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Adoptive Transfer of Immunity to *Plasmodium berghei* with Immune T and B Lymphocytes[1]

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Immunity to malarial infection may be transferred with immune lymphocytes. This study was designed to determine which lymphocyte type is responsible for the adoptive transfer of immunity to malarial infection. In one set of experiments, the ability of immune T and B lymphocytes, separated by passage through nylon-wool columns, to transfer immunity to infection was determined. In another experiment, the effect of killing T lymphocytes with anti-theta serum on the transfer of immunity was determined. The effect on the ability of immune lymphocyte suspensions to transfer immunity after B lymphocytes were removed from such suspensions by centrifugation on Ficoll-Hypaque gradients, after they had formed rosettes with sensitized, complement-coated sheep erythrocytes, was also determined. The ability of lymphocyte suspensions to adoptively transfer resistance to malarial infection was greatly impaired by the removal from the suspensions of differentiated B-type lymphocytes. Our results indicate that it is the differentiated B cell, most probably an antibody-producing cell, which lacks both theta antigen and the complement receptor that is responsible for conferring immunity to malaria.

Immunity to plasmodial infection has been attributed, at least in part, to the development of antiplasmodial globulin. The importance of immune globulins in conferring immunity to malaria infection has been established by the passive transfer of immune gamma globulin in humans (9) and in experimental animals (2, 6, 10). Protective antiplasmodial globulins act on mature schizonts (8) and on free merozoites (14).

Immunity to malarial infection may also be transferred with lymphocytes from immune animals (7, 17, 19). Stechschulte (19) found that both immune spleen and lymph node cells were effective in transferring immunity to *Plasmodium berghei*. Thoracic duct lymphocytes were not. The effective transfer of immunity with cells caused investigators to propose that the development of immunity to the plasmodia depended on cell-mediated immune responses, even though no attempt was made to identify which lymphocyte type(s) was responsible for conferring immunity. Further support for the theory that cell-mediated immune responses are important in the development of resistance to malarial infection come from studies involving the in vivo depletion of T cells. Antithymocyte serum abolished the innate resistance mechanisms of rats of different ages to primary infection with *P. berghei* but did not arrest antibody production (18). Neonatal thymectomy also reduced the protective immune response to *P. berghei* in rats (3, 20).

It is now known that the humoral response to most antigens requires the participation of T cells as well as B cells (16). T cells act as helpers in the humoral response to most antigens. Brown (4) postulated that impairment of T cell response by thymectomy or by antithymocyte serum treatment may render rodents incapable of making a rapid response to new antigenic variants of the plasmodia. He also reported (5) that lymphocyte suspensions from rats immune to *P. berghei* and depleted of B lymphocytes were capable of conferring immunity to *P. berghei* challenge. Brown interpreted these results as meaning that the immune T cells were acting as helpers in the humoral response. T cells were presumably sensitized to variant-specific antigens.

T cells may be killed in lymphocyte suspensions with anti-theta serum and complement. B cells may be removed from such suspensions by centrifugation on Ficoll-Hypaque gradients after they have formed rosettes with sensitized, complement-coated sheep erythrocytes.

T and B cells may also be separated after absorption onto nylon-wool columns. For unknown reasons, B lymphocytes stick preferentially to nylon wool (11). Epstein et al. (12)

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found that passage of human peripheral blood lymphocytes through long nylon-wool fiber columns at 37 C resulted in a two- to threefold depletion of immunoglobulin-bearing cells. Julius et al. (15) used 12-ml syringes packed with nylon wool and found that after one absorption, a murine spleen cell population containing 85 to 90% T cells with less than 5% immunoglobulin-bearing cells and with 10% unidentifiable cells could be obtained. B cells may be eluted from the nylon-wool columns after the effluent T cells have been removed; therefore, this method allows for the selective removal of specific lymphocytes and for their recombination. Trizio and Cudkowicz (21) evaluated the efficacy of the nylon-wool column depletion method in adoptive transfer experiments with irradiated recipients. They found that neither the effluent, predominantly T cell population, nor the adherent, predominantly B cell population, could be induced to produce anti-sheep erythrocyte plaques. T effluent cells were, however, able to synergize with bone marrow cells to produce plaque-forming cells, and adherent B cells were able to synergize with thymocytes to produce plaque-forming cells.

T cell killing with anti-theta serum, removal of rosette-forming B cells on Ficoll-Hypaque gradients, and passage of immune lymphocyte suspensions through nylon-wool columns were used in this study to determine, in adoptive transfer experiments, to what extent T and B lymphocytes from immune animals, alone or in combination, are able to adoptively transfer immunity to *P. berghei* in rats.

MATERIALS AND METHODS

Animals. All rats used in this study were Fischer 344 (CD*F) females obtained from Charles River Breeding Laboratories in Wilmington, Mass. All animals were housed in the animal facility of the Department of Microbiology, Ohio State University. They were allowed food and water ad libitum.

Donors in adoptive transfer experiments were 8-week-old female CD*F rats infected by intraperitoneal injection of 2×10^7 *P. berghei*-infected rat erythrocytes. These rats were challenged with 2×10^7 *P. berghei*-infected rat erythrocytes 1 week after they had cleared the parasites (20 to 25 days of clearance time). Forty-five to 50 days after challenge, the rats were killed and their spleens were removed. Lymphocytes from the spleens of these recovered donors were harvested as described in reference 13.

Normal animals, the same age as recovered donors, were maintained as described for recovered donors, and lymphocytes were harvested from their spleens as previously described (13).

Recipients in all experiments were 4-week-old CD*F females.

Titration of effective lymphocyte numbers for the transfer of protection against challenge with *P. berghei*. The number of lymphocytes that had to be transferred from an immune to a susceptible rat to confer resistance to *P. berghei* infection was determined in a series of experiments in which 2×10^6, 2×10^7, or 2×10^8 viable immune or normal lymphocytes were transferred to susceptible rats 1 week before challenge with *P. berghei*. Lymphocyte viability was determined by trypan blue exclusion. There were five rats in each experimental group. Percentage of parasitemia was determined by counting the number of infected cells per 10,000 erythrocytes in Giemsa-stained thin blood films.

Each concentration of cells conferred protection to *P. berghei* challenge, but the intraperitoneal injection of 2×10^8 immune lymphocytes conferred the most protection. A peak parasitemia of 2% was reached by day 3 in rats that received 2×10^8 immune lymphocytes. No parasites were observed in animals of this group after day 7. Peak parasitemia of 2% occurred at day 9 in animals that received 2×10^7 immune lymphocytes. Animals in this group took longer to clear parasites than did those that received 2×10^8 immune lymphocytes. Parasites were observed in animals in this group up to day 17. Parasitemia peaked at 10% at day 13 in animals that received 2×10^6 immune cells. Animals that received normal cells or no cells at all were not protected. These results are summarized in Fig. 1. A total of 2×10^8 viable splenic lymphocytes were used in all subsequent studies.

T cell depletion of lymphocyte suspensions by anti-theta serum killing. T cells were killed with brain-associated anti-theta and complement. Brain-associated anti-theta serum was prepared as described previously (13).

For transfer experiments, lymphocyte suspensions were adjusted to contain 10^8 viable cells/2 ml. Two milliliters of adsorbed anti-theta serum diluted 1:8 and 2 ml of adsorbed guinea pig complement diluted 1:4 were mixed with 2 ml of immune lymphocytes in a 15-ml tube. The tubes were incubated at 37 C for 45 min in a humid CO_2 incubator. After incubation, the cells were washed three times in Hanks balanced salt solution (HBSS). To inject five animals, 10 tubes were prepared. The packed lymphocytes were suspended to a final volume of 5 ml in

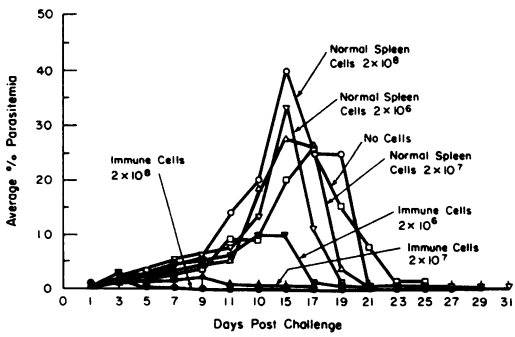

FIG. 1. *Titration of the number of lymphocytes required to transfer immunity to P. berghei injection to 4-week-old recipient rats.*

HBSS. Twenty-seven percent of the cells were killed with this method. Cell killing was measured by trypan blue exclusion.

Suspensions of 10^8 viable immune lymphocytes were treated with normal rabbit serum and guinea pig complement under the same conditions described for lymphocytes treated with anti-theta serum. Only 3.5% of the cells were killed by incubation of immune lymphocytes with normal rabbit serum and complement.

Five 4-week-old CD*F rats were each given 1.46×10^8 viable immune lymphocytes (the number of viable lymphocytes remaining after treatment of 2×10^8 lymphocytes with anti-theta serum and complement). Another group of five rats were each injected intraperitoneally with 1.98 viable immune lymphocytes, the number of viable lymphocytes left after 2×10^8 lymphocytes were treated with normal rabbit serum and complement. Control rats received 2×10^8 viable normal spleen cells or no cells at all. All animals were challenged 1 week later with 2×10^7 *P. berghei*-infected rat erythrocytes. Percentage of parasitemia was determined from Giemsa-stained blood films as described above.

B cell depletion after rosette formation with sensitized, complement-coated sheep erythrocytes. B cells will form rosettes with sensitized, complement-coated sheep erythrocytes (1). One hundred to 150 ml of a 5% suspension of washed sheep erythrocytes was prepared in HBSS. An equal volume of anti-sheep hemolysin, diluted 1:1,000, was added to the sheep cell suspension. This mixture was incubated with frequent mixing at 37 C for 30 min. The sensitized cells were washed twice in HBSS and resuspended to a 5% concentration in HBSS. Fresh mouse complement was diluted 1:10 in HBSS. An equal volume of diluted complement was added to the sensitized sheep erythrocytes. This mixture was incubated at 37 C for 30 min. The cells were washed three times in HBSS, and the sensitized, complement-coated sheep erythrocytes (EAC) were resuspended to a 5% concentration in HBSS. Three and one-half milliliters of immune lymphocyte suspension containing 10^8 viable immune lymphocytes was added to 3.5 ml of EAC in a 15-ml tube. The contents of the tubes were mixed and incubated at 37 C for 30 min. After incubation, the mixture contained 35% rosettes. The mixture (7 ml total) was layered onto 3 ml of Ficoll-Hypaque suspension. The mixtures were centrifuged at 2,000 rpm in an International centrifuge, model CM, for 20 min. Lymphocytes were harvested from the interface of the buffer and Ficoll-Hypaque layer. The cells were washed three times in HBSS. Sheep erythrocyte contamination was minimal. The harvested lymphocytes were tested for the presence of complement receptor lymphocytes (CRL). A 0.2-ml amount of a splenic lymphocyte suspension containing 5×10^6 viable lymphocytes/ml was incubated with 0.2 ml of EAC. The contents of the tubes were mixed and incubated at 37 C for 30 min. Rosettes were counted in a hemocytometer. The CRL-depleted population contained 2% rosettes. Since recovered rat spleens contain approximately 50% CRL, and since during treatment we accounted for the removal of 35% CRL, it is likely that some cells capable of forming rosettes were lost during centrifugation and washing.

The harvested cells were tested for the presence of T cells in a cytotoxicity assay described in reference 13. Forty percent of the remaining cells were identified as T cells. Four 4-week-old rats were each given intraperitoneal injections of 1.3×10^8 viable lymphocytes depleted of CRL. (This number represents the number of cells that would be left of the standard inoculum of 2×10^8 cells after 35% of the CRLs had been removed.) The rats were challenged 1 week later with 2×10^7 *P. berghei*-infected rat erythrocytes. Percentage of parasitemia was calculated from the number of cells infected per 10,000 cells examined in Giemsa-stained blood films.

Separation of T and B lymphocytes on nylon-wool columns. Prior to use, glass and nylon wool were soaked in normal saline for 1 h at 37 C. The wools were rinsed three times in doubly distilled, demineralized water and wrung dry. The wools were placed in separate beakers and covered with double-distilled, demineralized water. The beakers of wool were incubated at 37 C for 1 week with daily changes of water. This amount of washing caused extensive loss of glass-wool fiber. After these washings, glass and nylon wool were wrung out and placed on a sheet of aluminum foil and dried in a 37 C incubator.

A 0.6-g portion of nylon wool or 0.8 g of glass wool was packed into the barrels of 10-ml syringes. The plungers were replaced, and the syringes were wrapped and sterilized.

Lymphocytes and macrophages were harvested from recovered rat spleens as described previously (13). The washed cells were resuspended in HBSS. A suspension containing 5×10^6 viable lymphocytes was made to determine the percentage of cells killed by anti-theta serum and of cells forming rosettes with EAC present before glass- and nylon-wool treatment. The cells were pelleted in a Sorvall RC-2B centrifuge at 1,000 rpm for 10 min. They were resuspended to a final concentration of 1.5×10^8 viable lymphocytes/2 ml in HBSS supplemented with 5% fetal calf serum (FCS medium).

To remove macrophages from the splenocyte suspensions containing 1.5×10^8 cells/2 ml, the cells were passed through glass-wool columns. Glass-wool columns were washed before use with 50 ml of FCS medium at room temperature. Harvested cells were pelleted by centrifugation at 1,000 rpm in a Sorvall RC-2B centrifuge. The cells were washed two times in HBSS and resuspended in HBSS. A viability count was made, and percent yields was calculated. From this count, a suspension containing 5×10^6 viable lymphocytes was made for use in evaluating the numbers of cells killed by anti-theta serum killing (T) and cells forming rosettes with EAC (B) in the glass-wool column effluents. The remaining suspension was adjusted in FCS medium to contain 1.5×10^8 viable cells/2 ml.

Prior to loading the cells onto the nylon-wool columns, each column was washed with 20 ml of warm (37 C) FCS medium, sealed with Parafilm, and incubated in an upright position in a 37 C humid incubator for 1 h. After incubation, pH gradients in the columns were corrected by rinsing 5 ml of FCS

medium through each column. Two milliliters of lymphocyte suspension was layered dropwise onto each column. The cells were washed into the column with 1 ml of FCS medium, added dropwise. Each syringe barrel was sealed with Parafilm and incubated at 37 C for 45 min. After incubation, effluent T cells were collected from the columns by passage through them of 25 ml of FCS medium. The columns were then washed by rapid passage of 100 ml of FCS medium. Adherent cells were then eluted by squeezing the nylon wool with stainless-steel forceps and rinsing with 8 ml of FCS medium. The nylon wool was squeezed and washed once more. Effluent and eluted cells were pelleted in the cold by centrifugation at 1,000 rpm for 10 min in a Sorvall RC-2B centrifuge. The cells were washed two times in HBSS, and each population was tested for the presence of T and B lymphocytes by anti-theta serum killing and rosette formation as previously described.

For adoptive transfer studies, a group of five 4-week-old female CD*F rats were each given 7×10^7 immune effluent T lymphocytes intraperitoneally. An inoculum of 7×10^7 cells was chosen because studies with adult recovered rats indicated that approximately 35% of the cells in the spleen could be killed with anti-theta serum and complement. The 7×10^7 amount is 35% of 2×10^8, the standard adoptive transfer inoculum.

Three 4-week-old rats received 10^8 (50% of 2×10^8) eluted B cells intraperitoneally. An inoculum of 10^8 eluted B cells was chosen because studies with adult recovered rats indicated that approximately 50% of the cells of the spleen were B cells by their ability to form complement receptor rosettes with sensitized erythrocytes. Another group of three rats each received 1.7×10^8 recombined T and B lymphocytes. All rats were challenged 1 week later by the intraperitoneal injection of 2×10^7 *P. berghei*-infected rat erythrocytes. Percentage of parasitemia for each rat was determined daily from Giemsa-stained blood films.

The nylon-wool experiments were repeated, but in the second run Dulbecco phosphate-buffered saline was used instead of HBSS. The same injection schedule was followed, but each group contained five animals.

RESULTS

The killing of T cells in immune lymphocyte suspensions with anti-theta serum and complement did not abolish the ability of the remaining cells to transfer immunity to *P. berghei* challenge. However, animals that received T-depleted immune lymphocyte suspensions cleared the parasites more slowly than did those animals that received lymphocytes that had been treated with normal rabbit serum and complement. Although infection levels were very low in the rats that received the T-depleted lymphocyte population, the animals did not clear the parasites until day 15. Animals that received normal serum-treated immune lymphocytes cleared the parasites by day 7. Transfer of T-depleted immune lymphocytes conferred more immunity than did the transfer of normal lymphocytes. Percentages of parasitemia in animals in the control groups were much higher than in animals that received T-depleted lymphocyte suspensions. Parasites were not completely cleared by animals in the control groups until day 23. These results are presented in Fig. 2.

Lymphocyte populations depleted of CRL were also effective in transferring immunity to *P. berghei* challenge. Peak parasitemia occurred at day 3 in animals that received CRL-depleted populations; however, the animals that received CRL-depleted populations maintained a low-level parasitemia until day 19, whereas the animals that received the undepleted population cleared all parasites by day 7. Animals that received no lymphocytes had much higher parasitemias than did animals

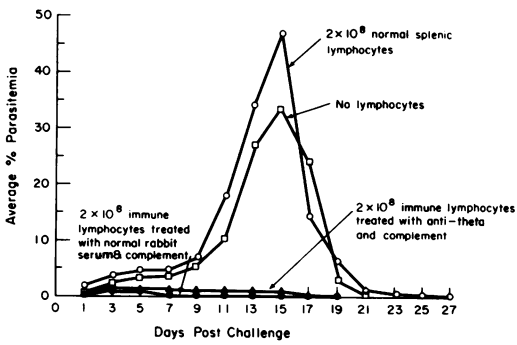

FIG. 2. *Effect of killing cells with anti-theta serum on the adoptive transfer of immunity by splenic lymphocytes.*

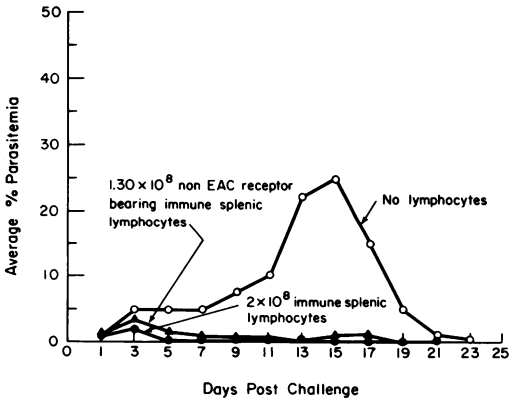

FIG. 3. *Effect of the removal of CRLs on the adoptive transfer of immunity to P. berghei infection by splenic lymphocytes.*

that received either CRL-depleted or undepleted immune lymphocyte populations. Parasites were not completely cleared until day 22 in these controls. These results are summarized in Fig. 3.

T cell depletion impaired the transfer of immunity to a lesser extent than did CRL depletion. Parasites were cleared in animals that received T-depleted populations at day 14, whereas those animals that received CRL-depleted populations took 19 days to clear parasites.

Glass- and nylon-wool columns were found to be effective for separating T and B lymphocytes, although separations were not perfect and many cells were lost during passage through the columns. An average of 65% of the total cell population was recovered after passage through glass wool. Passage of immune splenocyte populations through glass wool removed all traces of malarial pigment. The harvested population consisted exclusively of large and small lymphocytes. Some CRLs were also retained on the glass-wool columns. CRL percentages dropped from between 41 to 50% to between 21 and 31% after passage through glass-wool columns. Macrophages will form rosettes with sensitized, complement-coated sheep erythrocytes. Macrophages do not form rosettes in the absence of Ca^{2+} and Mg^{2+} ions (1). In experiments in which the percentage of CRLs was determined in recovered rat spleens in HBSS or with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, 46% of the cells of the recovered rat spleen formed rosettes in HBSS; 40% were formed in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. This 6% difference cannot account for an average loss of 44% CRLs after passage through glass wool.

Of the starting population of cells, 45% were recovered after passage through nylon wool. Seventy percent of the glass wool yield was recovered after one passage though nylon wool.

The effluent population from the nylon-wool columns contained an average of 2.8% CRLs. This represents a 96% decrease over starting values. This population was 83 to 92% T lymphocytes, as determined in cytotoxicity assays with anti-theta serum and complement. Five to 14% of the effluent cells had neither complement receptors nor the theta antigen.

The population eluted from nylon wool had twice as many CRLs as the population eluted from glass wool. The population eluted from nylon wool consisted of an average of 54% CRLs and 6% T cells, and approximately 40% of the cells could not be identified as T cells or as B cells. These results are summarized in Table 1.

Figure 4 shows that the capacity to transfer immunity to *P. berghei* challenge resided primarily in the eluted (B cell) population and in the combined effluent (T) and eluted (B) populations. Eluted cells (B) transferred immunity as effectively as did both populations combined. Animals that received either the eluted (B) population or the combined effluent and eluted populations had only one small peak of parasitemia, which occurred at day 3. These animals cleared all parasites by day 7, and no parasitemia was observed in these animals after that day. Parasitemia peaked at days 4, 7, 9, and 12 in animals that received the effluent T population. Parasitemias were higher in these animals than they were in animals that received

TABLE 1. *Evaluation of the efficacy of glass- and nylon-wool columns for separating T and B lymphocytes*[a]

Treatment	% T cells	% CRL (B)
Glass wool		
Before	35	41
	48	47
	51	44
	28	47
After	33	31
	44	25
	49	27
	44	21
Nylon wool		
T effluent	83	2.4
	89	1.0
	92	2.9
	89	5.0
B eluted	5.58	60
	6.00	42
	6.00	60
	6.00	54

[a] The data presented are from four separate experiments.

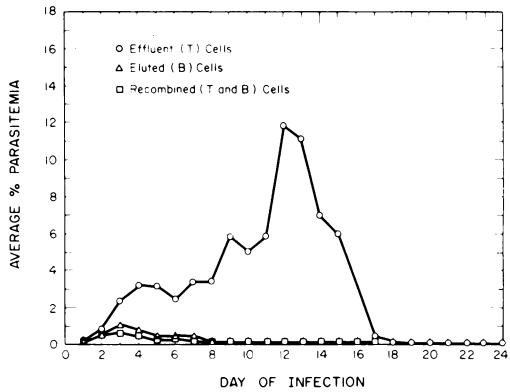

FIG. 4. *Average percentages of parasitemia of animals that received 7 × 10^7 effluent T cells (○), 10^8 eluted B and null cells (△), or 1.7 × 10^8 effluent and eluted cells combined (□).*

eluted (B) or the combined eluted and effluent cells. These animals did not have the ability to completely clear parasitemia, and, even though parasitemia was less than 1% at day 17, parasitemia persisted at this low level in some of these animals up to day 23. Some protection was transferred with effluent T cells, since parasitemias in these animals were milder than parasitemias in untreated controls.

DISCUSSION

Rats that received suspensions of immune splenic lymphocytes from which theta-bearing (T) and complement-receptor-bearing (B) cells had been removed had less ability to clear parasites than those rats that received the undepleted populations. Even those T and B cell-depleted suspensions, however, transferred a very substantial proportion of the ability to control the infection. One cell type was present in both depleted suspensions. This population lacked both theta antigen and the complement receptor. On transformation into antibody-secreting cells, B cells were no longer detectable as CRLs (1). As cells lacking theta and complement receptors were common to both depleted populations and as both depleted populations carried the ability to transfer immunity, it is reasonable to propose that the bulk of the immunity was transferred with the null cell population. This null cell population probably included antibody-secreting, transformed B cells that lacked both theta and complement receptors.

Brown (5) has suggested that T cells might be important as helpers in establishing variant-transcending immunity. He suggested that, during malarial infection, T cells become primed to a determinant common to all plasmodial variants but characteristic of the strain producing the infection. Each antigenic variant stimulates a separate B cell population. The sensitization of T cells with one variant type would produce an expanded T cell population capable of acting as helpers to all B cell clones responding to variants developed later in the infection. Since the young rats that received the T-depleted lymphocyte population in our studies were not immunologically mature, these animals would have only the immunity conferred by the differentiated B cells that they received. Because T cells were not present in this transferred population, these animals could not respond rapidly to antigenic variants and thus developed persistent parasitemias.

Rats that received immune lymphocytes from which CRLs had been removed also had longer persistent parasitemias than did animals that received the unselected immune lymphocyte suspensions. By the same type of reasoning as was applied to the situation in animals that received T-depleted lymphocyte populations, it may be proposed that these animals had only a static immunity due to the absence of the mature B cell which would be capable of responding to the new parasite variants that might have arisen during the infection.

Experiments with glass-wool- and nylon-wool-purified immune lymphocyte suspensions support the conclusions reached for populations of cells depleted of CRLs and T cells. The protective capacity of the immune spleen cell suspensions resided in the population eluted from nylon wool. This population of cells consisted primarily of null cells and CRLs. The effluent population (T cells) did not transfer as much protection as did the eluted population or both populations combined. There was some protection transferred by effluent T cells, as parasitemias were milder in young rats that received effluent T cells than they were in untreated control animals. The three peaks of parasitemia observed in these animals indicate that these animals lacked the cell type ultimately responsible for the clearance of parasites. These animals probably received T memory cells but were lacking B memory and antibody-secreting cells. The primed T cells were probably capable of acting as helpers in initiating a humoral response. Since the transferred preparations contained little *P. berghei* antigen, these transferred T cells probably populated the T-dependent areas of the spleen and the lymph nodes and were quiescent until antigen was introduced 1 week later. These T cells may have then become activated in a helper capacity. Some of the B cells of the recipients, then 5 weeks old, may have been capable of being turned on to produce antiplasmodial globulin. The process of establishing an antibody-secreting population in the recipients of the T effluent cells led to delayed clearance times. It is also possible that some of the null cells of the T effluent population were B memory cells or antibody-secreting cells, but, because such a small number of these cells was transferred, perhaps an effective antibody response could not be mounted and parasitemias were not rapidly cleared.

The animals that received the eluted population (CRLs and null cells) responded immediately to the introduced antigen. It is possible that this response could have been the production of antibody, which enabled the animals to clear all of the parasites by day 7. Since nylon wool retains B precursor and B memory cells as well as antibody-producing cells (15), the immunity that was transferred with the eluted B

population was most likely due to antibody-producing differentiated B cells and B memory cells. The fact that the methods that depleted lymphocyte suspensions of B cells impaired the transfer of immunity to *P. berghei* challenge more than did methods that depleted T cells indicates that the bulk of the protection resides in the differentiated B cell population. The nylon-wool experiments also indicate that T cells are not the ultimate effectors of immunity to malarial infection but are, rather, involved as helpers in the establishment of a humoral response. These results argue strongly for immunity transferrable by antibody-producing cells and not by T cells acting in classical cell-mediated immune responses.

LITERATURE CITED

1. **Bianco, C., R. Patrick, and V. Nussenzweig.** 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. Exp. Med. 132:702-722.
2. **Briggs, N. T., B. T. Wellde, and E. H. Sadun.** 1966. Effects of rat antiserum on the course of *Plasmodium berghei* infection in mice. Mil. Med. 131:1243-1249.
3. **Brown, I. N., A. C. Allison, and R. B. Taylor.** 1968. *Plasmodium berghei* infections in thymectomized rats. Nature (London) 219:292-293.
4. **Brown, K. N.** 1971. Protective immunity to malaria parasites; a model for the survival of cells in an immunologically hostile environment. Nature (London) 230:163-167.
5. **Brown, K. N.** 1974. Antigenic variation and immunity to malaria, p. 35-46. *In* Parasites in the immunized host: mechanisms of survival. Ciba Foundation Symposium 25 (new series). Associated Scientific Publishers, Amsterdam.
6. **Bruce-Chwatt, L. J., and F. D. Gibson.** 1956. Transplacental passage of *Plasmodium berghei* and passive transfer of immunity in rats and mice. Trans. R. Soc. Trop. Med. Hyg. 50:47-53.
7. **Cabrera, E. J., and N. E. Alger.** 1971. Transfer of adoptive immunity to *Plasmodium berghei:* a comparison of routes of injection. J. Protozool. 18:596-597.
8. **Cohen, S., and G. A. Butcher.** 1970. Properties of protective malarial antibody. Immunology 19:369-383.
9. **Cohen, S., I. McGregor, and S. Carrington.** 1961. Gamma globulin and acquired immunity to human malaria. Nature (London) 192:733.
10. **Diggs, C. L., and A. Osler.** 1969. Humoral immunity in rodent malaria. II. Inhibition of parasitemia by serum antibody. J. Immunol. 102:298-305.
11. **Eisen, S., H. J. Wedner, and C. W. Parker.** 1972. Isolation of pure human peripheral blood T lymphocytes using nylon wool columns. Immunol. Commun. 1:571-577.
12. **Epstein, L. B., M. H. Cline, and T. C. Merrigan.** 1971. The interaction of human macrophages and lymphocytes in the PHA-stimulated production of interferon. J. Clin. Invest. 50:744-753.
13. **Gravely, S. M., J. Hamburger, and J. P. Kreier.** 1976. T and B cell population changes in young and in adult rats infected with *Plasmodium berghei*. Infect. Immun. 14:178-183.
14. **Hamburger, J., and J. P. Kreier.** 1975. Antibody-mediated elimination of malaria parasites (*Plasmodium berghei*) in vivo. Infect. Immun. 12:339-345.
15. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645-649.
16. **Katz, D., and B. Benacerraf.** 1972. The regulatory influence of activated T cells on B cell responses to antigen. Adv. Immunol. 15:1-94.
17. **Phillips, R. S.** 1970. *Plasmodium berghei:* passive transfer of immunity by antisera and cells. Exp. Parasitol. 27:479-495.
18. **Spira, D. T., P. Silverman, and G. Gaines.** 1970. Antithymocyte serum effects on *Plasmodium berghei* infection in rats. Immunology 19:759-766.
19. **Stechschulte, D. J.** 1969. Cell-mediated immunity in rats infected with *Plasmodium berghei*. Mil. Med. 134:1147-1152.
20. **Stechschulte, D. J.** 1969. *Plasmodium berghei* infection in thymectomized rats. Proc. Soc. Exp. Biol. Med. 131:748-752.
21. **Trizio, D., and G. Cudkowicz.** 1974. Separation of T and B lymphocytes by nylon wool columns: evaluation of efficacy by functional assays *in vivo*. J. Immunol. 113:1093-1097.