-A mice were also found to have another defect, namely, the mononuclear cells did not increase in number in the thymus-dependent areas of the spleen of malaria infected mice as they did in other mice.²² On the other hand, Wood and Clark²³ have shown that the increased NK activity of the spleen and peritoneal cavity of mice infected with *Babesia microti* or *P. vinckei* did not appear to be associated with the effectiveness of the host response against these parasites, since NK activity reached maximum level when parasitaemia was low and had decreased by the time the parasites reached peak densities. In addition, mice pretreated with ⁸⁹Sr beta estradiol

experienced the same pattern of infection as did control mice, yet the infection in pretreated mice induced much lower levels of NK activity. The course of *B. microti* infection was unaltered in beige mice, which are genetically deficient in NK cells. Thus, it was interpreted that NK cells were irrelevant to the resolution of infection by these organisms.²³ Allison and Eugui²⁴ argued that there may be a subset of NK cells not depleted in bg/bg mice and that these may function as effector cells against erythrocytic forms of plasmodia. NK activity in beige mice may be augmented by BCG stimulation.²⁵ This process is known to confer non-specific resistance in mice (except the A-strain) against *P. berghei* infection.²⁶ According to Allison and Eugui,²⁴ a subset of NK cells unaffected by the bg/bg mutation and responsive to BCG (but defective in strain-A mice) could participate in immunity against malaria.

The role of NK cells in human malaria is not clearly understood. Increased NK activity in the peripheral blood cells, assayed against K562 target cells, has been observed in children with falciparum malaria.²⁷ This increased NK activity was shown to be correlated with the degree of parasitaemia and the interferon level. The lytic activity of peripheral blood mononuclear cells in malaria patients was not enhanced by the addition of exogenous interferon, indicating that these cells had been activated already by interferons produced during the course of illness.²⁷ Our study showed that purified 'null' cells with a receptor for the Fc portion of IgG in adult patients with falciparum malaria had normal lytic activity against K562 target cells during the acute phase of the illness; during convalescence, the lytic activity markedly decreased.²⁸

Though NK cells appear to be involved in non-specific immunity against malaria, it is not known what mechanisms the NK cells exert to produce this effect. It is

most likely that NK cells act by causing intra-erythrocytic death of malarial parasites. In 1944, Taliafero and Taliafero²⁹ observed the appearance of 'Crisis forms' of *P. brasilianum* in *Cebus capucinus* monkeys at the time when immunity was becoming established. A similar observation was made about mice infected with *P. chabaudi* or *B. microti*.³⁰ Electron-microscopic observations have confirmed that the 'crisis forms' of babesia were actually degenerating intra-erythrocytic parasites.³¹ It is possible that NK cells could mediate the intra-erythrocytic killing of the parasites. The mechanisms whereby the parasites are killed are not known. The working hypothesis forwarded by Allison and Eugui²⁴ is that a small amount of the IgG antibody would react with the antigen present on the surface of infected red cells which adhere to the vascular endothelium.³² Upon contact with effector cells in the peripheral blood, e.g., NK cells which bear Fc-IgG receptors, binding occurs between the malaria antigen on the infected red blood cells and the antibody, thus, subsequently triggering the production of free oxygen radicals including superoxide (O_2^-), free perhydroxyl radicals (HO_2) or free hydroxyl radicals ($OH\cdot$). These free oxygen radicals are known to exert oxidant stress leading to the death of parasites inside red blood cells. This concept is supported by recent findings of Clark and associates;^{33,34} they showed that the administration of free oxygen radical generators such as alloxan or t-butyl hydroperoxide into mice infected with *P. vinckei* caused a rapid reduction in parasitaemia. In the presence of iron, these reactive oxygen intermediates induce changes leading to autoxidation of polyunsaturated fatty acids leading to cell membrane damage and the death of the malaria parasites. Since iron is important in the free oxygen radical mediated killing of these parasites, chelation of iron could adversely affect the parasitocidal

effect of oxidant stress. This proves to be the case since administration of desferrioxamine inhibits the inhibits the alloxan-mediated killing of *P. vinckei* in mice.³⁴

To understand more fully the role of NK cells in the body's defence mechanisms against malaria parasites, it is important to design experiments showing that an enriched NK cell population can cause intra-erythrocytic death of *P. falciparum* *in vitro* and that this effect is enhanced by interferon in the presence of IgG antibody against malaria. The associated respiratory burst in NK cells should also be demonstrated and the NK-cell-mediated killing is suppressed by the addition of an iron chelator in the *in vitro* test system.

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