Circulating immune complexes in infants fed on cow's milk

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Delire *et al.*[1] have reported antigen–antibody (Ag–Ab) complexes in newborns, fed on cow's milk, 6 days after birth, whereas they were absent from breast-fed neonates. The method they used was the inhibition of latex–IgG agglutination by polyclonal rheumatoid factor (pRF), linked to the Ag–Ab complexes[2]. The presence of immune complexes was attributed to the passage through the placenta of maternal IgG with antibody activity against proteins in cow's milk. This interpretation was also based on the characterization of the Ag–Ab nature of the immune complex-like material. We have now used the same and a different method to study a similar population of newborns. Our results are very different from those of Delire *et al.* We conclude that the possible pathogenicity of immune complexes cannot be assessed on the basis of a single insensitive test and in the absence of symptoms of serum sickness.

We have studied a population of newborns similar to that examined by Delire *et al.*, 24 bottle-fed and 53 breast-fed, for the presence of immune complexes in cord sera and serum samples collected 6 days after birth. We have used the same method as Delire *et al.*[1] and, in addition, the Clq–SP assay of Hay *et al.*[3].

Our results obtained with the pFR-inhibition test were very different from those of Delire *et al.*; in fact we observed a very high incidence of immune complexes in umbilical cord sera, and no significant change 6 days after birth. Furthermore, there was no difference between breast-fed and bottle-fed neonates and the increase in the number of immune complexes was absolutely random (Table 1).

Table 1 Immune complexes detected by pRF-inhibition method in neonates

Subjects	*N*	Cord blood	Day 6
Breast-fed	53	20 (38%)	19 (36%)
Bottle-fed	24	5 (21%)	4 (17%)

In contrast, using the Clq–SP assay we found only three samples of cord sera positive for immune complexes and, 6 days after birth, only two sera were positive, both in the breast-fed group (Table 2).

These observations stress the fact that the same serum populations can give very different results with different methods for immune complex evaluation. In the Clq–SP assay, using *Staphylococcus aureus* protein A as detector, it is mainly IgG1 complexes that are identified, whereas the pRF-inhibition test has a wider spectrum of detection.

Table 2 Immune complexes detected by Clq–SP method in neonates

Subjects	*N*	Cord blood	Day 6
Breast-fed	53	3 (6%)	2 (4%)
Bottle-fed	24	0	0

The difference between our data and those of Delire could be due to the fact that the pFR-inhibition test used, although simple and easy to perform, has too subjective a reading, depends on the source of pFR used (which may affect agglutinating activity) and on the IgG levels on the surface of the latex particles. Furthermore, it is very important that the presence of immune complexes be linked with symptoms of serum sickness.

Thus, we believe that it is impossible to draw conclusions about the pathogenic role of Ag–Ab complexes on the basis of a single insensitive test and in the absence of characteristic symptoms.

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Binding of C3b proceeds by a transesterification reaction at the thiolester site

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The binding of C3b, the opsonic fragment of the third component of complement (C3), to bacterial surface structures mediates two events important in host defence: assembly of the C5b-C9 lytic complex and opsonic recognition by phagocytic cells (for reviews see refs 1, 2). These interactions proceed through a labile binding site[3] located on the α' chain of the C3b molecule, with the resultant formation of a covalent oxy-ester bond[4,5] in which the acyl group is contributed by the protein. By exposure of a titratable sulphydryl group[6,7] an internal thiolester of native C3 has also been localized to the α' chain. Studies with ^{14}C-methylamine, a nucleophile which is inherently reactive with a thiolester, have further indicated a stoichiometric (1:1) and covalent interaction, again within the α' chain[8–10]. After reaction of the native protein with ^{14}C-methylamine and radioalkylation of the exposed sulphydryl with ^{3}H-iodoacetic acid, a 35-residue tryptic peptide has been isolated that yields the sequence -Cys^{9}-Gly-Glu-Glu^{12}- on Edman degradation; tritium counts are released at step 9 (*S*[^{3}H]-(carboxymethyl)cysteine) and ^{14}C counts at step 12 (γ-glutamyl[^{14}C]methylamide)[8]. We now present data which directly demonstrate that the second glutamyl residue of the reactive thiolester can, on proteolytic cleavage of the protein, donate its carbonyl group in a transesterification reaction with appropriate acceptor molecules. These results provide a model for analysis of the interactions at the molecular level between surface constituents of microorganisms and C3b.

Small molecules such as oligosaccharides and amines have been shown to inhibit C3 uptake onto Sepharose–trypsin matrices[11,12], while other studies have shown direct incorporation of radiolabelled sugars and amino acids into nascent C3b and have localized the site of incorporation to the α' chain[13,14]. To define this site more precisely, purified human C3[15] was cleaved into fragments C3a and C3b in the fluid phase by limited tryptic digestion[16] in the presence of 100 mM ^{14}C-sucrose, ^{14}C-D-glucosamine or ^{14}C-glycerol (NEN; each diluted to a specific activity of 1.5 mCi mmol^{-1}). For all experiments, C3 was >80% native as assessed by sulphydryl group titration in the presence and absence of methylamine[8,16]. Controls for each reaction included native C3 without trypsin (C3) and pretrypsinized C3 (C3b), incubated in identical conditions of temperature

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