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Stabilization of homologous and heterologous cell-bound amplification convertases, C3bBb, by C3 nephritic factor

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Summary. C3 nephritic factor (C3NeF) may be found in the sera of patients with membranoproliferative glomerulonephritis or partial lipodystrophy. It is capable of activating the alternative pathway in normal human serum; purified C3NeF has been shown to bind to the amplification convertase of complement, C3bBb. The binding of C3NeF to C3bBb results in stabilization of the otherwise labile C3 convertase. Decay of the convertase is accompanied by release of Bi and C3NeF from C3b. To determine whether stabilization of C3bBb occurs by the binding of C3NeF to C3bBb itself, to C3b or Bb alone, homologous and heterologous cell-bound convertases were prepared with $C3^{hu}$, B^{hu}, $C3^{rat}$ and B^{rat} and exposed to C3NeF or properdin. It was found that properdin induced stabilization of $C3b^{hu}Bb^{hu}$, $C3b^{rat}Bb^{hu}$, $C3b^{hu}Bb^{rat}$ and $C3b^{rat}Bb^{rat}$ in a dose-dependent manner. On the other hand, nine out of ten C3NeF preparations were only capable of stabilizing $C3b^{hu}Bb^{hu}$ and $C3b^{rat}Bb^{hu}$ and not $C3b^{hu}Bb^{rat}$ and $C3b^{rat}Bb^{rat}$. To determine whether binding of [^{125}I]-C3NeF to the various convertases occurred, cell-bound convertase were prepared in the presence of excess B^{hu} and B^{rat}, washed and further incubated with [^{125}I]-C3NeF; the cells were then washed and the amount of cell-bound [^{125}I]-C3NeF was measured. As in the stabilization experiments C3NeF bound only to $C3^{hu}Bb^{hu}$ and $C3b^{rat}Bb^{hu}$. The binding of C3NeF was always directly related to the presence of Bb^{hu} in the convertase. The results obtained with nine out of ten C3NeF preparations suggest that C3NeF is an autoantibody directed against antigenic determinants on Bb^{hu}, which are exposed after interaction of Bb^{hu} with $C3b^{hu}$ or $C3b^{rat}$. One out of ten C3NeF preparations showed reactivity with both cell-bound C3b alone and cell-bound C3bBb. These reactivities could be separated by absorption with cell-bound $C3b^{hu}$.

Abbreviations: C3bBb, amplification convertase of complement formed from the major cleavage fragments of C3 and B; C3NeF, C3 nephritic factor; C3bBb (C3NeF), C3NeF-stabilized amplification C3 convertase; P, Properdin; EAC4b3b, sheep erythrocyte sensitized with rabbit antibody and bearing the major cleavage fragments of C4 and C3; $C3^{rat}$, rat C3; B^{rat}, rat B; VBS, isotonic veronal-buffered saline; GVB, VBS containing 0·1% gelatin; GVB^{++}, GVB containing 5×10^{-4} M magnesium and $1{\cdot}5 \times 10^{-4}$ M calcium; $DGVB^{++}$, half-isotonic GBV^{++} containing 2·5% dextrose; EDTA, ethylene diamine tetra-acetate; GVB-EDTA, GVB containing 0·04 M EDTA; DGVB-EDTA, DGVB containing 0·04 M EDTA.

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INTRODUCTION

The capacity of sera from patients with membranoproliferative glomerulonephritis (MPGN) (Spitzer, Vallota, Forristal, Sodura, Stitzel, Davis & West, 1969) to induce cleavage of C3 in normal human serum by the alternative pathway (Vallota, Götze,

Spiegelberg, West & Müller-Eberhard, 1974) was ascribed to an activity termed C3 nephritic factor (C3NeF) (Spitzer *et al.*, 1969). Subsequent investigations established that C3NeF acts by binding to (Schreiber, Götze & Müller-Eberhard, 1976; Daha, Austen & Fearon, 1977) and stabilization of the amplification convertase C3bBb (Daha, Fearon & Austen, 1976a) and further by protection of the convertase from decay dissociation by β_1H (Weiler, Daha, Austen & Fearon, 1976). The ability of C3NeF to bind to either cell-bound (Schreiber *et al.*, 1976) or fluid phase C3bBb (Daha *et al.*, 1977) meant that further purification of C3NeF to homogeneity was possible, thus providing the opportunity to determine its chain composition (Daha, Austen & Fearon, 1978; Scott, Amos, Sissons, Lachmann & Peters, 1978), and investigation of its immunoglobulin characteristics (Davis, Ziegler, Gelfand, Rosen & Alper, 1979). Treatment of purified C3NeF with pepsin and papain established reactivity of the Fab′ portion of C3NeF with its antigen present on the C3bBb complex (Daha & Van Es, 1979a). In accordance with this concept it was shown that C3NeF, once it was bound to either the fluid phase or cell-bound convertase (Daha & Van Es, 1979b), was capable of reacting with C1q and C1. The present investigation was carried out to determine the antigenic site(s) with which C3NeF reacts on the cell-bound C3bBb complex. The experiments performed with homologous and heterologous convertases prepared with $C3^{hu}$, B^{hu}, $C3^{rat}$ and B^{rat} suggest that C3NeF binds mainly to Bb^{hu} after the binding of Bb to either Ceb^{hu} or $C3b^{rat}$

MATERIALS AND METHODS

XM diaflo ultrafiltration membranes (Amicon Corp., Lexington Mass.), $N^{125}I$, $Na^{131}I$ (The Radiochemical Centre, Amersham); insolubilized lactoperoxidase (Worthington, Freehold, N.J.) and 30% H_2O_2 (Merck, Amsterdam, The Netherlands) were obtained as indicated. Isotonic veronal-buffered saline (VBS), pH 7·5, containing 0·1% gelatin (GVB); GVB, containing 5×10^{-4} M magnesium (Mg^{++}) and $1{\cdot}5 \times 10^{-4}$ M calcium (Ca^{++}) (GVB^{++}); half-isotonic GVB^{++}, containing 2·5% dextrose ($DGVB^{++}$); and half-isotonic GVB containing 0·04 M diamine tetra-acetate (EDTA) (DGVB-EDTA) were used as diluents (Nelson, Jensen, Gigli & Tamura, 1966).

Human B (Hunsicker, Ruddy & Austen, 1973), C3 (Tack & Prahl, 1976), $\bar{D}$ (Fearon & Austen, 1975), $\bar{P}$ (Fearon & Austen, 1975) and rat C3 (Daha, Stuffers-Heiman, Kijlstra & Van Es, 1979) were purified to homogeneity and measured as described. Rat B was purified to homogeneity from fresh plasma of rats by sequential chromatography on QAE-A50, SP-C50, gelfiltration on G-150 and repeat chromatography on QAE-A50 (Daha & Van Es, 1980). $EAC4b^{hu}$ were prepared and converted to $EAC4b^{hu}3b^{hu}$ (Daha *et al.*, 1979) or $EAC4b^{hu}3b^{rat}$ by interaction with the desired amount of $C3^{hu}$ or $C3^{rat}$. C3NeF from the sera of ten patients with membranoproliferative glomerulonephritis (Daha *et al.*, 1978) was purified by binding to $C3b^{hu}Bb^{hu}$ in the fluid phase. C3NeF functional activity was measured using $EAC4b^{hu}3b^{hu}$ intermediates in the presence of excess B^{hu} and $\bar{D}^{hu}$ (Daha *et al.*, 1977). C3NeF preparations, normal IgG and human B were radiolabelled with either ^{125}I or ^{131}I by the lactoperoxidase method (Thorell & Larsson, 1974) with specific activities ranging between 100,000 and 150,000 c.p.m./μg protein.

RESULTS

In order to determine the stabilizing effect of C3NeF on homologous and heterologous cell-bound convertase, the following experiments were performed. $EAC43b^{hu}$, two batches of $1{\cdot}2 \times 10^8$ cells bearing an average of 2300 C3b molecules per cell, were interacted with 0·05 μg B^{hu} and 40 ng $\bar{D}$ for 30 min at 30° in 1·2 ml $DGVB^{++}$. The cells were subsequently washed twice in ice-cold DGVB-EDTA and each batch was resuspended in 1·2 ml DGVB-EDTA. Aliquots of 0·1 ml were then added in duplicate to three two-fold dilutions of purified C3NeF in DGVB-EDTA or to DGVB-EDTA without C3NeF. The degree of stabilization by C3NeF was determined by subsequent incubation for 30 min at 30°; the residual convertase sites were measured after further incubation for 60 min at 37° in the presence of guinea-pig serum diluted 1:15 in GVB-EDTA as a source of C3-C9. The stabilizing effect of properdin on the convertases was determined according to the same protocol using the second batch of cell-bound convertases (Fig. 1). The stabilizing effect of C3NeF or properdin on convertases prepared with $EAC43^{hu}$, B^{rat} (5 μg/ml), $\bar{D}$ (40 ng/ml); $EAC43^{rat}$, B^{hu} (0·5 μg/ml), $\bar{D}$ (40 ng/ml); and $EAC43^{rat}$, B^{rat} (5 μg/ml), $\bar{D}$ (40 ng/ml) were determined in a similar fashion. The concentrations of B^{hu} and B^{rat} were chosen such that before exposure to C3NeF or properdin an average of 1·8 convertase sites/cell were available.

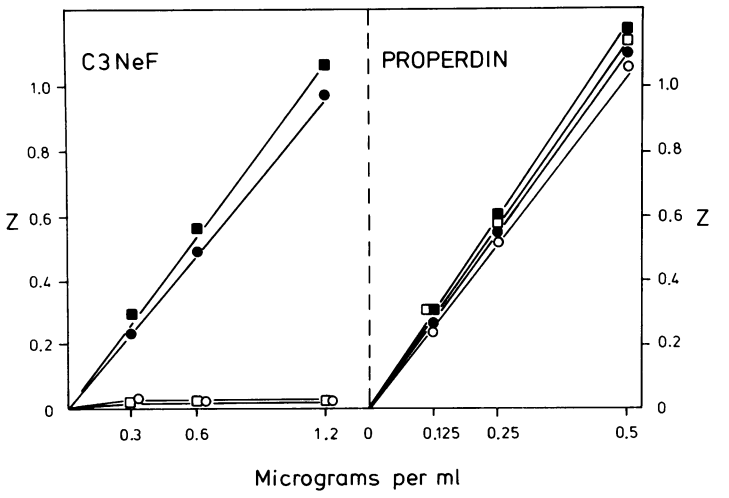

Figure 1. Degree of stabilization by C3NeF (left panel) or properdin (right panel) of cell-bound $C3b^{hu}Bb^{hu}$ (—■—), $C3b^{rat}Bb^{hu}$ (—●—), $C3b^{hu}Bb^{rat}$ (—□—) and $C3b^{rat}Bb^{rat}$ (—○—).

The results depicted in Fig. 1 indicate that properdin induced a dose-dependent protection of decay of the convertase sites. On the other hand C3NeF only induced stabilization of convertase sites prepared with $C3b^{hu}$ or $C3b^{rat}$ with B^{hu}. No stabilization could be detected for convertases prepared with B^{rat}.

Because the stabilizing potential of C3NeF was determined for only one point in time and minor stabilization by C3NeF could have escaped detection, kinetic experiments were now performed to determine the $T_{\frac{1}{2}}$ of decay for the homologous and heterologous convertases in the presence and absence of C3NeF.

For this purpose cell-bound $C3b^{hu}Bb^{hu}$, $C3b^{hu}Bb^{rat}$, $C3b^{rat}Bb^{hu}$ and $C3b^{rat}Bb^{rat}$ bearing an average of 1·8 sites per cell were prepared as described above, washed and resuspended in DGVB-EDTA alone or DGVB-EDTA containing 1·2 μg C3NeF per ml; the $T_{\frac{1}{2}}$ of decay was determined as described previously. The $T_{\frac{1}{2}}$ of decay of the various convertases listed in Table 1 indicate that C3NeF was capable of prolonging the decay of cell-bound $C3b^{hu}Bb^{hu}$ from $T_{\frac{1}{2}}=4$ min to $T_{\frac{1}{2}}=56$ min at 30°. C3NeF could also prolong the $T_{\frac{1}{2}}$ of decay of cell-bound $C3b^{rat}Bb^{hu}$ from $T_{\frac{1}{2}}=3$ min to a $T_{\frac{1}{2}}=47$ min. On the other hand C3NeF was not able to induce a change in the $T_{\frac{1}{2}}$ of either cell-bound $C3b^{hu}B-b^{rat}$ of $C3b^{rat}Bb^{rat}$.

Because there was a discrepancy between the stabilizing effect of C3NeF in the various convertases, and since it is known that binding of C3NeF is essential for its stabilizing effect, the binding of [^{125}I]-C3NeF to all four types of convertases was determined. For this purpose $EAC3b^{hu}$ and $EAC43b^{rat}$ bearing an average of 3000 C3b molecules per cell, were interacted with either 2·0 μg B^{hu}/ml or 20 μg B^{rat}/ml for 30 min at 30° in $DGVB^{++}$ and in the presence of 100 ng $\bar{D}$. The cells were washed with DGVB-EDTA and 2×10^8 intermediates were incubated in triplicate with 70 ng [^{125}I]-C3NeF for 30 min at 37°. Cell-bound and free [^{125}I]-C3NeF were then separated by centrifugation of the cells through 1 ml of 18% metrizamide in DGVB-EDTA for 10 min at 1500 *g*. The supernatant was discarded and the cell-bound radioactivity determined. As a control EAC4 and $EAC43b^{hu}$ or EAC43-b^{rat} were incubated with the same amount of [^{125}I]-C3NeF and treated similarly. The results shown in Table 2 indicate that C3NeF was bound to cell-bound $C3b^{hu}Bb^{hu}$ and $C3b^{rat}Bb^{hu}$. When compared

Table 1. Half-life of decay of homologous and heterologous amplification C3 convertases in the presence and absence of C3 nephritic factor

	$T_{\frac{1}{2}}$ (min)	
Convertase	Without C3NeF	With C3NeF
$C3b^{hu}Bb^{hu}$	5	56
$C3b^{rat}Bb^{hu}$	3	47
$C3b^{hu}Bb^{rat}$	3·5	3·4
$C3b^{rat}Bb^{rat}$	3	3·1

Table 2. Binding of [^{125}I]-C3NeF to homologous and heterologous amplification convertases

Intermediate	[^{125}I]-C3NeF bound (c.p.m.)
EAC43b^{hu}Bb^{hu}	5448*
EAC43b^{rat}Bb^{hu}	5317
EAC43b^{hu}Bb^{rat}	203
EAC43b^{rat}Bb^{rat}	198
EAC4	212
EAC43b^{hu}	197
EAC43b^{rat}	193

* Mean of three determinations.

with the control binding of [^{125}I]-C3NeF to cell-bound C3b^{hu}Bb^{rat} and C3b^{rat}Bb^{rat} was found to be absent. No significant difference was seen between the amount of radioactivity bound to EAC4, EAC43b^{hu} and EAC43b^{rat}.

The experiments described above suggest that binding to the convertase of [^{125}I]-C3NeF only occurs when Bb^{hu} is part of the convertase. To determine whether binding of [^{125}I]-C3NeF to cell-bound C3b^{hu}Bb^{hu} was correlated with the amount of binding of Bb^{hu} to C3b, the following experiment was performed: seven batches of 5×10^8 intermediates of EAC43b^{hu}, bearing 158 C3b molecules per cell, in 0·5 ml DGVB^{++} were incubated with 20, 10, 5, 2·5, 1·25, 0·6 or 0 μg [^{131}I]-B^{hu} all in the presence of 50 ng $\bar{D}$ and all with one dose of 80 ng [^{125}I]-C3NeF for 30 min at 30°. Cell-bound [^{131}I]-Bb and [^{125}I]-C3NeF were determined as before. The number of cell-bound [^{131}I]-Bb and [^{125}I]-C3NeF molecules were determined and the results were expressed as shown in Fig. 2. There was a linear relationship between binding of [^{131}I]-Bb and [^{125}I]-C3NeF to cell-bound C3b, with an average ratio of C3NeF: Bb of 0·98.

It is known (Daha, Fearon & Austen, 1976b) that when the density of cell-bound C3b is low only C3 convertases with a C3b:Bb ratio of 1:1 are formed. Because the possibility existed that cell-bound C3b also influences the binding of C3NeF to C3b-bound Bb, experiments were performed with EAC43b^{hu} bearing 158 molecules of C3b per intermediate and with EAC43b^{hu} bearing 480 molecules of C3b per intermediate. The experiments were performed exactly as described above for the previous experiment. The results, presented in Fig. 3, indicate that although the ratio of C3b:Bb varies from 0·8 to 3·2, the ratio of C3NeF:Bb remains fairly constant within a range of 0·9 to 1·05. The experiments described above were performed with ten C3NeF preparations from ten different individuals. It was found that nine preparations were only capable of reacting with cell-bound C3b^{hu}Bb^{hu} or C3b^{rat}Bb^{hu} but not with cell-bound C3b^{hu}. One of the ten C3NeF preparations also showed reactivity with cell-bound C3b^{hu} and not with cell-bound C3b^{rat}. Twenty percent of this purified radiolabelled [^{125}I]-C3NeF could be absorbed to cell-bound C3b^{hu} and recovered after elution from the ghosts. This

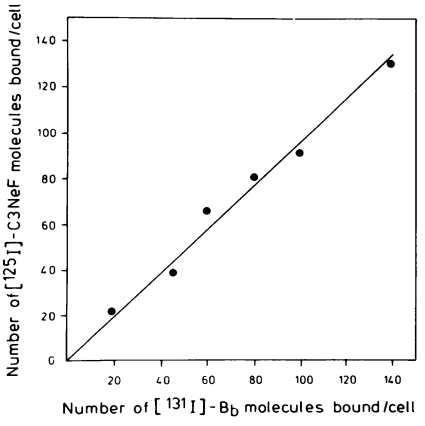

Figure 2. Relationship between binding of [^{125}I]-C3NeF and [^{131}I]-Bb to EAC43b^{hu} bearing 158 C3b-molecules/cell.

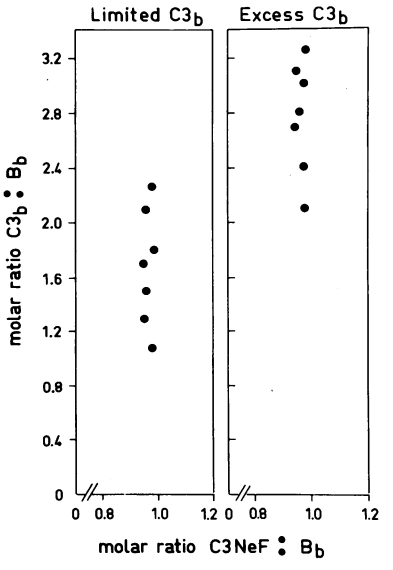

Figure 3. Molar ratio of C3NeF:Bb and C3b:Bb during interaction of EAC43b^{hu} bearing 158 (left panel) and 480 molecules C3b (right panel) per cell.

material was capable of binding to $C3b^{hu}Bb^{hu}$ and to induce stabilization. On the other hand it did not bind to $C3b^{rat}Bb^{hu}$ and also did not induce stabilization of this convertase. The remaining 80% of the [^{125}I]-C3NeF preparation was able to stabilize both $C3b^{hu}Bb^{hu}$ and $C3b^{rat}Bb^{hu}$. These experiments indicate that C3NeF is directed predominantly against convertase bound Bb^{hu}, and that within the same patient part of the C3NeF may react to cell-bound $C3b^{hu}$ alone and part to cell-bound $C3b^{hu}Bb^{hu}$ and $C3b^{rat}Bb^{hu}$.

DISCUSSION

Stabilization by C3NeF of the amplification convertase C3bBb occurs both in the fluid phase and on a solid surface (Schreiber *et al.*, 1976; Daha *et al.*, 1976a) and is dependent on the binding of C3NeF to the convertase (Schreiber *et al.*, 1976; Daha *et al.*, 1977). However, it was still not clear whether C3NeF binds to C3b,Bb or to both in the C3bBb complex. The inability of C3NeF to bind to C3b or Bb alone would suggest a higher binding affinity between C3NeF and C3bBb. This could indicate exposure of identical antigenic determinants on C3b as well as Bb in the C3bBb complex. Other possibilities however are the binding of C3NeF to one antigenic determinant exposed on C3bBb, on C3b alone or on Bb alone, due to conformational changes.

The isolation of rat B and the observation that it could interact with cell-bound $C3b^{hu}$ in the presence of $\bar{D}$ to form a convertase which was stabilized by properdin, which is known to bind to C3b (Fearon & Austen, 1975), made it possible to investigate the stabilizing effect of C3NeF on cell-bound convertases prepared with various likely combinations of cell-bound $C3b^{hu}$, $C3b^{rat}$, $Bb^{hu}$and Bb^{rat}. Although properdin was capable of stabilizing all possible convertases (Fig. 1), nine out of ten C3NeF preparations only stabilized those convertases which were prepared with B^{hu}. Even in a kinetic experiment stabilization of cell-bound $C3b^{hu}Bb^{rat}$ and $C3b^{rat}Bb^{rat}$ could not be detected (Table 1). Because the binding of C3b-bound Bb occurred at a 1:1 molar ratio, which had also been observed in the fluid phase (Daha *et al.*, 1977), the results suggested that C3NeF reacted only with Bb^{hu} when Bb^{hu} was part of a convertase. To exclude the possibility that C3NeF binding to C3b-bound Bb^{hu} was partially caused by the presence of C3b, convertases were prepared with various C3b:Bb ratios ranging between 0·8 and 3·2. Even under these conditions, the molar ratio of Bb:C3NeF remained fairly constant (Fig. 3). Although the ratio of C3b:Bb varied between 0·8 and 3·2 and an increase in neo-antigenic sites may have occurred, there was no detectable change in the Bb:C3NeF ratio, suggesting interaction of C3NeF with antigenic site(s) on Bb. Because neither fluid-phase C3b nor Bb inhibited C3NeF binding to C3b-bound Bb the conclusion may be drawn that most C3NeF preparations react with antigenic site(s) on Bb, which are uncovered after the interaction of Bb with C3.

On the other hand, one out of ten C3NeF preparations showed reactivity with both cell-bound $C3b^{hu}$ and cell-bound $C3b^{hu}Bb^{hu}$. Absorption with cell-bound $C3b^{hu}$ revealed that this [^{125}I]-C3NeF preparation contained at least two populations of C3NeF. One population, which reacted with cell-bound $C3b^{hu}$ and not with cell-bound $C3b^{rat}$, induced stabilization of $C3b^{hu}Bb^{hu}$ only. The major portion of this C3NeF preparation, however, was similar to the other ten preparations in that it stabilized an amplification convertase composed of either $C3b^{hu}$ or $C3b^{rat}$ with Bb^{hu}. The data presented in this paper suggest that most C3NeF preparations react with convertase-associated Bb^{hu}, but that C3NeF may also show reactivity with cell-bound $C3b^{hu}$ alone. Our experiments also indicate that binding of C3NeF to C3b or to Bb alone may induce stabilization. These results are compatible with a recently presented report that the classical C3 convertase $C\overline{42}$ may be stabilized with an heterologous antibody directed against the β-chain of C4 (Gorskei & Müller-Eberhard, 1980). Although it has been shown that F-$\overline{42}$, an autoantibody with specificity for the classical C3 convertase (Daha, Hazevoet, Van Es & Cats, 1980; Halbwachs, Leveille & Leibowitch, 1980), also stabilizes this convertase, its binding characteristics have not yet been determined.

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