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Immunity to Malaria after Recovery from Piroplasmosis in Mice

RECOVERY from infections caused by intraerythrocytic protozoa is usually followed by a period of resistance to challenge with the homologous parasite[1]. In some cases this resistance is confined to challenge with the homologous strain, in others it is confined to the same species[2] and more rarely extends to related species[3,4]. It has recently been reported that resistance to challenge can even transcend the generic barrier[5]. Cox and Milar[5] demonstrated that mice which had recovered from infection with the malaria parasite *Plasmodium chabaudi* resisted challenge with the rodent piroplasm *Babesia* (*Nuttallia*) *rodhaini* but that this protection did not apply to the malaria parasite *P. berghei*. We have already shown that *P. chabaudi* protects against *P. vinckei*, but not against *P. berghei*[3], and that *B. microti* protects against *B. rodhaini*[4]. Independently of Cox and Milar[5] we have found that mice which have recovered from infections with *Babesia* (*Nuttallia*) *microti* are resistant to challenge with the virulent malaria parasite *P. vinckei*. This work both confirms and extends the unexpected discovery made by Cox and Milar[5].

Mice were infected by intraperitoneal inoculations with *B. microti*. As is usual with this infection, the mice exhibited a patent parasitaemia which lasted about 21 days and then overcame the infection[4]. Approximately 14 days after recovery these mice were challenged with *P. vinckei*, as were matched controls which had received a single injection of uninfected blood at the time the experimental animals were infected. The control animals developed parasitaemias which rose rapidly and killed all the mice on the sixth to seventh day after infection. This is the normal pattern in this infection[6]. The mice which had recovered from infections with *B. microti* resisted the challenge and only a fleeting parasitaemia was seen. Additional experiments showed that (*a*) mice which had recovered from *P. vinckei* infections were protected against *B. microti*; (*b*) mice that had recovered from *P. chabaudi* were protected against *B. microti*; and (*c*) mice which had recovered from *B. microti* were protected against *P. chabaudi*.

It has hitherto been assumed that protective cross-immunity indicates some degree of phylogenetic affinity[3,4]. In the light of our results and those of Cox and Milar[5], this can no longer be accepted. Furthermore, the general assumption that immunity to malaria is fundamentally a strain specific phenomenon must also be modified. These results also indicate that antigenic variation[7] in malaria is probably a minor component in the complex immune response to these parasites. The possibility of a heterologous vaccine against malaria must once again be a possibility.

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Relationship between the Papain Sensitivity of Human γG Immunoglobulins and their Heavy Chain Subclass

WE have described the recognition of two conformational forms of γG immunoglobulin, distinguished by their differing susceptibility to proteolysis by papain in the absence of cysteine[1,2]. Myeloma γG globulins from different individuals were similarly characterized as sensitive or resistant to papain cleavage in these conditions. We report now (*a*) the results of papain sensitivity studies on myeloma proteins of the four known γG chain sub-classes; (*b*) the immunological relationship between the papain sensitive and resistant forms of normal γG immunoglobulin and these myeloma proteins; and (*c*) the carbohydrate composition of myeloma proteins of each sub-class. These studies show that it is only the γG1 and γG3 sub-class population of normal γG immunoglobulin which undergo papain proteolysis in the absence of cysteine. Susceptibility to cleavage by papain can thus be used for the preliminary assignment of myeloma proteins to the γG1, γG3, or γG2, γG4, sub-classes. Furthermore, γG2 and γG4 myeloma proteins can be distinguished from each other by their behaviour on incubation with papain in the presence of cysteine (at 37° C, for 4 h). Proteins of the latter sub-class are completely digested to Fab and Fc fragments in these conditions, whereas those of the former undergo only 10-20 per cent digestion.

Papain digestion of normal γG immunoglobulin in the absence of cysteine, for 4 h, followed by 'Sephadex *G*-150' gel-filtration allows the isolation of the intact papain resistant form and the Fab, Fc fragments of the papain sensitive form. The Fab and Fc fragments of the papain sensitive material, recovered in the second 'Sephadex *G*-150' fraction, were separated by subsequent sequential chromatography on CM and DEAE cellulose[3]. Further incubation of the resistant form, isolated in the first fraction, with papain in the presence of cysteine, for 6 h, effected a partial digestion, the resultant Fab and Fc fragments being isolated by a similar sequential chromatographic procedure.

Antisera capable of distinguishing the antigenic differences between the papain resistant population of normal γG immunoglobulin and the Fab Fc fragments of the papain sensitive population were raised in guinea-pigs using the technique of Dvorak[4]. By this procedure, the animal is rendered tolerant to antigenic determinants common to two related proteins and therefore antibody is only elicited to unshared determinants.

Thus an antiserum (A1) was raised by intravenous injection of the papain resistant protein (5 mg), isolated from normal γG immunoglobulin, followed immediately by subcutaneous injection of whole normal γG immunoglobulin (100 μg). The latter was administered, admixed with complete Freund's adjuvant (CFA), as two injections into the rear footpads. A test bleed after 4 weeks yielded an antiserum which did not react with the resistant form, but did react with whole γG immunoglobulin and with the Fab, Fc fragments obtained by papain digestion of the whole λG immunoglobulin in the absence of cysteine (Fig. 1). The papain sensitive form of normal γG immunoglobulin is thus shown to be immunologically distinguishable from the resistant form.