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The pathogenesis of *Plasmodium falciparum*-associated tissue lesions — what role for C3b receptors?

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Summary

Human tissue sections from cerebral and cerebellar cortices, the liver, the stomach pylorus, the kidney and the placenta, were examined for the presence of C3b receptors. This was carried out in order to determine whether these receptors play any role in the pathological effects of malarial infections on these tissues. The sheep erythrocyte/rabbit antibody/complement system was used. C3b receptors were only detected in the kidney glomeruli, but not in the other tissues. It is suggested that during malarial infections complement may be indirectly involved in the pathogenesis of tissue damage in the organs examined by triggering the activation and participation of other body systems.

Résumé

Les coupes des tissus humains dérivées des cortices cérébraux et cérébelleux, du foie, du pylore de l'estomac, du rein et de la placenta sont étudiées pour la présence des récepteurs de C3b. Cette étude était faite pour déterminer si ces récepteurs jouent un rôle pathologique sur ces tissus au cours du paludisme. Le système d'érythrocyte du mouton/l'anticorps du lapin/le complément est utilisé. Les récepteurs de C3b sont découverts seulement dans les glomérules du rein mais pas dans les autres tissus. On suggère qu'au cours du paludisme, le complément est peut-être impliqué indirectement dans le pathogenèse des dégâts de tissu dans les organs étudiés. Cela est possible en provoquant l'activation et la participation des autres systèmes du corps.

Introduction

Receptors for activated third component of complement, C3b, which are present in some human tissues, like the kidneys, the spleen and the skin [1–4] are believed to play a significant role in the deposition of complement-containing immune complexes in such diseases as nephritis and polyradiculoneuritis. Complement thus plays more than an inflammatory role, as it also enhances the deposition of immune complexes, the degradation of which initiates tissue damage.

The nerve fascicles of peripheral nerve tissues have C3b receptors with a lower binding activity than those found in the spleen and kidney tissues [4]. Glomerular localization of C3b-coated sheep erythrocytes in tissue sections also required far more erythrocyte-bound C3b than does splenic germinal centre localization [5]. In view of these findings and the apparent preferential deposition of immune complexes in the kidneys, the presence and comparative distribution of C3b receptors in the human brain, liver, stomach pylorus and placenta were investigated in this study.

These organs are favoured sites for the sequestration of erythrocytes parasitized by mature forms of *Plasmodium falciparum* and severe infections are often associated with haemorrhage and vascular lesions in these organs and the kidneys [6–10]. Similar observations have been made in Aotus monkeys experimentally infected with *P. falciparum* [11,12]. Complement may be involved both in the mechanism of sequestration and the pathogenesis of the lesions in all these organs. Do C3b receptors have any role to play?

Materials and methods

Autopsy specimens from automobile accident victims were obtained within 24 h of death from the Adeoyo State Hospital, Ibadan. All autopsy tissues investigated, including the kidney, were obtained from each of the accident victims studied. Similarly, tissues were also obtained within 24 h of death from the Department of Pathology, University College Hospital, Ibadan. Tissues were taken from patients who died of diseases that did not affect the organs under investigation. Normally delivered placentae were obtained at delivery from the Labour Ward of the University College Hospital, Ibadan. Cryostat sections (6 μm thick) were cut from tissues frozen in liquid nitrogen. Specimen samples of cryostat sections fixed and stained with haematoxylin and eosin were examined to ascertain that they were histologically sound and to preclude the presence of erythrocytes.

Amboceptor (A) was raised locally by immunizing rabbits with a 20% suspension of sheep erythrocytes (E) in saline. Sera were collected 14 days from the first day of immunization, to provide early antibody. Treatment of sera with 5% 2-mercaptoethanol, confirmed that the antibody was mostly of the IgM class. Guinea-pig serum was the source of complement.

One per cent suspensions of (i) sensitized sheep erythrocytes (EA) and (ii) complement-coated sensitized sheep erythrocytes (EAC), were prepared in gelatin veronal buffer (GVB) according to the method described previously [13]. The EAC preparation was used as the indicator cell system. Tissue sections were washed for 5 min in PBS, pH 7.2, before being used in haemadsorption experiments.

While tissue sections from the brain, the liver, the stomach pylorus and the placenta served as test tissues, the kidney sections were used as controls. For haemadsorption, microculture slides with a single concavity (10 mm diameter) were used. A thin layer of high vacuum silicon grease was spread around the concavity before filling it with thoroughly resuspended indicator cells, using a Pasteur pipette. If stored at −70°C or −20°C, the tissue sections were allowed to warm to room temperature (23°C ± 2°C), for 10 min before use. The glass coverslip bearing the tissue section was placed on the microculture slide, such that the tissue was submerged in the indicator cell system in the centre of the concavity. The coverslip was then pressed down to adhere tightly to the microculture slide, with the aid of the silicon grease. Care was taken in laying down the coverslip, in order to avoid trapping of air bubbles which would interfere with reading of the slides. This system formed a closed chamber. The microculture slide was then inverted, with the coverslip down so that the EAC suspension thoroughly bathed the tissue section. The preparation was placed in a moist chamber and left at room temperature for 30 min. After incubation, the slide was turned over so that the coverslip was up. The system was left in that position for another 20 min to allow erythrocytes on the glass and those loosely adhering to the tissue to detach. Control preparations were made similarly, but 1% suspensions of EA and E were substituted for EAC. The preparations were examined under the ordinary light microscope at ×10 and ×40 magnifications. Tissue sections with adsorbed erythrocytes were taken as positive reactions.

Results

EAC strongly adhered to the glomeruli in the kidney sections (Fig. 1). Conversely, EAC did not adhere to tissue sections of the cerebral and cerebellar cortices, the liver and the stomach pylorus (Fig. 2). There was dotting of EAC on the placental sections (Fig. 3), but there was no localized dense adsorption of EAC as obtained for the kidney sections.

EA and E also did not adhere to the kidney, brain, liver and gut tissue sections. While E did not adhere to placental tissue sections, EA was found dotted in a similar pattern as EAC. Figure 4 depicts findings with EA and E as exemplified with kidney section.

Discussion

The presence of C3b receptors in human renal glomeruli is thought to be significant in the deposition of immune complexes bearing complement in immunologically related renal diseases [2,5]. C3b receptors are present in the peripheral nerve tissues, while C3b and C3d receptors can be found in the white and red pulps of the spleen [1,3,4]. However, differences have been observed in the binding

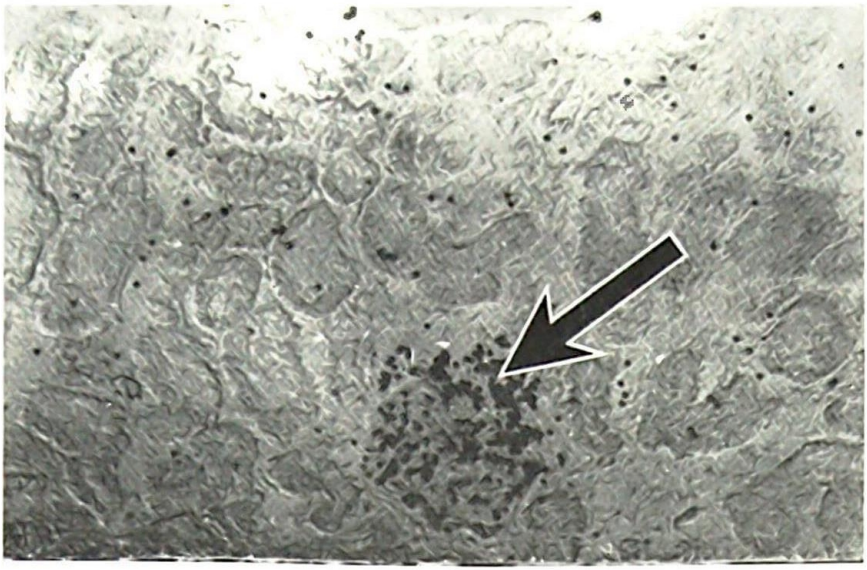

Fig. 1. Adsorption of EAC (complement-bearing sheep erythrocytes sensitized with rabbit antibodies) to a renal glomerulus in an unstained section of human kidney (×34). Site of EAC deposition arrowed.

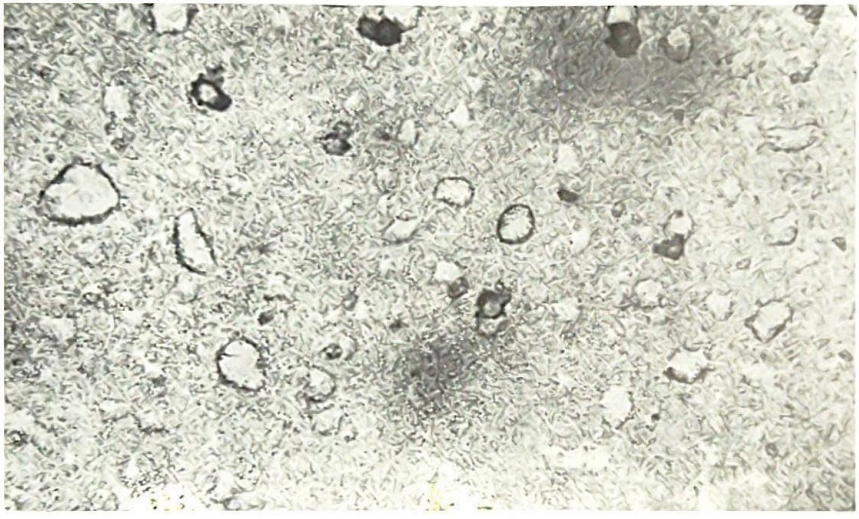

Fig. 2. EAC on an unstained section of cerebral cortex (×8). No adhering sheep erythrocytes.

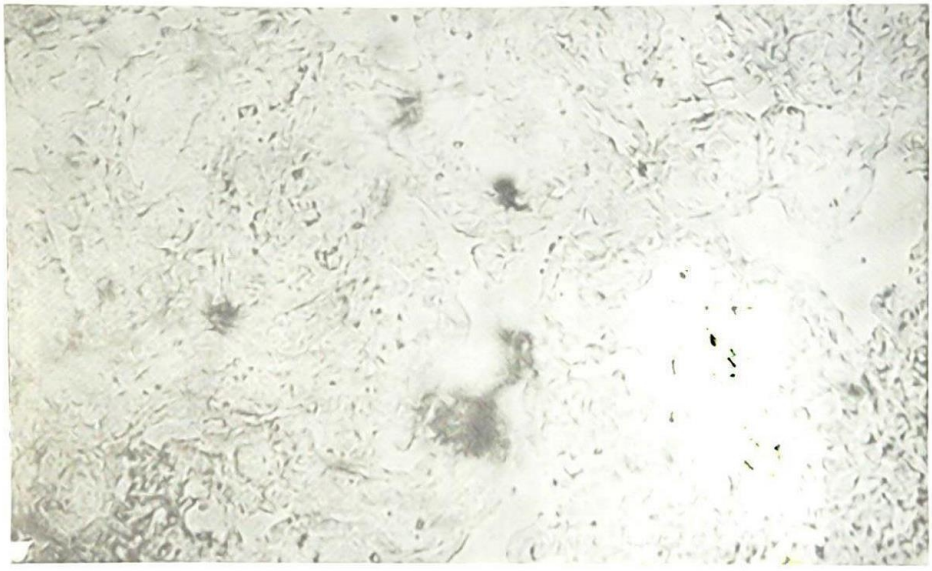

Fig. 3. EAC on an unstained section of the placenta (×8). Note the dotting of sheep erythrocytes, but no dense adsorption as seen on the kidney section shown in Fig. 1.

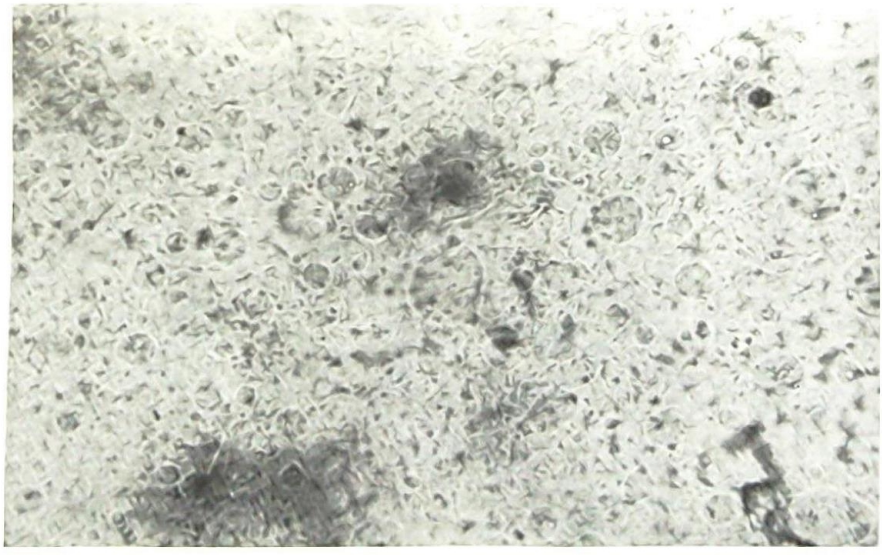

Fig. 4. EA on an unstained kidney section (×8). No sheep erythrocytes adhered to the glomerulus. Similar results were obtained for other tissues.

activities of the C3b receptors present in these tissues [4,5]. These observations show that the binding activity and perhaps the distribution of C3b receptors vary and appear to decrease from the spleen to the kidney and to the peripheral nerve tissues in that order.

Immune complexes and complement have been implicated in the pathogenesis of most renal lesions associated with malarial infections [14–16]. The presence of renal C3b receptors further supported the role of C3b-coated immune complexes in kidney damage during malaria infections. Although some observations indicate that the activation of complement may be the cause of vascular damage in cerebral malaria, the exact role of complement in the initiation and production of cerebral haemorrhage remains unclear [17,18]. The failure to demonstrate C3b receptors in the brain tissues in the present study, suggests that complement may not be directly involved in initiating cerebral vascular damage by functioning in immune complex deposition and retention. Complement action could, by its activation of other systems, like the coagulation and kinin systems, be causing the accumulation of phagocytic cells by chemotaxis.

Similarly, the absence of C3b receptors in the liver sections may indicate that the destruction of malaria-infected erythrocytes by the liver phagocytic cells is not complement-mediated. Vascular damage in the gut during *P. falciparum* infections may also not be initiated by complement-bearing immune complexes, but by other mechanisms.

Application of EAC and EA suspensions on placental tissue sections produced identical results. It would appear that the localization of the few erythrocytes on the sections were due to Fc pieces of the sensitizing antibody which was the common factor in both preparations. The rabbit anti-sheep erythrocyte antiserum used in this study contained mostly IgM antibodies and any IgG antibodies present would be in very small amounts. As only IgG antibodies cross the placenta via their Fc portions, it is conceivable that the small amount of IgG antibodies present in the antiserum may account for the dotting pattern of EAC and EA observed. Erythrocyte sequestration and placental lesions in acute *P. falciparum* malaria appear not to be initiated by immune complexes coated with complement, because C3b receptors were not demonstrable.

Among some of the organs known to have C3b receptors, the spleen would appear to have receptors with the highest binding activity, followed by the kidneys [4,5]. The apparent greater predilection for the kidneys in the deposition of C3b-bearing immune complexes may therefore be due to (1) its high vasculature, complex structure and functions and (2) the comparatively high binding activity of its C3b receptors. Except in renal lesions, the pathogenesis of *P. falciparum*-associated tissue lesions is most probably not initiated by immune complexes bearing activated complement. Complement, when activated, may participate in causing necrosis by activating other systems. It may also stimulate the recruitment of destructive lysosomal cells, which it induces to migrate to the site of complement activation.

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