Preliminary studies of artificial immunization of rats against *Plasmodium berghei* and adoptive transfer of this immunity by splenic T and T+B cells *

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Protective T lymphocyte and T+B lymphocyte responses in rats artificially immunized against P. berghei *have been demonstrated by adoptive transfer. The techniques used could be developed for detailed analysis of protective lymphocyte responses generated by various methods of immunization, and their relationship to immunity.*

In another paper (*4*) we discuss the role of T cells in the development of protective immunity in rats chronically infected with *Plasmodium berghei*. This paper reports similar experiments in rats immunized by methods other than chronic infection and indicates possible ways in which lymphocyte separation techniques can be exploited to elucidate responses to various artifical methods of immunization.

MATERIALS AND METHODS

The KSP11 strain of *P. berghei* was used throughout these studies. Parasite maintenance, reference stabilates, in-bred rats, infection and challenge, parasite counts, infectivity tests, and the preparation and separation of spleen cells on V26 experimental and control polymethylmetacrylic bead columns are described elsewhere (*4*).

Irradiation of parasitized erythrocytes

August rats were used as donors of infected erythrocytes. Three rats were infected from the reference stabilate and on day 6 their blood was used to infect batches of 15 male rats (weight, 45–70 g). Each batch of 15 rats provided sufficient material to inoculate 10 experimental rats with one dose of irradiated parasites.

A batch of 15 rats was bled 6 days after infection by cardiac puncture into citrate saline. All subsequent operations were carried out at 4°C. The cell suspension was centrifuged at 1500 *g* for 10 min and the packed cells were suspended in Krebs saline (*7*) plus 0.2% glucose (KG) and recentrifuged at 500 *g* for 15 min. The pale brown top layer of cells containing most of the parasitized cells plus some uninfected erythrocytes and many leucocytes was removed and suspended in 50 ml of KG. This suspension was divided into 3 aliquots containing approximately 10^8–10^9 parasitized cells per inoculum according to batch. Each aliquot was irradiated at 60 krad [a] (*11*) using a ^{60}Co gamma source and an exposure of not more than 3 min. The cells were then concentrated by centrifugation at 750 *g* for 10 min and suspended in 22 ml of KG, and 2.0 ml of this cell suspension were immediately inoculated intraperitoneally into each rat to be immunized. Examination of thin blood smears showed that infected erythrocytes were circulating in these animals more than 24 h after inoculation. Two mice were also given injections of the suspension (0.25 ml) as a test for infectivity of the irradiated parasites. None of the samples of irradiated cells used proved infective.

Immunization by infection

August rats were infected from the reference stabilate and their blood was used within 6 days to infect male rats (weight, 70–100 g) with 10^6 parasitized cells. On days 5, 6, 7, and 8 these rats were treated with sulfadiazine intraperitoneally at a rate of 20 mg/100 g of body weight to cure the infection. Before treatment 5–10% of the erythrocytes were

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[a] 1 rad = 10^{-2} gray.

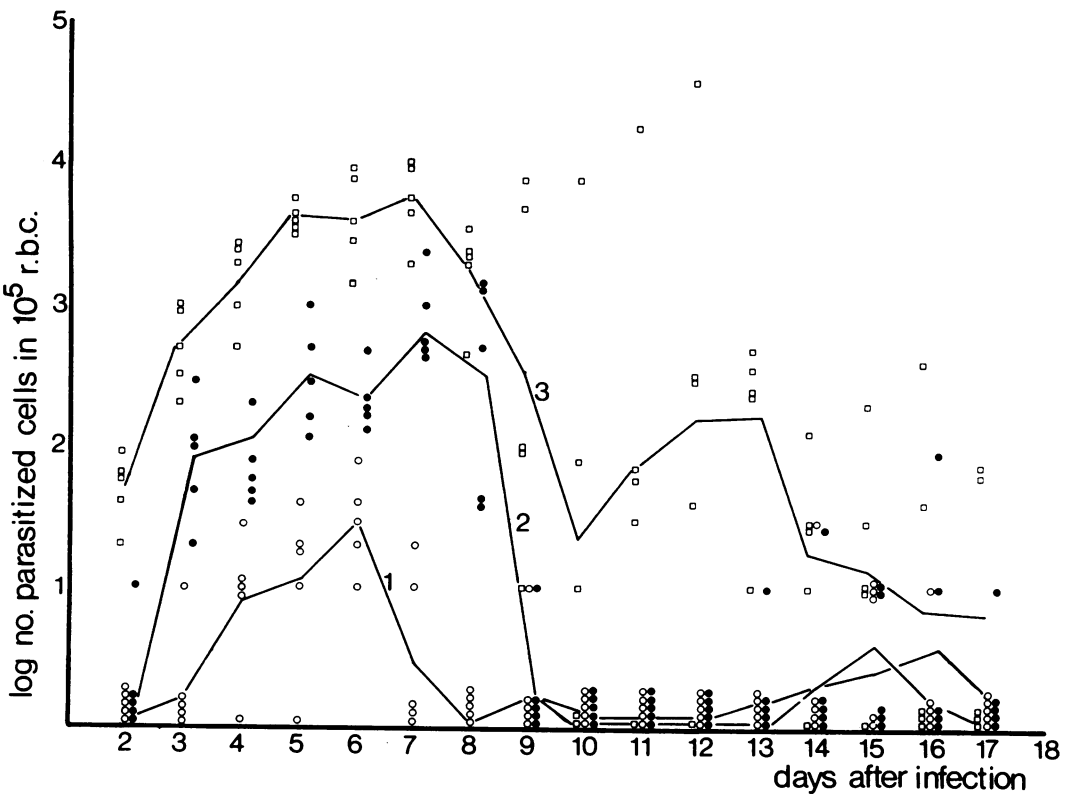

Fig. 1. Parasitaemia in rats equivalent to donors immunized with irradiated parasites (○), rats receiving spleen cells from immunized donors (●), and those receiving no cells (□). Spleen cells were transferred at a donor/recipient ratio of 1 : 1. Rats were challenged with 10^6 parasitized erythrocytes. Curves indicate geometric mean parasitaemias of donor equivalents (1), spleen cell recipients (2), and controls (3). Rats given injections of normal irradiated syngeneic erythrocytes rather than parasitized cells are not protected (unpublished observations by the authors). r.b.c = erythrocytes.

infected. The drug regimen used cleared parasites from the peripheral blood by day 8 of treatment.

Challenge infection

Rats (weight, 70–90 g) infected for 6 days from the reference stabilate were used as a source of parasites for the challenge inoculum of 10^6 parasitized erythrocytes given intraperitoneally to immunized animals.

RESULTS

Immunization with irradiated parasitized cells followed by transfer of unfractionated spleen cells

A group of 10 male rats (weight, 70–80 g) were immunized with irradiated parasites on days 0, 3, 8, 20, and 24, each rat receiving a total of approximately 10^8–10^9 parasitized erythrocytes. Spleen cells from 5 of the rats were transferred to intact male recipient rats (weight, 90–100 g) at a donor/recipient ratio of 1 : 1 on day 31. The recipients and the remaining 5 immunized rats were then challenged with 10^6 parasitized cells. The results are given in Fig. 1.

Both the directly challenged immunized rats and the recipients of spleen cells from immunized rats showed substantially better protection than the controls rats. It should be noted, however, that both the groups of immunized rats and recipients of spleen cells tended to show a mild recrudescence of the infection after day 12.

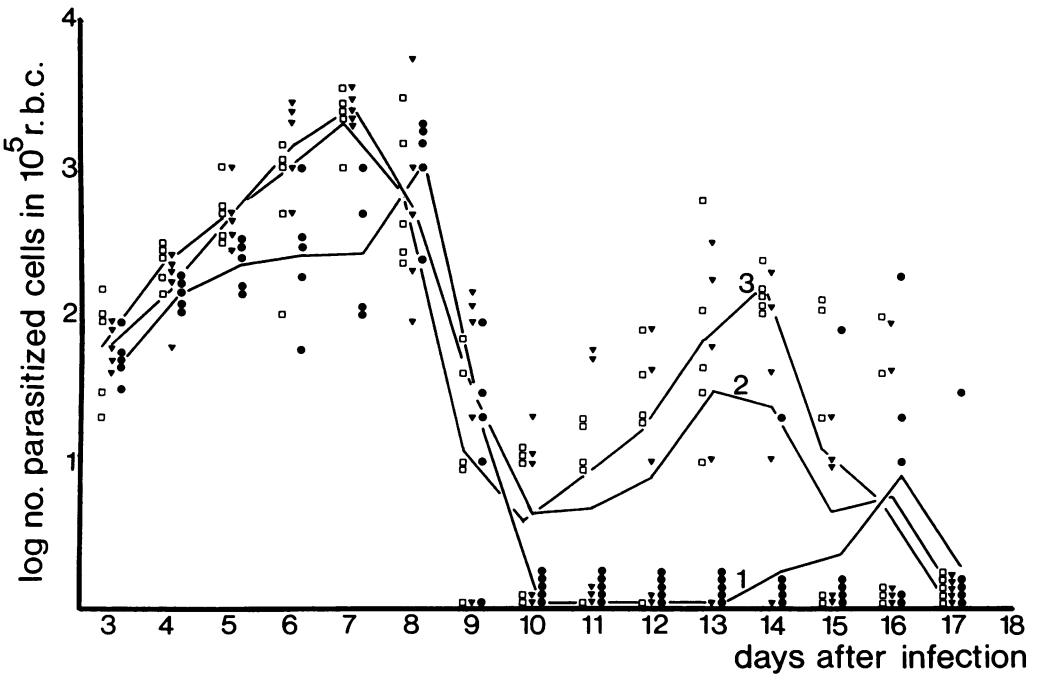

Fig. 2. Parasitaemia in rats receiving 4.6×10^7 unfractionated spleen cells (•), 4.2×10^6 T cells (▾), and no cells (▫) from donors immunized with irradiated parasites. The challenge dose was 10^6 parasitized erythrocytes. Curves indicate geometric mean parasitaemias in recipients of unfractionated spleen cells (1) and T cells (2), and in controls (3). r.b.c. = erythrocytes.

Immunization with irradiated parasitized cells followed by transfer of T cells and unfractionated spleen cells

In this experiment male donor rats (weight, 60–80 g) were immunized on days 0, 3, 8, 21, and 24, each rat receiving a total of approximately 6×10^8 irradiated parasitized red cells. On day 52 these rats were killed and 4.2×10^7 T cells, according to the criteria described elsewhere (*4*), or 4.6×10^7 unfractionated spleen cells were inoculated intraperitoneally into intact male recipient rats (weight, 130–140 g). The results are shown in Fig. 2.

As in the previous experiment, recipients of unfractionated spleen cells from immunized donors showed lower peak parasitaemia than the control rats and a more effective clearance of the first parasitaemia wave. Also, again as in the previous experiment, there was a tendency for the parasitaemia to recrudesce after day 12. Rats receiving T cells were no more successful than controls in curtailing the first parasitaemia wave but were able to restrict the second peak more effectively.

Immunization by infection and cure followed by a booster dose of irradiated parasites

A batch of 23 male rats (weight, 65–100 g) were immunized by means of infection and cure, as described above under materials and methods, day 0 being the day of infection. On days 20, 27, 34, and 41 these animals were given a booster dose of irradiated parasites, each rat receiving approximately 3×10^9 infected erythrocytes. On day 58, 5 of these rats were challenged with 10^6 parasitized cells in comparison with 5 control rats that received only sulfadiazine treatment. Spleen cells were isolated from the remaining rats on the same day. T and T+B cell populations were eluted from V26 experimental and control columns and inoculated into intact recipient rats, which were then challenged. Recipients received either 6×10^7 T cells, less than

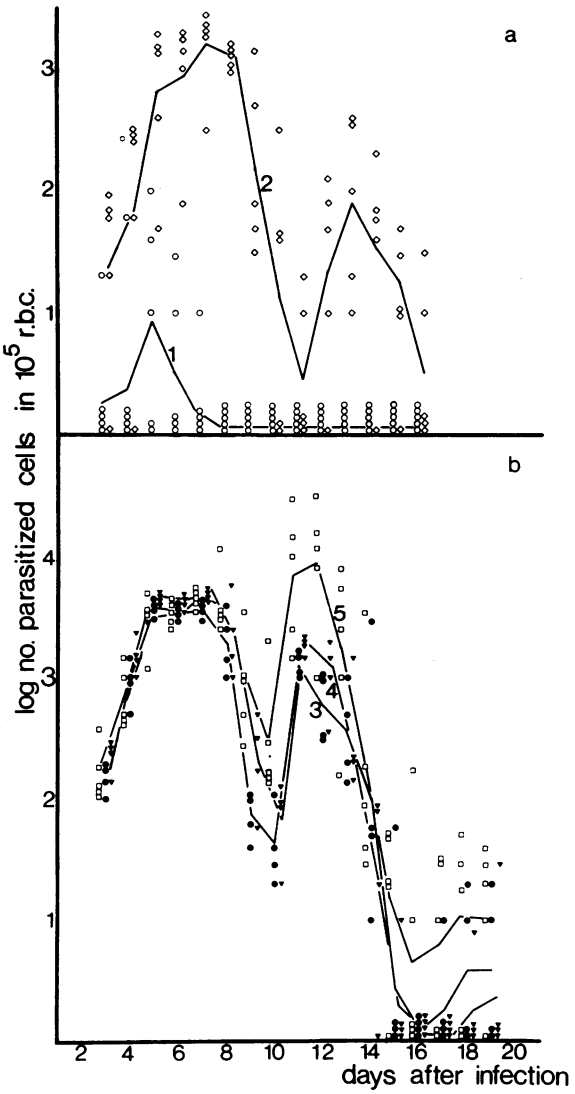

Fig. 3. (*a*) Parasitaemia in rats equivalent to donors immunized by means of drug-cured infection and irradiated parasites (○) and in drug-treated controls (◊). (*b*) Parasitaemia in recipients of 8×10^7 T+B cells (●), 6×10^7 T cells (▾), and no cells from such donors (□). The challenge dose for experimental rats was 10^6 parasitized cells. Donor equivalents and drug-treated controls at this time weighed approximately 250 g and the recipients 100 g. Curves indicate geometric mean parasitaemias in immunized donor equivalents (1), drug-treated controls (2), T+B cell recipients (3), T cell recipients (4), and controls (5). r.b.c. = erythrocytes.

1% of cells showing surface membrane immunoglobulin (SmIg), or 8×10^7 T+B cells, including 29% SmIg positive cells. The results are shown in Fig. 3.

Rats immunized by means of cured infection plus irradiated parasites showed a brief parasitaemia on challenge. However, in contrast to the animals in the previous experiment that were immunized with irradiated parasites alone, these rats showed no tendency towards a recrudescence of the parasitaemia after day 12. The presence of sensitized B cells in the transfer inocula provided no additional protective effect over that due to sensitized T cells for the spleen cell recipients in this experiment, unlike those in the previous experiments. Rats receiving sensitized T and T+B cells reacted in the same way, both the first and second waves of parasitaemia being restricted to some extent. A second experiment gave a very similar result and infectivity tests showed that immunized rats carried a subpatent infection 18 days after challenge.

DISCUSSION

The rats used in these experiments were immunized in two different ways: the first method involved immunization with 5 doses of irradiated parasites, the second involved immunization first by an infection, which was eradicated by drug therapy starting on day 6 when the parasitaemia level was between 5% and 10%, and then by administration of 4 booster doses of irradiated parasites. Every effort was made to ensure that the immunizing parasite populations were homologous but in the absence of a suitable *in vitro* test for antigenic variants in *P. berghei* this is uncertain.

The significance of antigenic homology has been shown in rhesus monkeys immunized against *P. knowlesi* malaria. With this host and parasite the results of subsequent challenge infection depend on whether or not the monkeys are sensitized and challenged with the same or a different antigenic variant (*3*, *8*). In *P. knowlesi*, unlike *P. berghei*, variant populations can be detected by a sensitive serological test. Antigenic variation has been demonstrated in *P. berghei* but only with relatively cumbersome *in vivo* tests (*1*, *2*, *5*). Consequently, although we attempted to ensure that the immunizing and challenge populations were homologous in the experiments described here it is not certain that this was in fact the case.

Rats immunized by irradiated parasites alone showed on challenge a considerable reduction in the

level of parasitaemia and in the duration of patent parasitaemia although there was a tendency for the infection to recrudesce after day 12. Following the second method of immunization, protective immunity was apparently stronger; parasitaemia was even more restricted and showed no tendency to recrudesce later.

Rats receiving unfractionated spleen cells from donors immunized with irradiated parasites alone behaved on challenge much like animals immunized in this way and challenged directly, although the first parasitaemia was more intense; however, the parasitaemia was slower to develop than in control animals. Like animals challenged directly after immunization, recipients of spleen cells often showed a recrudescence of parasitaemia after day 12. T cells alone were much less effective than T+B cells in transferring immunity but were able to restrict the second peak of parasitaemia to some extent.

Curiously, this difference between recipients of T+B and T cells was not shown by rats receiving cells from donors immunized first by infection and then with irradiated parasites. Among those animals there was no difference between T and T+B cell recipients, both groups behaving much like the recipients of T cells alone. This result is surprising in that animals equivalent to the cell donors were, when subjected to direct challenge, apparently better protected than donor equivalent animals immunized with irradiated parasites alone.

The fact that rats immunized with irradiated parasites alone and recipients of their spleen cells control the first parasitaemia well but show a tendency to relapse indicates, on the basis of arguments advanced by Brown et al. (*4*), that they are well primed at the B cell level to the immunizing and challenge antigenic variant but less well primed to cope with the breaking through of a new antigenic variant of the parasite. Rats immunized by infection and cure and irradiated parasites seemed well primed for coping with both primary parasitaemias and for preventing the recrudescence of infections. The cell transfer experiment indicated, however, that this ability to respond rapidly was not present, or at least was not retained, in the B cell population between immunization and challenge. (It has been demonstrated (4) that T+B cells from rats sensitized by chronic infections and eluted from V26 control columns can produce a greater protective effect than T cells alone, presumably owing to the presence of the B cells.) At least some " memory " is retained in the T cells since a protective effect was noted on transfer of spleen T cell suspensions. It would be interesting to test the effectiveness of the recirculating, long-lived, non-dividing T lymphocyte pool from these rats. Recirculating T cells are known to retain carrier memory (*6*) and protective T cell memory for *Salmonella* infections (*9*, *10*). The immunized animals described here differ from the chronically infected donors described elsewhere (*4*) in that parasites and their products are not continuously circulating in the blood and being retained by the spleen; thus there is probably a very much smaller tendency for the primed memory T cells in any number to be localized in the spleen at the time of cell transfer. This may be the reason why T cell suspensions were not very protective. Lack of B-cell memory in cells from drug-treated donors may be a consequence of B-cell paralysis induced by a massive release of antigen after drug treatment. It is also possible that sulfadiazine binding to parasite protein modifies its antigenicity or immunogenicity.

In summary, these limited experiments indicate that the two different methods of immunization against malaria can give, as expected, different degrees of protection. It seems likely that these differences, and differences shown by other methods of immunization, can be analysed at the cellular level by the techniques of lymphocyte fractionation and adoptive cell transfer.

RÉSUMÉ

ÉTUDES PRÉLIMINAIRES SUR L'IMMUNISATION ARTIFICIELLE DE RATS CONTRE *PLASMODIUM BERGHEI* ET TRANSFERT ADOPTIF DE CETTE IMMUNITÉ PAR DES CELLULES SPLÉNIQUES T ET T+B

Des rats ont été immunisés selon l'une ou l'autre des méthodes ci-après: i) par cinq inoculations de 10^8 à 10^9 *Plasmodium berghei* irradiés avec 60 krad, ou ii) par une infection à *P. berghei* guérie par un traitement à la sulfadiazine entre le cinquième et le huitième jour, à raison de 20 mg/100 g de poids corporel, puis par quatre inoculations de 10^8 à 10^9 parasites irradiés.

Pour évaluer les réponses protectrices on a pratiqué

soit l'épreuve directe à l'aide de 10^6 *P. berghei* provenant du même stabilat que les parasites utilisés pour l'immunisation, soit par transfert adoptif de cellules spléniques non fractionnées ou de cellules spléniques T à des receveurs syngéniques, lesquels étaient éprouvés ensuite.

Les rats immunisés avec des parasites irradiés seulement ont présenté une parasitémie faible et transitoire mais avec tendance à la recrudescence après le douzième jour. Chez les receveurs de cellules spléniques non fractionnées provenant de ces animaux, le résultat était à peu près le même, si ce n'est que la première parasitémie était plus intense. Les cellules T transféraient moins efficacement l'immunité, mais chez les rats qui en avaient reçu, le deuxième pic de parasitémie était plus faible que chez les témoins.

Les rats immunisés par infection et soumis à une injection de rappel de parasites irradiés ont également présenté une brève parasitémie lors de l'épreuve, mais l'infection n'avait aucune tendance à la recrudescence. Les tests d'infectivité ont montré que ces rats présentaient une infection subpatente. Chose surprenante, les receveurs de cellules spléniques non fractionnées provenant de ces rats n'étaient pas sensiblement plus capables de réduire la parasitémie que les receveurs de cellules T; c'est-à-dire que dans les deux groupes l'infection était un peu réduite par rapport à celle des témoins.

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