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C3b inactivator in normal mice and mice infected with *Plasmodium berghei berghei*

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Summary

C3b inactivator levels were assayed in clean albino mice and mice infected with *Plasmodium berghei berghei*. Infected animals (63.1%) had low C3b inactivator levels when compared with 45% of controls with low levels. In the low titre range, infected mice had a significantly lower mean level ($P < 0.001$) of the factor than controls. Conversely, in the high titre range, the mean C3b inactivator level is significantly ($P < 0.001$) higher in infected than in control mice. Lower levels of the protein were associated with low grade parasitaemia, while raised levels were found in animals with higher parasitaemia. Low grade malaria parasitaemia predisposes to lowered C3b inactivator levels that would enhance persistence of deposited immune complexes, and subsequent tissue damage where C3b receptors are present.

Résumé

On a estimé les niveaux de 'C3b inactivator' chez les souris albinos et chez les souris infectées avec *Plasmodium berghei berghei*. 63.1% des animaux infectés ont les niveaux bas comparés à 45% des témoins des niveaux bas. Dans les variations des titres bas, les souris infectées ont la moyenne significative ($P < 0.001$) bas du facteur que les témoins. Mais, dans les variations des titres élevés, la moyenne de niveau de 'C3b inactivator' est significativement ($P < 0.001$) plus élevé chez les souris infectées que les témoins. Les niveaux des protéines moins élevées sont associés avec le grade bas du parasite alors qu'on a trouvé les niveaux élevées chez les animaux avec les plus hauts grades du parasite. Les grades bas du parasite paludisme prédisposent au niveau baissé de 'C3b inactivator' qui pourrait améliorer la persistence des complexes d'immunité déposés et les dégâts subséquents où les recepteurs de C3b sont présents.

Introduction

The C3b inactivator is a normal serum protein that modulates the activity of the activated third component of complement C3b [1]. As one of the regulatory proteins of the complement cascade, it functions in preventing C3 depletion, mainly through the C3b feedback cycle. It does this by splitting C3b into other sub-fractions, thereby blocking further C3b participation in the reaction sequence [2].

Okerengwo *et al.* [3] found a general deficiency of the protein in malarial infections particularly in childhood nephrosis. The finding implied that the basic defect in childhood nephrosis may be an acquired deficiency of the C3b inactivator during malarial infections. This is because the nephropathy is usually associated with malarial infections, especially *P. malariae*, in Nigerian children [4,5].

C3b inactivator was first demonstrated in the guinea-pig [6] and later in man [7,8]. In order to reascertain the role of C3b inactivator in the immunopathogenesis of the nephropathy associated with malarial infections, the factor was investigated in mice during *Plasmodium berghei berghei* infections. The findings are reported in this paper.

Materials and methods

Sera from 10 adult male and female mice were pooled separately. Each pooled serum was inactivated at 56°C for 30 min and then adsorbed with one-fifth its volume of washed, packed

sheep erythrocytes. The pooled sera were used as standard male and female mice C3b inactivator sera. A standard human C3b inactivator serum was similarly prepared by pooling 5 ml of serum from each of five adult blood donors. The human standard serum was included in the tests.

The activities and titres of C3b inactivator in the three standard sera were determined as described in detail by Okerengwo *et al.* [3]. Briefly, a 1% suspension of sheep erythrocyte/rabbit antibody-complement (EAC) complex was prepared using locally raised antibody serum (amboceptor-A) to sheep erythrocytes (E). Fresh guinea-pig serum was used as a source of complement (C). The activity of each standard serum was determined by testing its ability to inhibit immune adherence haemagglutination (I-AHA) of EAC and human group O^+ erythrocytes (HO^+). Tests were performed in titration plates using 0.025 ml amounts of EAC suspension, 0.05 ml of 1% HO^+ suspension and 0.025 ml of each undiluted standard serum. Serial doubling dilutions of each standard serum were then tested to determine the highest dilution that would inhibit EAC/HO^+ haemagglutination. The titre of each standard serum was expressed as the reciprocal of that highest dilution.

Michaelis's barbital sodium acetate buffer, pH 7.2, containing 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 0.1% gelatin, was used as diluent in the tests.

On day 0, 10 clean male and 10 clean female albino mice were infected intraperitoneally with 0.1 ml inoculum of 1×10^5 *Plasmodium berghei berghei* infected erythrocytes from one donor mouse. Tail blood films were made from each animal on day 4. The films were stained with Leishman dye and the percentage parasitaemia was assessed by microscope counts. Parasitaemia ranged between 2.5% and 11.6%. Infected mice were bled by cardiac puncture and sera collected after allowing 1 h for erythrocyte retraction at room temperature. Ten non-infected male and 10 non-infected female mice, which served as controls (injected with distilled water on day 0), were similarly bled and sera collected.

The test and control sera were inactivated at 56°C for 30 min and adsorbed with one-fifth their volumes of washed packed sheep erythrocytes. The activity and titre of C3b inactivator in each test and control serum was determined as for the standard sera.

Results

The C3b inactivator titre was expressed as the reciprocal of the highest dilution of serum that showed inhibition of I-AHA. The standard pooled human, male and female mice sera all had titres of 64.

Figure 1 shows the frequency distribution patterns of C3b inactivator titres in infected and control mice. In each group, the animals fell into three categories: negligible, low and high titres. A titre of 32 or less was generally considered low, while a titre of 0–4 was taken as negligible within the low titre range. Titres of 64 and above were regarded as high.

Titres not exceeding 4 were found in 26.3% of infected mice while 36.8% had titres ranging from 8 to 32 and in another 36.8% titres ranged from 64 to 256^+. Generally, 63.1% of infected mice had low levels of C3b inactivator, while only 36.8% had high levels of the protein.

In the control group, however, while 15% had negligible titres (≤ 4), 30% had titres ranging between 8 and 32. Altogether, 45% of the control mice had low levels and 55% had high levels of 64 and above.

Figure 1 shows that mice with high C3b inactivator levels also had higher levels of malaria parasitaemia (8.5–11.6%). On the other hand, low grade parasitaemias (2.4–6.3%) were asso-

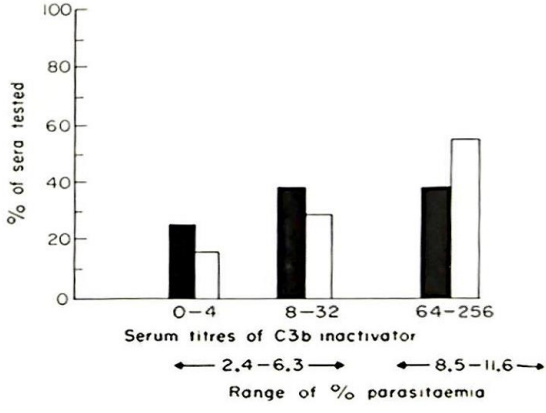

Fig. 1. Frequency distribution patterns of C3b inactivator in infected (■) and control (□) mice.

Table 1. A comparison of the geometric means in infected and control mice at low and high titre ranges

		Infected mice	Control mice
Low titre	Mean	10.47	22.38
	s.d.	2.75	1.41
		($t = 12.0303$; $P < 0.001$)	
High titre	Mean	102.32	91.20
	s.d.	1.81	1.38
		($t = 14.0759$; $P < 0.001$)	

ciated with low levels of C3b inactivator. Using Student's *t*-test, the mean % parasitaemia in mice with high C3b inactivator levels was significantly higher than the mean % parasitaemia in mice with low levels of the protein ($t = 5.5180$; $P < 0.001$).

Table 1 compares the geometric means of C3b inactivator in infected and control mice at low and high titre ranges. At low titres, infected mice had a statistically significant lower mean level than the control ($t = 12.0303$; $P < 0.001$). In the high titre range, however, infected mice had a significantly higher mean level than the control mice ($t = 14.0759$; $P < 0.001$).

Discussion

Experimental animal models are often used to confirm observations made in humans, and vice versa. Further details on such findings are better studied in the animal models because of the ease of manipulation of experimental conditions. The serum protein, C3b inactivator, was first demonstrated in the guinea-pig by Nelson *et al.* [6] and later the presence of the protein in man was reported [7–9].

In this study, it has been shown that the albino mice have serum C3b inactivator at the same normal levels as humans. There was no sex-related difference in the levels of the protein, as was also observed for man [10].

A higher percentage of infected mice had low levels of C3b inactivator, when compared with controls having low levels. Furthermore, at low titres infected mice had a significantly lower mean level of the factor than the controls. Generally, this observation agrees with a previous report that malarial conditions are associated with low C3b inactivator levels in man [3]. However, it was significant that infected mice in the low titre category had a comparatively lower range of percentage parasitaemia. Conversely, high titres were associated with higher parasitaemia and significantly raised C3b inactivator levels. These findings seem to indicate that while apparently low grade malaria parasite infections are associated with depressed levels of the protein, high grade and rather acute infections give rise to significantly higher levels of C3b inactivator. In man, the levels of Factor B, C3b inactivator and other normal serum proteins are also elevated following most acute infections or surgery as a result of an acute phase reaction [11,12].

While the depression in C3b inactivator levels in infected mice may be attributable to malaria infection, it is rather difficult to explain the negligible titre levels observed in three of the apparently healthy control mice.

It is conceivable, however, that in healthy conditions mice, like man, may have either high or low normal levels since the factor is a normal component of serum [3].

The present study has confirmed that low grade malaria infections, usually obtained in human *P. malariae* infections, predispose to depressions of the C3b inactivator protein. Such depressions would enhance the persistence of deposited immune complexes in any tissues (e.g. the kidneys) with appropriate receptors, like the C3b receptors, and result in subsequent tissue damage.

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