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Babesia microti and *Plasmodium berghei yoelii* infections in nude mice

THE precise nature of the responses to *Babesia* spp. and *Plasmodium* spp. which lead to their elimination from the host and subsequent acquisition of immunity is uncertain. A degree of thymus dependence is involved, as shown by the aggravation of infection by neonatal thymectomy[1,2], anti-thymocyte serum[3] and anti-lymphocyte serum[4,5]. For instance, parasitaemias due to *P. berghei* were higher in neonatally thymectomised rats than in controls, and lasted about twice as long, with higher mortality[1,2].

The recent availability of congenitally hypothymic (nude *nu/nu*) mice allows a more critical appraisal of the importance of the role of thymus competence in the elimination of these parasites. *B. microti* and *P. berghei yoelii* produce transient parasitaemias in normal mice, followed by recovery and resistance to challenge with the same species of parasite[6,7]. We have investigated these infections in nude mice.

Groups of three nude mice or normal littermates 6–8 weeks of age were infected by intraperitoneal inoculation with 10^6 erythrocytes parasitised by either *B. microti* (King's 67 strain, provided by Dr F. E. G. Cox) or *P. berghei yoelii* (17 × strain, from Dr N. Wedderburn). The nude mice were housed in germ-free isolators. Tail blood smears were stained with Giemsa, and the number of parasitised cells in 400 erythrocytes counted. The course of the *B. microti* infection is summarised in Fig. 1*a*, and of *P. berghei yoelii* in Fig. 1*b*. Means and ranges are indicated.

Following a latent period that was the same in all cases, there was a transient parasitaemia in littermate control mice and a persistent parasitaemia in nude mice. Apparent differences in the nature of this persistence of each parasite can be explained in terms of preference for different developmental stages of the red cell series, for whereas *B. microti* seems largely to be restricted to mature red cells *P. berghei yoelii* parasitises only reticulocytes. Nearly all the reticulocytes in the *P. berghei yoelii* infected nude mice contained parasites from day 10. Thus the rise in reticulocyte number with worsening anaemia, rather than any difference in the response of nude mice to the two organisms, is likely to be responsible for the overall parasitaemia remaining stable with one (Fig. 1*a*) and increasing with the other (Fig. 1*b*). A high and stable percentage of the susceptible red cell stage was infected by each organism until the nude mice eventually died or were killed.

In contrast to our findings, neonatally thymectomised rats took only twice as long as normal littermates to clear *P. berghei* from the peripheral circulation[1,2]. There is evidence that neonatal thymectomy in rats does not completely eliminate T lymphocytes[8]. Thus, in keeping with data obtained with anti-thymocyte serum[3], the thymus dependence of elimination of *Plasmodium* spp. from rodents appears to be more complete than earlier work suggests.

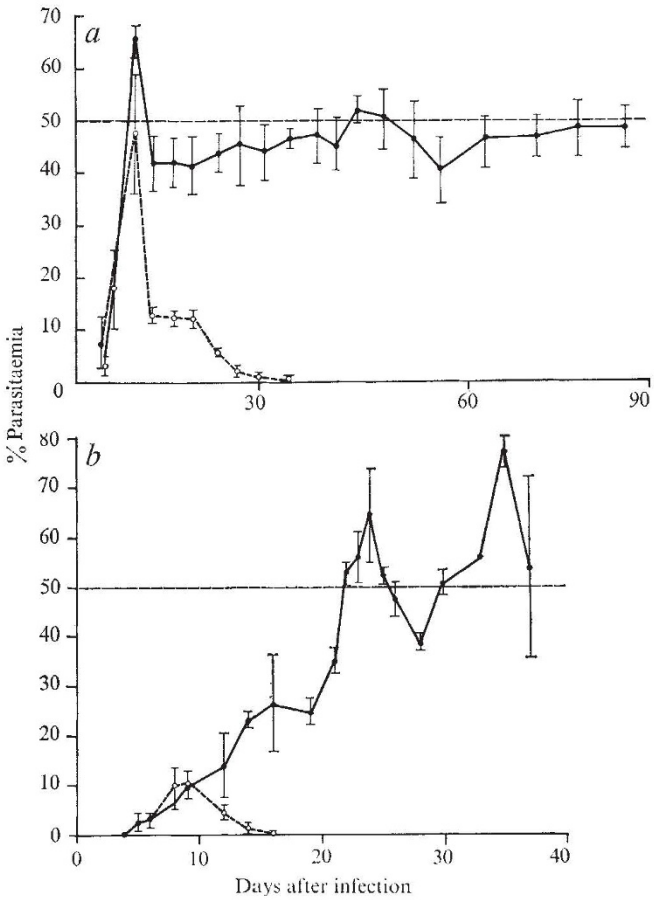

Fig. 1 *a*, *Babesia microti* infection in nude mice and normal littermates. *b*, *Plasmodium berghei yoelii* infection in nude mice and normal littermates. ●, *nu/nu*; ○, *nu/+*.

The results obtained with *B. microti* in nude mice (Fig. 1*a*) were similar to those found on giving anti-lymphocyte serum to hamsters before and during infection with this parasite[5]. This confirms an absolute dependence on the thymus for elimination of this parasite as well as *P. berghei yoelii*. Nude mice may be a more suitable model because they seem to survive persistent parasitaemias of *B. microti* more successfully than do anti-lymphocyte serum treated hamsters. On day 80, twice as long as any hamster survived, nude mice were apparently quite healthy after maintaining a 50% parasitaemia for the previous 70 d. In addition the problem of anti-lymphocyte serum standardisation inherent in the hamster model is eliminated.

In summary, the present data confirm that elimination of *B. microti* and *P. berghei yoelii* from the circulation of mice does not occur without thymus competence. It remains to be established whether the thymus-derived cells exert a helper effect in the production of a particular class or subclass of antibody required for parasite elimination, or form part of another mechanism of immunity. Further experiments are in progress to test these possibilities. Clearly the parasitised nude mouse could prove to be a useful model for further immunological research in this field.

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Evidence for oligomeric IgA production by peripheral rat lymph nodes

A DISTINCTIVE characteristic of IgA is its polymorphic nature with regard to the molecular size. It occurs in secretions mostly as a dimer or tetramer[1,2] while serum IgA has a polydisperse weight distribution in many species[1]. It is mostly monomeric in human serum (7S) (ref. 3), mostly polymeric in the mouse[4] and in the dog[5], but in both these and other species several molecular sizes occur in the blood[1].

When extending studies on natural anti-hapten antibodies in the rat[6] we made the unexpected finding that natural anti-3-iodo-4-hydroxy-5-nitrophenylacetyl (NIP) of the thoracic duct lymph had sedimentation characteristics (9S–13S) similar to the secretory IgA antibodies that we could demonstrate in rabbit colostrum[7] and to the oligomeric IgA produced by mouse spleen cultures[8]. Natural antibody in the blood serum of the same rats sedimented like 19S IgM. This finding prompted us to study sedimentation patterns of anti-NIP antibodies in the blood and the lymph of immunised rats.

Rats were immunised with 10 μg NIP-SIII pneumococcal polysaccharide[8] or 500 μg of NIP chicken globulin (CG)[8] in complete Freund's adjuvant (CFA). As we wanted the antibody production to take place preferentially in the peripheral lymph nodes draining to the thoracic duct the antigen was injected into the hind foot pads. The rats were bled and cannulated (abdominal thoracic duct) 8–9 d after immunisation. Thoracic duct lymph samples were clarified by spinning for one hour at 50,000*g*. The lymph and the serum samples were titrated with NIP-T_4 bacteriophages as described earlier[9]. They were centrifuged through a continuous and linear 5–20% sucrose gradient in 12 ml tubes in rotor SW-41 Ti of a Beckman L 3–50 ultracentrifuge for 14 h (39,000 r.p.m.). A total of 25 fractions were collected and titrated with NIP-T_4. Sedimentation constants ($S_{20,w}$) were derived according to McEwen[10].

The predominant antibody peaks of the blood serum had sedimentation constants of 19S and 7S. A minor 9S component could usually be detected (Fig. 1*a*). Antibodies of the thoracic duct had major 19S and 7S fractions but they also had considerable 13S and 11S fractions (Fig. 1*b*); 9S antibody could be seen as a distinct peak in some but not all lymph samples. We have found this phenomenon in all the 14 serum-lymph pairs studied so far.

In some experiments we incubated diluted lymph before centrifugation with rabbit anti-rat IgA. Anti-IgA is known to displace or eliminate IgA but not IgG or IgM antibodies in the sedimentation pattern[8]. Data in Fig. 1 show that the 9S, 11S and 13S peaks were displaced by our anti-IgA and were thus very probably IgA.

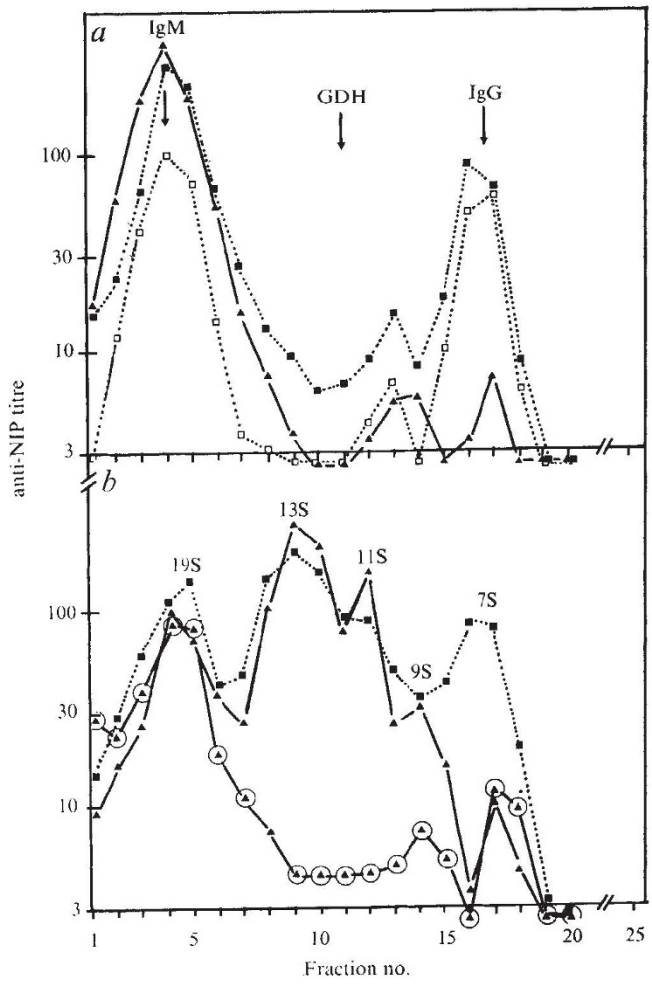

Fig. 1 Sedimentation pattern of anti-NIP, *a*, in the serum and, *b*, thoracic duct lymph of rats after immunisation with NIP-CG and NIP-SIII. ——, NIP-CG immunisation; . . . . , NIP-SHI/ immunisation; encircled triangles, sample mixed with anti-IgA. Different symbols indicate different rats. Rabbit anti-DNP and glutamate dehydrogenase (GDH) were used as reference compounds, their maximum activity is indicated by arrows.

Table 1 Characteristics of the antibody classes that could be separated from the thoracic duct of immunised rats and lymph node culture fluids

Sedimentation constant	Estimated Stokes' radius (Å)	Ig class	Determined molecular weight	Molecular weight of the theoretical human IgA polymer that would come closest to the observed molecular weight (see text)
19S	107	IgM	860,000	
13S	91	IgA	515,000	520,000
11S	80	IgA	380,000	370,000
9.4S	70	IgA	280,000	320,000

The total IgA of several lymphs and sera was determined by the method of Mancini, Carbonara and Heremans[11]. We found that concentrations in the lymph fluid were 4–12 times higher than in the serum of the same animal. In the ultracentrifugation the IgA of the lymph fluids was fairly evenly distributed from the 7S to 15S fractions while the serum IgA was distributed from the 7S to 11S fractions.

The Stokes's radii of the four heaviest antibody fractions were determined by gel filtration according to Laurent and Killander[12]. They were calculated as means of two gel filtration runs, through a Sepharose 6B column (1.5 × 79 cm). Reference proteins in these runs included rat serum albumin, hyperimmune rabbit anti-DNP (IgG) and glutamate dehydrogenase. Their radii were assumed to be 35,51 and 60 Å respectively[13,14]. The fractions from the gel filtration were rerun in ultracentrifugation. Molecular weights (Table 1) were calculated using the $S_{20,w}$ values and the Stokes radii in the formula

$$M=(6\pi\eta_{20,w}Na_{20,w})/(1-\bar{\nu}\rho_{20,w})$$

in which $\eta_{20,w}$ and $\rho_{20,w}$ are the viscosity and density of water at 20° C, N is Avogardo's constant and a is Stokes's radius. The