Studies on the protective immune response to *Plasmodium chabaudi* in mice.

by

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CONTENTS

Page

SUMMARY

Inbred strains of C57Bl and NIH mice infected with the A/S strain of Plasmodium chabaudi usually developed high parasitaemias but infections were rarely fatal in immunocompetent mice and in most mice the parasites could be eradicated within 53 days or less. The immune response of C57Bl and NIH mice to infection with the A/S strain of P.chabaudi was studied. The principle method used in this study for investigating the immune response of the mice was to examine the immunity conferred on syngeneic mice, either X-irradiated or non-irradiated, by transferring to them lymphoid cells or serum from immune or semi-immune donors. The lymphoid cell populations examined were unfractionated spleen cells, nylon wool column enriched subpopulations of thymus-derived lymphocytes (T cells) and the so-called bursa-derived lymphocytes (B cells), bone marrow cells and phagocytic cells. In the course of these experiments observations were made on the effect of X-irradiation on the subsequent growth and multiplication of the parasite.

In addition, an in vitro assay for antibody-dependent cell mediated cytotoxicity was used to investigate the activity of splenic K cells during malaria infection. K cells are lymphoid cells which may include lymphocytes of an undefined category, but possess receptors for the Fc portion of antibody on their surface and have the ability to non-specifically lyse target cells coated in antibodies.

a) The adoptive transfer of immunity to P.chabaudi with immune spleen cells.

Spleen cells from mice which had previously been infected with P.chabaudi were able to confer some immunity on syngeneic mice which had been irradiated with 600 or 800 rads. The protection was detected as a

shortened patent parasitaemia in immune cell recipients compared to controls. The early experiments indicated the value of using irradiated recipients rather than non-irradiated recipients. In irradiated mice, a) smaller numbers of immune cells were required to promote detectable immunity than in non-irradiated mice, b) there was an amplification of the difference in the duration of primary parasitaemias in recipients of immune cells and normal cells compared to non-irradiated mice and c) as the irradiated host is immunodepressed, the protective effect of donor cells can be examined with a reduced contribution by the hosts own immune system.

An initial non-specific resistance to P.chabaudi infection was observed in irradiated mice, although the infection in most of these mice was subsequently more severe than in non-irradiated mice. The non-specific resistance could be reduced or abolished by injecting lymphoid cells into mice shortly after irradiation or by infecting irradiated mice more than 15 days after irradiation. Other workers suggest that following irradiation, the reticulo-endothelial system is stimulated at the time that the non-specific resistance to P.chabaudi was observed.

b) The adoptive transfer of immunity in syngeneic mice with enriched subpopulations of splenic immune T cells, B.cells, bone marrow cells and phagocytes.

Immunity to P.chabaudi could be adoptively transferred with enriched spleen subpopulations of immune T cells or immune B cells in mice which had been irradiated with 600 or 300 rads. The protective effects of unfractionated immune cells was, however, usually better than that of either immune T or B cell subpopulations. In most experiments enriched immune T cell recipients were more likely to suffer relapsing patent parasitaemias than either enriched immune B cell recipients or

unfractionated immune cell recipients. In one experiment a comparison was made of the course of P.chabaudi infection in mice which had been irradiated with either 600 rads or 300 rads and which received injections of different immune cells. A dose of 600 rads permits the immune system of mice to recover from the effects of irradiation, but a dose of 300 rads is lethal to mice unless lymphoid cells are injected after irradiation. It was found that in recipients of enriched immune T or B cells, which had been irradiated with 600 rads, the parasitaemia became subpatent before their equivalents irradiated with 300 rads, but that there was little difference in parasitaemias between recipients of unfractionated immune cells given 600 or 300 rads. Experiments in which enriched immune T cells and B cells were recombined and injected into syngeneic mice gave inconclusive results as to whether the immune subpopulations acted synergistically. Similar experiments in which immune subpopulations of lymphoid cells were recombined with normal subpopulations of lymphoid cells demonstrated that the latter cells did not enhance the protective effect of the former cells. Bone marrow cells from immune mice were able to confer some protection on syngeneic recipients, but were not as protective as enriched immune T cells or B cells. The results obtained in adoptive transfer experiments using phagocytic cells from the spleen of immune mice depended on the length of time spleen cells were incubated in petri-dishes at 37°C before harvesting the phagocytes. Using C57Bl mice, phagocytes harvested after 16 hours incubation were as protective as unfractionated immune cells in a cell transfer experiment, but phagocytes harvested after 16 hours incubation were not protective. Examination of NIH phagocytic cells after 2,5 hours incubation at 37°C, which were as protective as unfractionated immune spleen cells in a cell transfer experiment, demonstrated that the petri-dish adherent cells may have contained B lymphocytes.

c) The passive transfer of immunity with serum from P.chabaudi infected mice.

The passive transfer of serum from C57Bl mice which had previously been infected with P.chabaudi to normal or irradiated syngeneic mice demonstrated that the serum recipients were initially protected from infection. Irradiated mice, however, were delayed longer in the onset of parasitaemia compared to non-irradiated mice. Using NIH mice, sera were collected from unfractionated immune spleen cell recipients, enriched immune T cell recipients and normal spleen cell recipients on the 11th day of a P.chabaudi infection, just after peak parasitaemia, and also on the 14th day of infection. On day 14, all immune cell recipients and most of the enriched immune T cell recipients had become subpatent but all normal cell recipients still had patent infections. Sera collected from the different spleen cell recipients on the 11th day of infection and passively transferred to irradiated mice demonstrated little protection. Sera collected on the 14th day of infection, however, reflected the immune status of the donors in their protective properties in mice infected with P.chabaudi. The serum from unfractionated immune cell recipients was the most protective of the 3 sera when compared to normal NIH serum and the serum from enriched immune T cell recipients was slightly protective, but the serum from normal cell recipients produced an enhanced infection in mice infected with P.chabaudi.

d) Antibody-dependent cell-mediated cytotoxicity of spleen cells in P.chabaudi infected mice.

In a preliminary investigation of K cell activity in the spleens of P.chabaudi infected mice, it was found that there was an increased activity of K cells collected at around peak parasitaemia compared to the activity of K cells in non-infected mice, and that this increased activity could also be found in mice which had recently become subpatent. As the

target cell for antibody-dependent cell-mediated cytotoxicity employed was the chick red blood cell, it is not known whether the K cell is involved in the killing of P.chabaudi parasites.

These results suggest that both T cells and B cells and antibody may be important in the immune response to P.chabaudi in mice. Primed T cells may act as helper cells in the production of malarial antibodies, but, as enriched primed T cells could confer protection on immunodepressed mice, it is possible that a cell-mediated mechanism of immunity may also exist.

INTRODUCTION

1.1. General

Malaria is a major parasitic disease of man which can seriously influence the economy of endemic areas by its effect on the health of communities. Over one million people die each year in Africa alone of malaria and many more experience morbidity as a result of infection.

The parasite responsible for the disease is classified as the genus Plasmodium, suborder Haemosporina, order Eucoccida, subphylum Sporozoa, phylum Protozoa as revised by Honigberg, Balamuth, Bovee, Corliss, Gojdics, Hall, Kudd, Levine, Loeblich, Weiser and Weinrich (1964). Parasites which are closely related to the Plasmodidae are the suborders Haemoproteidae and Leucocytozoidae.

Plasmodidae are intracellular parasites for most of their life cycle and have 2 hosts - vertebrates and female anopheline mosquitoes. The geographical distribution of malaria is restricted to warm, humid areas which are suited to the development of the parasite in the mosquito.

In the vertebrate host there are 2 schizogonic cycles, one in fixed tissue cells and the other in red blood cells. A schematic diagram of the life cycle is given in Figure 1. A feeding mosquito injects sporozoites into the blood of a vertebrate from its salivary glands, and the sporozoites leave the blood probably within an hour (Fairley, 1947; Nussenzweig, Vanderberg, Senabria and Most, 1972). The sporozoite enters liver parenchymal cells in the case of mammals and, initially, lymphoid macrophage cells in the case of birds and reptiles. The sporozoites develop into exoerythrocytic schizonts and merozoites are released which invade erythrocytes of the blood. The

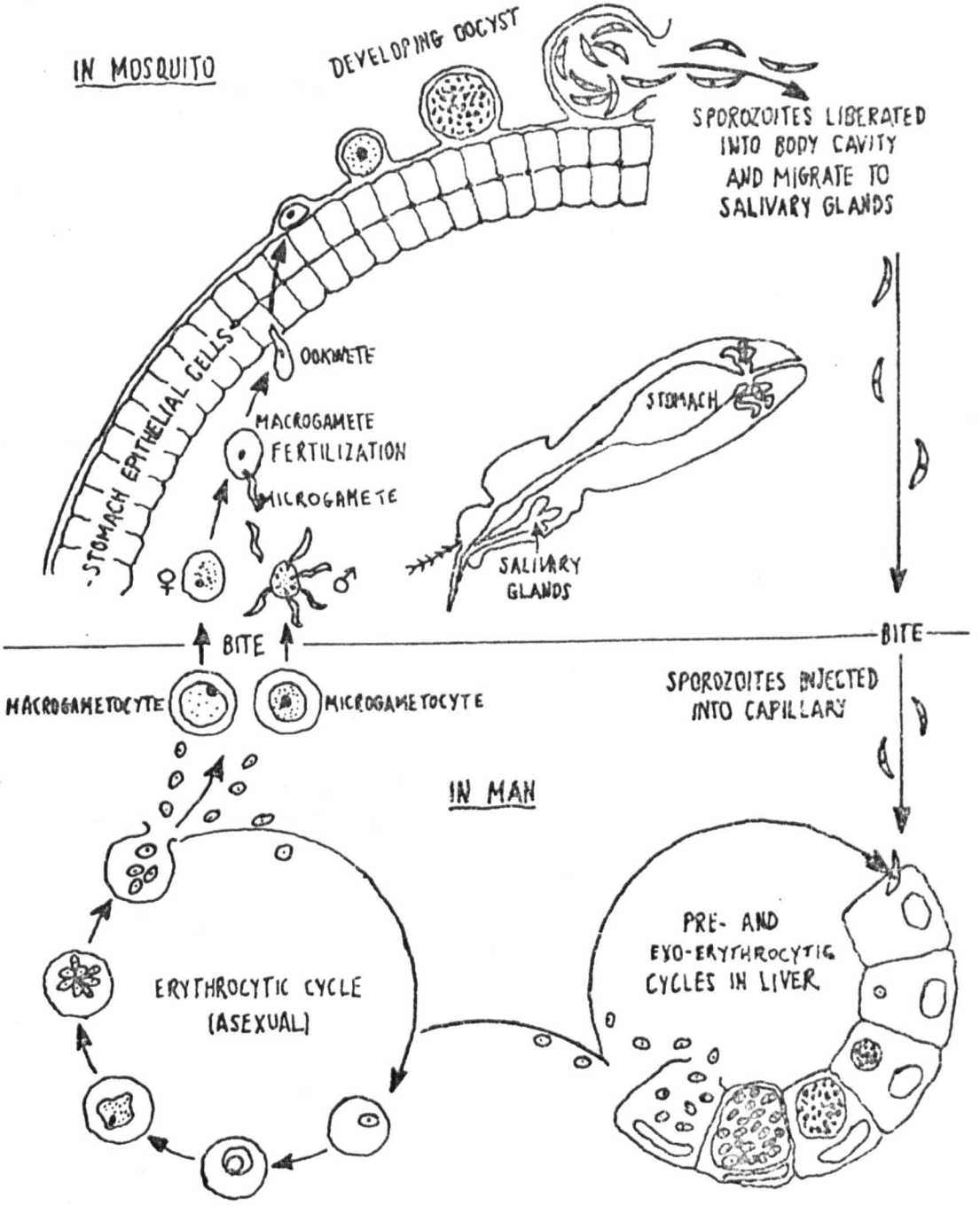


Figure 1.

The growth cycle of the malaria parasite

earliest form of parasite seen in Giemsa's stained smears of erythrocytes, resembles a signet ring and is called the "ring stage" or young trophozoite. The parasite enlarges, producing malarial pigment, until it goes through an erythrocytic schizogony, and merozoites are released into the blood stream which reinvade susceptible erythrocytes.

Gametocytes rather than erythrocytic schizonts can develop after merozoite invasion of erythrocytes. Gametocytes do not develop further until ingested by a female mosquito. In the stomach of the mosquito, male gametocytes (microgametocytes) produce after nuclear division several male gametes by a process known as "exflagellation". The male gametes swim free until they meet a female gamete (macrogamete). A single microgamete penetrates the macrogamete to form a zygote. The zygote elongates, becomes mobile (ookinete) and penetrates the epithelial lining of the stomach to form an oocyst. Sporogony occurs inside the oocyst and it eventually bursts, and many liberated sporozoites disperse to penetrate the salivary glands, where they remain until the mosquito feeds.

Laveran (1880) observed the malaria parasite within the erythrocytes of infected humans and Ross (1898) first described stages in mosquitoes. The entire development of the vector stages of the parasite was observed by Grassi and colleagues in 1889 (from Garnham, 1966). The exo-erythrocytic stages were first observed in mammals by Garnham (1947) and Shortt and Garnham (1948).

Four species of Plasmodium can infect man: P.falciparum, P.vivax, P.malariae and P.ovale. Of these four, P.falciparum is undoubtedly the most important as it is responsible for "malignant tertian" malaria or falciparum malaria which causes most fatalities

and morbidity of all four species. P.vivax is the most widely distributed species, occurring in both tropical and more temperate areas, and is the cause of "benign tertian" malaria or vivax malaria which is not usually fatal. P.malariae is not as common as P.vivax although it has a wide distribution; unlike P.falciparum or P.vivax which have 48 hour growth cycle in the erythrocytic stages, P.malariae has a 72 hour growth cycle and is known as "quartan" malaria. P.ovale has the most restricted distribution of these species and is the rarest of them to infect man. The growth cycle in the blood takes 48 hours for P.ovale. Like P.vivax, P.malariae and P.ovale are not usually fatal.

The symptoms of malaria infection include a high fever with shivering, often accompanied by body pains, vomiting and diarrhoea. These symptoms are caused by the release of toxic products from ruptured red blood cells. The infection is accompanied by anaemia, commonly the cause of death in falciparum malaria. The blood flow to body organs is decreased during infection. The spleen and liver become enlarged and many red blood cells and parasitized red blood cells are removed from the circulation by phagocytes. Malarial pigment is also removed from the circulation by the spleen and liver which causes these organs to be discoloured. Necrosis occurs in the kidneys which may be the result of a combination of a reduced blood flow to the organs and the deposition of antigen-antibody complexes. Haemorrhaging also occurs in the kidneys due to the rupture of blood capillaries caused by the blockage of capillaries by late trophozoites and schizonts. The blockage of blood capillaries throughout the body by parasites also increases the anoxia already resulting from a decreased blood flow and is partly responsible for the pathogenicity of P.falciparum.

For practical and ethical reasons it is often difficult to

experiment with human malarias. There are problems in adapting human malarias to different hosts, such as monkeys, because malaria parasites are host specific. Several non-human malarias, therefore, have been used for research purposes. For example, two primate malarias which naturally infect monkeys (Macaca irus), P.cynomolgi and P.knowlesi, are commonly used. Both of these species are able to infect man. P.gallinaceum, a parasite of jungle fowl, is often used in experiments with chickens.

1.2. Rodent species of malaria.

The first species of rodent malaria parasite to be identified was named P.berghei by Vincke and Lips (1948). This parasite was found in Thamnomys surdaster and was infective to laboratory mice and rats with a preference for development in immature erythrocytes, and having a 24 hour growth cycle in the blood. Four years later another rodent malaria, P.vinckei (Rodhain, 1952), was identified from the sporozoites of the mosquito Anopheles dureni. Rodhain believed the natural host of P.vinckei to be T.surdaster and in fact an infection in these animals, and laboratory mice, was often fatal. For P.vinckei to be adapted to Laboratory rats and hamsters, it was usually necessary to splenectomize animals. P. vinckei has a 24 hour growth cycle in the blood but, unlike P.berghei, it has no preference for immature red blood cells. Landau (1965) described a further two rodent parasites in the blood of Thamnomys rutilans, one parasite resembling P.berghei and named P.berghei yoelii and the other was named P.chabaudi. P.b.yoelii differed from P.berghei in aspects of both the exo-erythrocytic and mosquito development (Landau and Killick-Kendrick, 1966) and was later renamed P.yoelii yoelii (Killick-Kendrick, 1974). P.chabaudi, as described by

Landau, differs from P.berghei by its preference for mature erythrocytes, its larger nuclei and by the smaller numbers of nuclei in erythrocytic schizonts. Also, as with P.vinckei, it was difficult to establish infections in laboratory rats and hamsters. P.chabaudi, in fact, so resembled P.vinckei that it was classified as a subspecies of P.vinckei (P.vinckei chabaudi) by Bafort (1968). Carter and Walliker (1975) showed by morphological and enzymic differences that in cloning the parasites of P.chabaudi described by Landau, it could be shown that there were actually 2 different parasite populations present, a species named P.chabaudi and a subspecies named P.vinckei petteri. Both of these parasites have been found in T.rutilans, unlike P.vinckei. P.v.petteri is morphologically similar to the P.vinckei group, the erythrocytic trophozoite having a regular outline, few amoeboid forms, golden pigment and similar characteristic enzyme forms. P.chabaudi usually has a smaller trophozoite than the P.vinckei group, is more often amoeboid, with little pigment and a denser blue cytoplasm. Schizonts of P.chabaudi are different morphologically from those of P.vinckei and are usually smaller, as are the sporozoites. The enzyme forms of P.chabaudi are also different from that of the P.vinckei group.

The discovery of rodent malarias has been used to great advantage in malaria research and undoubtedly they have contributed much to progress in this field in recent years. Apart from financial considerations, rodents are easier to maintain than higher animals and can be used more readily for large scale investigations in chemotherapy and immunology. It should be pointed out, however, that extrapolation of results using a rodent malaria model to the human situation should be done with caution, and it is probable that investigations with simian malarias are often more suitable for this purpose.

1.3. Review of the immunology of malaria

Most work o n the immunology of malaria concerns the asexual stage of the blood and since the results presented in this study concern only this stage, the review will concentrate mainly on the immunology of this stage.

Immunity to malaria can be divided into 2 categories: innate immunity and acquired immunity. Innate immunity is often related to the genetic constitution of the host. For example, with human malaria it has been found that young children with the sickle cell trait are less susceptible to infection with P.falciparum (Allison, 1957) and young children deficient in the enzyme glucose-6-phosphate dehydrogenase are also less susceptible to infection (Allison and Clyde, 1961). Malaria parasites may require receptor sites on the surface of erythrocytes for entry into these cells and the Duffy blood group could be associated with such receptors: Duffy blood group negative human erythrocytes are resistant to infection by P.knowlesi in vitro whereas Duffy blood group positive human erythrocytes are susceptible to infection (Miller, Mason, Dvorak, McGinniss and Rothman, 1975). Moreover, negroes with a Duffy negative genotype (Fy Fy) are resistant to infection with P.vivax, which may correlate with the fact that the Duffy negative genotype occurs in high frequency in West Africa where P.vivax infection is rare (Miller, Mason, Clyde and McGinniss, 1976).

Increased resistance to P.berghei infection with age has been demonstrated in rats (Zuckerman and Yoeli, 1953). It has been reported that serum factors are not responsible for this age resistance (Smalley, 1975) although there is also evidence to the contrary (Tosta and Filho, 1976).

There is little known about the immune response to sporozoites. As the infective dose of sporozoites is probably only present in the bloodstream for less than 1 hour (Fairley, 1947; Nussenweig et al., 1972), it is possible that this is insufficient time to initiate an immune response, assuming the sporozoites are viable. It has been reported, however, that if a second dose of infective P.berghei sporozoites was injected into rats 8 - 36 hours after a primary dose, then the number of secondary exo-erythrocytic forms which develop from the second inoculum is less than half that expected for the number of sporozoites injected (Verhave, 1975). Acquired immunity to the sporozoite stage of infection can be induced in animals and man by the injection of sporozoite antigen. Russell, Mulligan and Mohan (1942) immunized chickens against P.gallinaceum with ground and dried, or ultraviolet light killed sporozoites. Excellent immunity to a homologous sporozoite challenge can be induced by X-irradiated sporozoites of P.berghei in mice (Nussenzweig, Vanderberg, Most and Orton, 1969), P.falciparum in man (Clyde, McCarthy, Miller and Hornick, 1973) and P.vivax in man (McCarthy and Clyde, 1977). Antibodies are produced as a result of sporozoite immunization which give a tail like precipitation with sporozoites in vitro, known as the circumsporozoite precipitation reaction or C.S.P. (Vanderberg, Nussenzweig and Most, 1969). The C.S.P. reaction has been demonstrated to parallel the level of immunity in P.vivax infections (McCarthy and Clyde, 1977) but it has been shown that the peak protection after immunization with X-irradiated sporozoites of P.berghei occurred before any C.S.P. antibody was detectable (Spitalny and Nussenzweig, 1973). It has also been shown that B cell deficient mice could be effectively immunized against sporozoite challenge with P.berghei without the appearance of C.S.P. antibody, but T cell deficient

mice could not be protected (Chen, Tigelaar and Weinbaum, 1977), suggesting that T cells may be more important in the immune response to sporozoites. Interferon may also have a role in the protection against sporozoite challenge, since mice treated with interferon inducers such as Newcastle Disease virus or statalon have been shown to be resistant to sporozoite challenge (Jahiel, Nussenzweig, Vanderberg and Vilcek, 1968).

Most evidence for immunity to the exo-erythrocytic forms of infection suggest that there may not be an effective immune response to this stage. Shortt and Garnham (1948) demonstrated that if homologous viable P.vivax sporozoites were injected into an immune human, the exo-erythrocytic forms appeared normal in the liver, although no patent parasitaemia developed. Similar results were obtained with P.berghei in rats by Yoeli (1966). Verhave (1975), however, demonstrated that the number of exo-erythrocytic forms was decreased in rats infected with the blood stages of P.berghei.

The persistence of malaria parasites in infected hosts for long periods with occasional patent relapses although the host may be clinically immune led to the theory of "premunition", which states that a low level of parasites was required to remain in the host in order to stimulate a strong immunity against reinfection (Sergent, Sergent and Donatien, 1933).

The importance of plasma factors in the protection against the blood stages of malarial infection was discovered by Coggeshall and Kumm (1937), who demonstrated the passive transfer of immunity to P.knowlesi and P.inui infections in monkeys by injecting serum from chronically infected (i.e. "immune") monkeys into monkeys suffering acute attacks of malaria: it was found that the parasitaemia and mortality in recipients was depressed. The passive transfer of protection with

serum has been demonstrated with avian malarias (Taliaferro and Taliaferro, 1940; Manwell and Goldstein, 1940) and rodent malarias (Zuckerman and Golenser, 1970; Phillips and Jones, 1972; Diggs and Osler, 1969; Jayawardena, Targett, Davies, Leuchars and Carter, 1975a; Hamburger and Kreier, 1975). The IgG fraction of immunoglobulin from the serum of immune donors can also be protective in humans (Cohen, McGregor and Carrington, 1961), monkeys (Butcher, Cohen and Garnham, 1970) and rodents (Diggs and Osler, 1969; Phillips and Jones, 1972). There is evidence that newborn children in malarious areas have high levels of [illegible] malarial antibodies which suggests that these antibodies cross between the mother and the placenta (McGregor and Wilson, 1971). Little is known about the complement requirement for immunity to malaria infection, but it has been reported that the third component is not required for immunity to P.berghei (Diggs, Shen, Briggs, Laudenslayer and Webb, 1972).

The reticulo-endothelial system has been recognised as being important for the removal of parasites from the blood during acute infection (Taliaferro and Cannon, 1936) and this has led to the theory that opsonizing antibodies could be important for enhancing phagocytosis. Increased phagocytosis of malaria parasites by macrophages in vitro has been observed with schizonts of P.knowlesi (Brown, Brown, Trigg, Phillips and Hills, 1970a) and free parasites of P.berghei (Chow and Kreier, 1972; Hamburger and Kreier, 1975). Immune serum confers less protection to splenectomized rats compared with intact rats against P.berghei infection (Brown and Phillips, 1974), suggesting the spleen is important for the phagocytosis of malaria parasites. Removal of the spleen can prolong infection in non-immune subjects and increase fatalities as has been shown with P.berghei infections in rats (Zuckerman and Yoeli, 1953) and P.inui in monkeys (Wyler, Miller and Schmidt, 1977). During a

primary infection, the spleen becomes enlarged and has an increased number of phagocytic cells and lymphocytes, and is more active in removing parasites from the circulation than the liver or bone marrow (Taliaferro and Cannon, 1936). The spleen, however, may be involved in the anaemia which is associated with malaria infection (McGhee, 1960; Zuckerman, 1960). This could result from stimulation of the reticuloendothelial system during malaria infection (Cantrell and Elko, 1970), producing a non-specific phagocytosis of red cells, or from the production of auto-antibodies against normal red blood cells by the infected host as observed in malaria infections (Zuckerman, 1960; McGhee, 1976; Lustig, Nussenzweig and Nussenzweig, 1977), although there is also evidence against the production of auto-antibodies (George, Stokes, Wicker and Conrad, 1966).

Lymphocytes of the spleen are involved in the development of a protective immune response to malarial infection. The adoptive transfer of lymphocytes from rats immune to P.berghei infection was found to confer protection on syngeneic recipients (Stechschulte, 1969; Roberts and Tracey-Patte, 1969; Phillips, 1970; Cabrera and Alger, 1971) and protective antibodies could be produced in these recipients (Phillips, 1970; Phillips and Jones, 1972). Similarly, immunity can be adoptively transferred between syngeneic mice with spleen cells from mice immune to P.yoelii (Jayawardena et al., 1975a). It has become clear that thymus-derived lymphocytes or T cells are necessary for the establishment of immunity. Removal of the thymus (Brown, Allison and Taylor, 1968; Stechschulte, 1969b; Chapman and Hanson, 1971; Jayawardena, Targett, Carter, Leuchars and Davies, 1977) enhanced rodent malarial infections as did treatment of rodents with anti-thymocyte serum (Spira, Silverman and Gaines, 1970; Brown, 1971; Barker and Powers, 1971; Bruce-Chwatt Dorrell and Tipley, 1972). In other investigations, however, with

fulminating infections of P.berghei, there is evidence that the depletion of T cells by thymectomy (Wright, 1968) or anti-thymocyte serum treatment (Sheagren and Monaco, 1969; Wright, Masembe and Bazira, 1971) allows rodents to survive longer, or has no effect at all on the course of infection (Seitz, 1976). T cells, therefore, may be involved in the pathogenesis of malaria infection. The stimulation of T cells occurs during P.yoelii infection (Jayawardena, Targett, Leuchars, Carter, Doenhoff and Davies, 1975b; Weinbaum, Evans and Tigelaar, 1976a). Delayed hypersensitivity reactions in the skin, associated with cell mediated immunity, have been demonstrated in monkeys immune to P.knowlesi (Phillips, Wolstencraft, Brown, Brown and Dumonde, 1970) and in the foot-pad during infection of cyclophosphamide treated mice with P.yoelii (Finerty and Krehl, 1976). Immunity can be adoptively transferred with enriched T cells from rats previously infected with P.berghei (Brown, Jarra and Hills, 1976; Gravely and Kreier, 1976). The lysis of P.berghei parasites in vitro in the presence of lymphoid cells with or without immune serum has been claimed in one investigation (Coleman, Renricca, Stout, Brissette and Smith, 1975). Also associated with a cell mediated type of immunity, a macrophage inhibition factor has been demonstrated as a result of P.yoelii infections in mice (Coleman, Bruce and Renricca, 1976).

The immune response to malaria infection may be complicated by the occurrence of immunosuppression during infection. The antibody response to heterologous antigens can be depressed during acute malaria infection (Salaman, Wedderburn and Bruce-Chwatt, 1969; Greenwood, Playfair and Torrigiani, 1971) and chronic infection (Wedderburn, Turk and Hutt, 1975; McBride, Micklem and Ure, 1977). The depression of the antibody response to tetanus toxoid occurred in young children in

malarious areas who had at least once during the investigation shown patent malaria infection (McGregor and Barr, 1962). A decrease in cell-mediated immunity has also been observed in mice infected with P.berghei, as they failed to reject skin grafts (Jerusalem, 1968; Sengers Jerusalem and Doesburg, 1971). Greenwood et al (1971), however, observed no change in skin graft rejection by mice infected with P. yoelii. The difference in results obtained with rodent malarias with skin graft rejection may be related to the results of Jayawardena et al. (1975b) who demonstrated that T cell activity was increased on a size of spleen basis (although not at the cellular level) during P.yoelii infections in mice whereas it was decreased during P.berghei infections, as measured by reactivity to a nonspecific mitogen. Nonspecific mitogens had a depressed effect on blood lymphocytes from malarious children (Moore, Hayworth and Brown, 1974) and on splenic lymphocytes in P.berghei infected rats (Spira, Golenser and Gery, 1976). Mice infected with rodent malaria have been found to be less able to produce an immune response against concurrent infections of the parasitic worm Trichurus muris (Phillips, Selby and Wakelin, 1974). Trypanosoma musculi (Cox, 1975) or oncogenic viruses (Salaman et al., 1969).

Greenwood, Brown, De Jesus and Holborrow (1972) attributed immunosuppression to the decrease in efficiency of lymphoid cells in transporting immune complexes to germinal centres of lymphoid tissue. Other workers have suggested that macrophages become defective in the initiation of antibody production to heterologous antigens (Loose, Cook and Di Lunzio, 1972; Warren and Weidanz, 1976). The depletion of T and B lymphocytes in lymphoid tissue during acute infection may affect the overall immune response (Krettli and Nussenzweig, 1974; Gravely, Hamburger and Kreier, 1976). The increased activity of splenic

suppressor cells to heterologous antigens during malaria infection should probably be considered as being an important mechanism of immunosuppression (Jayawardena, 1977). The high levels of gamma-globulin production associated with malaria infection (Cohen, McGregor and Carrington, 1961; Poels and van Niekerk, 1977), could be the result of nonspecific stimulation of B cells, perhaps by a B cell mitogen associated with malarial antigen (Greenwood and Vick, 1975), causing a reduced capacity of B cells to respond to any antigens which are introduced to the immune system. Alternatively, plasma cells, possibly resulting from the recruitment of B cells by a mitogen, have been shown to invade thymus dependent areas of lymphoid tissue during P.berghei infection in mice and may prevent the proliferation of T cells or their interaction with macrophages (Poels and van Niekerk, 1977). In no case, however, has immunosuppression been associated with the parasite itself. There is some indirect evidence that suppressor cells in the spleen of P.inui infected monkeys may prevent the eradication of infection since monkeys splenectomized during a chronic infection were able to eradicate their infection before non-splenectomized monkeys (Wyler et al., 1976). Similarly, rats infected with P.berghei and subsequently injected with spleen cells from syngeneic donors which had recovered from infection, took longer to eradicate their infection than controls (Phillips, Brown and Hill, in press).

The presence of soluble antigens circulating in the blood may also affect the immune response to the malaria parasite itself, possibly by antigenic competition which could produce a reduced immune response by the host, or by blocking an effector immune response to the parasite itself (Wilson, 1974). Soluble antigens were first described by Eaton (1939) who showed with an in vitro complement fixation test that, in P.knowlesi infected monkeys, the level of soluble antigen increased during

an acute infection and fell with a decrease in parasitaemia. Soluble antigens have been found to appear during P.falciparum infections as shown by precipitation test (McGregor, Turner, Williams and Hall, 1968) and these antigens have been physio-chemically and serologically characterized (Wilson, 1974). There is evidence that antigen-antibody complexes formed during P.falciparum and P.malariae infections are important factors in the high incidence of progressive nephropathies in malarious areas (Houba, Lambert, Voller and Soyanwo, 1975).

Antigenic variation of malarial parasites is a phenomenon which may be at least partially responsible for the prolonged survival of the blood stages of the parasite in an infected host. Using agglutinating serum with schizont infected cells obtained from various relapses during a P.knowlesi infection in rhesus monkeys, Brown and Brown (1965) demonstrated that different antigenic determinants appeared on the surface of schizont infected cells of different relapse populations. This suggests that the host must produce antibodies continually to counter the production of new parasite variants. Some evidence for antigenic variation by P.falciparum has also been obtained using the same serological test (Voller, 1971). There is indirect evidence from in vivo studies on P.berghei (Briggs, Wellde and Sadum, 1968) and P. cynomolgi bastianelli (Voller and Rossan, 1969) that these two parasites can also undergo antigenic variation.

One of the main objectives of research in malarial immunology is to produce vaccines which can effectively immunize man. Probably the most successful type of vaccine which has been developed to date is that containing X-irradiated sporozoites, mentioned previously, which can give complete intra-species protection against mosquito borne infections. Employing antigen from the erythrocytic stages of infection,

several lines of approach have been examined in vaccination studies. Partial protection was achieved with heat or formalin killed avian intraerythrocytic parasites (Gingrich, 1941) and with heat killed P.berghei intraerythrocitic parasites (D'Antonio, 1972). Resistance to infection could be produced in rats and mice infected with X-irradiated erythrocytic stages of P.berghei (Corradetti, 1966; Wellde and Sadum, 1967). Soluble antigens of P.berghei could also give protection in rodents (Jerusalem, Weiss and Poels, 1971; Hamburger and Zuckerman, 1976), but in these studies enhancement of parasitaemia could also be obtained in vaccinated animals. Carter and Chen (1976) using X-irradiated infected blood from P.gallinaceum infected chickens enriched in gametocytes, not only obtained shorter patent parasitaemias on challenge, but also demonstrated that the number of oocysts developing in mosquitoes after feeding on immunized chickens during an infective challenge was greatly reduced. This result introduced the possibility of the development of a vaccine which could break the transmission of malaria. Nonspecific resistance to P.yoelii and P.vinckei infection in mice could be obtained by injecting mice with 2×10^7 live organisms of Bacillus Calmette-Guerin (B.C.G.) (Clark, Allison and Cox, 1976). Interferon inducers such as Newcastle Disease virus may protect rodents against the blood stages of P.berghei (Schultz, Huang and Gordon, 1968).

Owing to the lack of immunogenicity of malarial antigen, adjuvants have been employed to enhance the immune response to the antigen. Vaccines consisting of soluble P.berghei antigen in conjunction with either saponin, hexylamine, Bordetella pertussis, levamisole and polyinosinic-polycytidylic acid produced lower mortality rates, reduced parasitaemias and shortened the course of infection in young rats (Desowitz, 1975). Good protection can be achieved with injections of

Freund's complete adjuvant (F.C.A.) incorporating dead schizonts of P.knowlesi in monkeys (Freund, Thomson, Sommer, Walter and Schenkel, 1945; Targett and Fulton, 1965; Brown, Brown and Hills, 1970b) freeze-dried P.knowlesi material in monkeys (Cabrera, Speer, Schenkel, Barr and Silverman, 1976), and with merozoites (Mitchell, Butcher and Cohen, 1974). The protection obtained with P.knowlesi antigen incorporated in F.C.A. is not strain specific but is species specific (Brown et al, 1970b). Other workers have found no protective effect of P.berghei antigen incorporated in F.C.A. with rodents (Desowitz, 1975; Reisen and Hillis, 1975).

To be effective, antimalarial vaccines may have to produce an immunity to infection which is effective against a wide spectrum of antigenically different parasites and, possibly, also an immunity that overcomes any adverse effects which the parasite may have on the host's immune system, such as immunosuppression.

Vaccines would necessarily promote an immune response to the antigen without producing undesirable immunological effects on the host, such as auto-immune reactions and granulomas caused by F.C.A. (Herbert, 1973), and for widespread use must be produced efficiently and economically.

Rationale for experimental studies

Most immunological studies of rodent malarias in recent years have involved the use of P.berghei or P.yoelii. There has been a certain amount of controversy with regard to the mechanisms of immunity and to the involvement of the different types of lymphoid cells. Very little information is available on immunological studies with P.chabaudi although a certain amount of knowledge has been acquired on this subject

using the closely related species P.vinckei. It was of interest, therefore, to compare the results of studies on the mechanisms of immunity to P.chabaudi with those of the other rodent malarias.

The role of T cells, B cells, bone marrow and phagocytic cells in the immune response to P.berghei or P.yoelii in rats and mice has been examined in adoptive transfer studies. In these studies, lymphoid cells or subpopulations of lymphoid cells were transferred from previously infected rodents into inbred syngeneic recipients. Similar experiments were carried out in this study using inbred strains of mice and P.chabaudi as the host and parasite models, respectively. In order to observe as far as possible the protective effect of primed donor lymphoid cells in recipient mice, recipients were first immunodepressed by X-irradiation, either partially or completely, before the injection of donor cells.

In further experiments, the development of humoral immunity in cell recipients was examined, and in addition, the effect on the growth and multiplication of parasites in X-irradiated mice was also observed.

As it has recently been suggested that cytotoxic cells may be responsible for the killing of malaria parasites (Coleman et al., 1975), in vitro experiments were also performed in which the non-specific antibody-dependent cell-mediated cytotoxicity of lymphoid cells from the spleens of malaria infected mice was compared to that of lymphoid cells from the spleens of normal mice.

As well as providing a comparison with investigations of the immune response to other rodent malarias, immunological studies with P.chabaudi may provide a suitable model system, the results of which could be extrapolated to malarias of higher animals and man. Knowledge of the mechanisms of immunity in mammals may lead to the development of

antimalarial vaccines which preferentially stimulate the cells which are responsible directly for killing malaria parasites or are responsible for inducing the killing of malaria parasites by the production of cytotoxic factors or antibody.

CHAPTER 2

MATERIALS AND METHODS

Parasite

Plasmodium chabaudi, the A/S strain, was isolated and cloned as previously described (Walliker, Carter and Morgan, 1973), and supplied by Dr. Walliker (University of Edinburgh). Infected blood was routinely passaged every 3 or 4 days in mice. Blood was normally removed by cardiac puncture from infected mice under ether anaesthesia with a syringe containing heparin (Evans Medical Ltd. or B.D.H. Chemicals Ltd.) in Hank's balanced salt solution (B.S.S.). The final concentration of heparin was adjusted to 10 or 17 units per ml. Heparinized blood was diluted, if necessary, in Hank's B.S.S. or medium 199 (Gibco-Biocult) for injecting intraperitoneally or intravenously into recipient mice as indicated in the text. Infected blood was kept on ice at 4°C before injection.

Storage and recovery of infected blood

Parasite populations which were not required for current experiments were frozen at -80°C for medium term preservation or at -196°C for long term preservation. Infected blood was added to an equal volume of 20% glycerol in 25% foetal calf serum (f.c.s., Gibco-Biocult) Krebs Ringer Solution (Krebs and Eggleston, 1940) and was aliquoted into capillary tubes or glass vials which were sealed on a bunsen flame. For freezing to -80°C the blood was placed in a thermos flask containing solid carbon dioxide. Flasks containing liquid nitrogen were used for preservation at -196°C. Before blood was placed into liquid nitrogen, it was left overnight in a thermos flask containing solid carbon dioxide.

Infected blood was rapidly thawed, by immersion of the glass container into water at room temperature, and drawn into a syringe for injection into mice.

Mice

The strains of mice used were inbred NIH mice (Anglia Laboratory Animals) and inbred C57Bl mice (Centre for Tropical Veterinary Medicine, Easter Bush). Mice were maintained in thermostatically controlled rooms at 21° ± 2°C with 12 hours light from 0800 - 2000h. and with a constant supply of Diet 41 (Oxoid)and water. Unless otherwise stated, the water contained 3g/litre of terramycin (Pfizer).

Rabbits

The New Zealand strain of rabbit was used (Ranch Rabbits, Crawley Down). The rabbits were maintained in the same way as mice except that they were supplied with Diet 18 (Oxoid) and had no terramycin in their drinking water.

X-irradiation of mice

Mice were exposed to 600 or 800 rads whole body X-irradiation using a Siemans II X-ray machine (300 K.V., 12 m.A., Thorens I filter, field size of 20 x 15 cm., source distance 50cm, producing approximately 112 rads per minute).

Adoptive transfer of lymphoid cells

Lymphoid cell suspensions were prepared from pooled spleens or bone marrow cells. For the preparation of lymphoid cell suspensions, spleens or hind legs were removed from mice which had been killed by cervical dislocation. These mice had been infected once or infected

and then reinfected homologously on one or more occasions. Lymphoid cells obtained from these animals are referred to as immune cells and lymphoid cells obtained from previously uninfected animals are referred to as normal cells. Within 1 hour or on the day following irradiation, irradiated mice were injected with syngeneic lymphoid cells either intravenously or intraperitoneally as indicated in the text.

Preparation of lymphoid cell suspensions and fractionation into subpopulations.

Spleens were removed from mice aseptically, and placed in a 9cm petri-dish containing 10ml of 5% f.c.s. medium 199 + 25mM Hepes with 10 units/ml of heparin, 100 units/ml of penicillin + 100µg/ml streptomycin (Glaxo). This supplemented medium 199 is referred to as 5% f.c.s. 199. The spleens were gently disrupted through a metal sieve (mesh size 60) into the 5% f.c.s. 199. Clumps of cells were broken up by passage through successively, a 21 gauge and 25 gauge needle. The cell suspensions were washed three times by centrifugation (250g) in 15ml conical centrifuge tubes and resuspended in 5% f.c.s. 199.

Enriched subpopulations of immune spleen cells were obtained by the nylon wool filtration method, based on the techniques of Julius, Simpson and Herzenberg (1973) and Trizio and Cudkowicz (1974).

Red blood cells were removed from spleen cell suspensions by adding 9ml of 0.83% NH_4Cl in Tris buffer, pH 7.4, to one ml of spleen cell suspensions containing approximately 10^9 cells. After 5 minutes incubation at room temperature or at 37°C, the cells were washed 3 times in 5% f.c.s. 199 and finally resuspended in 5ml of 5% f.c.s. 199. A glass wool column, made with a 5ml plastic syringe barrel filled to the 4ml mark with glass wool, was clamped in a vertical position and 30ml of

Hanks B.S.S. followed by 5ml of 5% f.c.s. 199 was washed through the column. The spleen cell suspension, freed of red blood cells, was passed through the glasswool column and washed out with 5% f.c.s. 199 at room temperature. The glasswool column removes some phagocytic cells, clumps of cells, dead cells and cell debris. After centrifuging once more, the cells were resuspended to give 1-2 x 10^8 cells/ml in 20% f.c.s. 199 warmed to 37°C. Two ml of this cell suspension were run into a nylon wool column in a vertical position which had been pre-incubated for 1.5 hours at 37°C, bathed in 20% f.c.s. 199. This column consisted of the barrel of a 10ml plastic syringe filled to the 6ml mark with tightly packed strands of scrubbed nylon wool (Travenol Laboratories). Before incubation at 37°C for 1.5 hours, the column was washed through with 15ml of 20% f.c.s. 199 and during incubation it was sealed at the top with parafilm and at the bottom with a 25 gauge needle stuck in a rubber stopper. A few drops of medium were added to cover the nylon wool after the cells had been dropwise run into the column, which was then resealed and again incubated at 37°C for 45 minutes. The non-adherent cells were run out dropwise from the syringe. These cells were washed out with 25ml of 20% f.c.s. 199 warmed to 37°C and are referred to as the enriched T cell subpopulation. Adherent cells were removed from the column by mechanical agitation of the nylon wool bathed in 20. f.c.s. 199, using a plunger from a 2ml plastic syringe to squeeze adherent cells out of the column. The released adherent cells were collected in 10ml of medium and these cells are referred to as the enriched B cell subpopulation. Both cell subpopulations were washed, resuspended in 5% f.c.s. 199 and kept on ice until injected into recipients. Cells passed through glass wool but not nylon wool columns are referred to as unfractionated spleen cells.

Enriched populations of strongly adherent cells were obtained by incubation at 37°C of 10^7 spleen cells in 10ml of 10% f.c.s. 199 in a 9cm plastic petri-dish. For a short incubation period of 1.5 - 2.5 hours, the petri-dishes were left in a 37°C incubator. For longer incubation periods, the petri-dishes were placed inside a dessicator with a 5% CO_2 + 95% air gas phase at 37°C. After incubation, non-adherent cells were removed by repeated washing of the surface of the petri-dish, and adherent cells were then released from the plastic surface by rubbing with a 2ml plastic syringe plunger into a small volume of 10% f.c.s. 199. The cells were washed 3 times in medium.

Bone marrow cells were obtained from the hind legs of mice. Marrow cells were forced out of bones with 1ml of 5% f.c.s. 199 from a 1ml plastic syringe. The cells were passed through the syringe once or twice to break up clumps and the cell suspension was washed 3 times and resuspended in 5% f.c.s. 199.

Cell viability

Viabilities of preparations of cells were measured by the trypan blue exclusion method (Weir, 1973). One volume of 0.2% trypan blue solution was added to 9 volumes of a cell suspension in 5% f.c.s. 199. The suspension was incubated for 5 minutes at room temperature and then a drop of suspension was put onto a glass slide and covered with a coverslip. The slide was examined under a light microscope with a x25 objective lens and x10 eye piece and viable cells were counted as those not taking up the dye. Viabilities of cells passed through glass wool and/or nylon wool columns were usually over 90% and always over 85%. Cell numbers for injection into mice were altered to take viability into account.

Immunofluorescence microscopy for identification of immunoglobulin-bearing cells.

One of the characteristics of B cells is the presence of immunoglobulin on the surface of these cells. B cells can be detected using ultraviolet microscopy by tagging the surface immunoglobulin with fluorescein conjugated antisera directed against the immunoglobulin, whereas T cells, although they may have some immunoglobulin in the cell membrane, do not have a sufficient amount available on the surface to demonstrate staining (Raff, 1970).

Cell suspensions were washed once and resuspended to give 5 x 10^6 - 1 x 10^7 cells in 0.25ml phosphate buffered saline (P.B.S.), pH 7.3. To each 0.25ml of cells was added 25µl of 1/10 fluorescein conjugated horse anti-mouse immunoglobulin (Progressive Laboratories Inc., Baltimore) diluted in P.B.S. After 45 minutes incubation on ice, the cells were washed 3 times in P.B.S. and resuspended to 0.1ml in P.B.S.

A drop of cell suspension was pipetted onto a clean glass slide, covered with a coverslip and placed under a Leitz Ortholux II microscope set for ultraviolet illumination, with a Wotan HBO 50W mercury lamp, 2 x KP490 exciting filters, a TK510 dichroic beam splitting mirror and a K515 suppression filter. Cells were examined under water immersion with a x50 objective lens and a x8 eyepiece. Numbers of immunoglobulin-bearing cells were detected by strong fluorescent staining under ultraviolet light.

Determination of T cell numbers

Suspensions of spleen cells in 10% f.c.s. 199, containing 10^6 cells were pelleted and 0.1ml of 1/40 rabbit anti-mouse thymocyte serum or normal rabbit serum diluted in 10% f.c.s. 199 was added to each pellet. The pellets were resuspended then incubated for 30 minutes at 37°C and

0.1ml of 1/10 guinea-pig complement (Wellcome) was added. After a further 45 minutes incubation the cell suspensions were washed once in 10% f.c.s. 199 and cell viabilities were checked with trypan blue.

Determination of numbers of phagocytic cells

A drop of cell suspension containing 5×10^6 cells/ml was mixed with a drop of polystyrene latex particles containing 2×10^9 particles/ml (1.8μ diameter, Dow Chemicals) on a clean glass slide. A coverslip was placed on top of the mixture and the slide was incubated for 10 minutes at 37°C. The phagocytic cells which took up latex particles were counted under a light microscope with oil immersion using a x100 objective lens and a x10 eye piece.

Preparation of sera

Mice and rabbits were exsanguinated under ether anaesthesia. Serum was removed from the blood after allowing it to clot for 1 hour at 37°C or overnight at 4°C. Any blood cells remaining in the serum were removed by centrifuging the serum and separating the supernatant from the pellet.

Preparation of rabbit anti-mouse thymocyte serum

Rabbit anti-mouse thymocyte serum was prepared by a method based on that of Rose, Parrott and Bruce (1976). A rabbit was injected intravenously 3 times with washed CBA thymocytes. Two weeks after the first injection of 3×10^8 cells, a second injection of 4×10^8 cells was given which was followed 1 week later by a third immunization of 6×10^8 thymocytes. The serum was heat-inactivated at 56°C for 20 minutes and exhaustively absorbed with CBA mouse red blood cells and liver cells, and this was repeated with NIH mouse red blood cells and liver cells.

Preparation of rabbit anti-chick red blood cell (C.R.B.C.) serum

A six month old rabbit was injected intravenously on alternate days over 18 days with 2ml of a 10% suspension of C.R.B.C.'s in P.B.S. and was exsanguinated on day 21. Control rabbit serum was obtained from the same rabbit, bled from the ear before immunization. The sera were heat-inactivated at 56°C for 30 minutes.

Examination of blood parasitaemia

Blood smears were made from the tails of mice after pricking the tip of the tail with a lance. If necessary, the lance was sterilized in alcohol between pricks, in order to reduce the possibility of passing infected red cells between mice. Fixing and staining was carried out in Coplin jars. Smears were left in 100% methanol for 5 minutes for fixing. They were then immersed in 10% Giemsa's stain (B.D.H. Chemicals) in a phosphate buffer, pH 7.2 (3g Na_2HPO_4 + 0.6g KH_2PO_4 per litre of distilled water). After 20 minutes the smears were removed, washed under the tap and allowed to dry. Parasitaemias were counted under oil immersion with a x100 objective and a x10 eye piece, and calculated as the number of parasitized cells per 10,000 red blood cells. A field which would normally be counted contained between 150 and 200 cells and for each count 2 or 3 fields would be examined. For parasitaemias below 1% more fields were examined and a negative parasitaemia would be considered to have been found if no parasites were observed in 50 fields. Parasitaemias were normally illustrated on graphs as $\log_{10}$ geometric mean parasitized red blood cells (p.r.b.c.) per 10^5 red blood cells unless otherwise stated in the text. Vertical bars giving standard deviations were placed on graphs where it was thought necessary and only when there were more than 4 mice in a group at the beginning of an experiment. On graphs where much data

was presented, standard deviations were put in only at critical stages of an experiment in order to maintain the clarity of the graphs.

Assay procedure for antibody-dependent cell mediated cytotoxity (K cell activity)

Mouse spleen cells were prepared as described previously, in 5% f.c.s. 199, after which they were incubated in 9cm petri-dishes for 2 hours at 37°C to remove adherent cells, and then they were washed and resuspended in 5% f.c.s. 199.

C.R.B.C.'s were obtained from 6-12 weeks old Leghorn chickens by cardiac puncture under ether anaesthesia and washed in P.B.S. 3 times. A suspension of 2×10^8 C.R.B.C.'s/ml in P.B.S. was prepared and 0.1ml of this suspension was added to 0.1ml of 100µCi ^{51}Cr labelled sodium chromate with specific activity in the range 100 - 350µCi/mg chromium (Radiochemical, Amersham). After incubation for 1 hour at room temperature the labelled C.R.B.C.'s were washed 3 times in P.B.S. and adjusted to 5×10^5 cells/ml in 5% f.c.s. 199.

Culture tubes were 15ml glass screw top tubes (Flow Laboratories) and each culture contained 0.4ml which consisted of 0.1ml C.R.B.C.'s, 0.1ml 5% f.c.s. 199, 0.1ml spleen cells and 0.1ml diluted rabbit anti-C.R.B.C serum or normal rabbit serum. Controls consisted of 10^6 C.R.B.C's and antiserum or normal serum in a volume of 0.4ml The cells were incubated at 37°C in 5% CO_2 + 95% air for 16 hours. After incubation, the cells were centrifuged and 200µl of supernatant was removed with an Oxford pipette. Both supernatants and pellets were counted on a gamma counter (I.C.N., Autogamma 500). The cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = \frac{2 \times \text{supernatant count} \times 100}{(\text{pellet} + \text{supernatant}) \text{ count}}$$

CHAPTER 3

The course of parasitaemia of P.chabaudi in laboratory mice

A full description of the blood stages of P.chabaudi is given by Carter and Walliker (1975) for laboratory mice and for the natural host, the thicket rat (Thamnomys rutilans). Some of the main characteristics of P.chabaudi, as described by Carter and Walliker, are presented in Chapter 1.2.

In this study, two strains of mice were used to investigate the effect on P.chabaudi of the immune response: the C57Bl and NIH strains. A typical pattern of mean parasitaemia is given for both strains of mice in Figures 2(a) and (b), respectively.

Six C57Bl males, 12 weeks old, were injected i.p. with 1×10^5 P.chabaudi p.r.b.c. and their parasitaemias were monitored for 32 days (Figure 2(a)). The mean parasitaemia rose steadily to a peak between days 7 and 9 of infection and then decreased. Between days 15 and 19 there was another increase in mean parasitaemia, peaking on day 19, after which the parasitaemias decreased until the mice became subpatent between days 23 and 27. One mouse had a brief patent relapse on day 30 but all other mice remained subpatent until day 32. The peak parasitaemias recorded ranged from 27% to 56% and none of the mice died from infection.

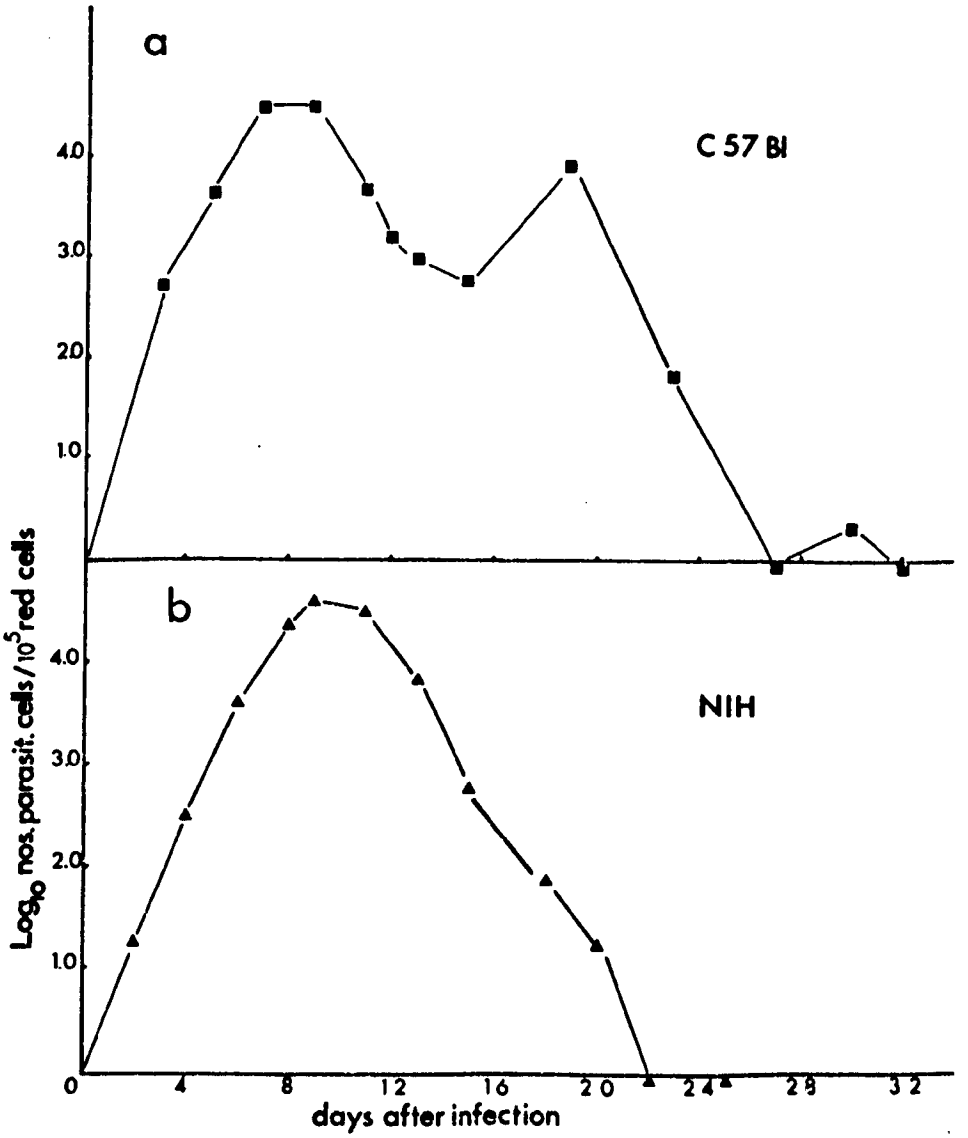
Groups of C57Bl females, splenectomized at different times after infection showed no recrudescence of parasitaemia 63 days after infection with 10^6 P.chabaudi p.r.b.c., suggesting that the infection was probably eradicated by this time. Only 1 mouse out of 4 had a recrudescence if splenectomized 53 days after infection.

Fig. 2(a).

The course of parasitaemia in C57Bl mice infected i.p. with 10^5 P.chabaudi p.r.b.c.(-■— ■-)

Fig. 2(b).

The course of parasitaemia in NIH mice injected i.p. with 2×10^6 P.chabaudi p.r.b.c.(-▲— ▲-)


a
C 57 Bl
4.0
3.0
2.0
1.0
b
NIH
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
32
days after infection
Log10 nos. parasit. cells / 10^5 red cells

Four NIH males, 13 weeks old, were injected i.p. with 2×10^6 P.chabaudi p.r.b.c. (Figure 2b). The parasitaemia came to a peak on day 9 and decreased until all mice were subpatent by day 22. The mice became subpatent between days 18 and 22. The peak parasitaemias ranged between 42% and 55%. Although there was no second peak in the mean parasitaemia with NIH mice, the downward slope of the graph after peak parasitaemia is humped after day 15, as a result of 1 of the 4 mice showing a brief increase in parasitaemia on day 18 and another showing a levelling off of parasitaemia around day 18. None of the mice in this experiment died from infection.

Death as a result of infection from the A/S strain of P.chabaudi in mice seldom occurred in immunocompetent mice, even if a very high parasite inoculum was given. For example, C57Bl mice could be infected with enough parasitized red cells to initiate an infection with a parasitaemia of 15% and survive the rapid onset of a peak parasitaemia.

Although no direct comparisons were made, there appeared to be little difference in parasitaemia between the 2 strains of mice employed, between mice 8 - 52 weeks old or between male and female mice.

Immunity to P.chabaudi, as measured by the ability of lymphoid spleen cells from previously infected animals to confer protection against infection on immunologically naive recipients (Phillips and Jones, 1972), was found to be retained in C57Bl mice for at least 112 days after infection and 148 days after reinfection. NIH mice were found in one experiment to retain immunity up to 173 days after reinfection. Reinfection of C57Bl or NIH mice after 4 or 5 months respectively, resulted in peak parasitaemias of 1 - 2%, demonstrating that the mice were still resistant to infection.

The asexual blood stage of P.chabaudi developed synchronously in C57Bl and NIH mice until peak parasitaemia. Schizogony occurred between 2200h and 0200h. During the early stages of infection there appeared to be no preference for reticulocytes. Five C57Bl males, 8 weeks old, infected with 10^5 p.r.b.c., appeared to have few reticulocytes parasitized, as observed in Giemsa's stained blood smears, until around peak parasitaemia when a reticulocytosis was evident (Table 1). The percentage of parasitized reticulocytes between days 7 and 10 increased with the increase in the numbers of reticulocytes in the peripheral blood. After peak parasitaemia there was a disturbance of the synchrony of infection, most marked between days 9 and 10 in this experiment.

From Giemsa's stained blood smears, the ring forms of the parasite were most abundant during the morning, with single and double chromatin dots. The rings developed into trophozoites during the late morning and afternoon. As late trophozoites developed into schizonts there was a tendency for them to withdraw from the peripheral blood, resulting in a noticeable decrease in blood parasitaemia. For example, in one experiment a C57Bl mouse with a parasitaemia of 17% at 1200h, had a drop in parasitaemia to 12% between 1200h and 2130h. The number of merozoites varied between 4 and 16 but very few schizonts were seen in the peripheral circulation with more than 8 nuclei.

From Table 1, it is evident that mature gametocytes were rarely seen in the peripheral circulation during the early stages of infection and in this experiment none were seen until day 7 of infection, which coincided with peak parasitaemia, when 1 or 2 gametocytes were usually seen per 10^3 red cells. The ratio of gametocytes to the percentage parasitaemia increased between day 7 and day 14 from .003 to .04 as the parasitaemia decreased. Mature gametocytes are strongly pigmented and easily distinguishable from other forms of the parasite.

Days of infection	% parasitaemia	% reticulocytes	% parasitized reticulocytes	ratio % parasit. retics: % parasitaemia	ratio % parasit gametocytes: % parasitaemia
3	0.21	1.7	0	0	0
5	5.1	2.7	0.06	0.01	0
7	4 2	6.0	0.86	0.02	0.003
9	2 6	19.9	6.6	0.25	0.0075
10	9.2	25.4	2.0	0.21	0.013
14	2.9	43.2	-	-	0.04

Table 1. The levels of reticulocytes, parasitized reticulocytes and gametocytes during the primary patent parasitaemia in C57Bl mice injected i.p. with 1×10^5 P.chabaudi p.r.b.c.

CHAPTER 4

Adoptive transfer of immunity to *P.chabaudi* using irradiated and non-irradiated mice as recipients

Billingham, Brent and Medawar (1954) referred to the transfer of immunity by lymphoid cells from animals which had previously received an antigenic stimulus to non-reactive hosts as "adoptive" immunity. Immunity can be transferred between animals in such a way that cell mediated and humoral immunity can be demonstrated in recipient animals (Billingham, Brent and Medawar, 1954; Mitchison, 1957).

In recent years, several workers have described the adoptive transfer of immunity to *P.berghei* infection in inbred rats (Stechschulte, 1969a; Roberts and Tracey Patte, 1969; Phillips, 1970; Cabrera and Alger, 1971) and to *P.yoelii* in inbred mice (Jayawardena et al., 1975a). All of these workers demonstrated that lymphoid cells from previously infected rodents could confer protection on syngeneic recipients against infection. The spleen, in particular, has been found to be a suitable source of immune cells, which may not be surprising in view of the importance of the spleen in the control and elimination of infection (Zuckerman and Yoeli, 1953; Todorovic, Ferris and Ristic, 1967).

Experiments were carried out to investigate the adoptive transfer of immunity of spleen cells from mice previously infected with *P.chabaudi* to syngeneic recipients. These experiments were performed using both normal mice and mice which had been exposed to 600 rads whole body X-irradiation. Lethal or sublethal X-irradiation treatment of an animal results in a depression of the immune response of that animal to an antigenic stimulus administered shortly after irradiation (Taliaferro and Taliaferro, 1951). The immunodepression of mice could

enable one to examine mainly the effects of syngeneic donor lymphoid cells in these mice on a P.chabaudi infection as there would be a substantially reduced contribution to the immune response to infection from the host's own immune system.

Experimental Procedure

Both C57Bl and NIH inbred mice were used in these experiments.

In the first experiment to be described, groups of 5 - 7 9 weeks old C57Bl males were either non-irradiated or were given 600 rads 2 days before infection. Each of the mice in these groups was injected with either 4×10^6 or 4×10^7 immune spleen cells intravenously (i.v.) or, 4×10^6 or 4×10^7 normal spleen cells i.v., on the day following irradiation. The immune spleen cells came from 5 C57Bl males which had been infected 3 times, the last time being 13 days before their spleens were used. The normal spleen cells came from 5 C57Bl males which were 9 weeks old. Recipients were injected intraperitoneally (i.p.) with 10^5 P.chabaudi p.r.b.c. In this experiment the mice received no terramycin in their drinking water.

In a second experiment, 9 weeks old NIH males in groups of 3 - 6 were used. The mice were either irradiated with 600 rads or non-irradiated and were injected with either 7×10^6 immune cells or 7×10^6 normal cells i.v., 1 hour after mice were irradiated. Recipients were injected with 2×10^6 P.chabaudi p.r.b.c. immediately after the injection of spleen cells. The immune spleen cells came from 3 NIH males which had been infected 3 times previously, the last time being 173 days before the spleens were used. Two NIH males, which were 9 weeks old and had not previously been infected, acted as normal cell donors.

Results

The mean parasitaemias are given for C57Bl irradiated and non-irradiated spleen cell recipients in Figures 3(a) and 3(b). There was a clear demonstration of protection in irradiated but not non-irradiated recipients of 4×10^6 immune spleen cells. Irradiated recipients of 4×10^7 immune spleen cells were also clearly protected and these mice became subpatent on day 20, 5 days before irradiated recipients of 4×10^6 immune spleen cells. Non-irradiated recipients of 4×10^7 immune spleen cells became subpapent on day 16 whereas non-irradiated recipients of 4×10^7 normal spleen cells became subpatent on day 23. Thus protection could be conferred on non-irradiated mice with a larger number of immune spleen cells.

Two important points emerge from these results. Firstly, the use of irradiated mice amplifies the difference in primary parasitaemia patterns between immune spleen cell recipients and normal spleen cell recipients as can be seen in Figures 3(a) and 3(b), Secondly, the onset of a patent parasitaemia in most irradiated mice occurred after that in the non-irradiated mice, indicating an initial resistance to infection in irradiated mice injected with either immune or normal spleen cells. Possibly associated with this resistance is the fact that irradiated immune cell recipients of 4×10^6 immune spleen cells had lower peak parasitaemias than irradiated recipients of 4×10^7 immune spleen cells (although the latter became subpatent before the former), although this could also have been at least partially due to an increase in the number of parasitized splenic erythrocytes being transferred with 4×10^7 immune spleen cells.

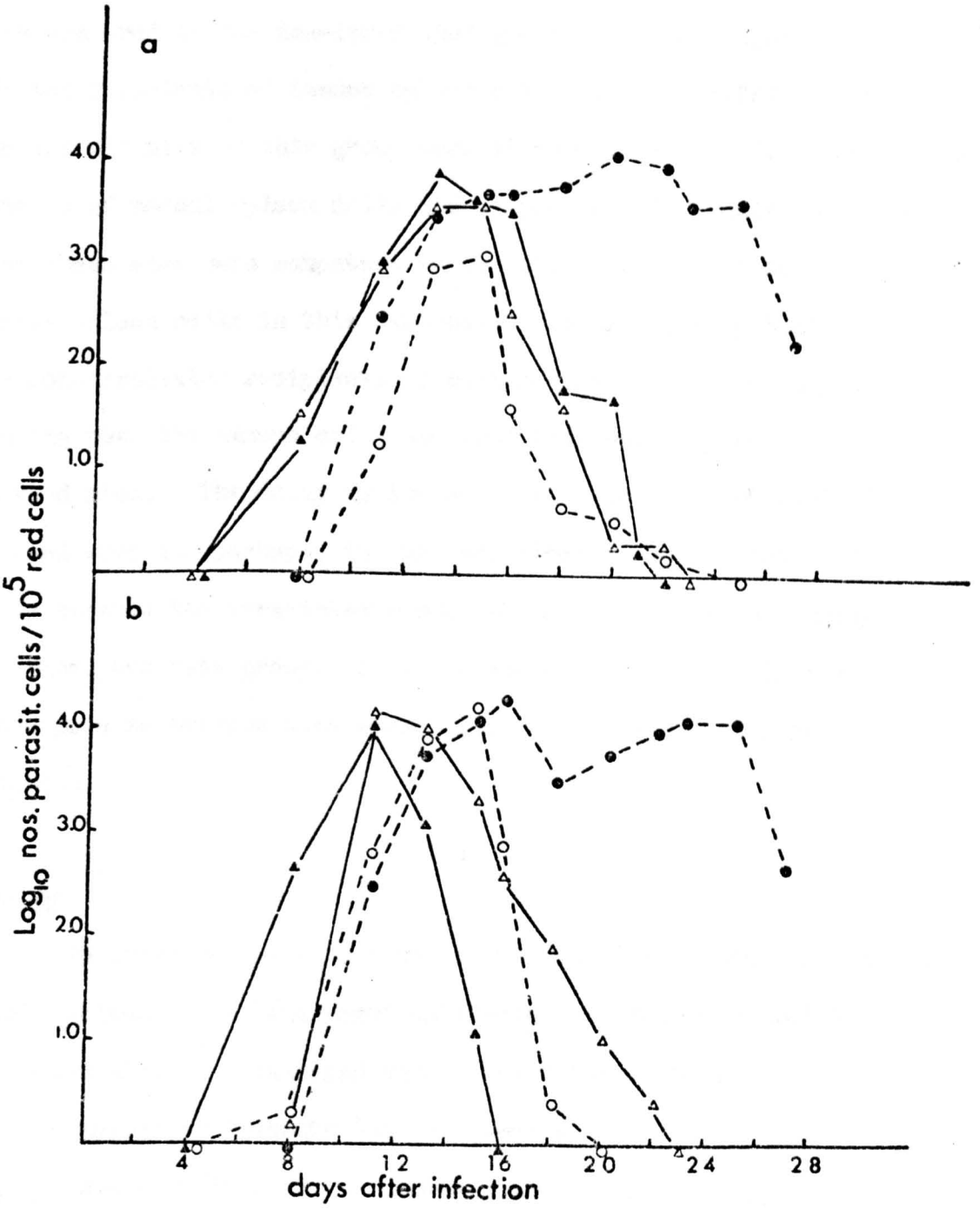
In the second experiment, with NIH mice, there was once more an amplification in the patterns of parasitaemia in the irradiated groups

Fig. 3(a).

The course of parasitaemia in C57Bl mice which were irradiated with 600 rads or were non-irradiated, and which were injected i.v. with 4×10^6 immune or normal spleen cells and injected i.p. with 1×10^5 P.chabaudi p.r.b.c.: --●--●-- 600 rads and normal spleen cells; --○--○-- 600 rads and immune spleen cells; —▲—▲— non-irradiated and immune spleen cells; -△—△- non-irradiated and normal spleen cells.

Fig. 3(b).

The course of parasitaemia in C57Bl mice which were irradiated with 600 rads or which were non-irradiated and which were injected i.v. with 4×10^7 immune or normal spleen cells and injected i.p. with 1×10^5 P.chabaudi p.r.b.c.: --●--●-- 600 rads and normal spleen cells; --○--○-- 600 rads and immune spleen cells; —▲—▲— non-irradiated and immune spleen cells; -△—△- non-irradiated and normal spleen cells.


a
b
4.0
3.0
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Log10 nos. parasit. cells / 10^5 red cells
4
8
12
16
20
24
28
days after infection

of mice compared to the non-irradiated groups of mice (Figure 4). The irradiated recipients of immune spleen cells were protected to some degree and all mice in this group were subpatent on day 13. Irradiated recipients of normal spleen cells had a more severe parasitaemia and none of these mice were subpatent by day 25. Non-irradiated recipients of immune spleen cells in this experiment had become subpatent 7 days before non-irradiated recipients of normal spleen cells, on day 15, indicating that the immune cells had conferred some protection on non-irradiated mice. The delay in the onset of patent parasitaemia in irradiated mice is, perhaps, in this experiment, not so clear cut, possibly because the irradiated normal spleen cell recipient group had only 3 mice, and both groups of non-irradiated mice had only 4 mice, but perhaps also because mice were infected on the same day as irradiation.

Discussion

In these experiments immunity was adoptively transferred with syngeneic spleen cells from previously infected mice to normal C57 and NIH mice and also to irradiated mice of both these strains. Such adoptive transfer of immunity has also been demonstrated in rats with P.Berghei (Stechschulte, 1969a; Roberts and Tracey Patte, 1969; Phillips, 1970; Cabrera and Alger, 1971) and in mice with P. yoelii (Jayawardena et al., 1975a).

The use of irradiated animals would appear to be advantageous for three reasons.

Firstly, a smaller number of immune cells was required for the expression of immunity in irradiated recipients than in non-irradiated mice, which is fortunate, as the number of cells which are obtained by

Fig. 4.

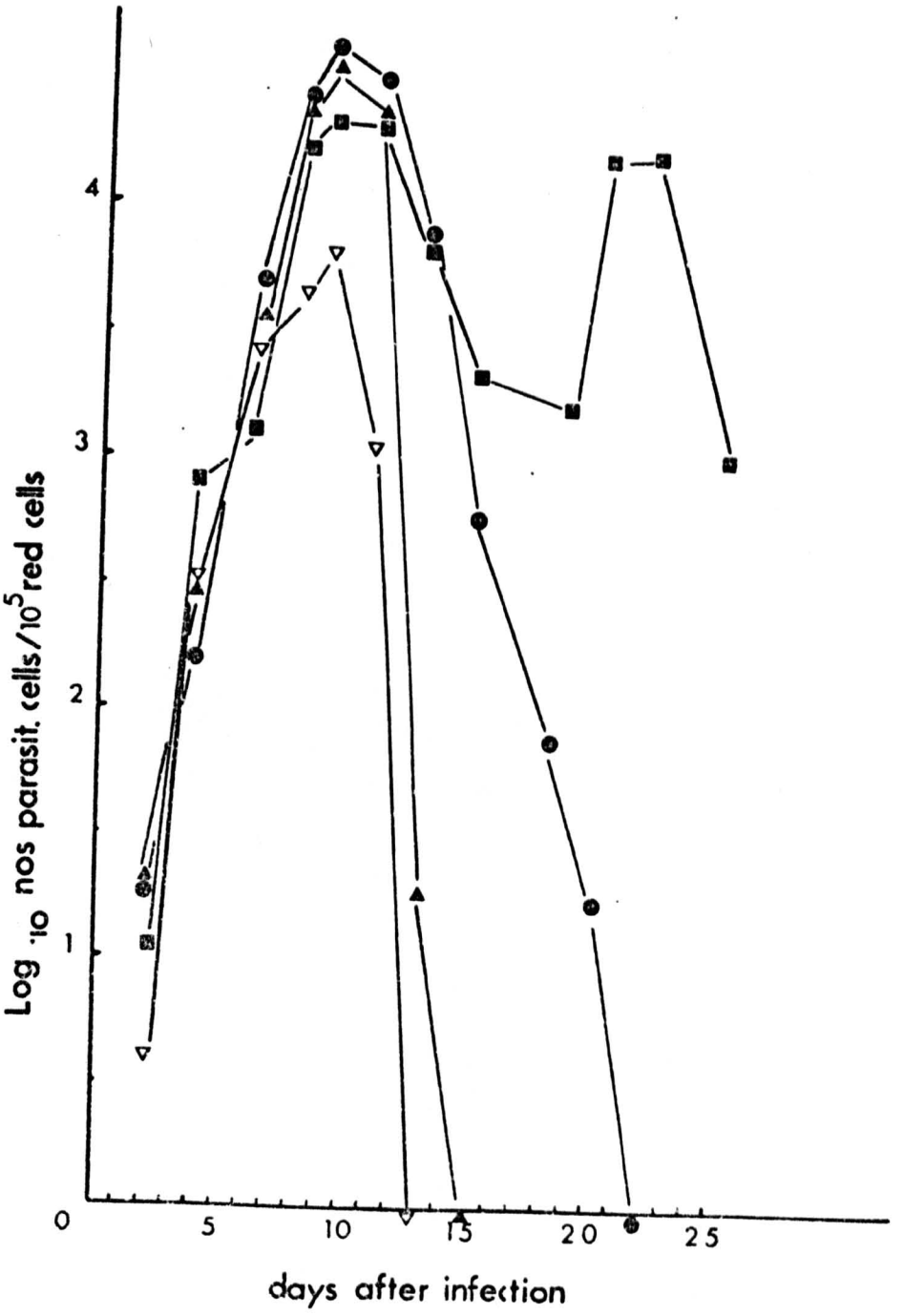
The course of parasitaemia in 600 rads irradiated and non-irradiated NIH mice which were injected i.v. with 7 x 10^6 immune or normal spleen cells and injected i.p. with 2 x 10^6 P.chabaudi p.r.b.c.

-■— ■- normal spleen cells - 600 rads recipients

-▽ — ▽- immune spleen cells - 600 rads recipients

-● — ●- normal spleen cells - non-irradiated recipients

-▲ - ▲- immune spleen cells - non-irradiated recipients


Log ·10 nos parasit. cells/10^5 red cells
0
1
2
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4
5
10
15
20
25
days after infection

fractionation is relatively small. This suggests that relatively small numbers of immune cells are more actively able to express immunity in irradiated mice than in non-irradiated mice. Irradiated animals given sensitized lymphoid cells within 24 hours of irradiation produce higher titres of specific antibody than do non-irradiated animals receiving the same cells, after the sensitizing antigen has been administered. (Mitchison, 1957; Harris, Harris, Beale and Smith, 1954; Cochrane and Dixon, 1962; Kornfeld and Weyzen, 1968). One possible reason for higher antibody titres in irradiated recipients, suggested by Cochrane and Dixon, is that due to the depletion of lymphoid tissue of radiosensitive lymphoid cells, the transferred sensitized lymphoid cells are able to proliferate more easily and subsequently give a better antibody response than in non-irradiated recipients. As the establishment of immunity to malaria has been associated with increased levels of protective antibody in P.berghei infected rats (Phillips and Jones, 1972), the above reasoning may provide one explanation as to why irradiated recipients of immune spleen cells can demonstrate some immunity to infection with a smaller number of transferred immune spleen cells than that required for protection in non-irradiated animals. Alternatively, irradiation may deplete mice of radiosensitive suppressor cells which control the immune response (Dutton, 1975).

The second advantage of the use of irradiated mice is the amplification of the difference in parasitaemias between irradiated recipients of immune cells and normal spleen cells. Irradiated animals which did not receive immune spleen cells had prolonged and more severe parasitaemias after infection with P.chabaudi, probably as a result of the immunodepressed host not being able to mount an efficient immune response with a small number of transferred unprimed lymphoid cells.

Thirdly, the experimental system is an extremely useful one with which to examine the protective activity of unfractionated lymphoid cells or fractionated subpopulations of lymphoid cells, and therefore, it could possibly increase knowledge of the mechanisms of immunity to P.chabaudi in mice.

Taliaferro, Taliaferro and Simmons (1945) described a higher susceptibility in irradiated birds to infection with P.gallinaceum or P.lophurae . Singer (1953), however, showed that irradiated mice had lower parasitaemias than non-irradiated mice when infected with P.berghei, although a reduction in the numbers of reticulocytes, in irradiated animals, which P.berghei preferentially invades, was believed to be the cause of this nonspecific resistance to infection. The initial resistance to P.chabaudi in irradiated mice may be related to a reduction in the number of reticulocytes, but this is unlikely as mice which have a phenylhydrazine induced reticulocytosis have a less acute P.chabaudi infection (Ott, 1968). However, it is possible that P.chabaudi could have a predilection for red blood cells of a particular age distribution which are depleted following X-irradiation.

Owing to the interesting nature of the initial nonspecific resistance of irradiated mice to P.chabaudi infection, and because it may have some importance in the immune response to infection, further investigations were made of the relationship between irradiation of mice and the subsequent infection of these mice (see Chapter 8).

CHAPTER 5

Transfer of immunity with fractionated enriched T cell and enriched B cell subpopulations of immune spleen cells

The role of different lymphoid cell types in the immune response to malarial infection has been the subject of controversy in recent years. In this respect, two main lines of investigation have been employed: prior to infection, animals have been depleted of lymphoid cell types and / or injected with spleen, lymph node, bone marrow or peritoneal lymphoid cells. The role of thymus-derived lymphocytes or T cells has been examined in animals by depleting them of T cells by thymectomy or by treatment with anti-thymocyte serum. Neonatal thymectomy (Brown et al., 1968; Stechschulte, 1969b) or anti-thymocyte serum treatment (Spira et al., 1970; Brown, 1971) of rats produces more acute P.berghei infections. Mice treated with anti-thymocyte serum and infected with a self-limiting strain of P.berghei (Barker and Powers, 1971) or with P. yoelii (Bruce-Chwatt, Dorrell and Tipley, 1972) also suffer more acute infections. Neonatally thymectomized hamsters (Wright, 1968) and antilymphocyte serum treated hamsters (Wright et al., 1971) or mice (Sheagren and Monace, 1969) survive longer with fulminating infections of P.berghei, suggesting that T cells could be involved in the pathogenesis of the infection. Congenitally athymic nude mice have more persistent and increased parasitaemias of P.yoelii than phenotypically normal mice (Clark and Allison, 1974; Weinbaum Evans and Tigelaar, 1976b). Bursa-derived lymphocytes or B cells have been removed from mice using an anti-μchain serum, and as a result suffered a markedly increased parasitaemia and mortality(Weinbaum, Evans and Tigelaar, 1976b). In other attempts to understand the role of T and B cells, either separately or in cooperation with each other, enriched populations of immune T or immune B cells were transferred to

syngeneic rats which were then infected with P.berghei (Brown et al., 1976; Gravely and Kreier, 1976) and to mice which were infected with P.yoelii (Jayawardena et al., 1975a). The results of these adoptive transfer studies suggested that both T cells and B cells are required for immunity to these rodent plasmodia.

In Chapter 2 it was seen that immunity to P.chabaudi infection could be transferred to non-immune irradiated mice with spleen cells from previously infected mice. The infection in the irradiated mice which were not reconstituted with immune spleen cells was enhanced because irradiation treatment depressed the host's own immune response. Thus, it would be possible to examine the protective effect of adoptively transferred subpopulations of enriched T cells or B cells in irradiated mice in the absence of a significant immune response from the host. Previous workers have either used completely immunocompetent animals (Brown et al., 1976; Gravely and Kreier, 1976) or animals competent in B cell function (Jayawardena et al., 1975a).

Subpopulations of enriched immune splenic T and B cells, separated on nylon wool columns (see Materials and Methods), were transferred to irradiated mice in an attempt to examine the protective effect of these cell subpopulations. It was hoped that these experiments would provide information on possible mechanisms of immunity to P.chabaudi .

Experiment 5.1.

Groups of 6 or 7 NIH female mice, 12 weeks old, were irradiated with 800 rads on day 0 and injected i.v. 1 hour later with either 1×10^6 unfractionated immune cells, 1×10^6 enriched immune T cells, 1×10^6 enriched immune B cells or 1×10^6 normal cells.

Immune cells were obtained from the spleens of 2 NIH females, infected on days -70 and -37 and normal cells were obtained from the spleens of 2, 12 weeks old NIH females. All mice were injected i.p. with 1 x 10^5 P.chabaudi p.r.b.c. immediately after the injection of spleen cells.

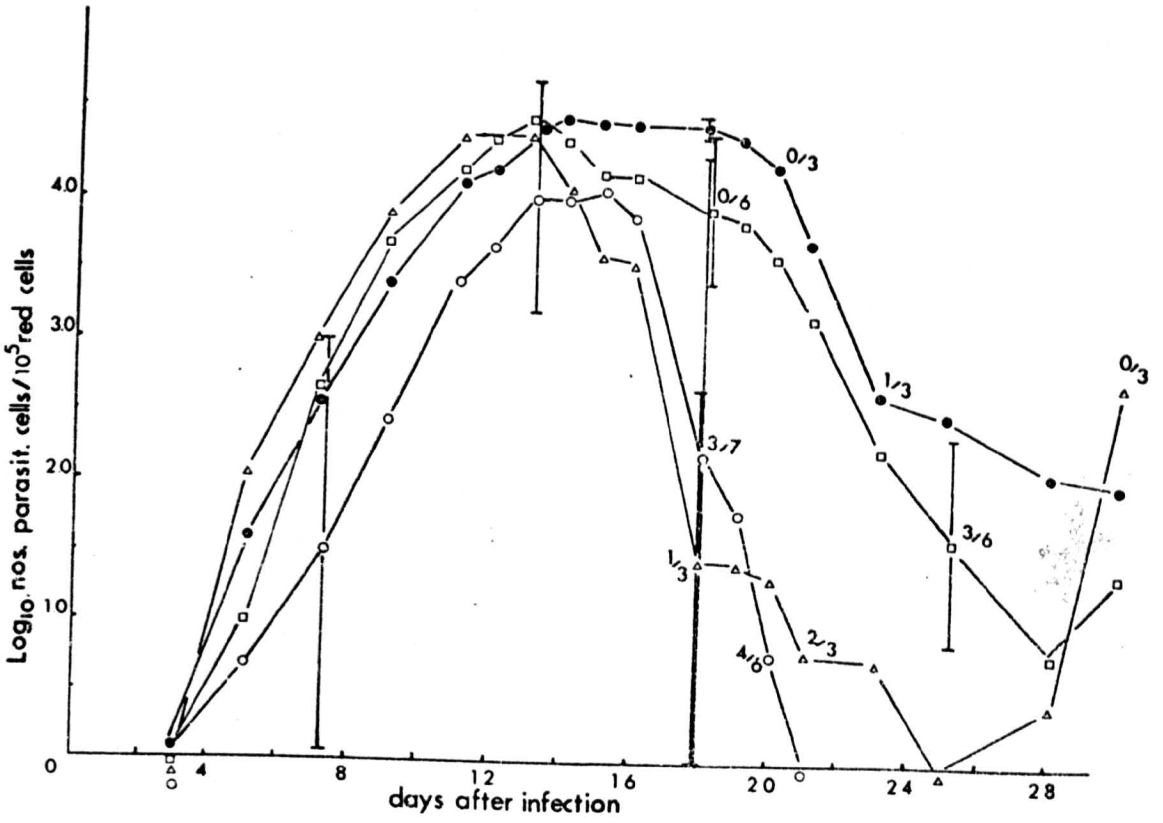
The number of immunoglobulin bearing cells dropped from 19% for the unfractionated immune cells to 1.5% in the enriched T cell subpopulations and increased to 66% in the enriched B cell subpopulation. The numbers of phagocytic cells were 16%, 5% and 2% for the unfractionated immune cells, the enriched immune B cells and the enriched immune T cells, respectively.

The geometric mean parasitaemias for spleen cell recipients with appropriately placed standard deviations are given in Figure 5. The best protection was obtained with unfractionated immune donor cells as the mice in this group became subpatent on day 21 and remained so throughout the rest of the observation period. Enriched immune B cell recipients, containing a threefold increase in numbers of B cells, appeared to be the next best protected group, becoming subpatent on day 25, although it should be pointed out that there were only 3 survivors in this group out of 6 mice by day 25. All of these 3 survivors had relapsed by day 30. The enriched immune T cell recipients were the least protected of the three groups of mice receiving immune cells. At no time during the first 30 days of infection were more than 3 mice out of 6 survivors subpatent. As expected, normal spleen cell recipients had the most persistent parasitaemias and only 3 mice survived out of 6 in this group.

In conclusion, it is evident from this experiment that the unfractionated immune cells, when adoptively transferred to 800 rads

Figure 5.

Course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads which were injected i.v. with either 1 x 10^6 unfractionated immune spleen cells -○— ○-, 1 x 10^6 enriched immune T cells -□ - □- , 1 x 10^6 enriched immune B cells -△ — △- , or 1 x 10^6 normal spleen cells -● — ●-. The mice were injected i.p. with 1 x 10^5 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group/ the total number of surviving mice in that group on a particular day .


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
days after infection
0/3
0/6
1/3
3/7
1/3
3/6
2/3
4/6
0/3

irradiated mice, conferred a higher degree of immunity than an enriched sub-population of immune B cells, which in turn may have conferred a higher degree of immunity than enriched immune T cells.

Experiment 5.2.

In a similar experiment, 16 weeks old NIH females were injected i.v. with with either 2 x 10^6 unfractionated immune cells, enriched immune T cells, enriched immune B cells or normal cells in groups of 6, 1 hour after irradiation with 800 rads on day 0. Immune spleen cells came from 2 NIH females which had been infected on days -135 and -16. Normal spleen cells came from 2 uninfected 25 weeks old NIH females. The irradiated recipients were injected i.p. on the same day with 2 x 10^6 P.chabaudi p.r.b.c.

The mean parasitaemias are given in Figure 6. Enriched immune T cells, enriched B cells and unfractionated immune cells contained 2%, 57% and 26% immunoglobulin-bearing cells, respectively. As in experiment 5.1 the recipients of unfractionated cells had shorter patent parasitaemias than any other group, becoming subpatent by day 12. The recipients of enriched immune T or immune B cells had similar parasitaemias, becoming subpatent by day 14 and in contrast to experiment 5.1, none of the mice in these 2 groups relapsed during the observation period of 26 days. Once again normal spleen cell recipients were the least protected mice, having prolonged primary parasitaemias.

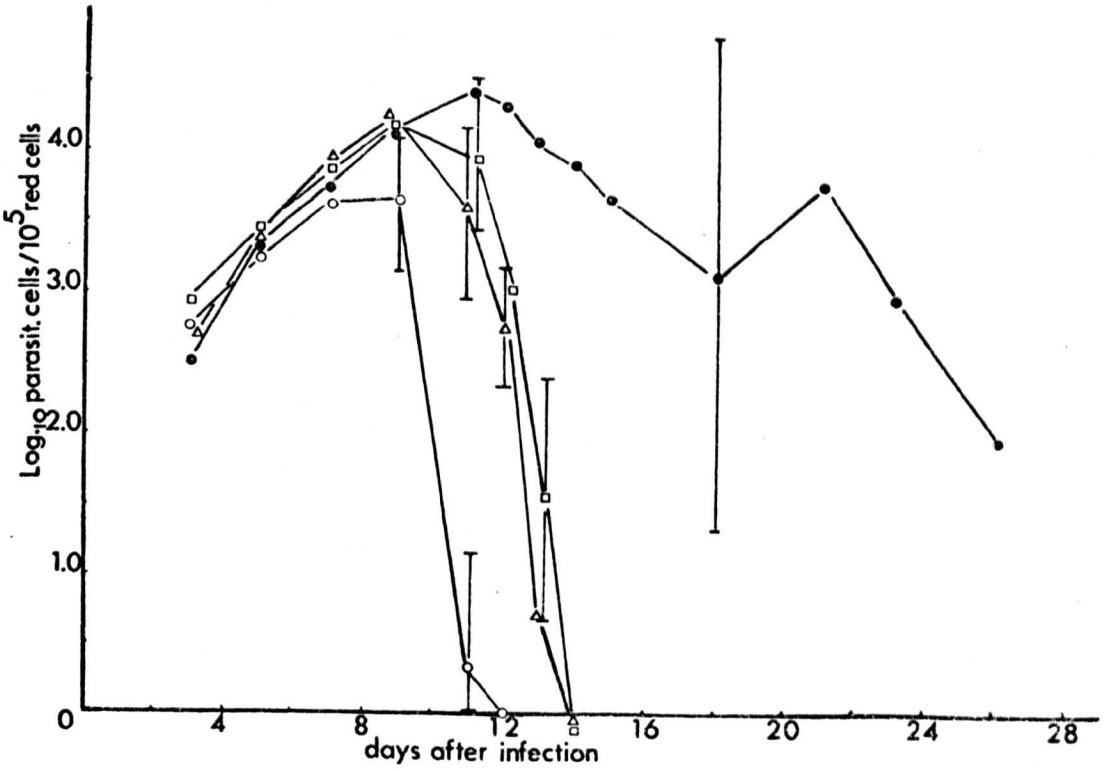
Cell transfer experiment using mice irradiated with 600 rads

Experiment 5.3.

Experiments similar to experiments 5.1. and 5.2. were performed using recipient mice irradiated with 600 rads. In one experiment to be described, the cell recipients were 32 weeks old C57Bl

Fig. 6.

Course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads and injected iv. with either 2×10^6 unfractionated immune cells -○ — ○-, 2×10^6 enriched immune T cells -□ — □-, 2×10^6 enriched immune B cells -△ — △-, or 2×10^6 normal spleen cells -● — ●-. The mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c.


Log10 parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
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4
8
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16
20
24
28
days after infection

females. The procedure in this experiment differed from experiments 5.1. and 5.2 in that the mice were irradiated 48 hours before infection and 20 hours before spleen cells were injected, and the spleen cells were injected i.p. Groups of 6 mice were injected i.p. with 1×10^6 lymphoid cells and infected by the same route with 1×10^6 P.chabaudi p.r.b.c. on the following day. Immune cells came from the spleens of 5 C57Bl females infected on days -70, -50, -38 and -21 and normal cells came from the spleens of 6 previously uninfected 14 weeks old C57Bl females.

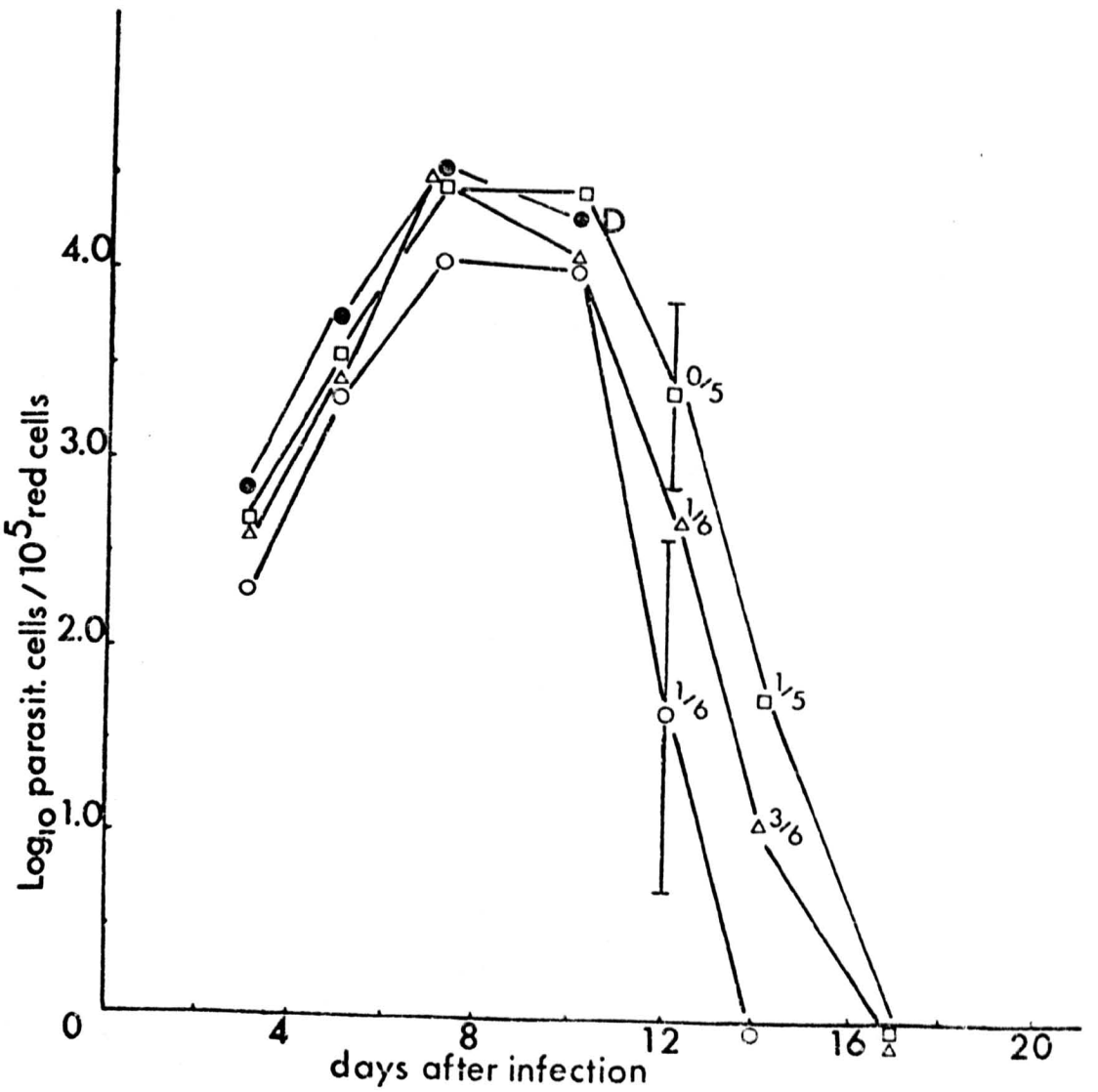
As in the previous experiments, the irradiated mice received either unfractionated immune spleen cells, enriched immune T cells, enriched immune B cells or unfractionated normal cells.

The mean parasitaemias for these groups are presented in Figure 7. The levels of parasitaemia were followed for 67 days.

The unfractionated immune cell recipients once more were the best protected group, becoming subpatent on day 14 of infection, and all mice in this group survived the infection. The enriched immune T cell recipients and the enriched immune B cell recipients had a similar pattern of primary parasitaemia, with mice in both groups becoming subpatent by day 17. There were only 3 survivors out of 6 mice, however, in each of these groups. Most mice in this experiment had relapses in parasitaemia 4 - 12 days after becoming subpatent but relapses in unfractionated immune cell recipients were of a shorter duration than in enriched immune T or immune B cell recipients. Between day 50 and day 67, a count of 2 parasites per 10,000 red blood cells was observed for one immune B cell recipient on day 50 and a count of 3 - 5 parasites per 10,000 red blood cells was observed for one unfractionated immune cell recipient between days 62 - 67, but all

Figure 7.

The course of parasitaemia of P.chabaudi in C57Bl mice irradiated with 600 rads, injected i.p. with either 1 x 10^6 unfractionated immune spleen cells -○ — ○-, 1 x 10^6 enriched immune T cells -□ — □-, 1 x 10^6 enriched immune B cells -△ — △-, or 1 x 10^6 normal spleen cells -● — ●-. The mice were injected i.p. with 1 x 10^6 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice in that group, on a particular day


4.0
3.0
2.0
1.0
0
Log_{10} parasit. cells / 10^5 red cells
4
8
12
16
20
days after infection
0/5
1/6
1/5
1/6
3/6

other mice in these 2 groups remained subpatent. Of the 3 surviving enriched immune T cell recipients, 2 had relapses between days 50 and 67 with parasitaemias reaching as high at 1% and 2.5%.

The results of this chapter will be discussed together with the results of chapter 6.

CHAPTER 6

Adoptive transfer of immunity with immune cell subpopulations: comparison of immunity in mice irradiated with 600 and 800 rads

In the previous chapter it was demonstrated that immunity could be adoptively transferred with enriched subpopulations of immune T or immune B cells to C57Bl mice which had been irradiated with 600 rads and in NIH mice which had been irradiated with 800 rads. The results of these experiments were essentially similar, with unfractionated immune spleen cells providing the best protection, but enriched immune T or B cells provided some protection.

Eight hundred rads is a lethal level of radiation for mice unless irradiated animals are reconstituted with viable syngeneic lymphoid cells. The immune response to infection in mice given 800 rads must, therefore, be to a large extent dependent on the lymphoid cells injected. Mice receiving a dose of 600 rads generally survive without reconstitution with lymphoid cells and can also survive P.chabaudi infection (see chapter 7). This suggests that C57Bl and NIH mice recover from the immunosuppressive effect of 600 rads sufficiently to control an infection of P.chabaudi.

It was therefore of interest in the type of experiments performed in chapter 5, to compare the results obtained after injecting the same cell populations into mice irradiated with either 600 or 800 rads. In this way it may be possible to assess the contribution of the hosts own immune system to protection against P.chabaudi in mice irradiated with sublethal doses of X-rays.

The experiment was carried out with NIH mice as it was also

desirable to demonstrate protection with subpopulations of enriched immune T and immune B cells in NIH mice given 600 rads.

Experimental procedure

For both doses of irradiation, 16 weeks old NIH females were injected i.v. in groups of 5 or 6 mice with either 1×10^6 unfractionated immune cells, 1×10^6 enriched immune B cells, 1×10^6 enriched immune T cells or 1×10^6 normal spleen cells. The immune cells came from the spleens of 3 NIH females which had been infected 3 times with P.chabaudi, the last time being on day -65. The normal cells came from the spleens of 2 NIH females which were 16 weeks old. The recipients were injected i.p. with 1×10^5 P.chabaudi p.r.b.c. immediately after the injection of spleen cells.

The number of immunoglobulin-bearing cells in the unfractionated immune cells, the enriched immune B cells and the enriched immune T cells was 24%, 52% and 1.3%, respectively.

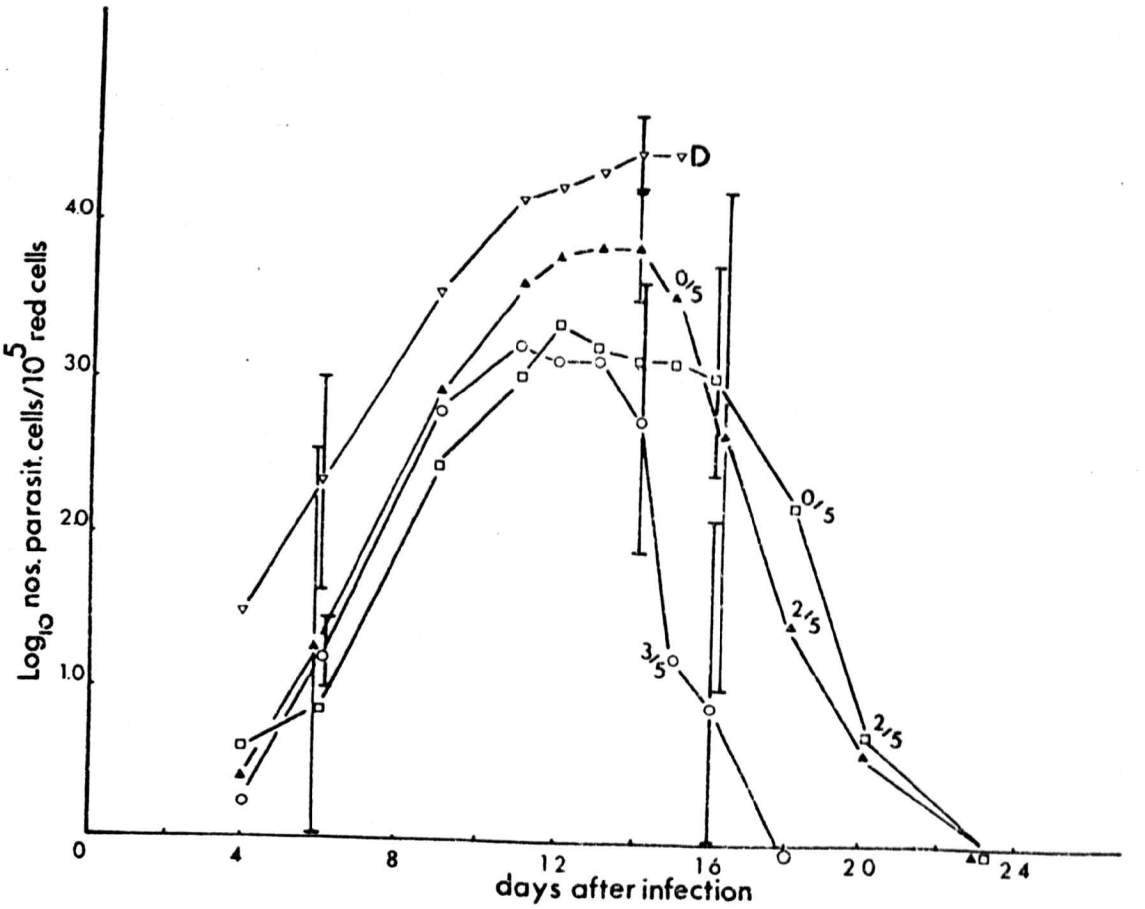
Figures 8 and 9 illustrate the primary patent parasitaemias for mice given 800 rads and 600 rads, respectively.

For mice given 800 rads the mean parasitaemias were essentially similar to those in experiment 5.2. The unfractionated immune cell recipients had shorter patent parasitaemias, becoming subpatent by day 18, whereas the 2 groups receiving enriched subpopulations of immune cells did not become subpatent until day 23.

Between days 35 and 47, 3 out of 5 enriched immune T cell recipients relapsed, although none of the enriched B cell or unfractionated immune cell recipients did so. Normal cell recipients given 800 rads died between days 11 and 18 of the acute infections suffered.

Fig. 8.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads, which were injected i.v. with either 1 x 10^6 unfractionated immune spleen cells -○— ○- , 1 x 10^6 enriched immune T cells -□— □- , 1 x 10^6 enriched immune B cells -▲— ▲- or 1 x 10^6 normal spleen cells -▽— ▽- . The mice were injected i.p. with 1 x 10^5 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice of that group, on a particular day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4.0
3.0
2.0
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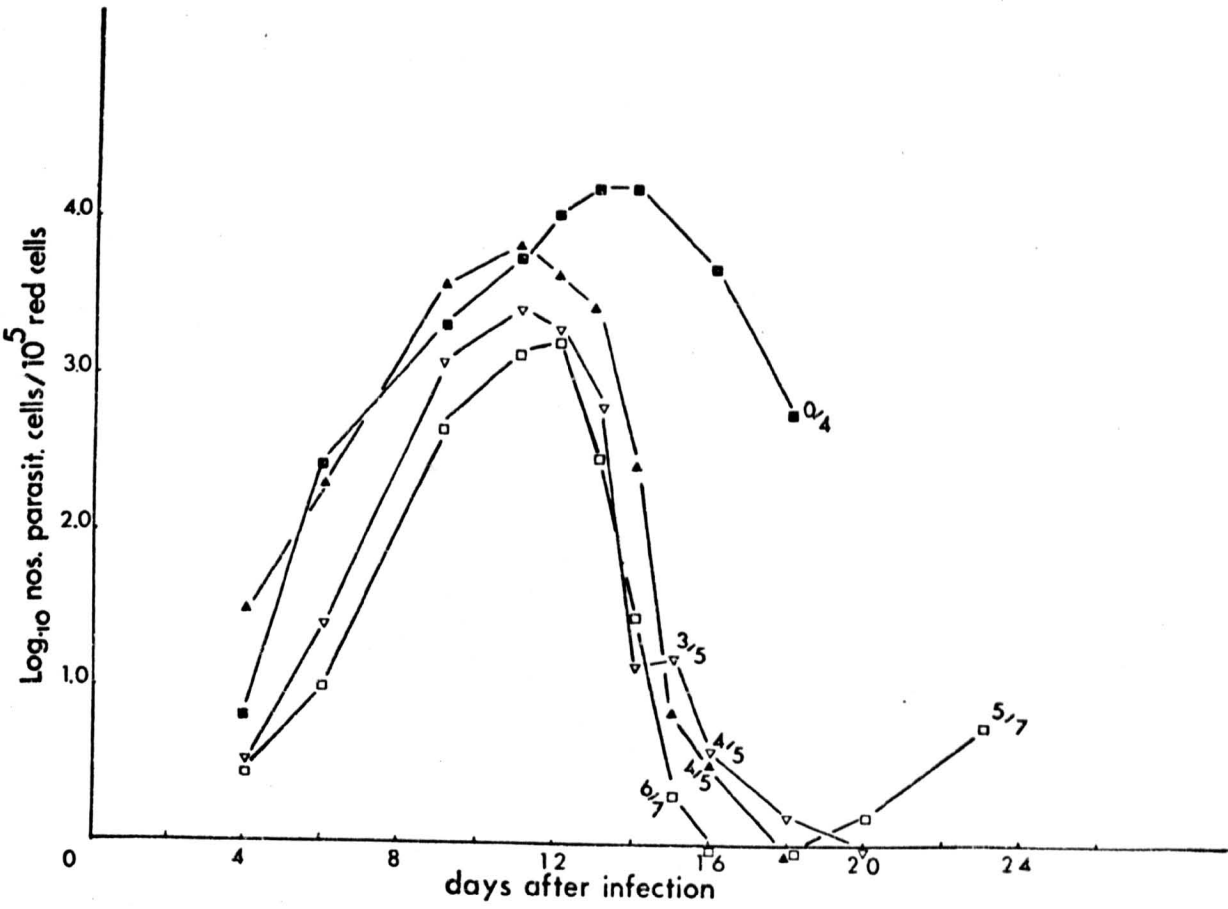
4
8
12
16
20
24
days after infection
Log10 nos. parasit. cells/10^5 red cells

Fig. 9.

The course of parasitaemia of P.chabaudi in NIH mice, irradiated with 600 rads, which were injected i.v. with either 1 x 10^6 unfractionated immune spleen cells -▽— ▽- , 1 x 10^6 enriched immune T cells -□— □- ,1 x 10^6 enriched immune B cells -▲— ▲- or 1 x 10^6 normal spleen cells -■— ■- . The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice of that group

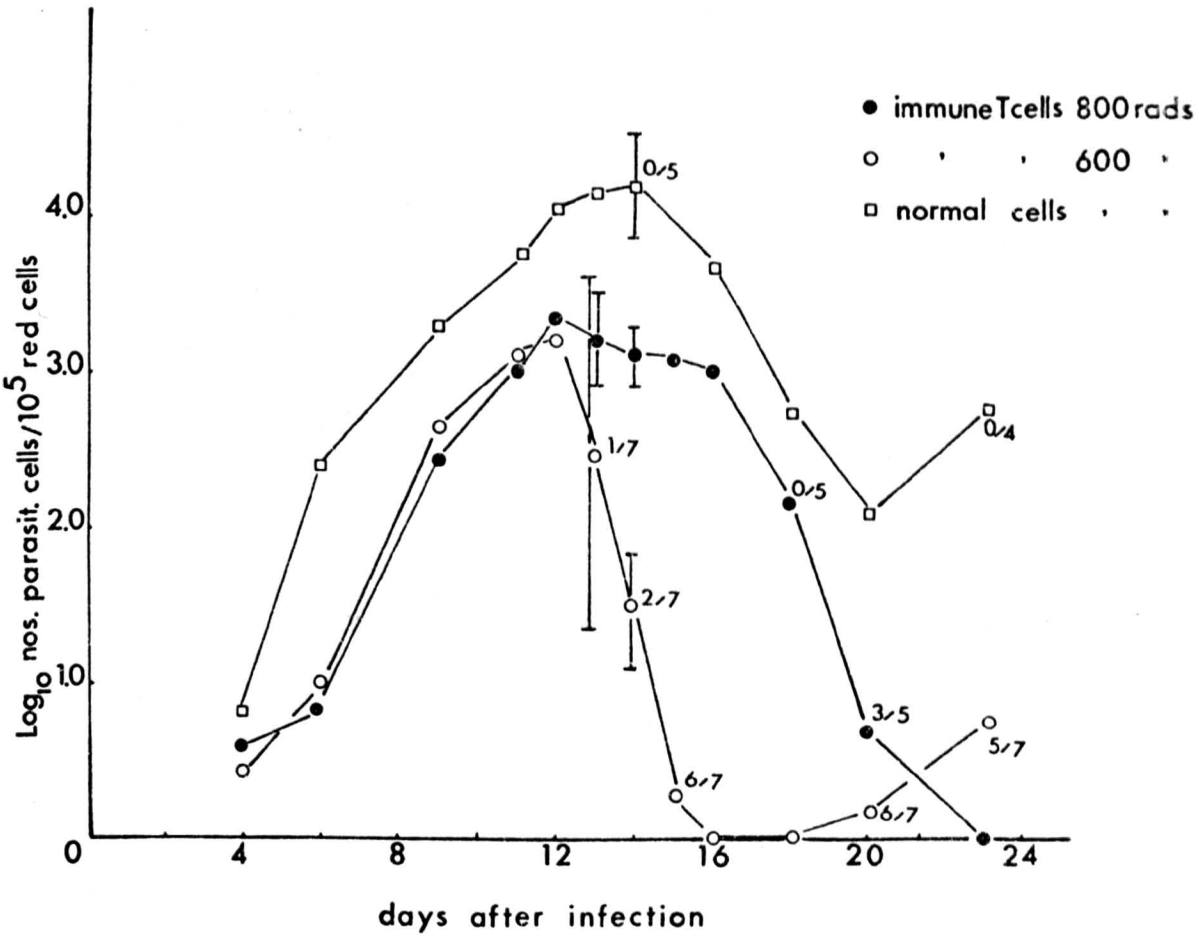

Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
days after infection
0/4
3/5
4/5
4/5
6/7
5/7

In mice given 600 rads, recipients of normal cells were again the least protected of the groups (Figure 9), although 3 out of 5 mice survived the infection. In contrast to the mice given 800 rads, the primary patent parasitaemia in recipients of the different preparations of immune cells followed a similar course. The primary parasitaemia in one mouse of the unfractionated immune cell recipients and one of the enriched immune B cell recipients were delayed in becoming subpatent. This gives the impression in Figure 9, that the enriched immune T cell group had slightly better protection, but apart from the 2 individual mice from the unfractionated immune cell group and the enriched immune B cell group, there was little difference between the groups receiving immune cells. The enriched immune T cell recipients relapsed between days 20 and 42, whereas only 1 mouse from the unfractionated immune cell group relapsed and none of the enriched immune B cell group relapsed.

A comparison of the course of parasitaemias of the enriched immune T cell recipients given either 600 rads or 800 rads is given in Figure 10. Also included is the course of parasitaemia for normal cell recipients given 600 rads. The enriched immune T cell recipients given 600 rads became subpatent on day 16, eight days before those given 800 rads. By day 23, however, 2 of the 600 rad group had relapsed. Within 13 days of becoming subpatent, 3 of the 7 enriched immune T cell recipients given 600 rads had relapsed, while only 1 of those given 800 rads had relapsed within 15 days of becoming subpatent. By day 35 all but one of the enriched immune T cell recipients given 600 rads had relapsed whereas only one of those given 800 rads had done so. None of the recipients of normal cells given 600 rads became subpatent within the first 30 days of infection and 3 animals in this group had died by day 30.

Fig. 10.

Comparison of parasitaemias of P.chabaudi in NIH mice irradiated with 600 or 800 rads, injected i.v. with 1 x 10^6 enriched immune T cells, and also 600 rads irradiated mice injected i.v. with 1 x 10^6 normal spleen cells. The mice were injected i.p. with 1 x 10^5 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice in that group, on a particular day


immune T cells 800 rads
600
normal cells
Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
days after infection
0/5
1/7
2/7
6/7
0/5
0/4
3/5
5/7
6/7

As with recipients of enriched immune T cells, enriched immune B cell recipients given 600 rads became subpatent before those given 800 rads. There was little difference, however, between the unfractionated immune cell recipients given 600 rads or 800 rads except that the mice given 600 rads tended to have higher peak parasitaemias.

Discussion

Subpopulations of immune cells containing enriched immune T or enriched immune B cells, derived from the spleens of mice previously infected with P.chabaudi, were able to confer a degree of immunity against P.chabaudi infection on syngeneic C57Bl and NIH mice. Both strains of mice given 600 rads could be protected by enriched immune cell subpopulations and NIH mice given 800 rads could also be protected. No experiments were carried out with C57Bl mice given 800 rads. There is no reason to believe that results would have been obtained different from those with NIH mice.

In 3 experiments in which NIH mice were given 800 rads, the protective effect of unfractionated immune cells was always better than that of enriched immune T or immune B cells. The enriched immune T cell and immune B cell recipients became subpatent in 2 of these experiments about the same time, but only after the unfractionated immune cell recipients had become subpatent. In the third experiment (experiment 5.1) a different result was obtained. Little or no protection was apparent during the first 30 days of infection with enriched immune T cell recipients. As there were only 3 surviving enriched immune B cell recipients, it is difficult to comment on the immune status of these mice, but 2 of them had relapsed within 7 days of becoming subpatent. All the unfractionated immune cell recipients became subpatent in this experiment and remained so for at least 16 days

after the primary patent parasitaemia.

C57Bl mice irradiated with 600 rads could also be conferred with a degree of immunity with enriched immune T or B cells, but as with NIH mice given 800 rads, better protection was conferred with unfractionated immune cells. (This experiment demonstrated that immunity could be transferred by injecting immune cells i.p. There were, however, more deaths in this experiment than one would have normally expected with mice given 600 rads and this may be a reflection of the route of injection of lymphoid cells. A high number of deaths was a common feature of experiments in which lymphoid cells were injected i.p., especially if mice were lethally irradiated.).

In NIH mice given 600 rads, the unfractionated immune cell recipients failed to give better protection than recipients of enriched immune T or immune B cells during the primary patent parasitaemia although, in the same experiment, recipients of unfractionated immune cells given 800 rads had a higher degree of protection conferred on them than recipients of enriched immune T or B cells. (Results of 1 experiment with NIH mice given 600 rads were, however, similar to those obtained in all experiments with mice given 800 rads).

Overall, the results of these experiments suggest that for the most efficient protection of irradiated mice against P.chabaudi infection with immune syngeneic cells, a population containing large proportions of T cells and B cells is required. The fact that enriched subpopulations of immune T or immune B cells imparted some protection to recipients implies that both T cells and B cells may be required for immunity. Since protection can be transferred from mice which have probably eradicated an infection of P.chabaudi, the active lymphoid cells are

probably memory T and B cells (Claman, Chaperon and Triplett, 1966; Roelants and Askonas, 1972).

Gravely and Kreier (1976) demonstrated that enriched immune T cells, also obtained from nylon wool columns, from rats previously infected with P.berghei, although conferring some immunity on syngeneic recipients were not as protective as enriched immune B cells or recombined enriched immune T and B cells. It is noted, however, that in these experiments, smaller numbers of enriched immune T cells than enriched immune B cells or recombined immune cells were used. Using immuno-absorbent columns to separate splenic T and B lymphocytes, Brown et al. (1976) found that enriched immune T cells protected rats from P.berghei infection, but that immune T cells in the presence of immune B cells would give better protection. Jayawardena et al. (1975a) demonstrated that immunity against P.yoelii could be obtained in mice injected with syngeneic, anti θ + complement treated immune spleen cell suspensions. This immunity could also be demonstrated in T cell deprived mice and these authors implied that T cells were not directly responsible for killing parasites, presumably by acting as cytotoxic cells. If this is the case, an explanation must be sought for the protective effect of enriched immune T cell subpopulations and the role of T cells themselves in this protection. Brown (1971) has argued that T cells may act as helper cells for the production of protective antibody in the immune response to malaria parasites. It has been demonstrated in thymectomized animals (Brown et al., 1968; Stechschulte, 1969b) and animals treated with anti-thymocyte serum (Spira et al., 1970; Brown, 1971) that thymus derived cells and presumably T cells are required for immunity to non-fulminating P.berghei infections. Antibody has also been shown to be important in immunity to infection (Diggs & Osler, 1969; Cohen and Butcher, 1970; Phillips and Jones, 1972).

From the results of Gravely and Kreier (1976), Brown et al (1976), it has been suggested that memory T cells in an enriched immune T cell preparation act as helper cells to host B cells in promoting immunity. Since in this report irradiated and hence immunosuppressed mice were used, it is less likely that cooperation of donor cells with the host's own immune system would occur. There is evidence, however, of the existence of radioresistant T cells (Sprent, Anderson and Miller, 1974; Kataska and Sado, 1975; Kadish and Basch, 1975) and B cells (Pilarski and Cunningham, 1974) and such radioresistant cells may contribute to the immune response against infection.

When NIH mice were given 600 rads, it was found that the enriched immune T cell recipients demonstrated the same degree of immunity as unfractionated immune cell recipients during the primary parasitaemia, whereas in all experiments where mice were given 800 rads, recipients of enriched immune T cells demonstrated a weaker immunity than recipients of unfractionated immune cells. It is possible, therefore, that the immune T cells in mice given 600 rads copperate with radioresistant B cells or the recovering host's B cell population to generate a protective immune response. Evidence for this comes from the fact that enriched immune T cell recipients given 600 rads, became subpatent with regard to their primary parasitaemias before their 800 rads equivalents.

With mice given 800 rads, there is less likelihood for there to be cooperation between donor cells and host cells, because of the irreparable damage to the immune system which requires additional donor lymphoid cells for recovery, although such cooperation cannot be excluded at this stage. The consistency of results with mice given 800 rads perhaps suggests that cooperation is more likely to occur between

donor lymphoid cells. Viability tests on enriched T cell subpopulations from nylon wool columns treated with anti-thymocyte serum + complement, showed that 72 - 86% of these cells were dead (and, therefore, presumably T cells) and 1 - 3% were immunoglobulin-bearing cells as judged by fluorescent antibody tests. It is not known whether such a small percentage of immunoglobulin-bearing cells represents a sufficient number to produce protection in recipients with primed T cells.

A similar argument could be applied to reasons for the protective effect of enriched immune B cells. There was a higher percentage of T cells in the enriched immune B cell fractions (up to 13%) than there was of B cells in the enriched immune T cell fractions. In this case, however, there would be fewer numbers of T cells in an enriched immune B cell fraction to act as helper cells in order to activate the clones of memory B cells than there would be in a preparation of unfractionated immune cells. As a result there could be a decreased efficiency of protective antibody production in enriched immune B cell recipients compared to unfractionated immune cell recipients (Arrenbrecht and Mitchell, 1975).

A population of extremely adherent cells, such as the glass adherent immunoglobulin secretory cells which resemble macrophages (Löwy, Teplitz and Bussard, 1975) may be important effector cells in the immune response to malaria, but may remain adhered to nylon wool. Experiments were carried out (see chapter 7) with groups of mice receiving enriched immune T + immune B cells in a 1:1 ratio. The results of these experiments varied somewhat but protection usually lay somewhere between that obtained with unfractionated immune cells and the enriched subpopulations of immune T or immune B cells. The reason for recombined immune T + immune B cells not giving as good protection as unfractionated

immune cells may be the result of a loss of certain nylon wool adherent cells. Alternatively, these results may be related to the proportions in which enriched immune T cells and immune B cells were added together before injection into recipients (chapter 7).

The observation in the experiments described in these 2 chapters of a weaker long term immunity in irradiated mice receiving enriched immune T cells may reflect an inability on the part of immune T cells to induce immunity strong enough to prevent relapse.

Relapses in malaria may arise for several reasons. Firstly, a state of immunodepression has been described during acute malaria infection (Salaman et al., 1969; Greenwood et al., 1971; Wedderburn, 1974; McBride et al., 1977) and during chronic malaria infection (McGregor and Barr, 1962; Wedderburn et al., 1975; McBride et al., 1977) which may produce relapses. Secondly, there is some indirect evidence that there may be suppression of the immune response to the malaria parasite induced by the spleen of monkeys infected chronically with P.inui, since splenectomized animals can eradicate their infections before intact animals (Wyler et al., 1977). Suppressor cells which inhibit the in vitro immune response to heterologous antigens have been detected in the spleen of mice infected with P.berghei (Jayawardena, 1977). Thirdly, malaria parasites can undergo antigenic variation (Brown and Brown, 1965) during infection, producing intra-strain antigenic variants which apparently have the ability to evade the immune response.

One of, or combinations of, the above phenomena may produce relapses in the enriched T cell recipients. For example, new parasite variants may appear faster than the memory B cells can produce antibody against them. Further, it is known that enriched T cell subpopulations

obtained by the passage of lymphoid cells through nylon wool columns contain suppressor cells (Tardieu and Daguillard, 1975). Suppressor cells in an enriched immune T cell subpopulation injected into recipients may be stimulated during the primary patent parasitaemia to inhibit partially the immune response, perhaps leaving the recipients of these cells prone to patent relapses. Alternatively, the recovering host's own immune system may have some effect on the appearance of relapses as it was observed that enriched immune T cell recipients given 600 rads usually relapsed before those given 800 rads. Once more, this could be an effect of suppressor cells from the host's immune system or it could be as a result of competition for position within lymphoid tissue between increasing numbers of virgin cells and memory cells.

Although, so far, much emphasis has been placed on the cooperation of T and B cells, a role for a cell-mediated type of immunity cannot be excluded to explain these results, especially in enriched immune T cell recipients. Clark et al. (1975) observed immunity to Babesia microti, P.yoelii and P.vinckei. Apparent intracellular death of parasites in mice previously immunized with B.C.G., but in the case of B.microti no anti-Babesial antibodies were detected, suggesting a cell-mediated type of immunity was in operation. Delayed hypersensitivity, which is associated with a cell-mediated type response, has been demonstrated in malaria infection (Phillips et al., 1970; Cabrera et al., 1976; Finerty andKrehl, 1976). A macrophage migration inhibition factor which also correlates with a cell-mediated type of immunity (Dumonde, Wolstencroft, Panayi, Mathew, Morley and Howson, 1969) is produced by lymphoid cells from mice previously infected with P.yoelii (Coleman et al., 1976). It is also possible that cytotoxic cells can lyse P.berghei parasites or parasitized cells in vitro even in the absence of immune serum (Coleman et al., 1975). Finerty and

Krehl (1976) demonstrated increased resistance to a virulent P.yoelii strain in mice treated with cyclophosphamide, which depletes lymphoid tissue of B cells and possibly other types of lymphoid cells (Turk and Poulter, 1972), and this was accompanied by an increased delayed hypersensitivity. Delayed hypersensitivity is inhibited by serum factors, possibly immune complexes, in the presence of B cells (Lagrange, Mackaness and Miller, 1974; Mackaness, Lagrange, Miller and Ishibashi, 1976). Such an inhibition of cell-mediated immunity may occur in malaria infections as suggested by the results of Finerty and Krehl (1976). The immune response which will ultimately be responsible for the control and elimination of an infection may depend on a balance between cell-mediated and humoral responses controlled by the relative proportions and/or activity of different lymphoid cell types in the lymphoid tissues.

In summary, the experiments in these 2 chapters provide evidence that T cells and B cells may both be required for an effective immune response to malaria infection and that probably memory cells of each type are produced, which when transferred to syngeneic irradiated recipients, enhance an immune response to infection in those recipients.

CHAPTER 7

A) Synergy between subpopulations of immune cells and other subpopulations of immune cells or normal cells

It was suggested earlier (pp 48-50) that the immunity to P.chadaudi infection which stems from subpopulations of enriched immune T or immune B cells could be a result of cooperation between the immune cell subpopulations and the recovering host's immune system, or between cells of the 2 immune cell subpopulations. Since sublethally irradiated and reconstituted mice often do eventually control an infection, it has been assumed that the lymphoid cells which are injected into the irradiated recipients play a role in controlling infection. However, there is no evidence to justify such an assumption and an experiment will be described investigating the contribution to immunity played by normal donor cells.

Experiments will then be described in which subpopulations of normal or immune cells were injected along with enriched immune T cells into irradiated mice. The object of these experiments was to examine for synergy between different cell preparations in recipients which could enhance the immunity obtained by injecting enriched immune T cells only.

Experiment 7.1

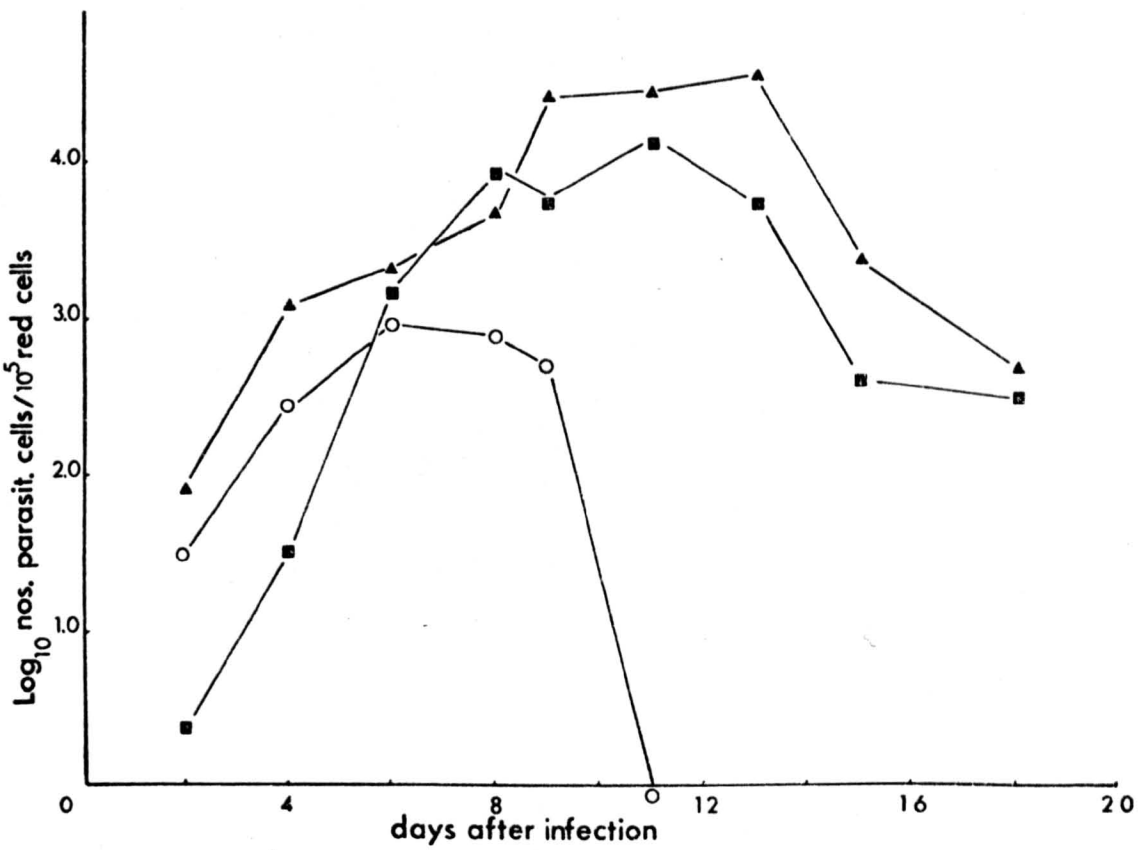
The contribution of normal donor cells to immunity in mice given 600 rads was examined by comparing the course of infection in irradiated mice reconstituted with normal spleen cells and irradiated mice which received no lymphoid cells.

Three mice out of 11 NIH females, nine weeks old, were injected i.v. with 1.4×10^7 normal spleen cells 1 hour after irradiation. A further 2 mice were injected with 1.4×10^7 immune spleen cells obtained from the spleens of mice infected on 3 previous occasions, the last time being 173 days before the spleens were used. The 6 remaining mice received no spleen cells. All mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c. on the same day.

The mean parasitaemias are illustrated in Figure 11. Irradiated recipients of immune spleen cells were subpatent on day 11 whereas none of the recipients of normal spleen cells or the mice not given cells became subpatent during the first 13 days of infection. There is no apparent evidence of protection conferred by normal spleen cells on irradiated recipients when compared to irradiated mice which received no spleen cells. In fact, these latter mice appeared to be more resistant to infection initially that the former. These results were confirmed in other experiments described in chapter 8. It was also observed in chapter 4 that C57Bl mice given 600 rads and receiving 4×10^7 normal spleen cells were no more immune to infection than irradiated mice given 4×10^6 normal spleen cells. Irradiated mice whether injected with normal spleen cells or not, however, can reduce their parasitaemias from as high as 44% (i.e. 4.6 on the $\log_{10}$ geometric mean scale) down to less than 1% (i.e. 2.0 on the $\log_{10}$ geometric mean scale). Most of these mice very quickly develop high level parasitaemias once again within 10 - 15 days of reaching their peak primary parasitaemia and the second peak is usually comparable in size with the first.

Fig. 11.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 600 rads, which were injected i.v. with either 1.4×10^7 normal spleen cells -▲—▲- , 1.4×10^7 immune spleen cells -○—○- , or were not injected with any spleen cells -■—■- . The mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c.


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
days after infection

Experiment 7.2

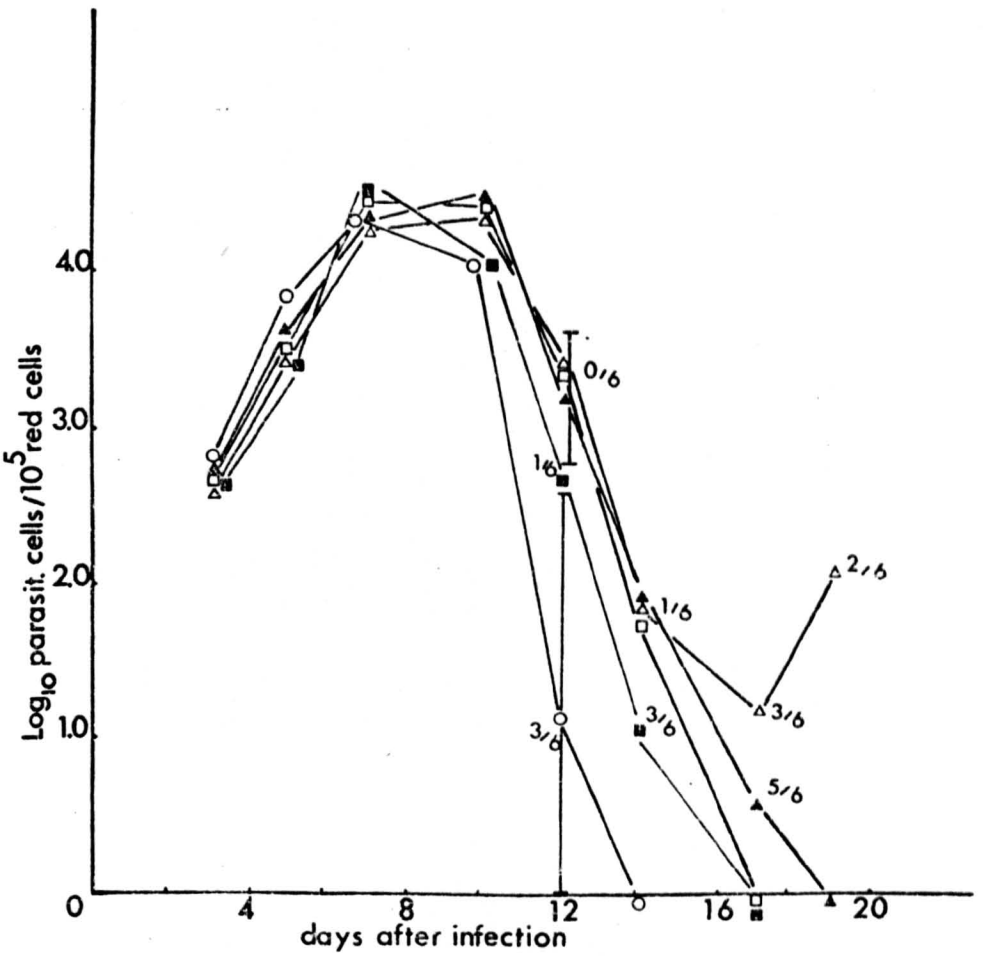
Synergy between enriched immune T cells and other subpopulations of spleen cells was examined by comparing parasitaemias in irradiated lymphoid cell recipients which received enriched immune T cells together with other selected subpopulations of cells. The first such experiment to be described was performed as part of experiment 5.3 from which details can be obtained.

Thirty-two weeks old C57Bl females were given 600 rads and injected 20 hours after irradiation with either 1×10^6 enriched immune T cells, 1×10^6 enriched immune T cells + 1×10^6 enriched immune B cells, 1×10^6 enriched immune T cells + 1×10^6 enriched normal B cells, 1×10^6 enriched immune B cells or 1×10^6 enriched normal B cells in groups of 6 mice. The mice were injected with 1×10^6 P.chabaudi p.r.b.c. on the following day.

The mean parasitaemias are presented in Figure 12. Irradiated recipients of enriched immune T + enriched immune B cells became subpatent on day 14, the same day as unfractionated immune cell recipients. Recipients of enriched immune T cells + enriched normal B cells did not become subpatent at this time, but along with the enriched immune T cell recipients and enriched immune B cell recipients became subpatent between days 14 and 17. Supplementing enriched immune T cells with enriched immune B cells, therefore, may have reduced the length of parasitaemia by up to 3 days but enriched normal B cells did not appear to have any enhancing effect on the protective properties of enriched immune T cells.

Fig. 12.

The course of parasitaemia of P.chabaudi in C57Bl mice which were irradiated with 600 rads and injected i.p. with either 1 x 10^6 enriched immune T cells -□—□- , 1 x 10^6 enriched immune T cells + 1 x 10^6 enriched immune B cells -○—○- , 1 x 10^6 enriched immune T cells + 1 x 10^6 enriched normal B cells -▲—▲- , 1 x 10^6 enriched immune B cells -■—■- , or 1 x 10^6 enriched normal B cells -△—△- . The mice were injected i.p. with 1 x 10^6 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice of that group, on a particular day


Log10 parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
days after infection
0/6
1/6
2/6
3/6
3/6
1/6
3/6
5/6

Experiment 7.3

The effect of immune bone marrow cells on infection in irradiated recipients given 800 rads was investigated and also the effect of supplementing enriched immune T cells with immune bone marrow cells. Details of the experiments are contained in experiment 5.2.

NIH females received the same total number of lymphoid cells i.v. (2×10^6), 1 hour after irradiation, in groups of 6 mice. Mice injected with enriched immune T cells + immune bone marrow cells were injected with 1×10^6 cells of each type. Other groups were injected with either enriched immune T cells, immune bone marrow cells or normal cells. The mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c. on the same day as cell transfer.

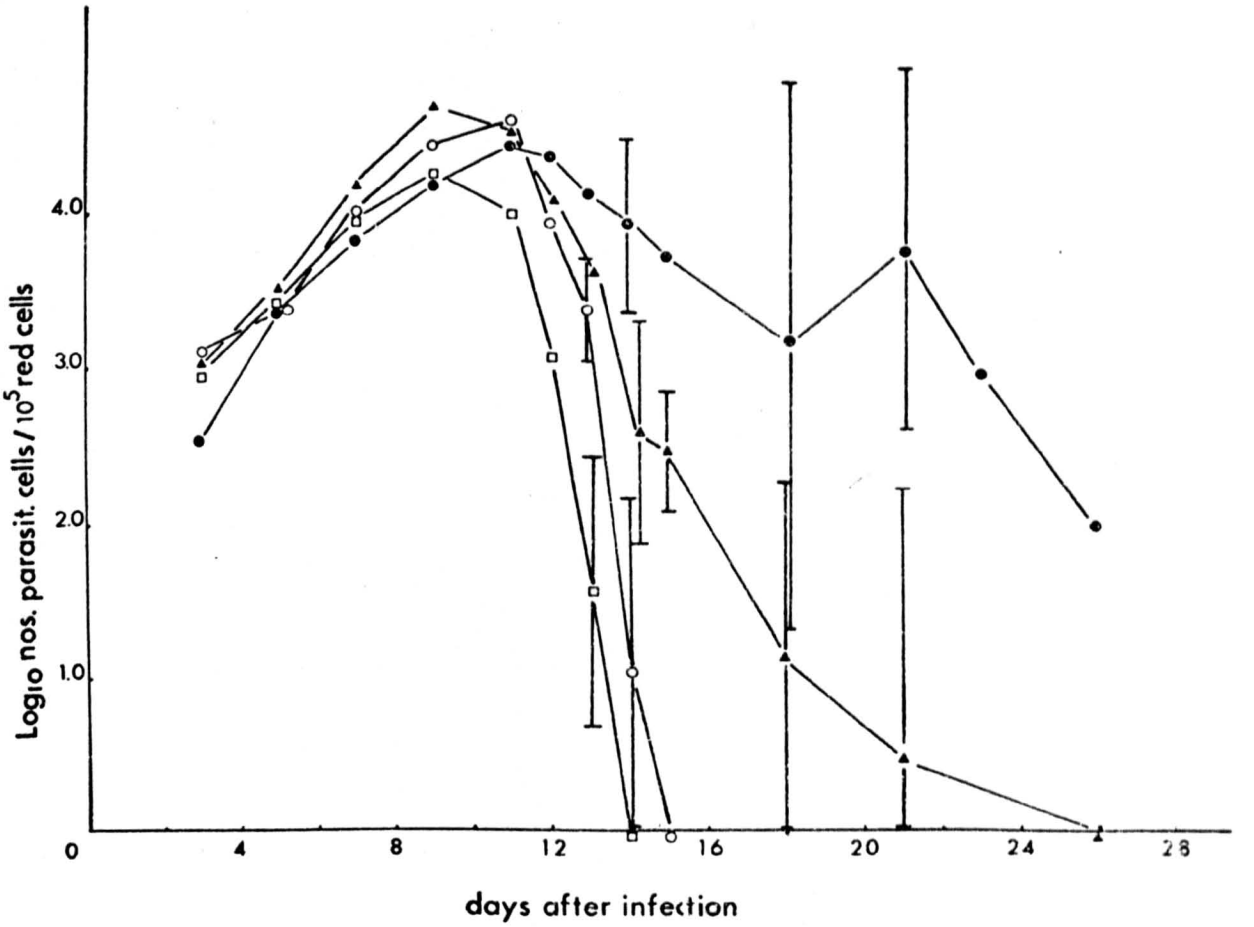
The mean parasitaemias are presented in Figure 13. It will be recalled from experiment 5.2 that unfractionated immune cell recipients became subpatent 2 days before enriched immune T cell recipients and enriched immune B cell recipients, on day 12. Recipients of enriched immune T cells + immune bone marrow cells did not become subpatent until day 15, one day after the enriched immune T cell recipients which became subpatent on day 14. Three recipients of immune bone marrow cells died during the infection, but the surviving mice were protected to some extent, becoming subpatent on day 26.

Experiment 7.4

A similar type of experiment to experiment 7.2. was carried out with NIH mice given 800 rads. For this experiment, however, all mice were injected with the same total number of cells as in experiment 7.3. Details of the experiment are given in experiment 5.1.

Fig. 13.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads and injected i.v. with either 2×10^6 enriched immune T cells -□ - □- , 1×10^6 enriched immune T cells + 1×10^6 immune bone marrow cells -○ — ○- , 2×10^6 immune bone marrow cells -▲ — ▲- , or normal spleen cells -○ — ○- . The mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c.


Log10 nos. parasit. cells / 10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
days after infection

NIH females were given 800 rads and injected 1 hour later with either 1×10^6 unfractionated immune cells, 1×10^6 enriched immune T + enriched immune B cells (5×10^5 cells of each subpopulation), 1×10^6 enriched immune T cells or 1×10^6 normal cells. The mice were injected i.p. with 1×10^5 P.chabaudi p.r.b.c. on the same day.

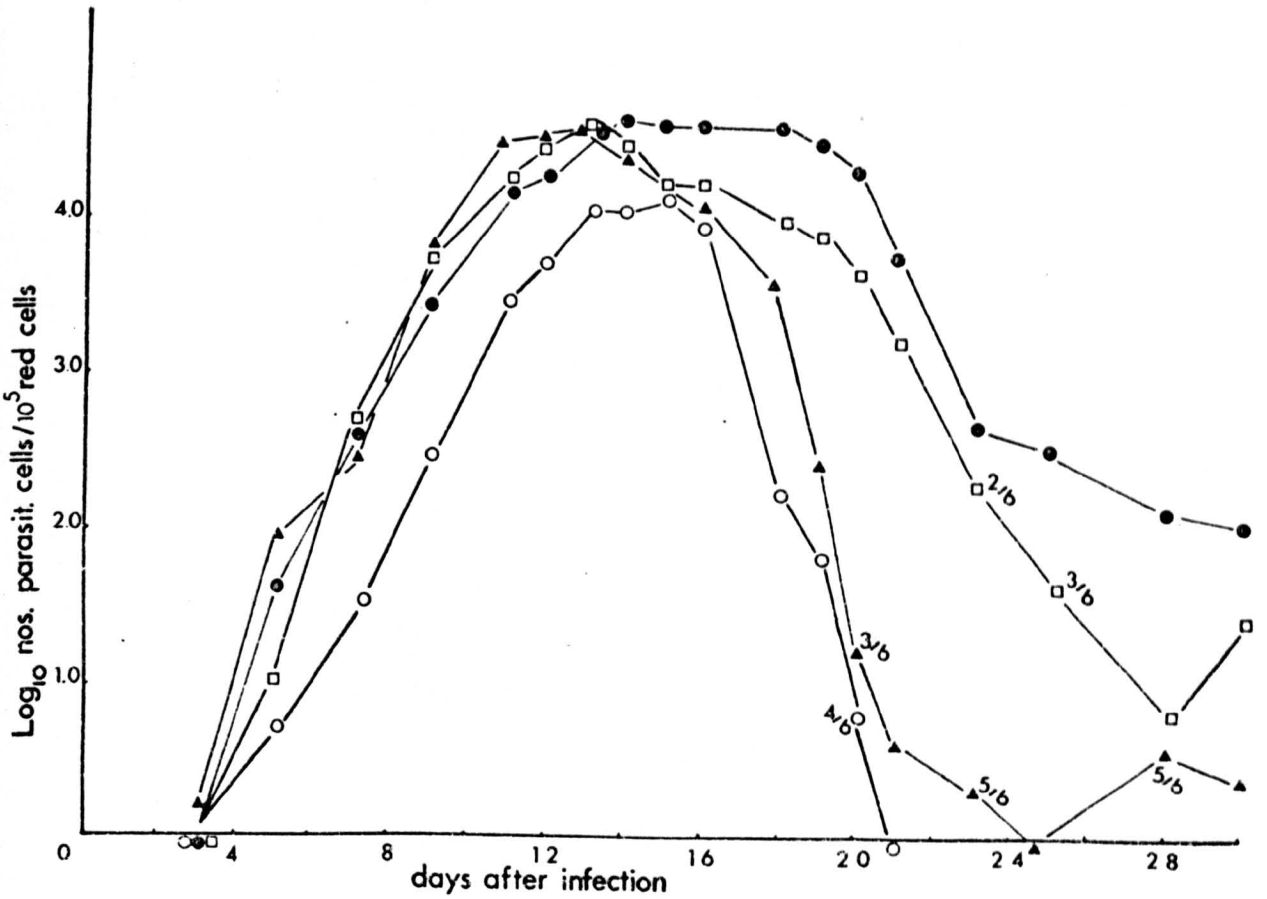
The mean parasitaemias are presented in Figure 14. The recipients of recombined enriched immune T + enriched immune B cells had a higher degree of immunity than enriched immune T cell recipients, but all did not become subpatent until day 25, 4 days after the immune unfractionated cell recipients. The recipients of enriched immune T + enriched immune B cells, in fact, were protected to a similar degree as recipients of enriched immune B cells (see experiment 5.1), although all of the former recipients survived whereas only 3 out of 6 enriched immune B cell recipients survived. All but one of the enriched immune T + enriched immune B cell recipients relapsed within 10 days after the primary parasitaemia had become subpatent, whereas none of the unfractionated immune cell recipients relapsed in this time.

Discussion

Sublethally irradiated C57Bl mice reconstituted with normal spleen cells were apparently not able to develop a higher degree of immunity than irradiated mice which were not reconstituted with lymphoid cells. Parasitaemia in both these groups of mice, however, did drop from a peak as high as 44% to levels less than 1%. It is probable that the development of immunity would be responsible for such a decrease in parasitaemia although other factors, such as the depletion of the type of cell that P.chabaudi preferentially invades, cannot be excluded. Normal spleen cells, therefore, appeared to have had little effect on the primary parasitaemia in mice irradiated with 600 rads and this implies

Fig. 14.

The course of parasitaemia of P.chabaudi in NIH mice which were irradiated with 800 rads and injected i.v. with either 1 x 10^6 unfractionated immune spleen cells -○— ○- , 5 x 10^5 enriched immune T cells + 5 x 10^5 enriched immune B cells -▲— ▲- , 1 x 10^6 enriched immune T cells -□— □- , or 1 x 10^6 normal spleen cells-●— ●-. The mice were injected i.p. with 1 x 10^5 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice in that group, on a particular day


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
days after infection
2/6
3/6
3/6
4/6
5/6
5/6

that the recovering host's immune system was mainly responsible for the protective immune response. The number of normal spleen cells which were injected could have been too small to give an effective immune response during the time it took for the host's immune system to recover sufficiently to mount a primary response. Alternatively, the level of parasite antigen required for the initiation of a protective immune response may not have been reached until the host's immune system had recovered sufficiently to respond to the antigen.

Enriched subpopulations of normal spleen cells did not enhance the protective properties of enriched immune T cells. There is some indirect evidence from the previous chapter, however, that enriched immune T cells may cooperate with the recovering host's immune system, since mice given 600 rads which received enriched immune T cells became subpatent after the primary parasitaemia before similar mice given 300 rads.

Two experiments have been described in which enriched immune T cells and enriched immune B cells were recombined and injected into recipients. In the first experiment, with C57Bl mice given 600 rads, the addition of 1×10^6 enriched immune B cells to 1×10^6 enriched immune T cells gave equivalent protection to 1×10^6 unfractionated immune cells and better protection than 1×10^6 enriched immune T cells. This suggests that the enriched immune B cells may have had an enhancing effect on the enriched immune T cells, but this could have been entirely an effect of increasing the number of immune cells in the recombined immune cell inoculum. Furthermore, double the number of recombined enriched immune T + B cells conferred protection on recipients which was only equal to that of 1×10^6 unfractionated cells.

In the second experiment, using NIH mice given 800 rads, the recipients of recombined immune cells received the same total number of cells as all other groups. It was found that although the recombined immune cells gave better protection than the same number of enriched immune T cells, the protection was not as good as that conferred by unfractionated immune cells. The recombined immune cells, in fact, appeared to confer a similar degree of protection in recipients to enriched immune B cells, although 3 of the 6 enriched immune B cell recipients died and none of the recombined immune cell recipients died.

Also in NIH mice given 800 rads, immune bone marrow cells did not enhance the protective effect of enriched immune T cells, although the immune bone marrow cells themselves were slightly protective.

Gravely and Kreier (1976) demonstrated that enriched immune T cells recombined with enriched immune B cells after separation on nylon wool columns were more protective against P.berghei than enriched immune T cells in syngeneic rats. As more than twice the number of recombined immune cells than enriched immune T cells alone were injected into recipients in the experiments of Gravely and Kreier, the enhanced protection in this instance could also be attributed to an additive effect of enriched immune T cells and enriched immune B cells and not to synergy between the subpopulations of cells.

No conclusive evidence has been found in these experiments, therefore, for synergy between different lymphoid cell types, to account for the superior protective effect against P.chabaudi of unfractionated immune cells in syngeneic recipients, when compared to enriched immune T cells or enriched immune B cells.

B) A comparison of the protection conferred by different numbers of unfractionated immune cells or enriched immune T cells

The experiments described so far have demonstrated that immunity can be adoptively transferred to NIH and C57Bl mice against P.chabaudi infection with numbers of lymphoid cells in the range of $1 \times 10^6 - 4 \times 10^6$. One of the problems of using X-irradiated mice is the possibility of animals dying during an experiment as a result of bacterial infection. In an effort to combat bacterial infection, irradiated mice received terramycin in their drinking water. Lethally irradiated mice must also have a minimum number of lymphoid cells injected in order to recover their immunocompetence. The number of splenic lymphoid cells which can be injected into irradiated recipients is, however, limited to the relatively small numbers which can be passed through and recovered from nylon wool columns.

An experiment was carried out in which a range of unfractionated immune cells and enriched immune T cells were injected into lethally irradiated mice, which were then infected with P.chabaudi. The object of this experiment was first to find the number of lymphoid cells required to be injected into lethally irradiated mice in order that the mice would survive, and secondly, to observe the minimal number of immune cells required to be injected in order to observe the expression of immunity. Another reason for performing this experiment, however, was to compare not only the protection conferred by the same number of enriched immune T cells and unfractionated immune cells, but also to examine how the difference in protection between groups injected with these cells varied with the numbers of lymphoid cells used.

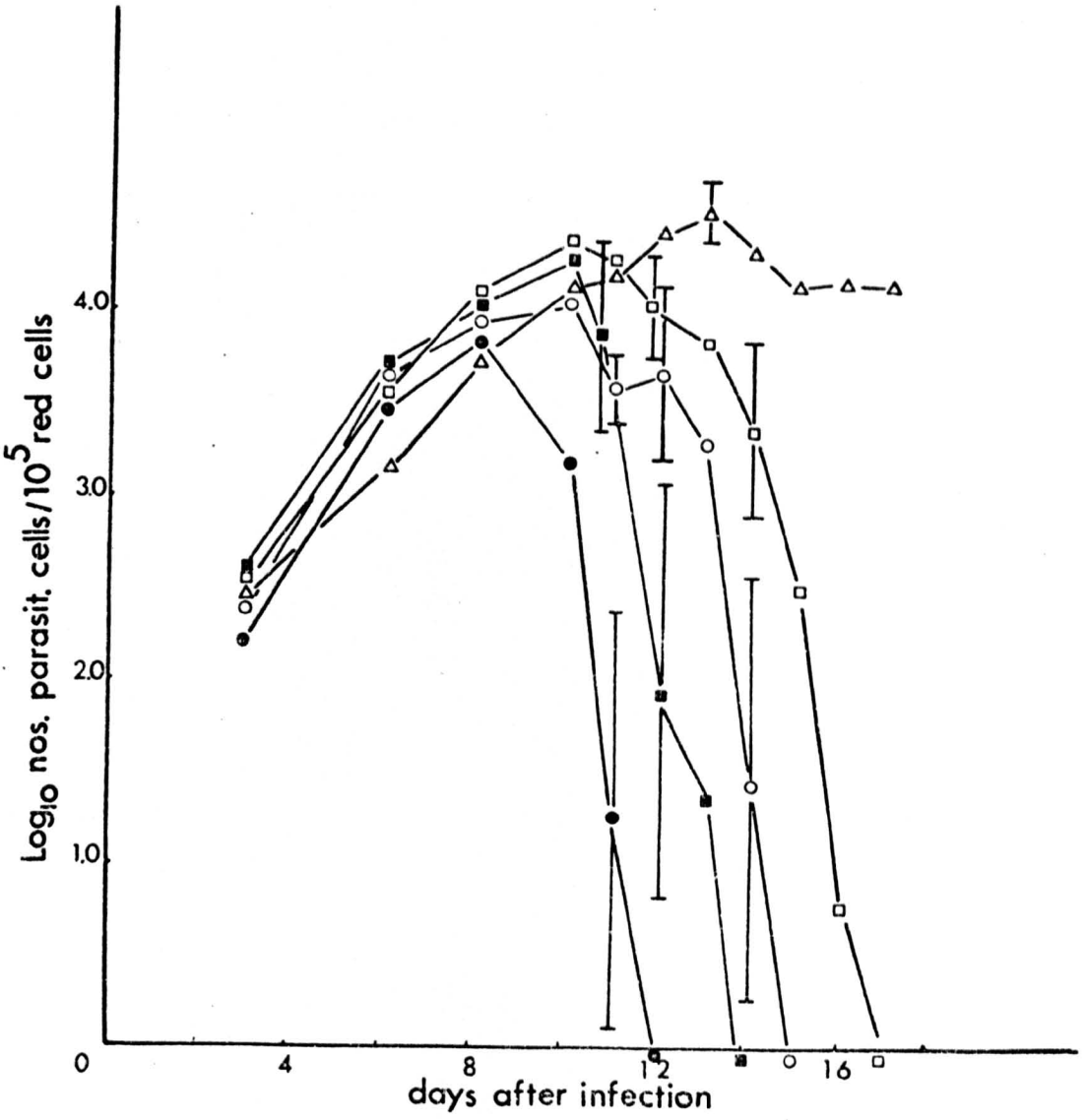
Experimental procedure

Groups of 6 NIH females, 19 weeks old, were injected i.v. 1 hour after 800 rads irradiation with 1 x 10^5, 1 x 10^6 or 1 x 10^7 unfractionated immune spleen cells or enriched immune T cells, or 1 x 10^7 normal spleen cells. Immune spleen cells came from 3 NIH females which had been infected 18 months, 14 months and 140 days before their spleens were used. Normal spleen cells came from 2 NIH females, which were the same age as the recipient mice. The cell recipients were injected i.p. with 2 x 10^6 P.chabaudi p.r.b.c. immediately after the injection of spleen cells. The percentage of immunoglobulin-bearing cells was 22%. 29.5% and 3% for the unfractionated immune cells, normal cells and enriched immune T cells, respectively.

The effect of different numbers of unfractionated immune cells or enriched immune T cells on the course of parasitaemia is shown in Figure 15. Most of the mice which received 1 x 10^5 immune cells died between days 11 and 13 and are not included in the graph. Groups of mice which received 1 x 10^6 or 1 x 10^7 unfractionated immune cells or enriched immune T cells were protected to some degree when compared to the control group which received 1 x 10^7 normal cells. Comparing recipients of 1 x 10^6 and 1 x 10^7 lymphoid cells, better protection was conferred by the higher number of immune cells, in a dose dependent manner. Mice receiving either 1 x 10^7 enriched immune T cells or unfractionated immune cells all became subpatent 3 days before their equivalents which received 1 x 10^6 lymphoid cells. Unfractionated immune cell recipients receiving 1 x 10^6 or 1 x 10^7 lymphoid cells became subpatent 2 days before their enriched immune T cell counterparts which had received the same number of lymphoid cells.

Fig. 15.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads and injected i.v. with either 1 x 10^7 unfractionated immune spleen cells –●–●–, 1 x 10^7 enriched immune T cells –■–■–, 1 x 10^6 unfractionated immune spleen cells –○–○–, 1 x 10^6 enriched immune T cells –□–□–, or 1 x 10^7 normal spleen cells –△–△–. The mice were injected i.p. with 2 x 10^6 P.chabaudi p.r.b.c.


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
days after infection

Discussion

It would seem to be undesirable to carry out experiments in which less than 1×10^6 spleen cells were transferred to each mouse given 800 rads, as all mice which received 1×10^5 lymphoid cells died and 2 mice of 6 from each group of mice which received 1×10^6 immune or normal spleen cells died. One animal from each group receiving 1×10^7 immune or normal spleen cells died, suggesting this would be a more suitable number of lymphoid cells to use in subsequent studies.

As the number of lymphoid cells transferred in certain adoptive transfer studies is increased, there can be a limit to the enhancement of the immune responses by numbers of cells, due to the increased numbers of suppressor T cells (Arrenbrecht and Mitchell, 1975). In this experiment, however, a difference of 2 days in the length of patent primary parasitaemia was observed between the unfractionated immune cell groups and the enriched immune T cell groups, receiving either 1×10^6 or 1×10^7 lymphoid cells. This suggests that there is no increased suppressive effect on the immune response of recipients of either group receiving the larger number of lymphoid cells, or, if there is any suppressive effect, it is similar in both groups.

It is interesting to note that the recipients of 1×10^7 enriched immune T cells became subpatent only 1 day before the recipients of 1×10^6 unfractionated immune cells. With a contamination of 3% immunoglobulin-bearing cells, mice which received 1×10^7 enriched immune T cells would have received approximately 3×10^5 B cells which is similar to the number of B cells the unfractionated immune cell recipients would have obtained. On the other hand, recipients of 1×10^7 unfractionated immune cells became subpatent 5 days before those which received 1×10^6 enriched immune T cells and since there would

have been nearly X100 more B cells in an unfractionated immune cell inoculum in this case, once again this may be an indication of the importance of the requirement of B cells in the immune response to P.chabaudi.

C) The role of phagocytic cells in the immune response in mice to P.chabaudi

Phagocytic cells of the reticulo-endothelial system play an important role in the removal of malaria parasites from the peripheral circulation (Taliaferro and Cannon, 1936). Evidence suggests that activated macrophages in the presence of immune serum have an enhanced capacity to phagocytose malaria parasites (Brown, 1971; Chow and Kreier, 1972). The adoptive transfer of peritoneal macrophages from rats immune to P.berghei infection did not protect syngeneic recipients from infection (Stechschulte, 1969a). Splenic macrophages, however, which are mainly responsible for the phagocytosis of parasites were not transferred in this study.

In experiments involving the use of nylon wool columns in the present study, cell suspensions were first passed through glass wool columns in order to remove most of the phagocytic cells as well as cell debris and clumps of cells. Comparison of the protective effect of immune cells passed through glass wool columns and those which were not passed through glass wool in mice was made in several experiments. One such experiment will be described in which the recipient mice were not irradiated.

In another approach, splenic phagocytic cells from immune mice were adoptively transferred to irradiated syngeneic recipients in order to

examine the protective effect of these cells against P.chabaudi infection and 2 of these experiments are described in detail.

a) Difference in the protective properties of glass wool filtered and unfiltered immune cells

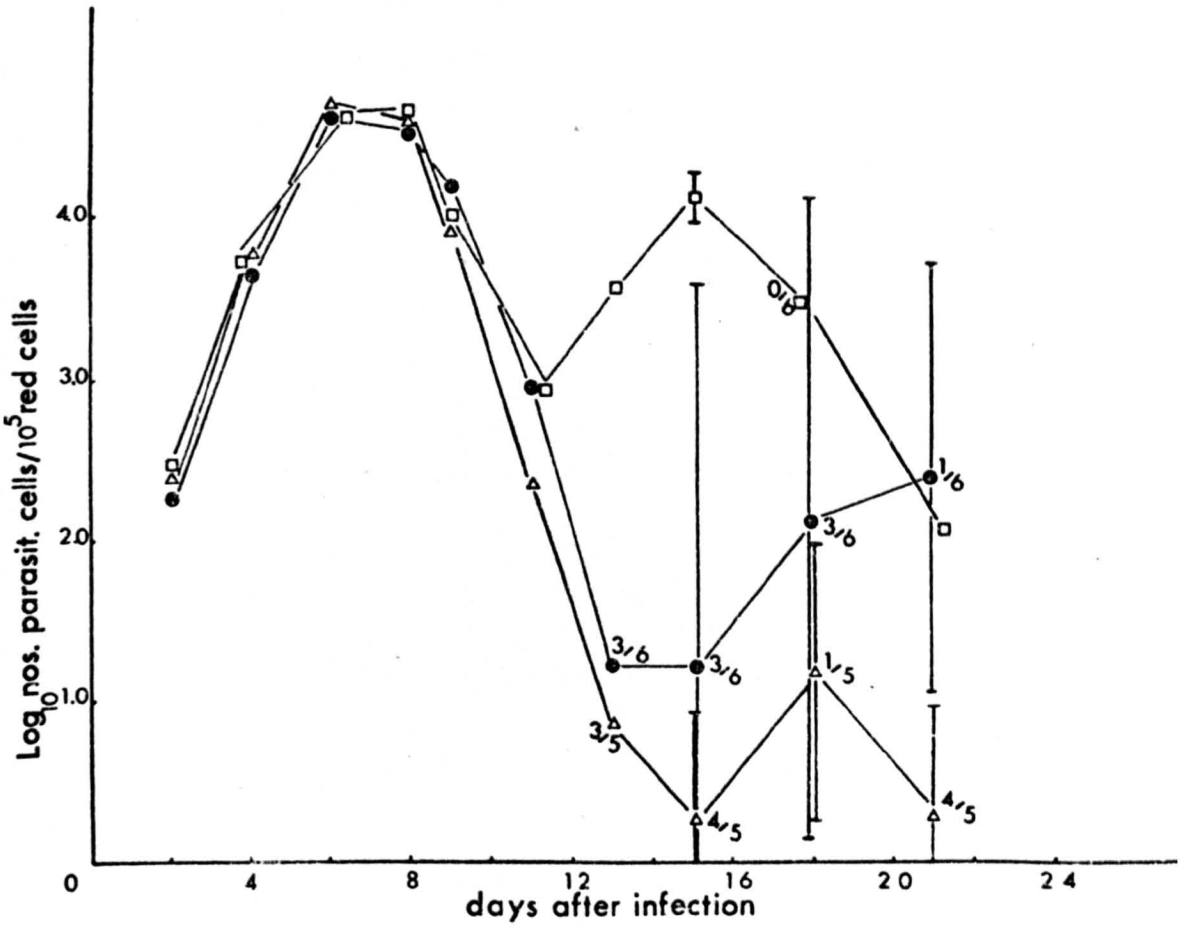
Populations of spleen cells were filtered through glass wool columns as described in Materials and Methods (Chapter 2). Twenty weeks old non-irradiated C57Bl males were injected i.v. with either 9×10^6 filtered immune cells, unfiltered immune cells or filtered normal cells. The immune cells came from the spleens of 4 C57Bl males which had been infected with P.chabaudi 89 days before the spleens were used. The normal cells came from 20 week-old C57Bl males. All mice were injected i.p. with 1×10^6 P.chabaudi p.r.b.c. immediately after the injection of spleen cells.

The mean parasitaemias for each group is presented in Figure 16. It can be seen that both glass wool filtered and unfiltered immune cells conferred better protection than glass wool filtered normal cells between days 11 and 19.

Previous experiments had shown that the filtration of spleen cells through glass wool columns could reduce the number of phagocytic cells from 15 - 20% to 5 - 10% as measured by the uptake of latex particles. The immunity conferred onnon-irradiated recipients in this experiment with primed lymphoid cells did not produce any more than about 5 days subpatency in recipients over the first 21 days, but there is a suggestion that the filtered immune cell recipientsware slightly better protected than the unfiltered immune cell recipients. The results certainly demonstrate that the glass wool filtered immune cells, which are to some extent depleted of phagocytic cells, were not less

Fig. 16.

The course of parasitaemia of P.chabaudi in non-irradiated C57Bl mice injected i.v. with either 9×10^6 glass wool filtered immune spleen cells -△- △- , 9×10^6 unfiltered immune spleen cells -●- ●- , or 9×10^6 glass wool filtered normal spleen cells -□- □- . The mice were injected i.p. with 1×10^6 P.chabaudi p.r.b.c. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice in that group, on a particular day


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
days after infection
0/6
1/6
3/6
3/6
3/6
1/5
3/5
4/5
4/5

efficient in conferring protection on syngeneic recipients than unfiltered cells.

b) The adoptive transfer of enriched splenic phagocytic cells from immune mice and normal mice

The spleen cell population which adheres to plastic petri-dishes and contains a high proportion of macrophages was harvested as described in Materials and Methods. In one experiment phagocytes were harvested after 16 hours incubation at 37°C. Twelve weeks old C57Bl females were used in this experiment. Spleen cells from 2 C57Bl females, immune donors which had been infected on 3 occasions, the last time being 55 days before spleen removal, were injected i.v. into the mice 20 hours after they had been irradiated with 600 rads. Spleen cells from 2 normal 12 weeks old C57Bl females were injected into other irradiated mice. Mice were injected with either 1×10^6 unfractionated immune cells, immune phagocytic cells or normal cells. The cell recipients were all injected i.p. with 5×10^6 P.chabaudi p.r.b.c. 48 hours after irradiation.

In another experiment, using NIH females given 600 rads, phagocytes were obtained after 2.5 hours incubation. Twenty hours after they were given 600 rads, 32 weeks old NIH mice were injected intravenously with either 1×10^6 immune phagocytic cells, unfractionated immune spleen cells or normal cells. Immune spleen cells came from 2 NIH females, infected on days -380 and -118, and normal spleen cells came from 2 previously uninfected 32 weeks old NIH females. The recipients were injected i.v. with 1×10^6 P.chabaudi p.r.b.c. on the same day as the injection of spleen cells.

Adherent cells from the spleens of immune NIH mice, harvested after 2.5 hours incubation at 37°C, were 68% phagocytic and those from the spleens of normal NIH mice were 72% phagocytic, as measured by the uptake of polystyrene latex particles. In this experiment immune adherent cells clearly had the ability to confer some protection on syngeneic recipients (Figure 17). There was very little difference between the course of primary parasitaemias for recipients of immune adherent cells and unfractionated immune spleen cells. A similar degree of protection was obtained with immune adherent spleen cells from immune C57Bl mice infected on days -76, -62 and -11, after 1.5 hours incubation of adherent cells at 37°C.

A somewhat different result was obtained with adherent cells using C57Bl mice if incubation at 37°C lasted 16 hours. As can be seen in Figure 18, immune adherent cells, which were 80% phagocytic, conferred little protection on recipients compared to immune unfractionated spleen cells, although around day 14 of infection there appeared to be a transitory protective effect.

Normal adherent cell recipients had parasitaemia patterns similar to unfractionated normal spleen cell recipients in both the experiments described.

Discussion

The difference in protective properties of immune adherent cells which were harvested after 2,5 hours or less in NIH and C57Bl mice and 16 hours in C57Bl mice, may be attributed to a loss of activation of immune macrophages after prolonged incubation *in vitro*. As immune adherent cells were 80% phagocytic after 16 hours incubation, it is unlikely that a defect in phagocytosis is responsible for such a loss of activation.

Fig. 17.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 600 rads and injected i.v. with 1 x 10^6 unfractionated immune spleen cells - □ - □ - , 1 x 10^6 immune phagocytic cells harvested after 2.5 hours incubation at 37°C - ▲ - ▲ - or normal spleen cells - △ - △ - , The mice were injected i.p. with P.chabaudi (10^6 p.r.b.c.).

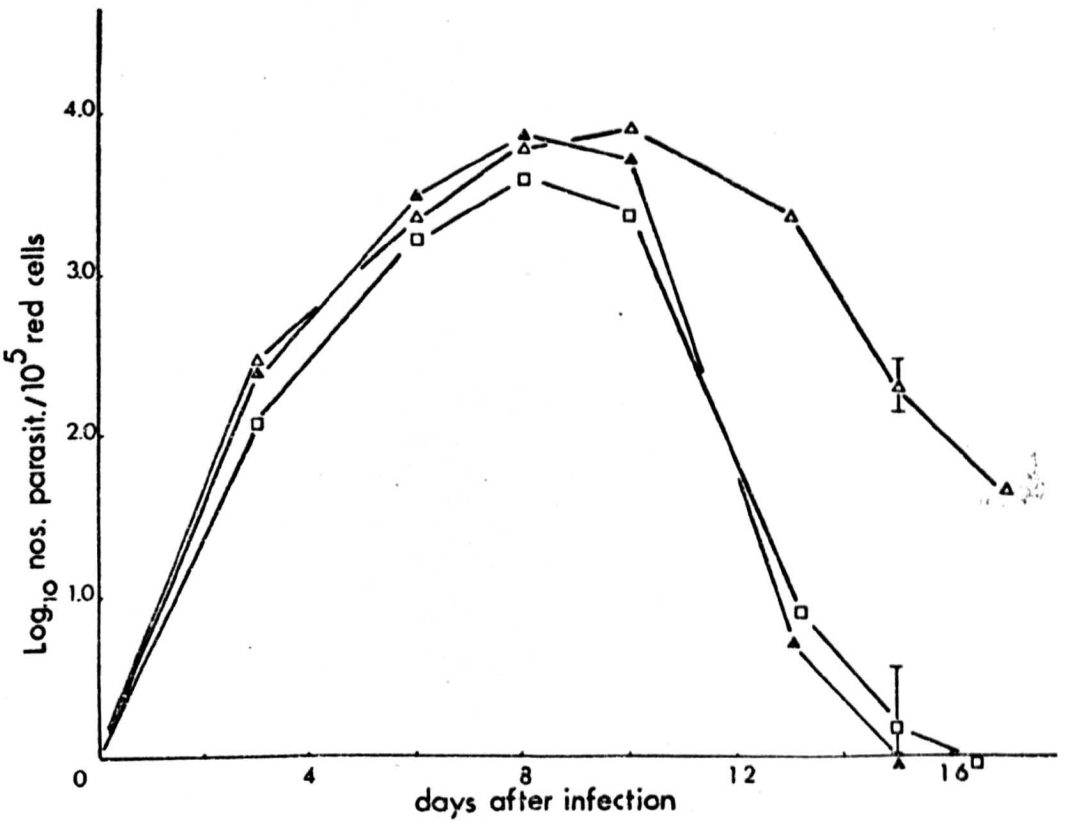
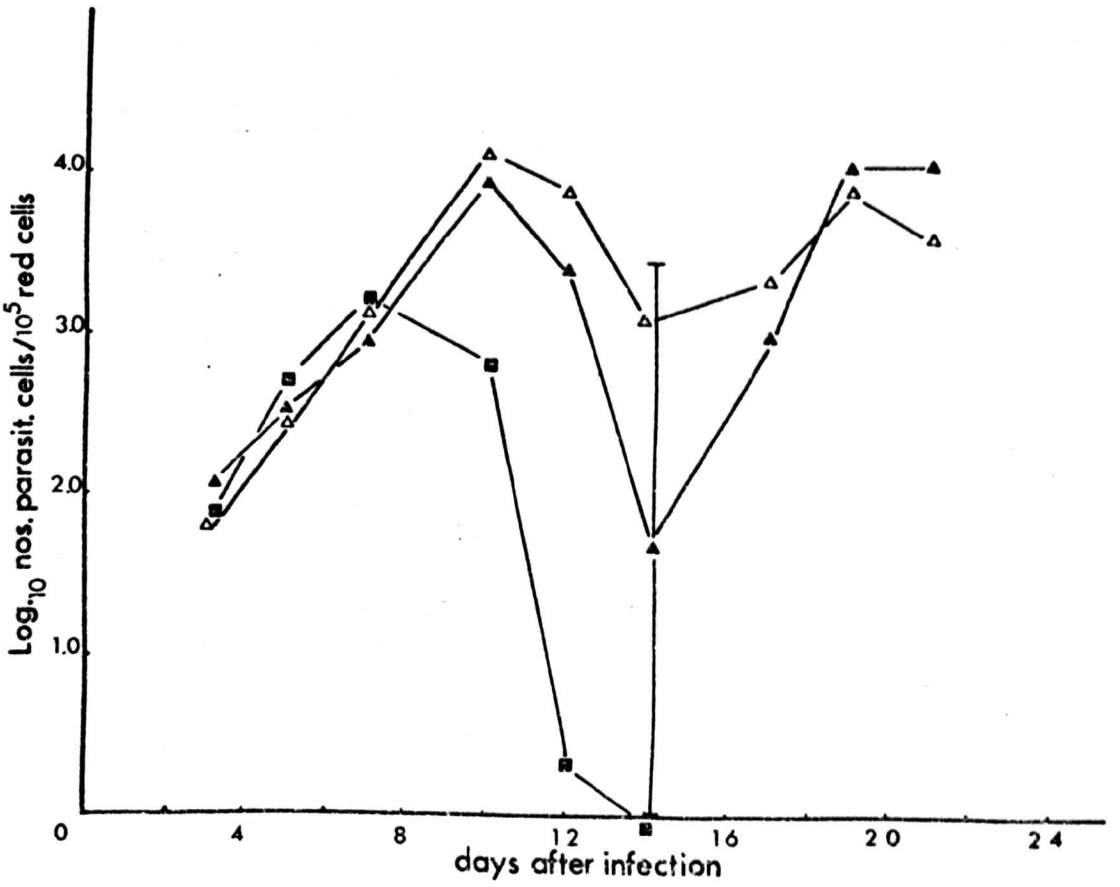

4.0
3.0
2.0
1.0
0
4
8
12
16
Log10 nos. parasit./10^5 red cells
days after infection

Fig. 18.

The course of parasitaemia of P.chabaudi in C57Bl mice irradiated with 600 rads and injected i.v. with 1 x 10^6 unfractionated immune spleen cells -■ - ■- , 1 x 10^6 immune phagocytic cells harvested after 16 hours incubation at 37°C -▲ - ▲-, or 1 x 10^6 normal spleen cells -△ - △- . The mice were injected i.p. with 1 x 10^6 P.chabaudi p.r.b.c.


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
days after infection

The immune adherent cells from the spleens of NIH mice were examined for numbers of immunoglobulin-bearing cells after 2.5 hours incubation at 37°C, and it was found that 25.5% of these cells were immunoglobulin-bearing compared to 28% of the unfractionated spleen cell population. The fluorescence, however, of many of the adherent cells was clearly not as bright as that shown by unfractionated immune cells. Cytophylic antibodies adhering to macrophages may have been at least partially responsible for the fluorescence observed with adherent cells, but it is also possible that B cells were present and if so, they may have contributed to the protection conferred by immune adherent cells. Phagocytic cells which secrete immunoglobulin have been detected which adhere to glass surfaces (Löwy et al., 1975). The inability of C57Bl immune adherent cells to confer much protection after 16 hours incubation at 37°C may be due to the fact that lymphocytes die rapidly in culture (Trowell, 1965) with 50 - 70% cells dead after 24 hours culture at 37°C. After only 2.5 hours incubation at 37°C the viability of non-adherent NIH spleen cells fell from 85% to 70%.

These experiments yield an unsatisfactory conclusion as to the protective effect of immune phagocytic cells and the subject should be examined in greater depth, especially in light of the fact that immune cells depleted of glass wool adherent cells are not less protective than unfiltered immune cells. It would perhaps be necessary to obtain more pure populations of phagocytes, which should be carefully screened for contaminating lymphocytes. For macrophages from immune mice to confer protection on recipients, larger numbers of these cells may be required, as the number of macrophages in the spleens of infected animals developing immunity increases greatly (Taliaferro and Cannon, 1936). It would also be desirable to isolate phagocytes at different times after infection of donor mice, as any active state of macrophages

in potentiating immunity may depend on the presence of parasite antigen in immune animals.

CHAPTER 8

Observations on resistance to infection in irradiated mice

The observation of a non-specific resistance to P.chabaudi infection in irradiated mice during the initial stages of the infection was further investigated. Experiments are described in this chapter, in which the numbers of lymphoid cells used to reconstitute irradiated mice and the time of infection in relation to irradiation were varied, in order to induce changes in this resistance and possibly to provide some insight into the reasons for the resistance. The effect of the combination of immune serum and the resistance to infection on the initial course of parasitaemia was also examined.

Experiment 1

Groups of 17 weeks old C57Bl males, each containing 6 - 7 mice were irradiated with 600 rads on either day -9 or day -2 before infection and reconstituted i.v. with either 1×10^5 or 6×10^6 bone marrow cells or 6×10^6 spleen cells on the day after irradiation. Another group of irradiated mice was given no lymphoid cells and a further group consisted of non-irradiated control mice. The mice were injected i.v. with 1×10^6 P.chabaudi p.r.b.c. on day 0. Mice had no terramycin in their drinking water.

As in the experiments described in chapter 4, irradiated mice challenged within 2 days of irradiation showed some initial resistance to infection when compared to non-irradiated controls. The parasitaemias, expressed as a percentage, are illustrated in Figures 19 and 20 for this experiment. All groups of mice irradiated on day -2 showed some resistance to infection as they had lower mean parasitaemias than the non-irradiated group up to at least day 6 of infection. The irradiated

Fig. 19.

The course of parasitaemia, expressed as a percentage, of P.chabaudi in C57Bl mice irradiated with 600 rads 2 days before infection and injected i.v. with either 6×10^6 bone marrow cells -○- ○- , 1×10^5 bone marrow cells -△- △- , or no lymphoid cells -●- ●- . Another group of mice was neither irradiated nor received any lymphoid cells --□--□--. The mice were injected i.v. with 1×10^6 P.chabaudi p.r.b.c.

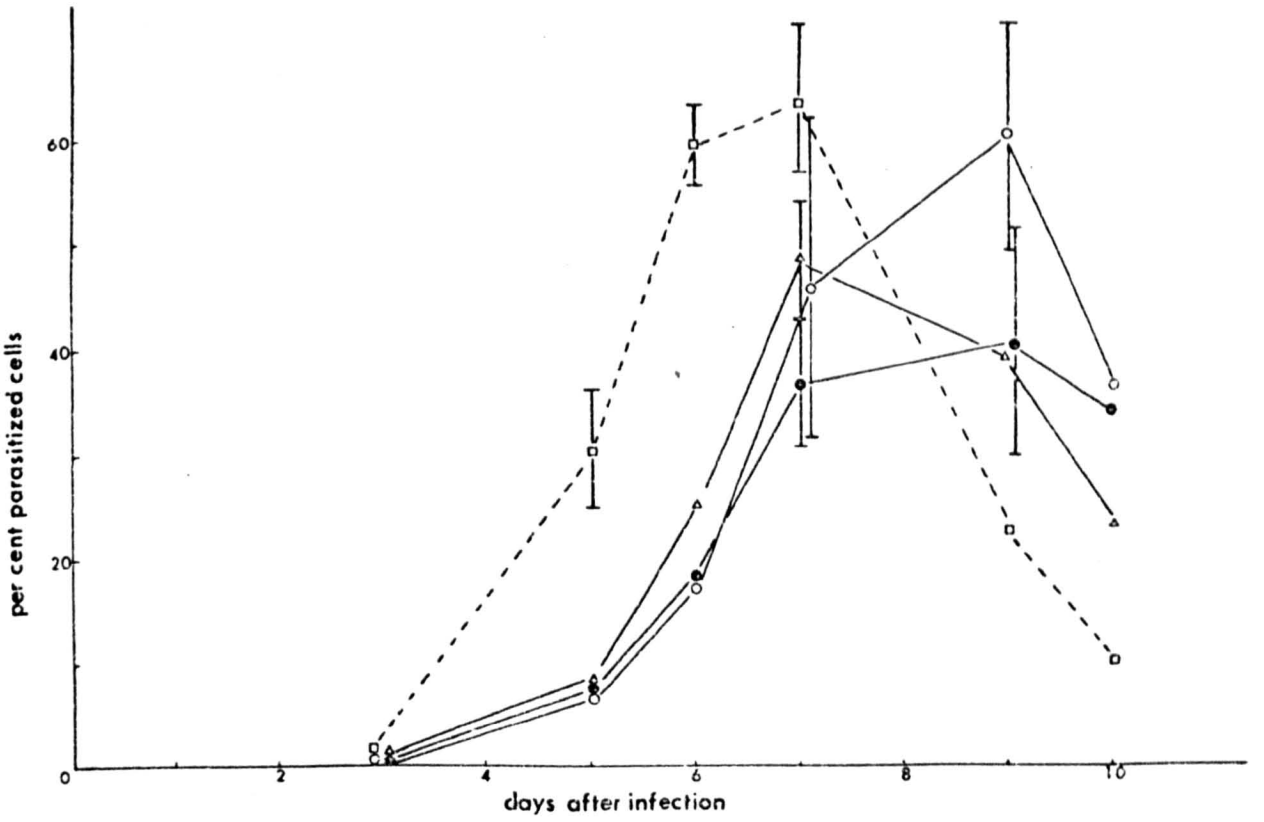
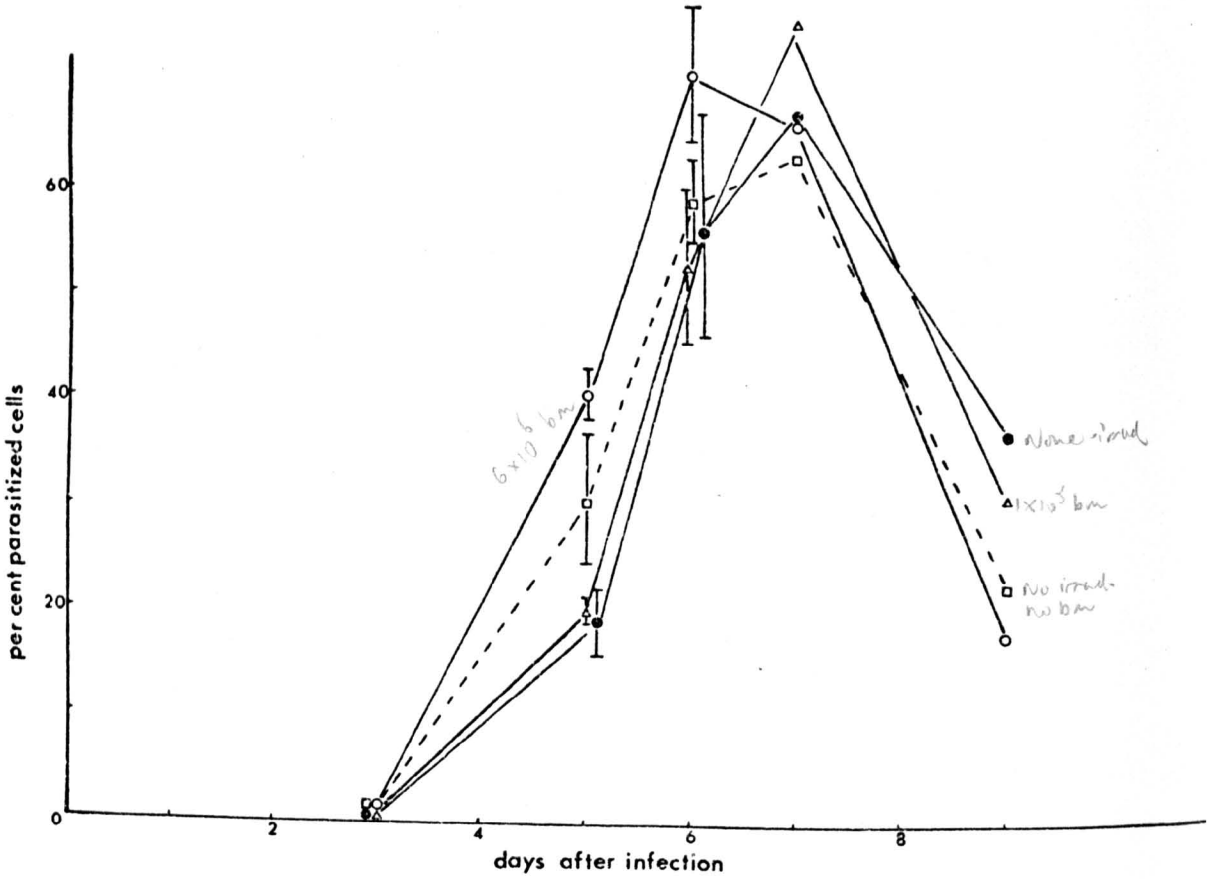

per cent parasitized cells
0
20
40
60
2
4
6
8
10
days after infection

Fig. 20.

The course of parasitaemia, expressed as a percentage, of P.chabaudi in C57Bl mice irradiated with 600 rads 9 days before infection and injected i.v. with either 6×10^6 bone marrow cells -○ — ○-, 1×10^5 bone marrow cells - △ - △ - , or no lymphoid cells - ● — ● - . Another group of mice was neither irradiated nor received any lymphoid cells - - □ - - □ - - . The mice were injected i.v. with 1×10^6 P.chabaudi p.r.b.c.


per cent parasitized cells
0
20
40
60
2
4
6
8
days after infection
6x10⁶ bm
none irrad
1x10⁵ bm
no irrad-
no bm

mice reconstituted with 6×10^6 normal spleen cells had a very similar course of parasitaemia to those mice reconstituted with 6×10^6 bone marrow cells and are not included in the graphs. Mice irradiated on day -2 and reconstituted with 6×10^6 bone marrow cells had generally higher peak parasitaemias than other irradiated mice and this may be important in view of the results shown in Figure 20. Mice irradiated on day -9 and reconstituted with 6×10^6 bone marrow cells had no resistance to infection and in fact had enhanced parasitaemias compared to the non-irradiated controls during the first 6 days of infection. The other groups of mice irradiated 9 days before infection still demonstrated an initial resistance to infection, although, compared to their day -2 counterparts, the resistance was reduced.

In a similar experiment, mice were irradiated on days -3 or -1, and essentially the same result was obtained, except that the resistance in mice irradiated 1 day before infection was not evident until day 5 of infection.

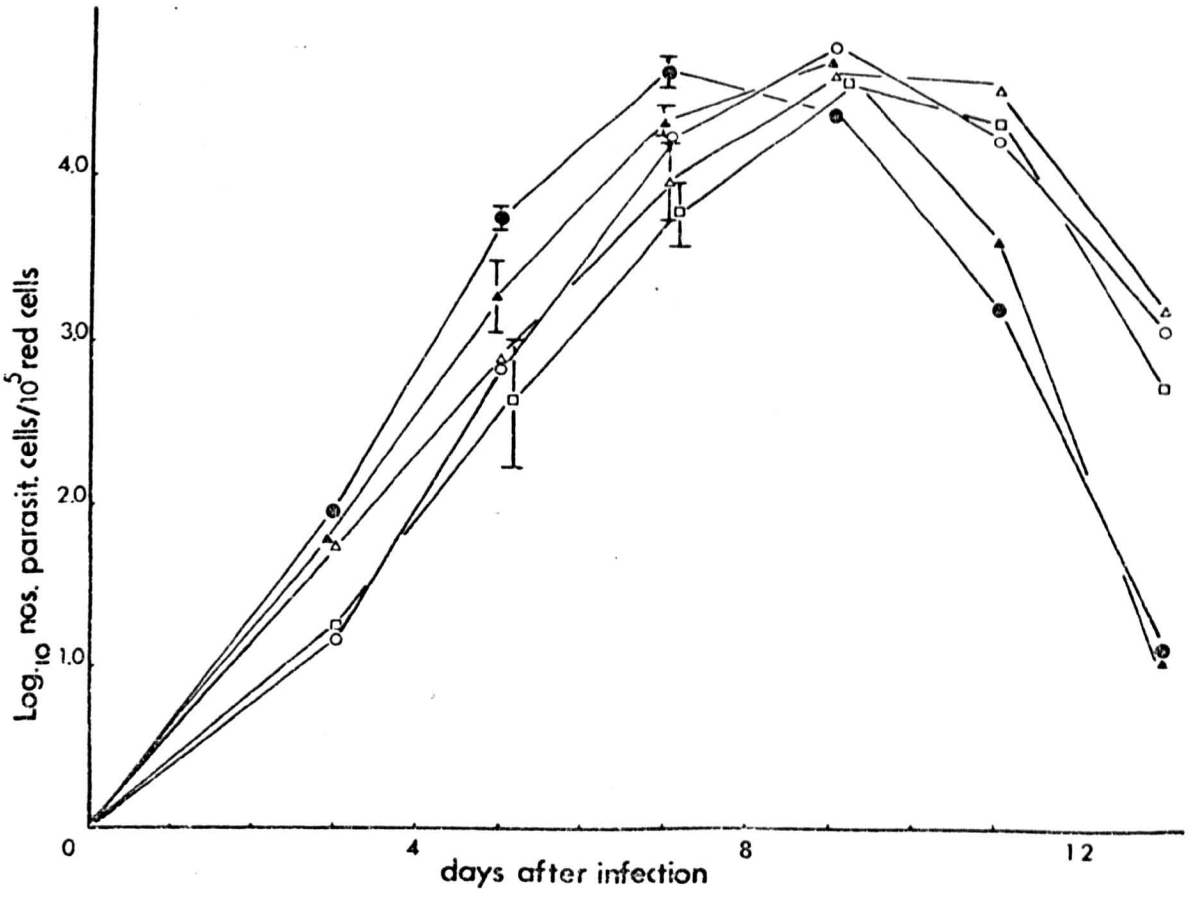
Experiment 2

As the resistance to infection in the previous experiment seemed to decrease in mice irradiated between days -2 and -9 before infection, mice were irradiated at various times before infection to examine this loss of resistance in greater detail. Accordingly, 10 weeks old C57Bl males were given 600 rads on day -22, day -15, day -8 or day -1 without reconstitution with lymphoid cells and all were injected i.v. with 1×10^5 *P.chabaudi* p.r.b.c. A control group of non-irradiated mice was also included. The mice received no terramycin in their drinking water.

The results of the experiment are shown in Figure 21. It is

Fig. 21.

The course of parasitaemia in C57Bl mice irradiated with 600 rads and injected i.v. 10^5 P.chabaudi p.r.b.c. either 22 days after irradiation -●- ●-, 15 days after irradiation -○- ○-, 8 days after irradiation -△- △-, or 1 day after irradiation -□- □-. Also included was a control group of non-irradiated mice -▲- ▲-.


Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
4
8
12
days after infection

evident that mice irradiated on day -22 have enhanced parasitaemias compared to non-irradiated controls. The other groups of irradiated mice demonstrated some initial resistance to infection but the resistance in mice irradiated on day -1 and day -8 lasted longer than for mice irradiated on day -15.

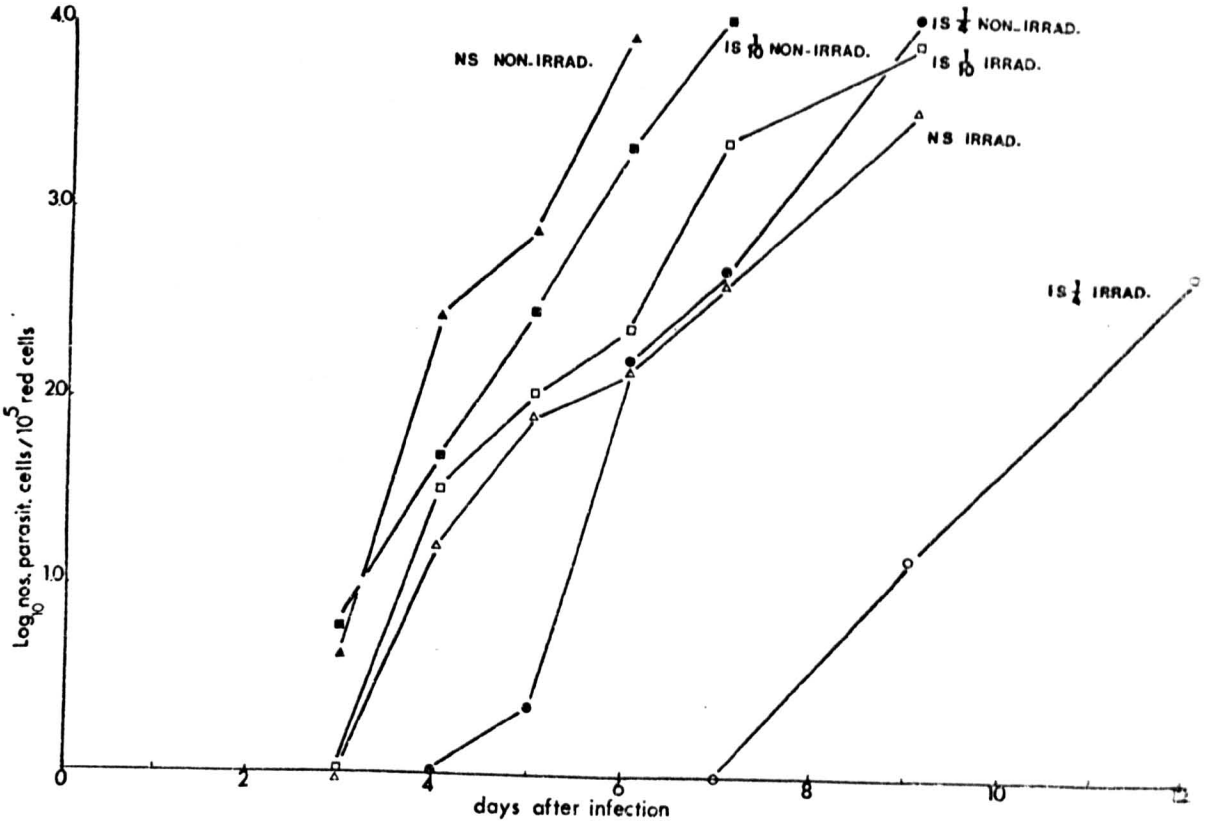
Experiment 3

The combined effect of immune serum and the non-specific resistance to P.chabaudi induced by irradiation in mice was investigated. The immune serum was obtained from mice which had been infected 35 days before sacrifice. One hour after 18 weeks old C57Bl males had been irradiated with 600 rads they were injected i.p. in groups of 3 along with non-irradiated animals, with 1 ml of a 1 in 4 or a 1 in 10 dilution in Hank's B.S.S. of immune serum or with undiluted normal serum. The mice were injected i.v. with 5×10^4 P.chabaudi p.r.b.c. on the same day. For each blood smear examined with a parasitaemia of less than 1%, 10,000 red blood cells were counted, if necessary, in search of parasites. The parasitaemias are presented in Figure 22 and individual parasitaemias are given in Table 2.

The irradiated recipients of 1 in 4 diluted immune serum are the best protected mice, showing no patent parasitaemia until after day 7, at least 3 days after their non-irradiated counterparts, which all remained subpatent until day 5. The non-irradiated mice receiving 1 in 4 diluted immune serum very quickly reached the level of parasitaemia of irradiated recipients of 1 in 10 immune serum or 1 in 10 normal serum, after patent parasitaemias had initially been observed. The resistance of irradiated mice is evident on day 3. Throughout the first 9 days of infection, the non-irradiated recipients of 1 in 10 immune serum had higher parasitaemias than irradiated mice which received normal serum.

Fig. 22.

The course of parasitaemia of P.chabaudi in C57Bl mice which were irradiated or non-irradiated and injected i.v. with a 1 in 4 or a 1 in 10 dilution of immune serum or undiluted normal serum: 1 in 4 immune serum - 600 rads recipients - ○ - ○ - ; 1 in 4 immune serum - non-irradiated recipients - ● - ● - ; 1 in 10 immune serum - 600 rads recipients - □ - □ - ; 1 in 10 immune serum - non-irradiated recipients - ■ - ■ - ; normal serum - 600 rads recipients - △ - △ - ; normal serum - non irradiated recipients - ▲ - ▲ - .


4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
Log10 nos. parasit. cells / 10^5 red cells
days after infection
NS NON-IRRAD.
IS 1/10 NON-IRRAD.
IS 1/4 NON-IRRAD.
IS 1/10 IRRAD.
NS IRRAD.
IS 1/4 IRRAD.

Day of infection	1 in 4 I.S. irradiated mice			1 in 4 I.S. non-irrad. mice			1 in 10 I.S. irradiated mice			1 in 10 I.S. non-irrad. mice			normal serum irradiated mice			normal serum non-irrad. mice		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	1
4	0	0	0	0	0	0	2	2	10	3	9	4	1	2	2	80	24	11
5	0	0	0	1	12	1	3	40	10	90	25	12	10	8	7	90	80	60
6	0	0	0	5	20	40	4	35	80	400	400	50	20	12	15	1100	500	900
7	0	0	0	30	70	50	80	200	700	1100	1900	800	30	50	40	3100	1400	3400
9	350	0	0	600	2600	1300	100	1200	3600	5900	5200	3600	300	600	300	6300	4200	5800
12	800	4	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. The number of parasitized cells per 10^4 r.b.c.'s in NIH mice irradiated with 600 rads or non-irradiated, and injected i.p. with 1ml of immune serum (I.S.) diluted 1 in 4 or 1 in 10 with Hanks B.S.S., or 1ml of normal NIH serum. The mice were injected i.v. with 5×10^4 P.chabaudi p.r.b.c.

Mice given Hank's B.S.S. (not shown on the graph) had parasitaemias similar to those given normal serum.

Discussion

The experiments described in this chapter were carried out in order to determine some of the conditions affecting the non-specific resistance to infection in irradiated C57Bl mice. Similar results have been obtained with NIH mice.

It was discovered that the length of time between irradiation and infection was important. As the period between resistance and inoculation of parasites was increased from 2 days to 22 days, the initial resistance to infection was lost and the infection could actually be enhanced during the initial stages. In one experiment which was not described, the resistance in mice infected 1 day after irradiation was not evident until day 5 of infection, suggesting that the mechanism which is responsible for the resistance may not influence the parasitaemia immediately after irradiation.

Non-specific resistance could be decreased by the injection of lymphoid cells into irradiated mice on the day after irradiation. It is not known whether there is any relationship between the enhancement of parasitaemia after reconstitution with lymphoid cells and the enhancement found in mice which received no lymphoid cells challenged 22 days after irradiation. It is probable that as lymphoid tissue in irradiated mice is repopulated with radioresistant stem cells of the haemopoietic system (Takada, Takada and Ambrus, 1971), a similar environment exists in mice by around day 22 as exists in mice reconstituted with lymphoid cells at an earlier stage before infection, which produces enhanced infections of P.chabaudi in mice treated in both ways.

Some possible reasons for the non-specific resistance to infection in irradiated mice are :

a) The growth of the parasite could be affected in the irradiated host, for example, by the production of toxic substances from cells damaged by irradiation.

b) Irradiation of the host depresses haemopoeisis and it is possible that the numbers of red cells of the type preferentially invaded by P.chabaudi are reduced.

c) There is a depletion of regulatory cells which control the immune response to the parasite.

d) There is a non-specific stimulation of the reticulo-endothelial system which removes parasites or parasitized cells from the circulation.

Examination of blood smears taken throughout a 24 hour cycle from infected mice showed no evidence that intracellular parasites developed from ring to schizont and reinvaded abnormally in irradiated mice. The numbers of merozoites produced in schizonts could have been affected, however.

Non-specific resistance to P.berghei in irradiated mice has been shown to be caused by a reduction in reticulocyte numbers (Singer, 1953). In order to investigate whether or not resistance to P.chabaudi in irradiated animals could be due to a reduction in reticulocyte numbers in irradiated mice, blood from infected animals was smeared on to slides covered with 1% cresyl blue in ethanol which stains for RNA (Emmel and Cowdrey, 1964). These smears were fixed and stained in Giemsa's stain for parasite examination. It was evident that in normal mice with approximately 1 - 2% reticulocytes in the blood, very few reticulocytes were invaded at low parasitaemia. It seemed, therefore, unlikely that

the initial decreased parasitaemia in irradiated mice was due to a decrease in reticulocyte numbers, although the parasite may preferentially invade blood cells which are intermediate between late reticulocytes and mature red cells, and which are not detected by staining with cresyl blue or Giemsa's stain. In a further experiment, whole blood was injected into irradiated and non-irradiated mice immediately before infection in order to examine whether the resistance would be still as apparent in irradiated mice injected with 'normal' blood cells. It was found that if 0.7ml of blood was injected into mice, irradiated recipients of whole blood were still as resistant to infection compared to non-irradiated recipients.

Resistance to Listeria monocytogenes and Brucella abortus infection has been demonstrated in irradiated mice and in athymic nude mice (Campbell, Martens, Cooper and McClatchy, 1974; Cheers and Waller, 1975; Chan, Longshawn and Skamene, 1977). Chan et al. postulated that the loss of regulatory suppressor T cells (Baker, 1975) may be responsible for resistance to bacterial infection since they are radiosensitive and are also depleted in nude mice (Okumara and Tada, 1971; Baker, 1975). Clark and Allison (1975), however, have reported enhanced P.yoelii infections in nude mice. Wyler et al. (1976) demonstrated indirect evidence for suppression by the spleen in the eradication of a subclinical P.inui infection. The adoptive transfer of spleen cells from NIH mice 15 days after irradiation, when these mice would have shown enhanced parasitaemias on infection, did not affect the course of parasitaemia in non-irradiated NIH mice.

Sljivic (1970a) demonstrated that the phagocytic function of the reticulo-endothelial system as measured by carbon clearance test, was increased by irradiation of mice. The clearance rate was increased

with increased doses of irradiation and could be reduced by injecting lymph node cells or bone marrow cells into irradiated mice, or by introducing antibodies into drinking water (Sljivic, 1970b). Since bone marrow or spleen cells also reduced or abolished the partial resistance to infection with P.chabaudi, it is possible that the mechanism for an increase in carbon clearance in irradiated mice is similar to that causing resistance to P.chabaudi. Irradiated mice given an antibiotic in this study, however, were still resistant to infection. Cheers and Waller (1975) observed an initial increased resistance to bacterial infection in irradiated mice and also that macrophages from irradiated mice were activated, having an increased capacity to ingest bacteria in vitro. The explanation put forward by Sljivic and by Cheers and Waller for their observations was that the reticulo-endothelial system was stimulated by the leakage of bacteria or bacterial endotoxin from the gut. The combination of the effect of the immune serum and the non-specific resistance to infection to give a much enhanced passive immunity during the initial stages of infection, suggests that activated macrophages may be involved in the phagocytosis of opsinized parasites as reported in previous studies (Brown, 1971; Criswell, Butler, Rossen and Knight, 1971; Chow and Kreier, 1972). It is known that stimulation of the reticulo-endothelial system occurs during malaria infections (Cox, Bilbey and Nicol, 1964; Lucia and Nussenzweig, 1969; Cantrell and Elko, 1970). It is not known whether this stimulation plays a significant role in the effector arm of immune mechanisms, although stimulation of the reticulo-endothelial system by Corynebacterium parvum makes mice resistant to P.berghei infection (Nussenzweig, 1967; Clark, Cox and Allison, 1977). If phagocytosis of parasites is involved in the non-specific resistance to infection, a decrease in this resistance or enhancement of infection

as the length of time increased after irradiation, may result from a blockade of the reticulo-endothelial system caused by the eventual uptake of dead cells and cellular debris by fixed macrophages after irradiation. The injection of lymphoid cells after irradiation may speed up this process. Such a blockade of the reticulo-endothelial system could result in a reduced phagocytosis or in a suppressed cellular or humoral response to antigen (Sabet, Newlin and Friedman, 1969).

Irradiated mice in cell transfer studies were all infected within 48 hours of irradiation and for the numbers of lymphoid cells injected, it is likely that these mice would initially demonstrate some resistance to infection. It is possible that where mice were injected with immune spleen cells, malarial antibodies would be produced. A similar situation would arise, therefore, to that in which irradiated animals injected with immune serum had an initially enhanced immunity while the protective effect of the immune serum lasted, except that malarial antibodies would be continually produced in recipients of immune spleen cells. Thus, irradiated immune spleen cell recipients would establish an enhanced immunity, which may at least partially explain why a smaller number of immune cells is required to confer a degree of immunity to irradiated mice than to non-irradiated mice.

CHAPTER 9

The anti-parasitic effect of serum from irradiated recipients of immune spleen cells or normal spleen cells during infection

Passive transfer experiments have demonstrated that immune serum provides a degree of protection against malaria infection. Such protection has been demonstrated with human malaria (Cohen et al., 1961), simian malaria (Coggeshall and Kumm, 1937; Butcher et al., 1970) and also rodent malaria (Zuckerman and Golenser, 1970; Phillips and Jones, 1972; Diggs and Osler, 1969; Jayawardena et al., 1975a; Hamburger and Kreier, 1975). The IgG fraction of immunoglobulin has been associated with this immunity (Cohen et al., 1961; Diggs and Osler, 1969; Butcher et al., 1970; Phillips and Jones, 1972) and the most protective antibody in P.berghei infected rats appears around the time of elimination of a malaria infection (Phillips and Jones, 1972).

In the previous chapter, the passive transfer of immunity with immune serum was demonstrated with P.chabaudi infections in irradiated and non-irradiated mice. It was found that better protection was obtained with immune serum in irradiated mice than in non-irradiated mice. For investigative purposes, therefore, irradiated mice were used to examine the protective effect of serum taken at different times during infection from irradiated recipients of either unfractionated immune spleen cells, enriched immune T cells or normal spleen cells. Mice from groups of cell recipients were sacrificed at 2 points during a primary parasitaemia, serum was collected and pooled for each group, and was passively transferred into irradiated recipients which had been infected with a small inoculum of parasites.

Experimental procedure

Eight months old NIH females were irradiated with 800 rads and 2 x 10^6 spleen cells were injected i.v. 1 hour later. Groups of 20 mice received either unfractionated immune cells, enriched immune T cells or normal cells. The immune cells came from 2 NIH female donors which had been infected 35 days before the spleens were removed. Normal cells came from the spleens of 2 non-immune 8months old NIH females. Spleen cell recipients were injected i.p.,on the same day as cell transfer, with 2 x 10^6 P.chabaudi p.r.b.c.

The development of parasitaemia was monitored in 5 or 6 mice from each group. On day 11 of infection, half of the mice in each group were sacrificed and bled for serum and on day 14 of infection, the remaining mice in each group were treated similarly. Sera from mice within each group were pooled on each day and stored at -20°C until required.

Twelve weeks old NIH females were given 600 rads and 20 hours later were infected i.v. with 10^4 P.chabaudi p.r.b.c. of the same parasite population used for infecting the spleen cell recipients. Shortly afterwards, the mice were injected i.v. with 0.5ml of pooled sera,collected from either the unfractionated immune cell recipients, the enriched immune T cell recipients or the normal cell recipients on days 11 and 14. A control group of irradiated mice was also included which received normal NIH serum. Smears from these mice were examined for parasites and, if necessary, 10,000 red cells were examined.

The numbers of immunoglobulin-bearing cells in spleen cell inocula were 1% and 34% for the enriched immune T cells and the unfractionated immune cells, respectively.

The mean primary parasitaemias for cell recipients are presented in Figure 23 and the arrows mark the times at which sera were collected. Recipients of unfractionated immune cells consistently had lower parasitaemias from days 12 - 14 than recipients of enriched immune T cells. Sera were collected just after the peak parasitaemia of recipients of immune cells and then on the day when a sample of 6 out of 10 remaining unfractionated immune cell recipients became subpatent.

The results of the passive transfer of sera collected on days 11 and 14 are given in Figures 24(a) and 24(b) respectively and in Tables 3(a) and 3(b) respectively. Generally mice which received serum from day 11 of infection showed little protection against infection when compared with mice which received normal serum except for the serum from mice which received unfractionated immune cells. On day 3, 4 out of 5 of the mice which received serum from unfractionated immune cell recipients were still subpatent whereas all but one of the mice in the other 2 groups which received sera from infected cell recipients were showing patent parasitaemias. One mouse, however, in each of the groups which received serum collected on day 11 from recipients of unfractionated immune cells or enriched immune T cells, showed low parasitaemias (Table 3(a)).

A different result was obtained with the passive transfer of sera collected from infected spleen cell recipients on day 14. There wasprotection throughout the time the parasitaemias were monitored in mice given serum from the unfractionated cell recipients (Figure 24(b) and Table 3(b)). No parasites were observed in any of these mice on day 3 after the passive transfer of serum and, up to day 7, less than 10 parasites per 10,000 red cells were counted for each mouse. Serum

Fig. 23.

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 800 rads and injected with either 2×10^6 unfractionated immune spleen cells -○—○- , 2×10^6 enriched immune T cells -□—□- , or 2×10^6 normal spleen cells -●—●- . The mice were injected i.p. with 2×10^6 P.chabaudi p.r.b.c. Sera were collected on days 11 and 14, marked with arrows

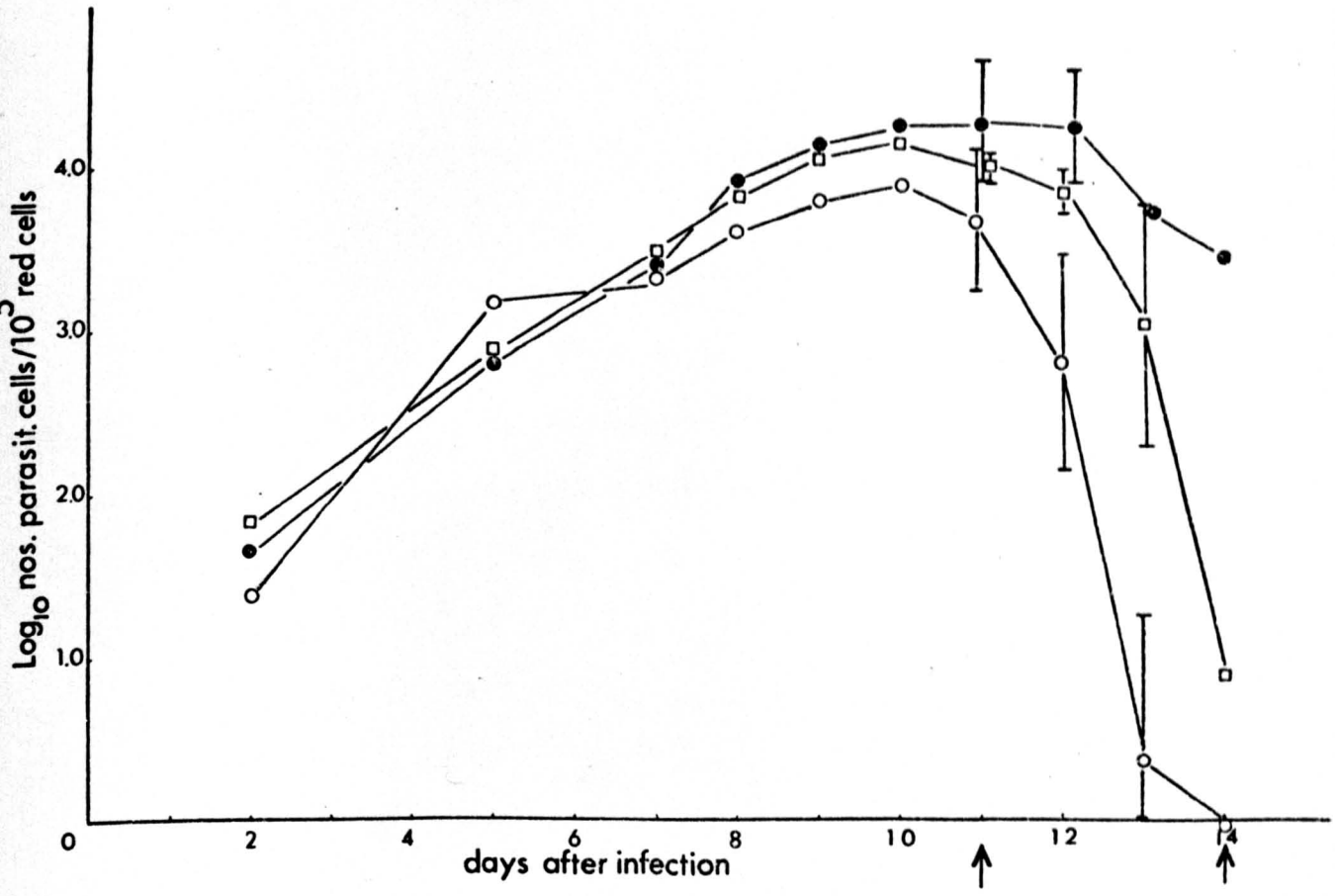
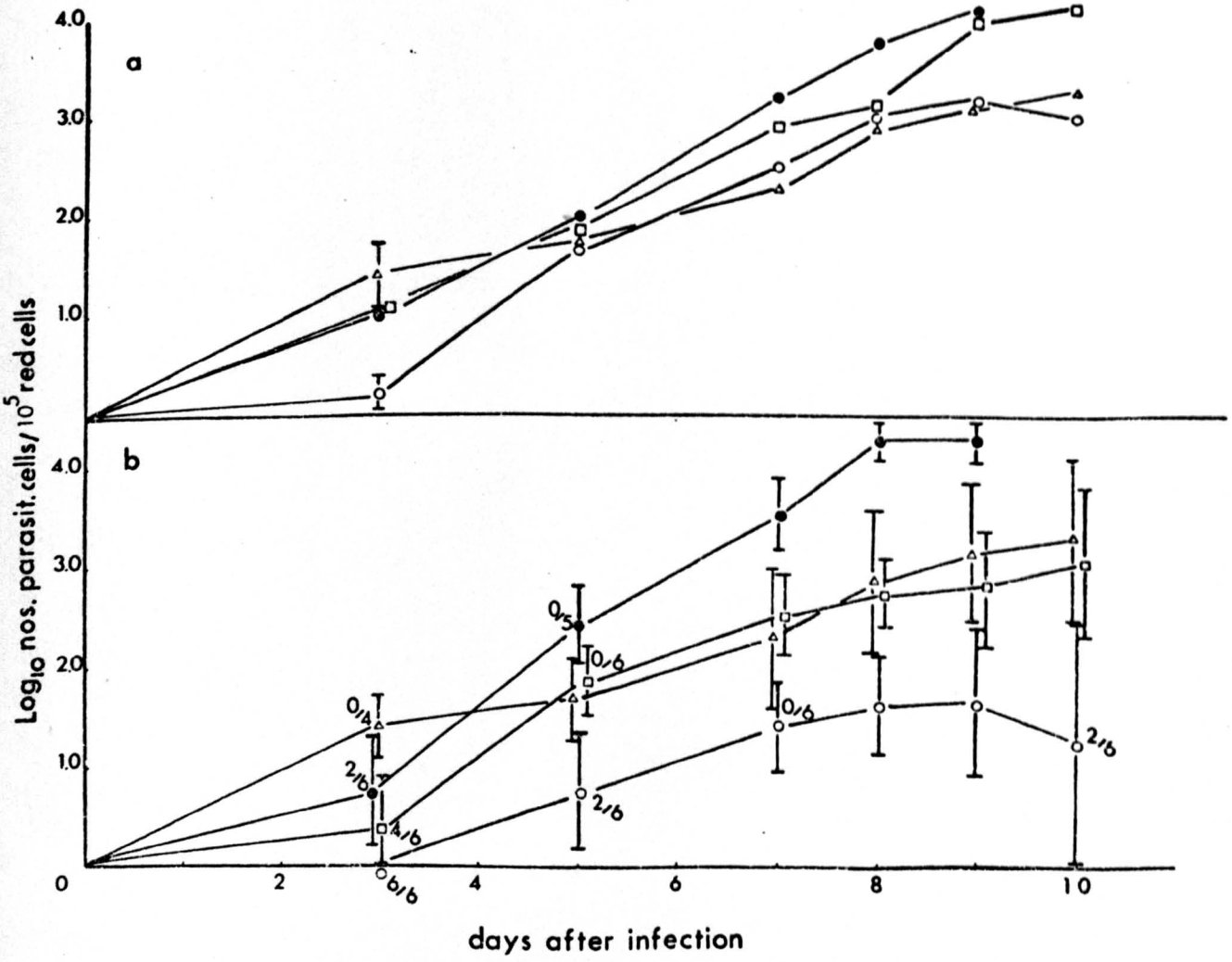

Log10 nos. parasit. cells/10^5 red cells
4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
14
days after infection

Fig. 24(a).

The course of parasitaemia of P.chabaudi in NIH mice irradiated with 600 rads and injected i.v. with 0.5ml of sera collected on the 11th day of a P.chabaudi infection in recipients of either 2×10^6 unfractionated immune spleen cells -○- ○-, 2×10^6 enriched immune T cells -□- □-, or 2×10^6 normal spleen cells -●- ●-. A control group of 600 rads-irradiated mice received 0.5ml of normal NIH serum -△- △-. The serum recipients were injected i.v. with 1×10^4 P.chabaudi p.r.b.c.

Fig. 24(b).

The same as Fig. 24(a) except that the sera were collected from spleen cell recipients on the 14th day of P.chabaudi infection. The fractions beside certain symbols represent the number of subpatent mice of a group / the total number of surviving mice in that group, on a particular day


a
b
4.0
3.0
2.0
1.0
0
2
4
6
8
10
Log_{10} nos. parasit. cells/10^5 red cells
days after infection
0/5
0/6
0/4
2/6
4/6
6/6
0/6
2/6
2/6

Day of infection	Sera from normal cell recips. mouse no:				Sera from immune cell recips. mouse no:					Sera from enr. immune T cell recips. mouse no:					normal serum mouse no:			
	1	2	3	4	1	2	3	4	5	1	2	3	4	5	1	2	3	4
3	4	2	0	1	0	0	1	0	0	0	1	5	1	2	3	6	1	2
5	40	30	1	8	0	9	20	6	14	3	1	30	30	30	4	20	2	3
7	600	700	D	10	1	D	100	60	150	50	4	800	300	70	6	200	20	10
8	900	3000		60	1		1300	200	400	100	2	1900	400	200	20	800	30	70
9	1600	3600		300	1		1900	300	800	500	D	2900	2000	400	30	1300	60	200
10	3300	4300		800	0		4400	400	1400	600		4300	2500	400	30	2300	80	300

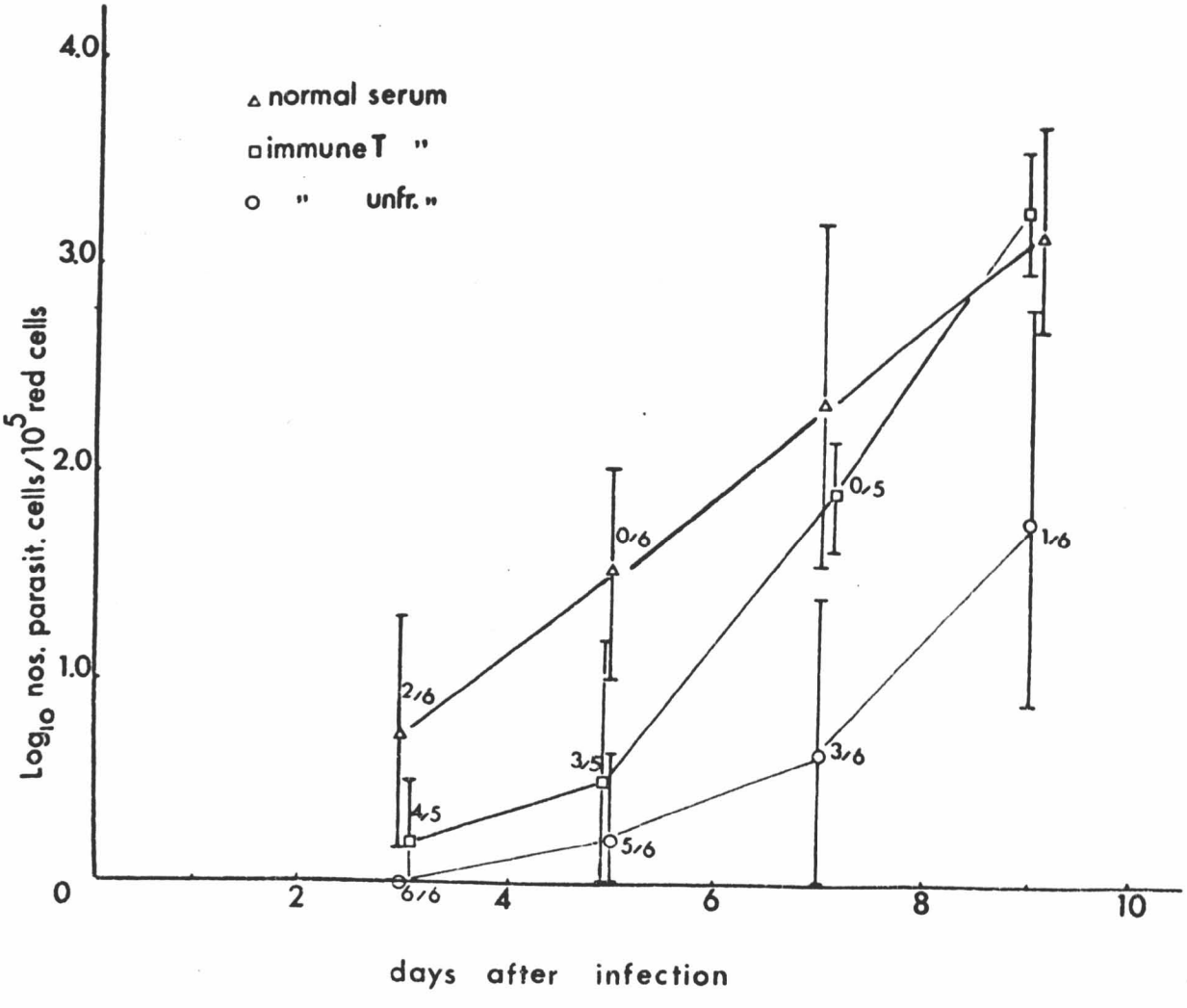
Table 3(a). The number of parasitized cells per 10^4 r.b.c.'s in NIH mice irradiated with 600 rads and injected i.v. with 0.5ml serum from spleen cell recipients on their 11th day of infection. The serum recipients were injected i.v. with 1×10^4 P.chabaudi p.r.b.c.

transferred from enriched immune T cell recipients also gave protection on day 3, as 4 out of 6 of the infected mice in this group showed no parasites. The mean parasitaemia in these latter recipients soon rose to the level of irradiated recipients of normal NIH serum. The mean parasitaemia appeared to be enhanced following the passive transfer of serum from normal spleen cell recipients although on day 3, 2 out of the 5 mice in this group were subpatent and none of the 4 control mice were subpatent at this time.

Obvious differences in the degree of protection to P.chabaudi between groups of mice which had serum passively transferred to them from spleen cell recipients infected for 14 days were often not statistically significant. In order to confirm the results obtained, the remaining sera collected on day 14 were injected into non-irradiated mice infected with a small inoculum of parasites. Unfortunately, there was no serum remaining from normal cell recipients. Groups of 5 or 6 NIH females, 22 weeks old were infected i.v. with 1×10^4 P.chabaudi p.r.b.c. The mice were injected i.v. immediately after with 0.5ml of normal serum from either infected unfractionated immune cell recipients or infected enriched immune T cell recipients, collected on day 14 of infection. The resultant parasitaemias are shown in Figure 25. The serum from unfractionated immune cell recipients was more protective than that of control mice receiving normal serum. Although the serum from enriched immune T cell recipients was not as protective as that from unfractionated immune cell recipients, it is clear that this serum was more protective than normal NIH serum from the number of mice which were subpatent in this group during the first 5 days of infection.

Fig. 25.

Repeat of experiment shown in Fig. 24(b) except that non-irradiated mice were used. NIH mice were injected i.v. with 0.5ml of sera collected on the 14th day of infection with P.chabaudi in unfractionated immune cell recipients -○ - ○- or enriched immune T cell recipients -□ - □- and also a control group which received normal NIH serum -△ - △- . The serum recipients were injected i.v. with 1 x 10^4 P.chabaudi p.r.b.c.


4.0
3.0
2.0
1.0
0
2
4
6
8
10
△ normal serum
□ immune T "
○ " unfr. "
Log10 nos. parasit. cells/10^5 red cells
days after infection
0/6
2/6
4/5
3/5
0/5
5/6
6/6
3/6
1/6

Identification of the stages of P.chabaudi affected by the host's immune response

Experiments were carried out in which both C57Bl and NIH mice which were immune to P.chabaudi were reinfected with a large number of parasites along with normal mice, and the parasitaemias were examined regularly over 48 hours.

In one such experiment in which C57Bl mice were used, the immune mice had been inoculated with P.chabaudi 52 days and 8 days before the start of the experiment. At 1045 hours, 2 immune and 2 normal mice were injected i.v. with 0.25ml or 0.3ml of infected blood concentrated by centrifugation and resuspended in half the volume of plasma. Each mouse received 1.25 - 1.46 x 10^8 parasitized cells, consisting mainly of ring stages, which gave an instant parasitaemia of about 2%. The growth and multiplication of the parasites was followed and is illustrated in Figure 26. In immune mice there was a decrease in parasitaemia over the next 48 hours with a loss of parasites between the trophozoite and schizont stage. In the control mice there was an overall increase in parasitaemia over the first 48 hours although there was a slight decrease during the development period between the late trophozoite stage and the schizont stage. The decrease in parasitaemia observed in controls between the late trophozoite and schizont stage was much smaller than that which occurred in immune mice. This result suggests that the immunity is directed against the late trophozoite and schizont stage and possibly the merozoite stage.

In a similar experiment, phagocytosis by the spleens and liver of C57Bl mice was examined in order to detect the parasite stages which were being removed from the circulation. The immune mice had been infected 162 days and 27 days before the start of the experiment and

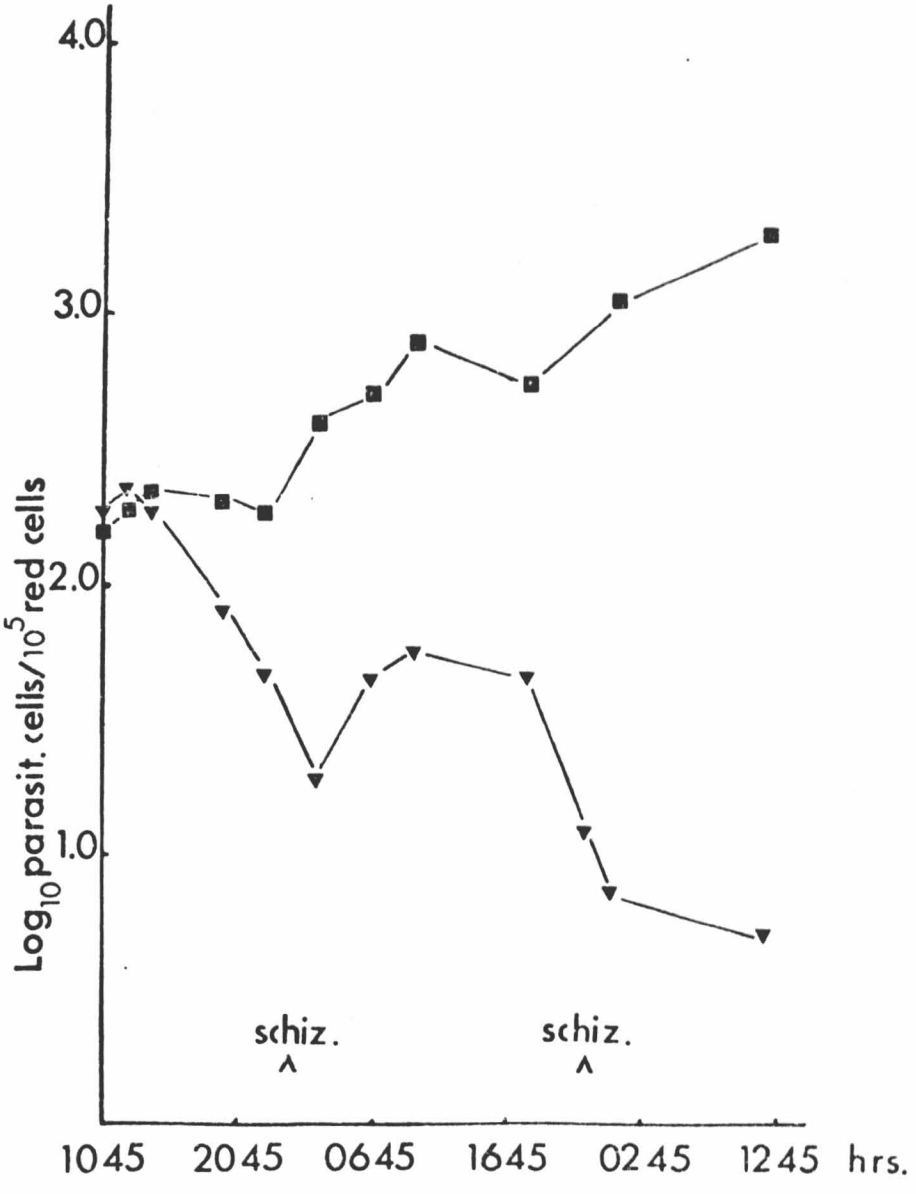


Figure 26.

The removal of P.chabaudi parasites from the blood of immune mice -▼— ▼- and non-immune controls -■ - ■ -.

were 33 weeks old. Five immune and 3 normal C57Bl mice were injected i.v. with 0.25ml of concentrated blood between 0945 and 1006 hours to give an instant parasitaemia of 4.5%. Individual mice were killed off between 2330 hours and 0200 hours with schizogony being around 0000 hours, and spleen and liver impression smears were made. There was an increase in parasitaemia from 5% to 17% after schizogony in control mice compared to a decrease from 5% to 1.4% in immune mice. There was no difference in the rate of growth between immune mice and control mice up to the schizont stage although as in the previous experiment there was a greater loss of parasites in the blood of immune mice than control mice from the late trophozoite to the schizont stage. Giemsa's stained liver and spleen smears showed that from just before to just after schizogony, phagocytic monocytes had taken up red cells containing late trophozoites and schizonts with usually only 1 parasitized cell being taken up by one phagocyte but larger numbers of parasitized cells were also taken up by single phagocytes. No phagocytosis of parasitized cells by polymorphs was observed in immune mice and there was no evidence of the phagocytosis of free merozoites either in mice. Although there was a slight withdrawal of late trophozoites from the peripheral circulation in control mice, no evidence of phagocytosis of parasitized red cells was found. A further experiment demonstrated that if the immune mice were sacrificed between the ring and late trophozoite stages then no parasitized cells were taken up by the spleen or liver.

The implication of these results using C57Bl mice immune to P.chabaudi infection is that the intracellular stages from late trophozoite to schizont are the only intracellular stages which are phagocytosed in the spleen and liver. Experiments performed using NIH mice gave similar results.

Discussion

The protective effect of passively transferred sera isolated from irradiated mice which had been the recipients of unfractionated immune spleen cells, enriched immune T cells or normal spleen cells reflected the immune status of the serum donors. Just as unfractionated immune cells were more protective than enriched immune T cells, serum collected from the infected recipients of the former cells on day 14 was more protective than serum collected from the recipients of the latter cells. Serum collected from normal cell recipients, which were the least protected of all mice, was not only the least protective of all sera collected from recipients on day 14 of infection, but appeared to enhance the parasitaemia when passively transferred to infected mice. It has been demonstrated previously that the protective effect of immune serum also reflected the immune status of the donor during P.berghei infection in rats (Phillips and Jones, 1972).

The development of increasingly effective immune serum between days 11 and 14 of infection of spleen cell recipients is most evident for unfractionated immune cell recipients, but also occurs to a lesser degree for enriched immune T cell recipients.

Although the protective effect of serum from enriched immune T cell recipients was short lived in this experiment, this does not mean to say that humoral factors, presumably malarial antibodies, have no major role in immunity to P.chabaudi infection. It is possible that the experimental system is not as sensitive as it might be and perhaps could be improved by decreasing the infective inoculum given to the recipients of sera. Also, as only excess malarial antibodies are obtained in the serum of donors, most of the protective antibodies produced in enriched immune T cell recipients may be actually involved

in the elimination of parasites or complexed to malarial antigens (Houba, Lambert and Soyanevo, 1975). The presence of antibody-antigen complexes can induce the production of suppressor cells which decrease the amount of antibody produced (Taylor and Basten, 1976). Antigenic variation of malarial parasites may affect the protective properties of the transferred serum (Brown and Brown, 1965). As immunity to P.knowlesi infection in monkeys increases, malarial antibodies can be produced in monkeys against parasite variants which have not previously been detected (Butcher and Cohen, 1972). Unfractionated immune cell recipients in the P.chabaudi system may, therefore, produce some cross-reacting antibody which, when passively transferred to infected recipients, would be at least partially protective against new variants of parasites arising in these infected animals. Enriched immune T cell recipients have fewer memory B cells or plasma cells transferred to them than do unfractionated immune cell recipients so that the former mice can be expected not only to have smaller amounts of antibody produced, but also a smaller spectrum of antibodies, including cross-reacting antibodies directed against new variants which may arise during infection.

In chapters 5 and 6 it was established that irradiated recipients of enriched immune T cells after infection with P.chabaudi were more likely to have parasitaemia relapses than irradiated recipients of unfractionated immune cells or enriched immune B cells. The decreased protective properties of serum from enriched immune T cell recipients compared to unfractionated immune cell recipients may continue into subpatency of infection, allowing more parasites to survive and multiply, leading to relapsing patent parasitaemias in enriched immune T cell recipients. (Unfortunately, due to the large number of mice required for this experiment, a group of enriched immune B cell recipients could not be included, although it would have been useful to

obtain some information on the protective activity of serum from such mice).

The fact that there is initially some protection from serum collected from enriched immune T cell recipients implies that humoral immunity has at least a partial role in immunity in these mice. However, the imbalance in the proportion of T cells to B cells in irradiated mice which receive enriched immune T cells could perhaps lead to an increased role for some type of cell-mediated immunity to infection and less of a humoral response (Finerty and Krehl, 1976 and see chapter 6 for discussion on the manifestations of cell-mediated immunity). This may be entirely an artificial situation, however, and unlikely to occur where there is a normal proportion of T cells to B cells.

Serum collected from normal cell recipients on day 15 may have enhanced parasitaemias due to some immunosuppressive factor in the serum although parasitaemias in serum donors were decreasing on day 15. The enhanced parasitaemia could have been caused by a dilution effect in the passive transfer similar to that observed by Jerusalem, Weiss and Poels (1971), who found that serum which was normally protective to mice infected with P.berghei, actually enhanced the parasitaemia when diluted.

The mechanism of humoral immunity to the asexual blood stage of malarial infection is still unclear. There is evidence that malarial antibodies adhere to extracellular parasites (Hamburger and Kreier, 1975) and to merozoites (Miller, Aikawa and Dvorak, 1975). Merozoites agglutinate in the presence of immune serum in vitro (Butcher and Cohen, 1970; Miller et al., 1976). Parasitized cells from the late trophzoite to the schizont stage of development of P.knowlesi parasites can agglutinate in the presence of immune serum, but earlier

stages in development of the parasite do not agglutinate (Brown and Brown, 1965). Observations with mice immune to P.chabaudi inoculated with around 10^8 parasites, demonstrated that the parasitaemia greatly decreased as parasites in the blood developed from late trophozoites to schizontscompared to control mice. Giemsa's stained impression smears of the spleen and liver showed that late trophozoites and schizonts were taken up by monocytes but no ring stages were taken up. Humoral immunity may, therefore, only be effective during a relatively short part of the parasite's asexual cycle in the blood.

Agglutination of P.knowlesi parasitized cells with serum from infected animals does not necessarily coincide with immunity in vivo (Butcher and Cohen, 1972; Brown and Hills, 1974). Some workers, however, have found some correlation with the immune status of malaria infected hosts and the inhibitory effect of sera from these hosts to the growth of parasites in culture (Butcher and Cohen,1972; Wilson and Phillips, 1976) while other workers found no correlation (Miller, Powers and Shiroishi, 1977).

There would appear to be no requirement for complement for in vitro inhibition of parasite multiplication (Cohen and Butcher, 1970; Brown et al., 1970) and there is no requirement for the third component of complement for immunity to P.berghei in vivo (Diggs, Shen, Briggs, Laudenslayer and Weber, 1972).

It has been reported that antibodies are produced during P.berghei infection which adhere to non-infected erythrocytes (Zuckerman, 1960; Lustig et al., 1977). These antibodies may be protective to the host by blocking receptors on erythrocytes which are required to be recognized by the penetrating erythrocyte, such as the receptor associated with the Duffy blood group probably required for P.vivax

invasion of human red blood cells (Miller, Mason, Clyde and McGuinness, 1976). Antibodies produced against the host's own normal erythrocytes, however, may lead to anaemia as hyperimmune serum to P.gallinaceum from chickens can protect chick embryos from infection but also produces slower increases in packed cell volume of blood than in embryos treated with normal serum (McGhee, 1976).

Opsonizing antibodies may be important in the elimination of parasites as there is evidence that macrophages show increased phagocytosis of parasites in the presence of immune serum (Zuckerman, 1947; Brown et al., 1970a; Chow and Kreier, 1972; Hamburger and Kreier, 1975). Preliminary experiments with the P.chabaudi system gave similar results although some normal red blood cells were taken up by macrophages. There is, however, one report of a cell-mediated effector mechanism which is possibly antibody-dependent in rodent malaria, as shown by the in vitro lysis of P.berghei parasitized red blood cells in the presence of spleen cells (Coleman et al., 1975).

Serum factors other than antibody could play a role in immunity to infection, as for example, in the killing of intracellular P.vinckei and P.yoelii parasites in B.C.G. immunized mice (Clark et al., 1976). The induction of interferon production in mice can result in the protection against infection by P.berghei sporozoites (Jaheil et al., 1968), although there is controversy as to whether interferon can be shown to have a significant effect on blood stage infections, with Jaheil et al. (1968) observing no protective effect and Schultz et al. (1968) observing protection in interferon treated mice.

The results of these experiments indicate the importance of humoral immunity against malaria infection and point to an antibody-dependent immune response which probably involves an effector cell, either the macrophage or some unknown type of cell.

CHAPTER 10

K cell activity during malaria infection

Although immunity to malaria infection can be passively transferred with immune serum (see previous chapter), the protection which is obtained in recipients is usually only temporary, probably as a result of the short half-life of immunoglobulin *in vivo*. Effector cells which are ultimately responsible for the killing and/or removal of parasites from the circulation may depend on the presence of malarial antibodies for their action. There is evidence that macrophages may be effector cells, especially in the presence of opsonizing antibodies (Brown et al., 1970; Chow and Kreier, 1972; also see chapter 9).

Another mechanism which could be involved in the death of malaria parasites is antibody-dependent cell-mediated cytotoxicity, a non-specific cytotoxic reaction of lymphoid cells on target cells in the presence of antibody directed against the target cell (MacLennan and Harding, 1970; Perlmann and Perlmann, 1970). The effector cells are lymphocytes (Perlmann and Perlmann, 1970; Calder, Urbaniak, Penhale and Irvine, 1974; Sanderson and Taylor, 1976) and are known as K cells. There is evidence that K cells are neither B cells (Wisloff and Freland, 1973; Calder et al., 1974) nor T cells (Van Boxel, Stobo, Paul and Green, 1972) and may belong to the population of "null cells" (Greenberg, Hudson, Shen and Roilt, 1973) which exists in small numbers in lymph nodes and spleen cells of normal animals (Stobo, Talal and Paul, 1972). The class of antibody responsible for lysis of target cells in antibody-dependent cell-mediated cytotoxic reactions with K cells is the IgG fraction of immunoglobulin (Calder et al., 1974) although immune complexes containing IgG may inhibit the reaction (Jewell and MacLennan, 1973).

The activity of K cells may be important in the control of tumour development in mice (Ghaffer, Calder and Irvine, 1976) and also in auto-immune disease (Calder, Penhale, MacLennan, Barnes and Irvine, 1973). Recent evidence has suggested that the K cell activity of peripheral lymphocytes is increased in children infected with P.falciparum (Greenwood, Oduloju and Stratton, 1977).

The K cell activity of spleen cells from mice infected with P.chabaudi was measured in order to determine whether the activity varies during a primary malaria infection. Chicken red blood cells (C.R.B.C.) with rabbit anti-CRBC serum have been used successfully in assaying K cell activity of mouse spleen cells (Ghaffer, Calder and Irvine, 1976) and were used in preliminary experiments in this study.

Experimental procedure

The technique for the assay of K cell activity is described in Materials and Methods in Chapter 2. Male C57Bl mice aged between 12 and 16 weeks old were injected with 5×10^6 P.chabaudi p.r.b.c. and the K cell activity of their spleens was compared to that of non-infected mice. Cultures containing rabbit anti-C.R.B.C. serum were performed at least in triplicate and control samples containing normal rabbit serum were performed in duplicate. Samples containing 1×10^6 C.R.B.C. instead of spleen cells were also included as a further control. The percentage cytotoxicity was calculated for each sample and mean values $\pm$ standard deviations were calculated. Student t-tests were performed on the results.

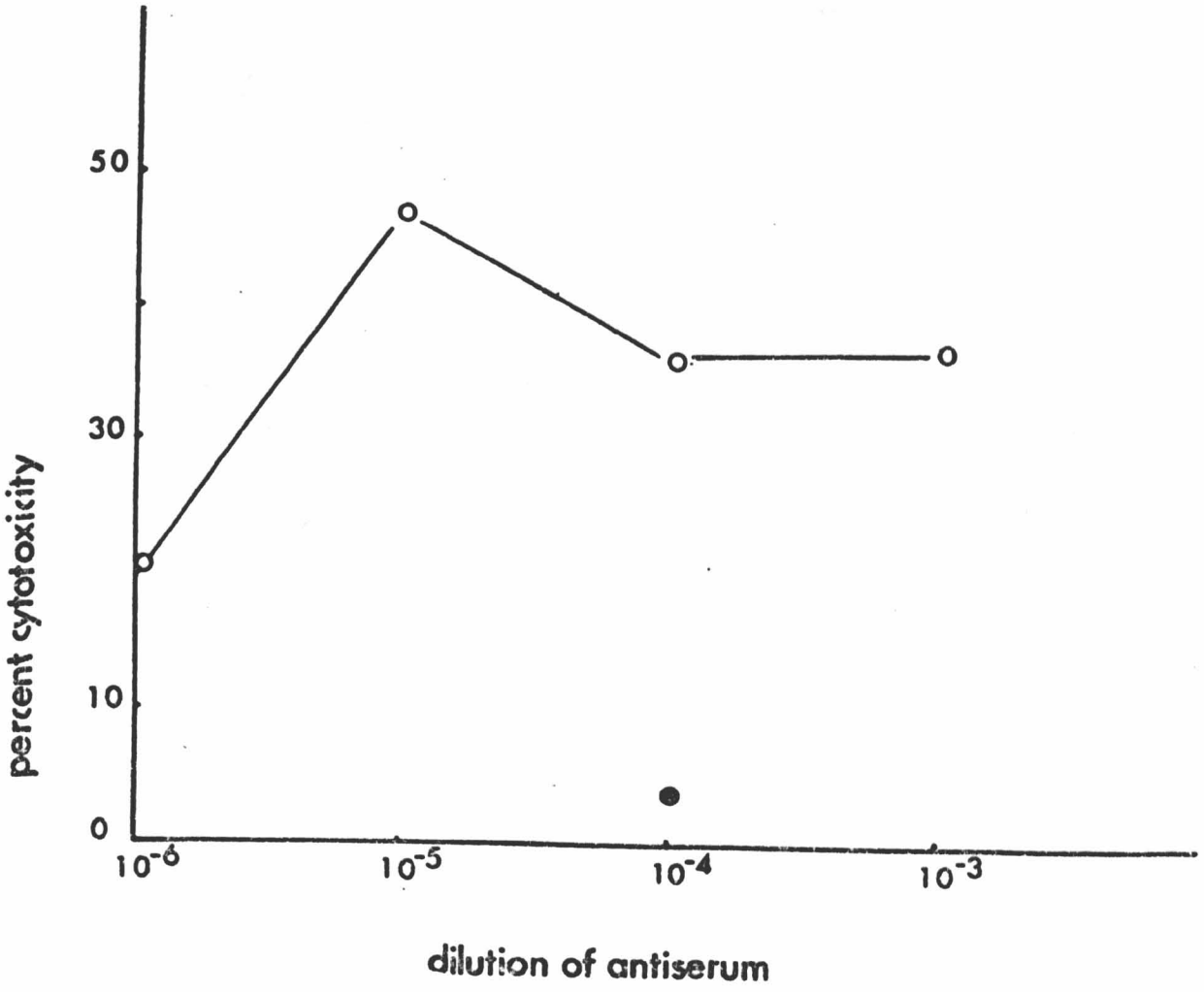
Results

In order to find a suitable dilution of rabbit anti-C.R.B.C. serum for use in subsequent experiments, dilutions of antiserum from 10^{-3} - 10^{-6} were used in an assay with spleen cells from 2 normal C57Bl mice at a spleen cell:C.R.B.C. ratio of 40:1. Cytotoxicity values are presented in Figure 27. The most efficient percentage cytotoxicity occurred with an antiserum dilution of 10^{-5} but with a further tenfold dilution the cytotoxicity value drops off considerably. A fairly steady value appeared between dilutions of 10^{-3} and 10^{-4} and for all subsequent experiments an antiserum dilution of 10^{-4} was used. Normal rabbit serum at a dilution of 10^{-4} gave the least percentage cytotoxocity. In all experiments cytotoxicity values for control samples containing normal rabbit serum were similar to that of controls without lymphocytes and/or antiserum.

Three initial experiments to measure K cell activity in P.chabaudi infected mice were performed using different lymphoid spleen cell:C.R.B.C. ratios and the results are shown in Table 4. The pooled spleens from 2 mice approaching peak parasitaemia on day 8 of infection (with parasites still growing synchronously) had higher cytotoxicity values than controls for 3 lymphoid cell:C.R.B.C. ratios of 10:1, 20:1 and 40:1. In a second experiment, using ratios of lymphoid cells: C.R.B.C. of 1.25:1, 2.5:1 and 5:1, spleen cells from mice on day 11 of infection, just after peak parasitaemia, had higher cytotoxicity values than controls. Spleen cells from mice which on day 15 of infection had recently become subpatent, in a third experiment, once more had higher cytotoxicity values than controls with ratios of 10:1 and 20:1, but at 40:1 there was a decrease in cytotoxicity values for spleen cells of infected mice, suggesting that the optimal ratio of spleen cells:C.R.B.C. was below 40:1.

Fig. 27.

Antibody-dependent cytotoxicity of pooled spleen cells from 2 C57Bl mice. Cultures containing 2×10^6 splenic lymphoid cells were incubated at 37°C with 5×10^4 C.R.B.C.'s labelled with ^{51}Cr, in the presence of rabbit anti-C.R.B.C. serum at concentrations of $10^{-3} \rightarrow 10^{-6}$ or normal rabbit serum at 10^{-4}. The percentage cytotoxicity of the spleen cells was calculated as % ^{51}Cr release in presence of antiserum minus % ^{51}Cr release in presence of normal serum -o- o- . The % ^{51}Cr release in the presence of normal serum (●) was the same as in controls containing C.R.B.C.'s and antiserum or normal serum but no spleen cells.


50
30
10
0
percent cytotoxicity
10^{-6}
10^{-5}
10^{-4}
10^{-3}
dilution of antiserum

Experiment No.	Parasitaemias	Ratio of spleen cells: C.R.B.C.	% cytotoxicity of inf. spleens + antiserum (10^{-4})	% cytotoxicity of inf. spleens + nor. serum (10^{-4})	% cytotoxicity of normal spleens + antiserum (10^{-4})	% cytotoxicity of normal spleens + nor.serum (10^{-4})
1 (day 8)	29%, 52%	10:1	30.9 ± 2.52	10.25 ± 0.78	17.45 ± 0.91	8.7 ± 1.98
		20:1	a 49.5 ± 10.95	10.2 ± o.28	32.0 ± 2.68	9.2 ± 1.13
		40:1	60.43 ± 8.67	8.75 ± 2.61	43.6 ± 2.85	7.9 ± 0.14
2 (day 11)	35%, 15% (decreasing)	1.25:1	12.3 ± 1.89	5.55 ± 0.49	7.8 ± 1.09	5.25 ± 0.07
		2.5:1	14.8 ± 1.94	7.4 ± 0.99	9.13 ± 1.7	5.85 ± 1.7
		5 :1	23.66 ± 2.45	5.1	8.8 ± 1.53	6.05 ± 0.2
3 (day 15)	0%, 0%	10:1	71.3 ± 10.61	4.7 ± 1.13	31.63 ± 5.16	6.2 ± 7.21
		20:1	71.6 ± 3.28	4.75	50.23 ± 13.08	5.35 ± 0.07
		40:1	a 55.7 ± 4.26	4.35 ± 2.05	53.1 ± 7.4	6.05 ± 1.2

Table 4. Antibody-dependent cell-mediated cytotoxicity in spleens of P.chabaudi infected C57Bl mice. Spleen cells from 2 mice were pooled and the ratio of spleen cells: C.R.B.C. was varied. a = not significant at p = 0.05

From these results it would appear that there was an increase in the non-specific activity of spleen cells of infected mice from at least just before peak parasitaemia until mice became subpatent.

Following these findings, preliminary experiments were carried out to determine at what point during infection the K cell activity varied from normal. Cytotoxicity values are given in Table 5 for individual experiments performed with mice at different stages of the primary parasitaemia.

The cytotoxicity of spleen cells from 2 infected mice with rising parasitaemias at 6% and 7% on the third day of infection was similar to that of controls. Spleen cells from mice with parasitaemias of 40% on the sixth day of infection had higher cytotoxicity values than controls. One of the 4 samples containing spleen cells from infected mice plus antiserum had a much lower cytotoxicity value than the other 3 in the group and as a result the observations were not significant at $p = 0.05$ but were significant at $p = 0.1$. If the sample in question was not included in the statistical calculations there was significance at the 0.05 level. In another experiment in which mice had parasitaemias of 60% on day 5 of infection there was no significant difference between cytotoxicity values compared to controls, although it should be noted in this experiment that samples incubated with normal rabbit serum had extremely high release of ^{51}Cr. Using mice with parasitaemias of 10% after peak parasitaemia on day 11 of infection, there was no difference in cytotoxicity values of spleen cells from infected mice and non-infected mice, possibly once again due to the unusually high ^{51}Cr release in samples containing normal rabbit serum. Spleen cells were also treated with NH_4Cl for lysis of mouse red blood cells in this experiment, to compare results of samples with and without

Parasitaemia of infected mice		Cytotoxicity of inf. spleens+antiserum (10^{-4})	Cytotoxicity of inf. spleens+nor. serum (10^{-4})	Cytotoxicity of nor. spleens + antiserum (10^{-4})	Cytotoxicity of nor. spleens + nor. serum (10^{-4})
6%, 7%	a	26.43 ± 3.06	7.3 ± 2.26	25.03 ± 4.04	9.1 ± 3.11
40%, 40%	a	50.07 ± 9.28	8.0 ± 0.99	36.62 ± 6.83	6.4
	b	(54.23 ± 5.06)			
60%, 60%	a	53.4 ± 2.19	28.05 ± 5.44	61.93 ± 8.6	26.35 ± 3.88
10%, 10% (decreasing)	a	58.26 ± 9.34	15.85 ± 10.39	57.37 ± 1.85	11.85 ± 1.67
	x	59.26 ± 2.46	12.35 ± 0.78	60.32 ± 4.49	18.15 ± 2.47
2%, 2% (decreasing)		46.66 ± 2.85	4.45 ± 0.92	19.75 ± 3.31	5.45 ± 0.49

Table 5. Antibody-dependent cell-mediated cytotoxicuty in spleens of P.chabaudi infected C57Bl mice. Spleen cells from 2 mice were pooled and the ratio of spleen cells:C.R.B.C. in culture was 20:1. The cytotoxicity values are given for mice with varying parasitaemias in different experiments.

a = not significant at p = 0.05

x = splenic red cells lysed prior to culture

b = mean calculated from 3 values instead of 4, one of the 4 values being unusually low. The result then became significant at p = 0.05

Blood leucocytes from infected donors				Blood leucocytes from uninfected donors	
Age of donor (years)	No. of parasites per field (approx. 5000cells/ field)	% ^{51}Cr release in presence of antiserum (10^{-4})	% ^{51}Cr release in presence of nor. serum (10^{-4})	% ^{51}Cr release in presence of antiserum	% ^{51}Cr release in presence of normal serum
-	25/1	67.3 ± 7.4	12.4 ± 3.3	55.1 ± 3.3	15.6 ± 5.6
1.5	20/1	80.1 ± 2.0	28.6 ± 16.7	78.3 ± 2.12	20.3 ± 3.8
-	100/1	83.5 ± 7.1	41.5 ± 6.1	82.1 ± 11.6	40.2 ± 7.5
-	50/1	60.1 ± 4.2	11.4 ± 0.9	66.0 ± 2.9	6.5 ± 2.0
12	45/1	42.9 ± 4.4	4.1 ± 4.4	50.4 ± 17.5	6.7 ± 5.4
-	80/1	62.8 ± 4.9	2.9 ± 0.4	54.5 ± 11.6	7.2 ± 10.4
1.5	45/1	50.4 ± 4.3	4.65 ± 2.1	56.3 ± 9.8	8.4 ± 2.1
2.0	10/1	66.8 ± 5.2	2.45 ± 0.7		
0.5	50/1	66.6 ± 11.7	10.0	57.5 ± 9.5	13.5 ± 4.5
2.0	300/1	64.9 ± 8.3	11.95 ± 5.86		
3.5	40/1	76.9 ± 9.6	9.6	70.4 ± 6.8	16.7 ± 4.5
2.0	200/1	51.6 ± 0.2	13.4 ± 2.5	56.4 ± 3.2	6.65 ± 2.3

Table 6. Antibody-dependent cell-mediated cytotoxicity of peripheral lymphoid cells from P.falciparum infected or uninfected donors. A lymphoid cell:C.R.B.C. ratio of 2:1 was used

mouse red blood cells, but no difference was found. The cytotoxicity value for spleen cells from infected mice was more than double that for spleen cells from uninfected mice after 13 days of infection when the infected mice had parasitaemias of 2% and 4%.

Similar experiments were carried out with lymphoid cells isolated from the blood of humans infected with P.falciparum at the M.R.C. Laboratories, Fajara, Gambia. Lymphoid cells were isolated by centrifugation of blood on Ficoll/Triosil gradients and the optimal lymphoid cell:C.R.B.C. ratio was found to be 2:1 for a rabbit antiserum dilution of 10^{-4}. The results of 1D experiments are presented in Table 6 and it was found that there was no significant difference between cytotoxicity values obtained for infected Gambians and uninfected Caucasians. Obviously no general conclusions can be drawn from such a small sample of people, although the net percentage cytotoxicity (% release in presence of antiserum - % release in presence of normal serum) gives values (50 - 70%) for most of the subjects examined which are within the range of most of 58 normal control subjects tested by Calder et al. (1974). Several problems were encountered in this study, including a tendency for C.R.B.C.'s to spontaneously lyse during experiments. This could have been as a result of using a different breed of chicken (Shavers) from that used in the study with mice, but it was more likely because C.R.B.C's were used which had been stored for up to five fays in Alsever's solution whereas fresh chick blood was used in each experiment involving mice.

Discussion

A preliminary investigation of antibody-dependent cell-mediated cytotoxicity (K cell activity) of spleen cells of C57Bl mice infected with P.chabaudi suggested that there was no decrease in K cell

activity during a primary infection, although other types of immune response can be depressed during malaria infection in mice (Salaman et al., 1969; Greenwood et al., 1971; Wedderburn, 1974; McBride et al., 1977). In fact, the results of several experiments suggested that there was an increase in K cell activity from just before peak parasitaemia until the parasitaemia became subpatent. Of 7 experiments where spleen cells were assayed for K cell activity from just before peak parasitaemia until subpatency, 5 demonstrated that there was an increase in K cell activity, and in at least 1 of the other 2 experiments results were obscured by the high spontaneous release of ^{51}Cr even in controls without spleen cells. This increase in cytotoxicity of spleen cells from infected mice to C.R.B.C. target cells in the presence of rabbit-anti-C.R.B.C. serum could be found in spleen cell:C.R.B.C. ratios of between 1.25 -40:1. On the basis of spleen size, there would have been a much greater K cell activity from just before peak parasitaemia onwards because the spleens of infected mice become much larger than those of non-infected mice (3-4 times larger at peak parasitaemia).

No difference was found in the K cell activity of blood lymphoid cells from P.falciparum infected humans, although there is really insufficient data on this study at present. Possible reasons for increased K cell activity not occurring in this case are that parasitaemias were much lower, peripheral lymphoid cells and not spleen cells were examined and a different host-parasite system was involved. Greenwood et al. (1977), however, have reported an increased K cell activity in the peripheral blood of children infected with P.falciparum.

Indirect evidence of possible cell-mediated immunity during the asexual stages of malaria infection has been found with the demonstration of delayed hypersensitivity (Phillips et al., 1970;

Finerty and Krehl, 1976), the stimulation of T cells in the presence of antigen *in vitro* (Weinbaum et al., 1976a) and the existence in the serum of infected animals of a macrophage inhibition factor (Coleman et al., 1976). Malaria parasites injected into mice immunized with B.C.G. show signs of intracellular death within 2 days of injection, suggesting that a cytotoxic cell or a cell releasing cytotoxic factors could be involved in the death of these parasites (Clark, Allison and Cox, 1976). There is, however, only one report of cell-mediated (and possibly antibody-dependent) cytotoxicity of malaria parasites *in vitro* (Coleman et al., 1975).

One possible reason for increased K cell activity during malaria infection is that there is an increase in the number of null cells in the peripheral blood of humans infected with *P.falciparum* (Wyler, 1976; Greenwood et al., 1977) and in the lymphoid tissue during acute *P.berghei* infection in mice (Krettli and Nussenzweig, 1974), and K cells have been associated with null cells (Greenberg et al., 1973).

There is no evidence from these experiments, however, that K cells are effector cells in the lysis of malaria parasites. If an antibody-dependent cytotoxic cell is involved in the lysis of parasites, eosinophils could not be discounted, as they and not K cells appear to be mainly responsible for the lysis of *Trypanosoma cruzi* parasites *in vitro* (Sanderson, Lopez, Bunn Morena, 1977). At a time when K cell activity was increased *in vitro*, it is likely that at that time in *P.chabaudi* infected animals there would be antibody-antigen complexes in the circulation. The presence of immune complexes in serum can inhibit K cell activity *in vitro* (Jewell and MacLennan, 1973) and, therefore, it is possible that because of the presence of antibody-antigen complexes in *P.chabaudi* infected mice, the *in vivo* activity

of K cells would be inhibited. It is possible that K cells are involved in the haemolytic anaemia which occurs during acute malarial infection, especially as auto-antibodies may be at least partly responsible for the destruction of non-parasitized red blood cells (Zuckerman, 1960; McGhee, 1976; Lustig et al., 1977).

The cell-mediated cytotoxicity of malaria parasites in vitro may be difficult to detect because of the artificial nature of the experimental conditions or, alternatively, because the parasite may be susceptible to lysis only during a very short part of its asexual development, such as the extracellular stage. The results in this chapter are only of a preliminary nature and further investigations should be carried out, with the experiments being extended to using malaria parasites as target cells in the presence of immune serum.

CHAPTER 11

Concluding discussion

The role of different splenic lymphoid cell types in the immune response to P.chabaudi in inbred C57Bl and NIH mice has been investigated by the adoptive transfer of immune lymphoid cells, the passive transfer of immune serum and by examining the in vitro activity of splenic K cells from infected mice.

Spleen cells from mice which had previously been infected with P.chabaudi could confer protection on irradiated and non-irradiated syngeneic recipients. Irradiated recipients were found to be more useful for subsequent investigations for 3 reasons: a) smaller numbers of immune cells were required to confer protection on irradiated than non-irradiated recipients b) the difference between irradiated recipients of immune cells and normal cells was amplified compared to non-irradiated recipients c) the immune response of irradiated mice is depressed so that the protective activity of donor lymphoid cells could be examined with a diminished contribution by the host's own immune system.

Irradiated mice were found to be initially resistant to P.chabaudi infection even if no lymphoid cells were injected into mice after irradiation. This phenomenon was investigated and the results demonstrated that the injection of spleen or bone marrow cells could decrease or prevent this initial resistance. Similarly when mice were infected 22 days after irradiation, instead of 2 days after irradiation, the initial resistance to infection was prevented. Irradiated mice injected with immune serum and infected with P.chabaudi 1 hour after irradiation were much more resistant to the parasite than non-irradiated

mice treated in the same way, suggesting that the mechanism of resistance amplified the effect of the immune serum. It is known that the reticulo-endothelial system of mice is stimulated by X-irradiation and the amplified effect of the immune serum in irradiated mice might result from an enhanced phagocytosis of opsonized parasites. Further experiments to examine the influence of the reticulo-endothelial system in irradiated mice on the initial resistance to infection may provide further information on this subject. The blockading of the reticulo-endothelial system would show whether or not it was involved in the removal of parasites. The uptake of parasites by the reticulo-endothelial system could be examined by radio-labelling of parasites, although such an approach would have to be well controlled as late trophozoites and schizonts of P.chabaudi probably withdraw into the deep tissues of most organs, including the spleen. Any stimulation of the reticulo-endothelial system in irradiated mice could be monitored by measuring the phagocytosis of carbon particles or antigen such as sheep red blood cells. It may also be possible to examine change of activity of phagocytic cells of the reticulo-endothelial system from irradiated mice in vitro.

Enriched subpopulations of immune T cells and immune B cells from mice previously infected with P.chabaudi could confer protection on mice irradiated with 600 rads or 800 rads. Unfractionated immune cells were found to be more protective than either enriched subpopulations of immune cells. Recipients of enriched immune T cells were found to be more susceptible to relapses than recipients of either enriched immune B cells or unfractionated immune cells. These results suggested that cooperation between T cells and B cells may be required for the most efficient protective immune response to P.chabaudi. Comparing the protection obtained with enriched immune T cells in mice given 600 rads or 800 rads, it was seen that mice given 600 rads became subpatent before

mice given 800 rads, thus implying that the enriched immune T cells could have been cooperating with cells of the recovering immune system in the mice given 600 rads.

The protective effect of serum passively transferred from recipients of enriched immune T cells or unfractionated immune cells, 14 days after infection, reflected the immune status of the immune cell recipients. Just as the unfractionated immune cell recipients had a higher degree of immunity to infection than enriched immune T cell recipients, so the serum taken from the former recipients was more protective than serum taken from the latter recipients.

The experimental system used in these adoptive transfer studies could probably be improved in several ways, in order to decrease the amount of cooperation between enriched subpopulations of cells from donor mice and radioresistant cells in the recipient mice as well as between the main type of cell in the enriched subpopulation and minority cells within this subpopulation. Recipient mice could be thymectomized and/or treated with an anti-B cell serum such as anti-μ-chain serum before irradiation to minimise the contribution of the cell recipients to the immune response. The nylon wool technique for the separation of T and B cells could also be improved by, for example, passing cells through the columns more than once. Other techniques for separating T and B cells should also be employed, such as immunoadsorbent columns (Brown et al., 1976), density gradient centrifugation (Mishell, Dutton and Raidt, 1971), electrophoresis (Platsoucas, Griffith and Catsimpoolas, 1976) or cell rosetting with heterologous erythrocytes (Gravely and Kreier, 1976). Enriched subpopulations of lymphocytes could be further purified by the <u>in vitro</u> lysis of small numbers of minority types of lymphocytes using antiserum + complement. The numbers of T cells and

B cells in the spleens of sacrificed recipient mice should also be periodically checked throughout experiments in order that, for example, the variations in numbers of B cells in mice injected with enriched immune T cells can be correlated with the immune status of the recipients. Relative numbers of T cells and B cells can be identified by viability tests on small numbers of cells treated with anti-θ thymocyte serum or anti-μ chain serum + complement, respectively, and also by examining, using ultraviolet transmission microscopy, cells treated with antisera conjugated with fluorescein.

The factor(s) responsible for the protective effect of serum from enriched immune T cell recipients and unfractionated immune cell recipients was not identified and therefore should be, although it is likely that the factor was malarial antibody. Experiments in which sera are passively transferred from immune cell recipients might be performed to determine the relationship between the protective effect of sera from enriched immune T cell recipients and the high incidence of relapse in these mice. In such experiments it would perhaps be interesting to infect mice which receive "immune" serum with parasite populations other than the original one used to infect the immune cell recipients, as this might provide information on the spectrum of protective malarial antibodies produced in immune cell recipients. The diversity of malarial antibodies in serum may be important in preventing patent relapses occurring.

Synergy between enriched immune T cells and enriched immune B cells was examined by recombining these subpopulations of cells, but the experiments gave inconclusive results and, therefore, deserve clarification. A problem arising from this type of experiment is that it may be difficult to distinguish between increased protection due to

synergy of donor cells or due to an actual increase in the total number of donor cells. The only clear cut result from these experiments was that there was no apparent synergy between enriched subpopulations of immune cells and donor unprimed lymphoid cells, although only comparatively small numbers of unprimed cells were used. Further experiments should be carried out to examine for synergy between different subpopulations of immune cells. More control groups of recipients given different numbers of only one of the enriched subpopulations of immune cells should be included to allow a better comparison with a group which receives both enriched subpopulations of immune cells. Varying the ratio of recombined enriched immune T cells and enriched immune B cells for injection into recipients may also be a useful exercise in examining for synergy since the only ratio employed in this study was 1:1.

No investigation was made in this study of the possibility of cell-mediated immunity occurring in immune cell recipients and more especially in recipients of enriched immune T cells, where such a mechanism might be more likely to occur than in unfractionated immune cell or enriched immune B cell recipients. Delayed hypersensitivity in rodent malarial infections can be measured by the injection of plasmodial antigen into the footpad of mice (Finerty and Krehl, 1976) and the macrophage migration inhibition factor can also be tested for (Coleman et al., 1976).

Some preliminary experiments were carried out to examine antibody-dependent cell-mediated cytotoxicity of the spleens of mice infected with P.chabaudi. An *in vitro* culture system was used in which the non-specific lysis of chick red blood cells in the presence of antiserum and spleen cells from infected mice was measured. Some technical difficulties were encountered in these experiments, one of

which was the fragility of the target cell employed. The results obtained, however, suggested that the cell responsible for the non-specific lysis of target cells in this assay, the K cell, had an increased activity during a primary parasitaemia. This experimental approach is, therefore, worth pursuing in order to find at what point during the parasitaemia the K cell activity varies from the normal and also how long the increased activity lasts during the later stages of infection. It would also be of interest to attempt to use this type of experimental system in order to examine for lysis of P.chabaudi parasitized cells or free parasites in the presence of spleen cells and immune serum, as has been done by Coleman et al. (1975) with P.berghei.

Irradiated animals have been used extensively for many years as a source of immunodepressed hosts for investigating the role of different syngeneic lymphoid cell types in the immune response to an antigen after adoptive transfer. This experimental system has been used successfully to demonstrate that lymphoid cells from mice immune to P.chabaudi can express immunity in syngeneic recipients. It was further used to demonstrate that both T cells and B cells are probably required for the most effective immunity in mice against P.chabaudi infection as has also been demonstrated in rats against P.berghei (Gravely and Kreier, 1976; Brown et al., 1976) and P.yoelii in mice (Jayawardena et al., 1977). The use of irradiated mice and P.chabaudi may prove to be an excellent model system for examining the mechanisms of immunity to P.chabaudi, the knowledge about which may be useful for the development of vaccines against malaria.

REFERENCES

Allison, A.C., 1957. Malaria in carriers of the sickle cell trait and in newborn children. Exp. Parasitol., 6, p.418.

Allison, A.C. and Clyde, D.F., 1961. Malaria in African children with deficient erythrocyte glucose-6-phosphate dehydrogenase. Brit. Med. J., (i) p.1346.

Arrenbrecht, S. and Mitchell, G.F., 1975. T cell dependent helper and suppressive influences in an adoptive IgG antibody response. Immunology, 28, p.485.

Bafort, J., 1968. Primary exo-erythrocyte forms of Plasmodium vinckei. Nature, 217, p.1264.

Baker, P.J., 1975. Homoeostatic control of antibody responses: a model based on the recognition of cell associated antibody by regulatory T cells. Transplant Rev., 26. p.3.

Barker, L.R. and Powers, K.G., 1971. Impairment of antibody response and recovery in malarial rodents by antilymphocyte serum. Nature, 229, p.429.

Billingham, R.E., Brent, L., Medawar, P.B., 1954. Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity. Proc. Roy. Soc., Lond. s.B., 143, p.58-80.

Briggs, N.T., Wellde, B.T. and Sadun, E.H., 1968. Variants of Plasmodium berghei resistant to passive transfer of immune serum. Exp. Parasitol., 22, p.338.

Brown I.N., Allison, A.C. and Taylor, R.B., 1968. Plasmodium berghei infections in thymectomized rats. Nature, 219, p.292.

Brown, I.N. and Phillips, R.S., 1974. Immunity of Plasmodium berghei in rats: passive serum transfer and the role of the spleen. Inf. and Immun., 10, p.1213.

Brown, K.N. and Brown, I.N., 1965. Immunity to malaria: antigenic variation in chronic infections of Plasmodium knowlesi. Nature, 268, p.1286.

Brown, K.N., Brown I.N. and Hills, L.A., 1970b. Immunity to malaria. I. Protection against Plasmodium knowlesi shown by monkeys sensitized by drug-suppressed infections or by dead parasites in Freund's adjuvant. Exp. Parasitol., 28, p.304.

Brown, K.N., Brown, I.N., Trigg, P.I., Phillips, R.S. and Hills, L.A., 1970a. Immunity to malaria. II. Serological response of monkeys sensitized by drug-suppressed infection or by dead parasitized cells in Freund's complete adjuvant. Exp. Parasitol., 28, p.318.

Brown, K.N., 1971. Protective immunity to malaria parasites provides a model for the survival of cells in an immunological hostile environment. Nature, 230, p.163.

Brown, K.N. and Hills, L.A., 1974. Antigenic variation and immunity to Plasmodium knowlesi: antibodies which induce antigenic variation and antibodies which destroy parasites. Trans. Roy. Soc. trop, Med. Hyg., 68, p.139.

Brown, K.N., Hills, L.A. and Jarra, W., 1976. Preliminary studies of artificial immunization of rats against Plasmodium berghei and adoptive transfer of this immunity by splenic T and T + B cells. Bull. W. Heal. Org., 54, p.149.

Bruce-Chwatt, L.J., Dorrell, J. and Tipley, E., 1972. Antilymphocyte serum and the course of rodent malaria in mice. Trans. Roy. Soc. trop. Med. Hyg., 66, p.522.

Butcher, G.A. and Cohen, S., 1970. Schizogony of Plasmodium knowlesi in the presence of normal and immune sera. Trans. Roy. Soc. Trop. Med. Hyg., 64, p.470.

Butcher, G.A., Cohen, S. and Garnham, P.C.C., 1970. Passive immunity in Plasmodium knowlesi malaria. Trans. Roy. Soc. Trop. Med. Hyg., 64, p.850.

Butcher, G.A. and Cohen, S., 1972. Antigenic variation and protective immunity in Plasmodium knowlesi. Immunology, 23, p.503.

Cabrera, E.J. and Alger, N.E., 1971. Transfer of adoptive immunity to Plasmodium berghei: a comparison of routes of injection. J. Protozool., 18, p.596.

Cabrera, E.J., Speer, C.A., Schenuel, R.H., Barr, M.L. and Silverman, P.H., 1976. Delayed dermal hypersensitivity in rhesus monkeys (Macaca mulatta) immunized against Plasmodium knowlesi. Z. Parasitenk., 50, p.31.

Calder, E.A., Penhale, W.J., McLeman, D., Barnes, E.W. and Irvine, W.J., 1973. Lymphocyte-dependent antibody-mediated cytotoxicity in Hashimoto thyroiditis. Clin. Exp. Immunol., 14, p.153.

Calder, E.A., Urbaniak, S.J., Penhale, W.J. and Irvine, W.J., 1974. Characterization of human lymphoid cell-mediated antibody-dependent cytotoxicity (LDAC). Clin. Exp. Immunol., 18, p.579.

Campbell, P.A., Martens, B.L., Cooper, H.R. and McClatchy, J.K., 1974. Requirement for bone marrow derived cells in resistance to Listeria. J. Immunol., 112, p.1407.

Cantrell, W. and Elko, E.E., 1970. Plasmodium berghei: phagocytic hyperactivity of infected rats. Exp. Parasitol., 28, p.291.

Carter, R. and Walliker, D., 1975. New observations on the malaria parasites of the Central African Republic - Plasmodium vinckei subsp. nov. and Plasmodium chabaudi Landau, 1965. Ann. trop. Med. Parasitol., 69, p.187.

Carter, R. and Chen, D.H., 1976. Malaria transmission blocked by immunization with gametes of the malaria parasites. Nature, 263, p.57.

Chan, C., Longshawn, P.A.C. and Skamene, E., 1977. Enhanced primary resistance to Listeria monocytogenes in T cell deprived mice. Immunology, 32, p.529.

Chapman, W.L. and Hanson, W.L., 1971. Plasmodium berghei infection in neonatally thymectomized hamsters. J. Parasitol., 57, p.24.

Cheers, C. and Waller, R., 1975. Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice. J. Immunol., 115, p.844.

Chen, D.H., Tigelaar, R.E. and Weinbaum, F.I., 1977. Immunity to sporozoite-induced malaria infection in mice. I. The effect of immunization of T and B cell-deficient mice. J. Immunol., 118, p.1322.

Chow, J.S. and Kreier, J.P., 1972. Plasmodium berghei: adherence and phagocytosis of rat macrophages in vitro. Exp. Parasitol., 31, p.13.

Claman, H.N., Chaperon, E.A. and Triplett, R.F., 1966. Thymus-marrow cell combinations. Synergism in antibody production. Proc. Soc. exp. Biol. Med., 122, p.1167.

Clark, I.A. and Allison, A.C., 1974. Babesia microti and Plasmodium yoelii infections in nude mice. Nature, 252, p.328.

Clark, I.A., Allison, A.C. and Cox, F.E.G., 1976. Protection of mice against Babesia and Plasmodium with B.C.G. Nature, 259, p.309.

Clark, I.A., Cox, F.E.G. and Allison, A.C., 1977. Protection of mice against Babesia spp and Plasmodium spp with killed Corynebacterium parvum. Parasitology, 74, p.9.

Clyde, D.F., McCarthy, V.C., Miller, R.M. and Hornick, R.G., 1973. Specificity of protection of man immunized against sporozoite induced falciparum malaria. Amer. J. Med. Sci., 266, p.398.

Cochrane, C.G. and Dixon, F.J., 1962. Antibody production by transferred cells. Adv. Immunol., 2, p.205.

Coggeshall, L.T. and Kumm, H.W. , 1937. Demonstration of passive immunity in experimental monkey malaria. J. Exp. Med., 66, p.177.

Cohen, S., McGregor, I.A. and Carrington, S.C., 1961. Gamma-globulin and acquired immunity to human malaria. Nature, 192, p.733,

Coleman, R.M., Renricca, N.J., Stout, J.P., Brissette, W.H. and Smith, D.M., 1975. Splenic mediated erythrocytic cytotoxicity in malaria. Immunology, 29, p.49

Coleman, R.M., Bruce, A and Renricca, N.J., 1976. Malaria-macrophage migration inhibition factor (M.I.F.). J. Parasitol., 62, p.137.

Corradetti, A., Verolini, F. and Bucci, A., 1966. Resista a Plasmodium berghei de parte de ratti albini precedementi immunizatti con Plasmodium berghei irradiato. Parassitologia, 8, p.133.

Cox, F.E.G., Bilbey, D.L.J. and Nicol. T., 1964. Reticulo-endothelial activity in mice infected with Plasmodium vinckei. J. Protozool., 11, p.229.

Cox, F.E.G., 1975. Enhanced Trypanosoma musculi infections in mice with concomitant malaria. Nature, 258, p.148.

Criswell, B.S., Butler, W.T., Rossen, R.D. and Knight, V., 1971. Murine malaria: the role of humoral factors and macrophages in the destruction of parasitized erythrocytes. J. Immunol., 107, p.212.

D'antonio, L.E., 1972. Plasmodium berghei: vaccination of mice against malaria with host-inactivated parasitized blood. Exp. Parasitol., 31, p.82.

Desowitz, R.S., 1975. Plasmodium berghei: immunogenic enhancement of antigen by adjuvant addition. Exp. Parasit., 38, p.6.

Diggs, C.L., Shen, H., Briggs, N.T., Laudenslayer, K. and Weber, R.M., 1972. Antibody mediated immunity to Plasmodium berghei independent of the third component of complement. Proc. Helminth. Soc. Wash., 39, p.456.

Diggs, C.L. and Osler, A.G., 1969. Humoral immunity in rodent malaria. II. Inhibition of parasitaemia by serum antibody. J. Immunol., 102, p.298.

Dumonde, D.C., Wolstencroft, R.A., Penayi, G.S., Mathew, M., Morley, J. and Howson, W.T., 1969. Lymphokines: non-antibody mediators of cellular immunity generated by lymphocyte activation. Nature, 224, p.38.

Dutton, R.W., 1975. Suppressor T cells. Transplan. Rev., 26, p.39.

Eaton, M.D., 1939. The soluble malarial antigen in the serum of monkeys infected with Plasmodium knowlesi. J. Exp. Med., 69, p.517.

Fairley, N.H., 1947. Sidelights on malaria obtained by subinoculation experiments. Trans. Roy. Soc. Trop. Med. Hyg., 40, p.621.

Finerty, J.F. and Krehl, E.P., 1976. Cyclophosphamide pretreatment and protection against malaria. Inf. and Immun., 14, p.1103.

Freund, J., Thomson, K.J., Sommer, H.E., Walter, A.W. and Schenkein, E.L., 1945. Immunization of rhesus monkeys against malarial infection (Plasmodium knowlesi) with killed parasites and adjuvants. Science, 102, p.202.

Garnham, P.C.C., 1947. Exo-erythrocytic schizogony in Plasmodium kochi Laveran. A preliminary work. Trans. Roy. Soc. Trop. Med. Hyg., 40, p.719.

Garnham, P.C.C., 1966. Malaria parasites and other haemosporidia. Blackwell Scientific Productions.

George, J.N., Stokes, E.F., Wicker, D.J. and Conrad, M.E., 1966. Studies on the mechanism of hemolysis in experimental malaria. Milit. Med., 131 (Suppl.), p.1217.

Ghaffer, A., Calder, E.A. and Irvine, W.J., 1976. K cell cytotoxicity against antibody coated chicken erythrocytes in tumour bearing mice - its development with progressively growing tumour and effect of immunization against the tumour. J. Immunol., 116, p.315.

Gingrich, W.D., 1941. Immunization of birds to Plasmodium cathemurium II. Inf. Dis., 68, p.46.

Gravely, S.M. and Kreier, J.P., 1976. Adoptive transfer of immunity to Plasmodium berghei with immune T and B lymphocytes. Inf. and Immun., 14, p.184.

Gravely, S.M., Hamburger, J. and Kreier, J.P., 1976. T and B cell population changes in young and in adult rats infected with Plasmodium berghei. Inf. and Immun., 14, p.178.

Greenberg, A.H., Hudson, L., Shen. C. and Roitt, I.M., 1973. Antibody-dependent cell-mediated cytotoxicity due to a "null" lymphoid cell. Nat. New Biol., 242, p.111.

Greenwood, B.M., Brown, J.C., De Jesus, D.G. and Holborow, E.J., 1971. Immunosuppression in murine malaria. II. The effect on reticulo-endothelial and germinal centre function. Clin. Exp. Immunol., 9, p.345.

Greenwood, B.M., Playfair, J.H.C. and Torrigiani, G., 1971. Immunosuppression in murine malaria. I. General characteristics. Clin. Exp. Immunol., 8, p.467.

Greenwood, B.M. and Vick, R.M., 1975. Evidence for a malaria mitogen in human malaria. Nature, 257, p.592.

Greenwood, B.M., Oduluju, A.J. and Stratton, D., 1977. Lymphocyte changes in acute malaria. Trans. Roy. Soc. Trop. Med. Hyg., 71, p.408.

Hamburger, J. and Kreier, J.P., 1975. Antibody-mediated elimination of malaria parasites (Plasmodium berghei) in vivo. Inf. and Immun., 12, p.339.

Hamburger, J. and Kreier, J.P., 1976. Plasmodium berghei: use of free blood stage parasites to demonstrate protective humoral activity in the serum of recovered rats. Exp. Parasitol., 40, p.158-169.

Hamburger, J. and Zuckerman, A., 1976. Plasmodium berghei: II. Immunobiological properties of fractions of a soluble extract. Exp. Parasitol., 39, p.479.

Harris, T.N., Harris, S., Beale, H.D. and Smith, J.J., 1954. Studies on the transfer of lymph node cells. IV. Effect of X-irradiation of recipient rabbits on the appearance of antibody after cell transfer. J. Exp. Med., 100, p.289.

Herbert, W.J., 1973. Mineral-oil adjuvants and the immunization of laboratory animals. Handbook of Experimental Immunology, edited by D.M. Weir, Blackwell Scientific Publications.

Honigberg, B.M., Balamuth, W., Bovee, E.L., Corliss, J.O., Gojdics, M., Hall, R.P., Kudd, R.R., Levine, N.D., Loeblich, A.R. JP., Weiser J. and Wenrich, D.H., 1964. A revised classification of the phylum Protozoa. J. Protozool., 11, p.7.

Houba, V., Lambert, P.H., Voller, A. and Soyanwo, M.A.D., 1976. Clinical and experimental investigation of immune complexes in malaria. Clin. Immunol. and Immunopathol., 6, p.1.

Jahiel. R.I., Nussenzweig, R.S., Vanderberg, J. and Vilcek, J., 1968. Antimalarial effect of interferon inducers at different stages of development of Plasmodium berghei. Nature, 220, p.710.

Jayawardena, A.N., Targett, G.A.T., Davies, A.J.S., Leuchars, E. and Carter, R., 1975a. The passive transfer of immunity to Plasmodium berghei yoelii. Trans. Roy. Soc. Trop. Med. Hyg., 69, p.426.

Jayawardena, A.N., Targett, G.A.T., Leuchars, E., Carter, R.L., Doenhoff, M.J. and Davies, A.J.S., 1975b. T cell activation in murine malaria. Nature, 258, p.149.

Jayawardena, A.N., Targett, G.A.T., Carter, R.L., Leuchars, E. and Davies, A.J.S., 1977. The immunological response of CBA mice to Plasmodium yoelii. Immunology, 32, p.849.

Jayawardena, A.N., 1977. T cell dependent effector and suppressor responses in experimental malaria. Intern. Congr. Protozool., p.72.

Jerusalem, C., 1968. Relationship between malaria infection (Plasmodium berghei) and malignant lymphoma in mice. Z. Tropenmed, Parasit., 19, p.94.

Jerusalem, C., Weiss, M.L. and Poels, L., 1971. Immunologic enhancement in malaria infection (Plasmodium berghei). J. Immunol., 107, p.260.

Jewell, D.P. and MacLennan, I.C.M., 1973. Circulating immune complexes in inflammatory bowel disease. Clin. Exp. Immunol. 14, p.219.

Julius, M.H., Simpson, E. and Herzenberg, L.A., 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol., 3, p.645.

Kadish, J.L. and Basch, R.S., 1975. Thymic regeneration after lethal irradiation. Evidence for an intra-thymic radioresistant T cell precursor. J. Immunol., 114, p.452.

Kataoka, Y. and Sado, T., 1975. The radiosensitivity of T and B lymphocytes in mice. Immunology, 29, p.121.

Killick-Kendrick, R., 1974. Parasitic protozoa of the blood of rodents. A revision of Plasmodium berghei. Parasitology, 69, p.225.

Kornfeld, L. and Weyzen, W.W.H., 1968. Antibody formation of transferred peritoneal cells and spleen cells of mice. I. Transfer of cells from immunized non-irradiated donors to syngeneic recipients with and without antigen. Immunology, 15, p.751.

Krebs, H.A. and Eggleston, L.V., 1940). The oxidation of pyruvate in pigeon breast muscle. Biochem. J., 34, p.442.

Krettli, A.V. and Nussenzweig, R.S., 1974. Depletion of T and B lymphocytes during malaria infections. Cell Immunol., 13, p.440.

Lagrange, P.H., Mackaness, G.B. and Miller, T.E., 1974. Potentiation of T cell mediated immunity by selective suppression of antibody formation with cyclophosphamide. J. Exp. Med., 139, p.1529.

Landau, I., 1965. Description de Plasmodium chabaudi n.sp., parasite de rongeurs africains. C.r. Acad. Sci., Paris, 260, p.3758.

Landau, I. and Killick-Kendrick, R., 1966. Rodent plasmodia of the Republique Centralafricaine: the sporogony and tissue stages of Plasmodium chabaudi and Plasmodium berghei yoelii. Trans. Roy. Soc. Trop. Med. Hyg., 60, p.633.

Laveran, A., 1880. Note sur un noveau parasite trouvé dans le sang de plusieurs mallades atteints de fièvre palustre. Bull. Accad. Med. Paris, 9, p.1235.

Loose, L.D., Cook, J.A. and Di Luzio, N.P., 1972. Malarial immunosuppression - a macrophage mediated effect. Proc. Helminthol. Soc. Wash., 39, p.484.

Löwy, I., Teplitz, R.L. and Bussard, A.E., 1975. Secretion of IgM antibodies by glass adherent fraction of immune spleen cells II. Cellular Immunology, 16, p.36-47.

Lucia, H.L. and Nussenzweig, R.S., 1969. Plasmodium chabaudi and Plasmodium vinckei - phagocytic activity of mouse reticulo-endothelial system. Exp. Parasitol., 25, p.319.

Lustig, H.J., Nussenzweig, V. and Nussenzweig, R.S., 1977. Erythrocyte membrane-associated immunoglobulins during malaria infection of mice. J. Immunol., 119, p.210.

Mackaness, G.B., Lagrange, P.H., Miller, T.E. and Ishibashi, T., 1976. Feedback inhibition of specifically sensitized lymphocytes. J. Exp. Med., 139, p.543.

Manwell, R.D. and Goldstein, F., 1940. Passive immunity in avian malaria. J. Exp. Med., 71, p.409.

McBride, J.S., Micklem, H.S. and Ure, J.M., 1977. Immunosuppression in murine malaria. I. Response to type III pneumoccal polysaccharide. Immunology, 32, p.635.

McCarthy, V.C. and Clyde, D.F. , 1977. Plasmodium vivax: correlation of circumsporozoite reaction with sporozoite-induced protective immunity in man. Exp. Parasitol., 41, p.167.

MacLennan, I.C.M. and Harding, B., 1970. The role of immunoglobulins in lymphocyte mediated cell damage in vitro. II. The mechanism of target cell damage by lymphoid cells from immunized rats. Immunology, 18, p.405.

McGhee, R.B., 1960. An autoimmune reaction produced in ducklings in response to injections of duck embryo blood infected with Plasmodium lophurae. J. Inf. Dis., 107, p.410.

McGhee, R.B., 1976. Plasmodium gallinaceum: response of malarious chicken embryos to injection of serum from hyperimmunized chickens. Exp. Parasitol., 39, p.88.

McGregor, I.A. and Barr, M., 1962. Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. Trans. Roy. Soc. Trop. Med. Hyg., 56, p.364.

McGregor, I.A., Turner, M.W., Williams, K. and Hall. P., 1968. Soluble antigens in the blood of African patients with severe Plasmodium falciparum. Lancet i, p.881.

McGregor, I.A. and Wilson, R.J.M., 1971. Precipitating antibodies and immunoglobulins in Plasmodium falciparum infections in the Gambia, W. Africa. Trans. Roy. Soc. Trop. Med. Hyg., 65, p.136.

Miller, L.H., Aikawa, M., Dvorak, J.A. 1975. Malaria (Plasmodium knowlesi)merozoites immunity and the surface coat. J. Immunol., 114, p.1237.

Miller, L.H., Mason, S.J., Dvorak, J.A., McGinniss, M.H. and Rothman, I.K., 1975. Erythrocyte receptors for Plasmodium knowlesi malaria: Duffy blood group determinants. Science, 189, p.561.

Miller, L.H., Mason, S.J., Clyde, D.F. and McGuinniss, M.H., 1976. The resistance factor to Plasmodium vivax in blacks. The Duffy blood group genotype, Fy Fy. N. Eng. J. Med., 295, p.302.

Miller, L.H., Powers, K.G. and Shiroisi, T., 1977. Plasmodium knowlesi functional immunity and antimerozoite antibodies in rhesus monkeys after repeated infection. Exp. Parasitol., 41, p.106.

Mishell, R.I., Dutton, P.W. and Raidt, D.J., 1970. Cell components in the immune response. I. Gradient separation of immune cells. Cell Immunol., 1, p.175.

Mitchison, N.A., 1957. Adoptive transfer of immune reactions by cells. J. Cell Comp. Physiol., 50 Suppl., p.247.

Mitchell, G.H., Butcher, G.A. and Cohen, S., 1974. A merozoite vaccine effective against Plasmodium knowlesi malaria. Nature, 252, p.311.

Moore, D.L., Heyworth, B. and Brown, J., 1974. P.H.A.-induced lymphocyte transformations in leucocytic cultures from malarious malnourished and control Gambian children. Clin. Exp. Immunol., 17, p.647.

Nussenzweig, R.S., 1967. Increased nonspecific resistance to malaria produced by administration of killed Corynebacterium parvum. Exp. . Parasit., 21, p.224.

Nussenzweig, R.S., Vanderberg, J.P., Most, H. and Orton, C., 1969. Specificity of protective immunity produced by X-irradiated Plasmodium berghei sporozoites. Nature, 222. p.488.

Nussenzweig, R.S., Vanderberg, J.P., Sanabria, Y. and Most, H., 1972. Plasmodium berghei: accelerated clearance of sporozoites from blood as part of immune mechanism in mice. Exp. Parasitol., 31, p.88.

Okumara, K. and Tada, T., 1971. Regulation of homocytotrophic antibody formation in the rat. VI. Inhibitory effect of thymocytes in the homocytotrophic response. J. Immunol., 107, p.1682.

Ott, K.J., 1968. Influence of reticulocytosis on the course of infection of Plasmodium chabaudi and Plasmodium berghei. J. Protozool., 15, p.365.

Perlmann, P. and Perlmann, H., 1970. Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes. Cell Immunol., 1 p.300.

Phillips, R.S., 1970. Plasmodium berghei: passive transfer of immunity by antisera and cells. Exp. Parasitol., 27, p.479.

Phillips, R.S., Wolstencroft, R.A., Brown, I.N., Brown, K.N. and Dumonde, D.C., 1970. Possible occurrence of cell-mediated immunity to Plasmodium knowlesi in chronically infected and Freund's complete adjuvant sensitized monkeys. Exp. Parasitol., 28, p.339.

Phillips, R.S. and Jones, V.E., 1972. Immunity to Plasmodium berghei in rats: maximum levels of protective antibody activity are associated with eradication with the infection. Parasitology, 64, p.117.

Phillips, R.S., Selby, G.R. and Wakelin, D., 1974. The effect of Plasmodium berghei and Trypanosome brucei infections on the immune expulsion of the nematode Trichuris muris from mice. Int. J. Parasitol., 4, p.409.

Phillips, R.S., Brown, K.N. and Hills, L.A., in press. Immunity to Plasmodium berghei in rats: reduced protective activity of immune spleen cells when transferred to recently infected rats.

Pillarski, L.M. and Cunningham, A.J., 1974. Host derived antibody forming cells in lethally irradiated mice. J. Immunol., 114, p.138.

Platsoucas, C.D., Griffith, A.L., Catsimpoolas, N., 1976. Density gradient electrophoresis of mouse spleen lymphocytes: separation of T and B cell fractions. J. Imm. Meth., 13, p.145.

Poels, L.G. and Van Niekerk, C.C., 1977. Plasmodium berghei: immunosuppression and hyperimmunoglobulinemia. Exp. Parasitol., 42, p.235.

Raff, M.L., 1970. Two distinct populations of peripheral lymphocytes in mice distinguishable by immunofluorescence. Immunology, 19, p.637.

Reisen, W.K. and Hillis, T.C., 1976. Failure to protect mus.musculis against Plasmodium berghei with footpad injections of killed parasites incorporated in Freund's complete adjuvant. J. Parasitol., 61, p.937.

Roberts, J.A. and Tracey-Patte, P., 1969. Adoptive transfer of immunity to Plasmodium berghei. J. Protozool., 16, p.728.

Rodhain, J., 1952. Plasmodium vinckei n.sp. Un deuxieme Plasmodium parasite de rongeurs sauvages au Katanga. Annal. Soc. Bel. Med. Trop., 32, p.275.

Roelants, G.E. and Askonas, B.A., 1972. Immunological B memory in thymus derived mice. Nat. New Biol., 239, p.63.

Rose, M.L., Parrott, D.M.V. and Bruce, R.G., 1976. Migration of lymphoblasts to the small intestine. I. Effect of Trichinella spiralis infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. Immunology, 31, p.723.

Ross, R., 1897. On some peculiar pigmented cells found in two mosquitos fed on malarial blood. Brit. Med. J., 2, p.1796.

Russell, P.F., Mulligan, H.W. and Mohan, B.N., 1942. Active immunization of fowls against sporozoites but not trophozoites of Plasmodium gallinaceum by injection of homologous sporozoites. J. Malar. Inst. India, 4, p.311.

Sabet, T., Newlin, C and Friedman, H., 1969. Effects of reticulo-endothelial system 'blockade' in antibody formation. I. Suppressed cellular and humoral haemolysin responses in mice infected with carbon particles. Immunology, 16, p.433.

Salaman, M.H., Wedderburn, N. and Bruce-Chwatt, L.J., 1969. The immunosuppressive effect of a murine plasmodium and its interaction with murine oncogenic viruses. J. Gen. Murobiol., 59, p.383.

Sanderson, C.J. and Taylor, G.A., 1976. Antibody-dependent cell-mediated cytotoxicity in the rat. The role of macrophages. Immunology, 30, p.117.

Sanderson, C.J., Lopez, A.F. and Bunn Moreno, M.M., 1977. Eosinophils and not lymphoid K cells kill Trypanosoma cruzi epimastigotes. Nature, 268, p.340.

Schultz, W.W., Huang, K.V. and Gordon, F.B., 1968. Role of interferon in experimental mouse malaria. Nature, 220, p.709.

Sengers, R.C.A., Jerusalem, C.R. and Doesburg, W.H., 1971. Murine malaria. IV. Disturbed immunological responsiveness during Plasmodium berghei infection. Exp. Parasitol., 30, p.41.

Seitz, H.M., 1976. Plasmodium berghei in isogeneic F_1 (C57Bl x DBA) mice. 3. Neonatal thymectomy and cell transfer experiments. Tropenmed. Parasit., 27, p.197.

Sergent, E., Sergent, E. and Donatien, A., 1933. Premunition et immunité dans le paludisme et dans d'autre maladies. Trans. Roy. Soc. Trop. Med. Hyg., 27, p.277.

Shortt, H.E. and Garnham, P.C.C., 1948. The pre-erythrocytic development of Plasmodium cynomolgi and Plasmodium vivax. Trans. Roy. Soc. Trop. Med. Hyg., 41, p.785.

Sheagren, J.N. and Monacco A.P., 1969. Protective effect of anti-lymphocyte serum on mice infected with Plasmodium berghei. Science, 164, p.1423.

Singer, I., 1953. The effect of X-irradiation on infection with Plasmodium berghei in the white mouse. J. Inf. Dis., 92, p.97.

Sljivic, V.S., 1970a. Radiation and the phagocytic function of the reticulo-endothelial system. I. Enhancement of the reticulo-endothelial system in X-irradiated mice. Brit. J. Exp. Pathol., 51, p.130.

Sljivic, V.S., 1970b. Radiation and the phagocytic function of the reticulo-endothelial system. II. Mechanism of reticulo-endothelial stimulation after irradiation. Brit. J. Exp. Pathol., 51, p.140.

Smalley, M.E., 1975. The nature of age immunity to Plasmodium berghei in the rat. Parasitology, 71, p.337.

Spira, D.T., Golenser, J. and Gery, I., 1976. The reactivity of spleen cells from malarious rats to non-specific mitogens. Clin. Exp. Immunol., 24, p.139.

Spira, D.T., Silverman, P.H. and Gaines, C., 1970. Anti-thymocyte serum effects on Plasmodium berghei in rats. Immunology, 19, p.759.

Spitalny, G.L. and Nussenzweig, R.S., 1973. Plasmodium berghei: relationship between protective immunity and anti-sporozoite (C.S.P.) antibody in mice. Exp. Parasitol., 33, p.168.

Sprent, J., Anderson, R.E., and Miller, J.F.A.P., 1974. Radiosensitivity of T and B lymphocytes. II. Effect of irradiation on response of T cells to alloantigens. Eur. J. Immunol., 4, p.204.

Stechschulte, D.J., 1969a. Cell-mediated immunity in rats infected with Plasmodium berghei. Milit. Med., 134, p.1147.

Stechschulte, D.J., 1969b. Effect on the reticulo-endothelial system and antibody synthesis in Plasmodium berghei infections of thymectomized rats. Proc. Soc. Exp. Biol. Med., 131, p.748.

Stobo, J.D., Talal, N. and Paul, W.E., 1972. Lymphocyte classes in New Zealand mice. II. Decreased frequency of lymphocytes lacking detectable Θ or immunoglobulin determinants. J. Immunol., 109, p.701.

Taliaferro, W.H.,and Cannon, P.R., 1936. The cellular reactions during primary infections and superinfection of Plasmodium brazilianum in Panamanian monkeys. J. Inf. Dis., 59, p.72.

Taliaferro, W.H. and Taliaferro, L.G., 1940. Active and passive immunity in chickens against Plasmodium lophurae. J. Inf. Dis., 66, p.153.

Taliaferro, W.H., Taliaferro, L.G. and Simmons, E.L., 1945. Increased parasitaemia in chicken malaria (Plasmodium gallinaceum and Plasmodium lophurae) following X-irradiation. J. Inf.Dis., 77, p.158.

Taliaferro, W.H. and Taliaferro, L.G., 1951. The effect of X-rays on immunity: a review. J. Immunol., 66, p.181.

Targett, G.A.T. and Fulton, J.D., 1965. Immunization of rhesus monkeys against Plasmodium knowlesi malaria. Exp. Parasitol., 17, p.180.

Tardieu, M. and Daguillard, D.F., 1975. Fractionated T lymphocytes can inhibit mitogenic response through dialyzable factors. Cell Immunol., 19, p.148.

Todorovic, R., Ferris, D. and Ristic, M., 1967. Roles of the spleen in acute plasmodial and babesial infections in rats. Parasitol., 21, p.354.

Tosta, C.E. and Filho, N., 1976. Non-antibody factor(s) in adult serum decreases the infectivity of Plasmodium berghei. Trans. Roy. Soc. Trop. Med. Hyg., 70, p.531.

Trizio, D. and Cudkowicz, G., 1974. Separation of T and B lymphocytes by nylon wool columns: evaluation of efficiency by functional assays in vivo. J. Immunol., 113, p.1093.

Trowell, O.A., 1965. Cells and tissues in Culture. Academic Press, edited by E.N. Willner.

Turk, J.L. and Poulter, L.W., 1972. Selective depletion of lymphoid tissue by Cyclophosphamide. Clin. Exp. Immunol., 10, p.285.

Van Boxel, J.A., Stobo, J.D., Paul, W.E. and Green, I., 1972. Antibody-dependent lymphoid cell-mediated cytotoxicity. No requirement for thymus derived lymphocyts. Science, 175, p.194.

Vanderberg, J., Nussenzweig, R.S. and Most, H., 1969. Protective immunity produced by the injection of X-irradiated sporozoites of Plasmodium berghei. V. In vitro effects of immune serum on sporozoites. Milit. Med., 134, p.1183.

Verhave, J.P., 1975. Immunization with sporozoites of Plasmodium berghei. Ph.D. thesis.

Vincke, I.H. and Lips, M., 1948. Un noveau Plasmodium d'un rongeur sauvage du Congo 'Plasmodium berghei n.sp.'. Annal. Soc. Bel. Med. Trop., 28, p.97.

Voller, A. and Rossan, R.N., 1969. Immunological studies with simian malarias. I. Antigenic variants of Plasmodium cynomolgi bastianelli. Trans. Roy. Soc. Trop. Med. Hyg., 63, p.46.

Voller, A., 1971. Variant specific schizont agglutinating antibodies in human malaria (Plasmodium falciparum) infections in Aotus. Trans. Roy. Soc. Trop. Med. Hyg., 65, p.2.

Walliker, D., Carter, R. and Morgan, S., 1973. Genetic recombination in Plasmodium berghei. Parasitology, 66, p.309.

Warren, H.S. and Weidanz, W.P., 1976. Malarial immunosuppression in vitro: adherent spleen cells are functionally defective as accessory cells in the response to horse erythrocytes. Eur. J. Immunol., 6, p.816.

Wedderburn, N., Turk, J.L. and Hutt, M.S.R., 1975. Chronic malarial infection in Balb/c mice. Effect on the immune response to sheep erythrocytes and histological changes in the liver and spleen. Trans. Roy. Soc. Trop. Med. Hyg., 69, p.463.

Weinbaum, F.I., Evans, C.B. and Tigelaar, R.E., 1976b. Immunity to Plasmodium berghei yoelii in mice. I. The course of infection in T cell and B cell deficient mice. J. Immunol., 117, p.1999.

Weinbaum, F.I., Evans, C.B. and Tigelaar, R.E., 1976a. In vitro assay for T cell immunity to malaria in mice. J. Immunol., 116, p.1280.

Weir, D.M., 1973. Handbook of Experimental Immunology; Blackwell Scientific Publications.

Weiss, M.L. and De Guisti, D.L., 1964. The effect of different sera in the culture medium on the behaviour of Plasmodium berghei following serial passage through tissue culture. J. Protozool., 11, p.224.

Wellde, B.T. and Sadun, E.H., 1967. Resistance produced in rats and mice by exposure to irradiated Plasmodium berghei. Exp. Parasitol., 21, p.310.

Wilson, R.J.M., 1974. Parasites in the immunized host: mechanisms of survival. Soluble antigens as blocking antigens. C.I.B.A. Foundation Symposium, 25, p.284.

Wilson, R.J.M. and Phillips, R.S., 1976. Method to test inhibitory antibodies in human sera to wild populations of Plasmodium falciparum. Nature, 263, p.133.

Wisloff, F. and Freland, S.S., 1973. Antibody-dependent cell-mediated cytotoxicity: no requirement for membrane bound immunoglobulin. Scan. J. Immunol., 2, p.151.

Wright, D.H., 1968. The effect of neonatal thymectomy on the survival of golden hamsters with Plasmodium berghei. Br. J. Exp. Pathol., 49, p.379.

Wright, D.H., Masembe, R.M. and Bazira, E.R., 1971. The effect of antithymocyte serum of golden hamsters and rats infected with Plasmodium berghei. Br. J. Exp. Pathol., 52, p.465.

Wyler, D.J., Miller, L.H. and Schmidt, L.H., 1977. Spleen function in quartan malaria (Plasmodium inui): evidence for both protective and suppressive roles in host defence. J. Inf. Dis., 135, p.86.

Wyler, D.J., 1976. Peripheral lymphocyte subpopulations in human falciparum malaria. Clin. Exp. Immunol., 23, p.471.

Yoeli, M., 1966. Patterns of immunity and resistance in rodent malaria infections. Bull. Soc. Pathol. Exot., 59, p.593.

Wyler, D.J., 1976. Peripheral lymphocyte subpopulations in human falciparum malaria. Clin. Exp. Immunol., 23, p.471.

Zuckerman, A., 1945. *In vitro* opsonic tests with *Plasmodium gallinaceum* and *Plasmodium lophurae*. J. Inf. Dis., 77, p.28.

Zuckerman, A. and Yoeli, M., 1953. Age and sex as factors influencing *Plasmodium berghei* infections in inbred and splenectomized rats. J. Inf. Dis., 94, p.225.

Zuckerman, A., 1960. Auto-antibody in rats with *P.berghei*. Nature, 185, p.189.

Zuckerman, A. and Golenser, J., 1970. The passive transfer of protection against *Plasmodium berghei* in rats. J. Parasitol., 56, p.378.