

C3b Inactivator in the Rheumatic Diseases

MEASUREMENT BY RADIAL IMMUNODIFFUSION AND BY INHIBITION OF FORMATION OF PROPERDIN PATHWAY C3 CONVERTASE

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ABSTRACT C3b inactivator (C3bINA) has been measured in biologic fluids by radial immunodiffusion using a monospecific antiserum prepared in rabbits, and by a hemolytic assay which measures the reduction in the capacity of EAC43 cells bearing limited C3b sites to form C3b , the alternative pathway C3 convertase. The radial immunodiffusion and hemolytic assays show a good correlation ($r = 0.86$ $P < 0.001$).

Measurement of C3bINA concentrations in the sera of patients with systemic lupus erythematosus showed that during exacerbations of disease activity C3bINA concentrations tended to be lower, usually in association with reductions in C4, C3, factor B, and properdin, and sometimes with reductions of the alternative pathway proteins, factor B, and properdin alone.

Supranormal values for C3bINA were found in the sera of 14 of 20 patients with seropositive rheumatoid arthritis and 3 of 9 seronegative patients, but none of 7 patients with degenerative joint disease. Synovial fluid concentrations of C3bINA, after correction for total synovial fluid protein and serum concentration of the enzyme, were significantly reduced in patients with rheumatoid arthritis compared to patients with degenerative joint disease ($P < 0.05$).

In both serum and synovial fluid from patients with rheumatoid arthritis, there was a good correlation between the concentrations of C3bINA and those of C3, factor B, and properdin, but not that of C4, suggesting

that levels of C3bINA may serve to modulate recruitment of the properdin amplification loop in this disease.

INTRODUCTION

During complement activation, C3 is cleaved by the classical pathway convertase (C42) (1) or the alternative pathway convertase (C3B) (2), into C3a anaphylatoxin, and C3b (1). C3b has several important biologic activities including the enhancement of phagocytosis (3) and immune adherence (4), continuation of the cytolytic sequence (5), and recruitment of the alternative pathway by complexing with factor B to form C3B which can then cleave more C3 (2). The apparent self-perpetuating feature of this recruitment is limited by the C3b inactivator (C3bINA),¹ a β -globulin with a mol wt of approximately 100,000 (6, 7) which cleaves C3b into two smaller fragments, C3c and C3d (8). Neither of these fragments possesses the biologic functions of the parent molecule, although certain lymphocytes and macrophages have C3d receptors (9-13). Thus, C3bINA plays a key role in the regulation of the complement system by blocking the cytolytic sequence, reducing immune adherence and phagocytosis, and preventing recruitment of the properdin amplification loop after activation of the classical pathway.

Activation of the complement system by the classical pathway in rheumatoid arthritis and systemic lupus

¹ *Abbreviations used in this paper:* C3bINA, C3b inactivator; DGVB⁺⁺, GVB⁺⁺ + D5W⁺⁺; D5W⁻⁻, 5% of dextrose in water with divalent cations; GVB⁺⁺, barbitol buffered saline with gelatin; SLE, systemic lupus erythematosus; Z' units, C3b inactivator activity.

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erythematosus has been shown by the demonstration of lowered total hemolytic complement levels (14-18), and reduction in individual components in synovial fluid and serum, respectively (15, 17, 18, 19-25), the presence of cleavage products (26, 27), immunofluorescence microscopy (28-32), and studies of the turnover rates of radio-labeled components (33-39). That activation of the alternative pathway also occurs has been demonstrated by the finding of cleavage fragments of factor B (40-41), reduced serum and synovial fluid concentrations of factor B (24, 34, 40-44) and properdin, immunofluorescence microscopy showing deposition of properdin and factor B (32, 45), and metabolic studies indicating hypercatabolism of these two proteins (38, 39, 46). It is unclear whether this alternative pathway involvement reflects primary direct activation independent of classical components or recruitment via the amplification loop after C3 cleavage induced by C4 $\bar{2}$. A possible mechanism whereby recruitment of the amplification might occur is consequent upon perturbations in levels of C3b inactivator which would allow C3b to persist, permitting it to complex with factor B.

Although functional assays for C3bINA have been described, they depend either on the inhibition of immune adherence agglutination (47), or enhancement of agglutination by bovine conglutinin (48), both of which are semiquantitative techniques. We have developed two new methods of measuring C3bINA in biologic fluids: (a) The activity of this enzyme has been measured by its capacity to cleave cell-bound C3b on EAC43, thereby inhibiting the formation of the alternative pathway convertase and subsequent lysis of the cells on exposure to factor B and C3 to C9; (b) Production of monospecific antibody has enabled us to measure C3bINA immunologically by radial immunodiffusion. Using these two assays, which correlate well, we have measured concentrations of C3bINA in the serum of patients with systemic lupus erythematosus and shown that depressions occur and may be related to disease activity; similarly, in synovial fluid from patients with rheumatoid arthritis, relative depressions of C3bINA are also found. In the latter instance, the extent of activation of the alternative pathway, manifested by reductions in the levels of properdin and factor B, is related to depressions of C3bINA.

METHODS

Functional assays

Buffers. Isotonic barbital buffered saline (pH 7.5) containing 0.15 mM Ca $^{++}$, 0.5 mM Mg $^{++}$, and 0.1% gelatin (GVB $^{++}$); 5% dextrose in water containing the same concentrations of divalent cations (D5W $^{++}$); and a mixture of equal volumes of GVB $^{++}$ and D5W $^{++}$ (DGVB $^{++}$) were prepared. DGVB $^{--}$ and GVB $^{--}$ were made as described above for DGVB $^{++}$ and GVB $^{++}$, but the divalent cations were omitted. A stock solution of 0.086 M EDTA, pH 7.5, was

diluted in GVB $^{--}$ to concentrations indicated, e.g., 0.04 M EDTA GVB $^{--}$ (49). For experiments in which pH was varied, DGVB $^{--}$ or 0.02 M EDTA DGVB $^{--}$ were adjusted directly with 1.0 N NaOH or 1.0 N HCl. For experiments in which ionicity was varied, DGVB $^{--}$ was adjusted by varying the ratios of GVB $^{--}$ to D5W $^{--}$.

Reagents. Guinea pig C $\bar{1}$ (49), human C2 (50), C3 (51), and factor B (52) were purified according to previously described techniques. Zymosan-treated human serum was prepared by incubating normal serum with boiled zymosan (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 5 mg/ml for 60 min at 37°C. Zymosan was removed by centrifugation at 4°C and the zymosan-treated serum aliquoted and stored at -70°C. Rat serum as a source of C3-C9 was obtained from Pel-Freez Biologicals, Inc., Rogers, Ark. DEAE or CM cellulose (DE52 or CM52, Whatman, Reeve Angel Co., Clifton, N. J.) and sephadex G200 (Pharmacia Fine Chemicals, Piscataway, N. J.) were obtained from the sources shown.

Cellular intermediates. For certain experiments EAC43 bearing limited amounts of C3 was prepared from EAC14, purified human C2, and limited amounts of purified C3 as previously described (53). For routine use, EAC43 was prepared by incubating EA at 2×10^8 /ml in a limiting dilution of zymosan-treated serum (54). The reagents were prewarmed to 30°C, mixed, and incubated for 30 min, and the reaction stopped by adding an equal volume of ice-cold 0.04 M EDTA GVB $^{--}$. After centrifugation at 4°C, the cells were resuspended in 0.04 M EDTA GVB $^{--}$ to remove the C $\bar{1}$ and incubated at 37°C for 2 h to allow the C2 sites to decay. The resulting EAC43 was again centrifuged at 4°C, washed once in 0.01 M EDTA GVB $^{--}$, once in DGVB $^{++}$, and standardized spectrophotometrically to a concentration of 1×10^8 /ml in DGVB $^{++}$ where it was stored at 0°C for up to 2 wk.

Measurement of C3b sites. To determine the average number of hemolytically active C3b sites per erythrocyte, 0.1 ml of EAC43 suspension in DGVB $^{++}$ was incubated with an equal volume of DGVB $^{++}$ containing a previously determined excess of factor B (8.0 μ g/ml) at 30°C for 30 min. The resulting EAC43B cells were incubated with 0.3 ml of rat serum diluted 1:15 in 0.04 M EDTA GVB $^{--}$ for 60 min at 37°C. 1.5 ml of 0.15 M sodium chloride was added, the tubes centrifuged, and the supernatant hemoglobin measured spectrophotometrically at 414 nm. Calculation of the proportion of cells lysed and the average number of hemolytic sites formed per cell was performed as previously described (50). A reagent blank from which factor B was omitted was consistently less than 3%.

Immunochemical measurement of C3bINA. C3bINA was purified by a previously published technique involving sequential DEAE, cellulose, CM cellulose, and sephadex G-200 chromatography (55). For immunization, the C3bINA was further purified on polyacrylamide disk-gel electrophoresis, the gels sliced, and slices containing C3bINA eluted in barbital buffered saline overnight. Approximately 100 μ g of antigen was emulsified in Freund's complete adjuvant, and injected subcutaneously at 2-wk intervals into two rabbits over several months. The antisera obtained between 2 and 4 wk after the final injection contained antibody to C3bINA, transferrin, and IgG. It was rendered monospecific by passage over a sepharose 4B column to which serum from a patient congenitally deficient in C3bINA (kindly supplied by Dr. Chester Alper) had been coupled by the cyanogen bromide method (56). The resulting antiserum was monospecific as judged by double diffusion in agarose and im-

munoelectrophoresis against human serum, and did not react with the serum from the congenitally deficient patient. It gave a line of identity with antisera previously described (55).

For radial immunodiffusion, the antiserum was incorporated at a final dilution of 1:8 in a 0.145-M sodium chloride, 0.05 M barbitol, 0.01 M EDTA buffer, pH 7.5, in a 1.5% agarose gel. 1.5-mm holes were cut in the gel, the samples loaded, and diffusion allowed to proceed for 48 h. The plates were then washed in 0.075 M sodium chloride for 24 h at room temperature and stained with 1% tannic acid in water. Maximal ring diameters were approximately 7 mm. A pool of serum obtained from three control subjects served as the 100% reference standard, and individual results were expressed as a percentage of this pool.

Clinical studies. 10-ml samples of blood were drawn into sterile Vacutainer tubes, allowed to clot for 1 h at room temperature, and the serum separated by centrifugation at 4°C and stored in equal portions at -70°C. Synovial fluid samples were aspirated from the knee joint into plastic disposable syringes, transferred to sterile test tubes, centrifuged at 4°C, and stored in equal portions at -70°C.

50 healthy laboratory personnel and medical students served as normal controls; 18 were females and 32 were males. Their ages ranged from 21 to 55 yr.

Serum and synovial fluid samples were taken from 20 patients with seropositive rheumatoid arthritis, 9 with seronegative rheumatoid arthritis, and 7 with degenerative joint disease. Criteria for these diagnoses were those previously used (25). Bilateral joint aspiration from two patients with degenerative joint disease increased the number of paired samples in this group to nine. Serum samples were obtained from 13 patients with systemic lupus erythematosus (SLE) when their disease was inactive as shown by clinical criteria and normal C3 concentrations, and again when the disease was clinically active and serum C3 concentrations were low. Serial serum samples taken over 2-3 yr were obtained from four of these patients. Patients with systemic lupus conformed to the provisional criteria established for this disease (57).

RESULTS

Hemolytic assay of C3bINA. The capacity of C3bINA, either purified or contained in normal human serum, to inactivate the hemolytic activity of C3b bound to EAC43 was assessed kinetically in the following experiment. To each of three tubes was added 1.5 ml EAC43 at 1×10^8 /ml DGVB⁻. After prewarming to 37°C, equal volumes of dilutions of either purified C3bINA, normal human serum, in 0.02 M EDTA DGVB⁻, or buffer alone, was added to one of the tubes. Samples (0.5 ml) were withdrawn from each reaction mixture at timed intervals up to 2 h and diluted in 5 ml ice-cold 0.01 M EDTA DGVB⁻. The treated EAC43 was centrifuged, washed once in 0.01 M EDTA DGVB⁻, once in DGVB⁻, once in DGVB⁺⁺, and finally resuspended to 1×10^8 /ml DGVB⁺⁺. Residual C3b present on the treated EAC43 was assessed by exposure of 0.1-ml samples to excess factor B and rat serum diluted in 0.04 M EDTA DGVB⁻ as described in Methods. A separate 0.1-ml portion of each EAC43 sample was lysed in distilled water to provide a 100% value and controls for lysis in

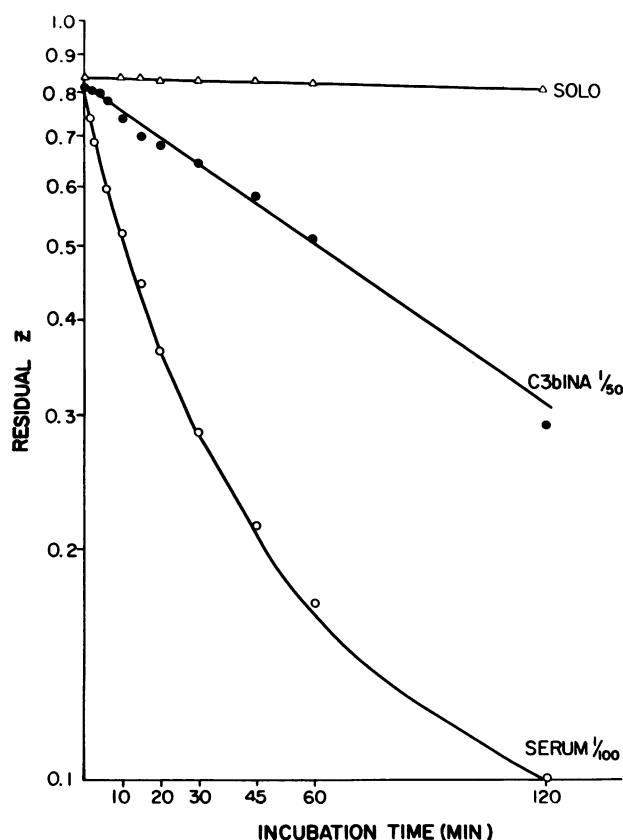


FIGURE 1 Inactivation of cell-bound C3b by C3bINA: EAC43 cells were incubated with functionally purified C3bINA (●—●), EDTA treated serum (○—○), or buffer alone, solo (Δ—Δ), and samples taken from the reaction mixture at various timed intervals. Residual sites (Z) are plotted on the ordinate and incubation time on the abscissa.

buffer alone or in the absence of factor B were also included. As shown in Fig. 1, the number of C3 sites destroyed by purified C3bINA or C3bINA contained in normal serum continued to increase for up to 2 h.

The temperature, pH, and ionicity of the assay buffer were varied in an attempt to achieve optimal conditions for the inactivation of cell-bound C3b. Serum was used as the source of C3bINA in these experiments, but other experiments using purified C3bINA gave similar results. The assay was performed at 0, 23, 30, and 37°C (Fig. 2). No inactivation of C3b was seen at 0°C whereas it was maximal at 37°C. In the pH range from 6.0 to 8.0, enhanced destruction of C3b was observed at the lower end of the range. The lowest ionic strength (seven parts D5W⁻ to one part GVB⁻) resulted in maximum inactivation of C3b. Since spontaneous lysis became excessive below pH 6.0 and the EAC43 tended to agglutinate at very low ionic strength, final conditions for the assay at 37°C, pH 6.0, and ionic strength equivalent to one part GVB⁻, three parts D5W⁻ were selected.

C3bINA activity may be expressed as Z' units (58), where Z' equals $-\ln(1 - \% \text{ inactivation})$, which is the same as $-\ln(\% \text{ lysis test sample} / \% \text{ lysis control})$. The control used in the denominator of this equation is the percentage lysis observed when EAC43 cells were incubated with buffer alone. In a kinetic assay, when the number of Z' observed at a given time was plotted against time, a straight line passing through the origin was observed (Fig. 3A). Similarly, if the number of Z' per hour was plotted versus input amounts of C3bINA, a linear relationship was also observed (Fig. 3B). For the assay of C3bINA in biologic fluids, a single dilution of the biologic fluid was incubated with an equal volume of EAC43 and sampled kinetically at 15, 30, 45, and 60 min. The number of units of C3bINA activity was defined as equal to the number of Z' generated per hour by undiluted test sample. Sera were assayed at a 1:1,000 dilution and synovial fluid at a 1:500 dilution. A standard reference serum pool, which was assayed each day, was arbitrarily assigned an activity of 1,000 U/ml (1,000 Z'/h) and day-to-day variation compensated for by multiplying the result obtained from the test sample by 1,000/ X where X was the result obtained for the reference standard.

Radial immunodiffusion assay. C3bINA concentrations among 50 normal individuals were distributed in

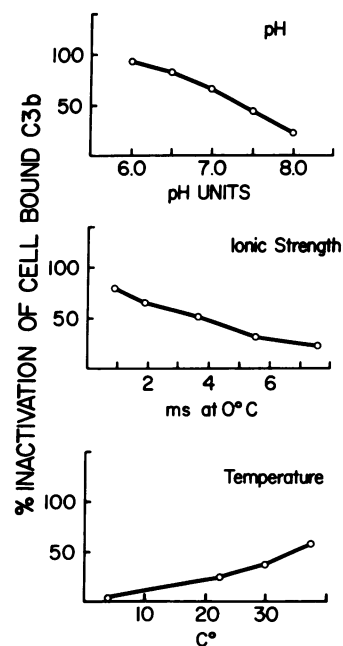


FIGURE 2 The effect of pH, ionic strength, and temperature on C3bINA activity. The percentage inactivation of cell bound C3b is shown on the ordinate, and changes in pH, ionic strength, and temperature ($^{\circ}\text{C}$) on the abscissa. Percentage inactivation is the ratio of number of Z inactivated divided by number of Z in control ($\times 100$).

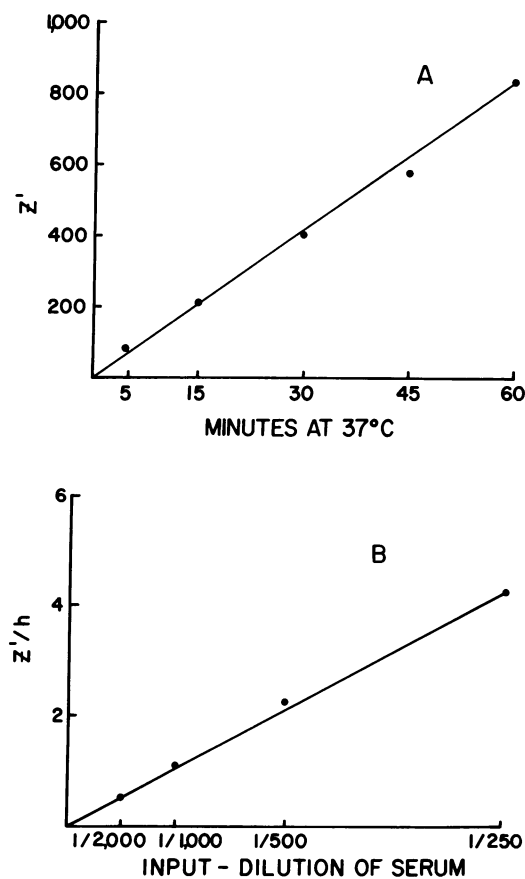


FIGURE 3 (A) Inactivation of cell bound C3b by a constant input C3bINA. Increasing destruction, expressed as Z' units, was observed with increasing incubation time. Z' is plotted on the ordinate and incubation time on the abscissa. The number of Z' is corrected for serum dilution. (B) Inactivation of cell bound C3b by varying inputs of C3bINA, during 1 h incubation at 37°C . Inactivation expressed as Z'/h is plotted on the ordinate and input of C3bINA, which was a dilution of serum, plotted on the abscissa. No correction for dilution has been used in this experiment.

a logarithmic fashion. After normalization, the mean was 82.1% of the standard pool, and the range of 2 SD was 57.9–116.5%. Serum samples taken from three normal subjects at weekly intervals over a 2-mo period showed that the immunochemical concentration of C3bINA did not fluctuate significantly. At room temperature, immunoreactivity in serum was stable for up to 3 days, at 4°C , up to 5 days, and at -20°C , up to 3 mo. Freezing and thawing three times produced no change in the immunochemical concentration of C3bINA.

The relationship between functional and immunochemical assays for 28 samples of serum or synovial fluid, selected because they represented a wide scatter of immunochemical C3bINA concentrations, was investigated. Using the least squares method, a good correlation was

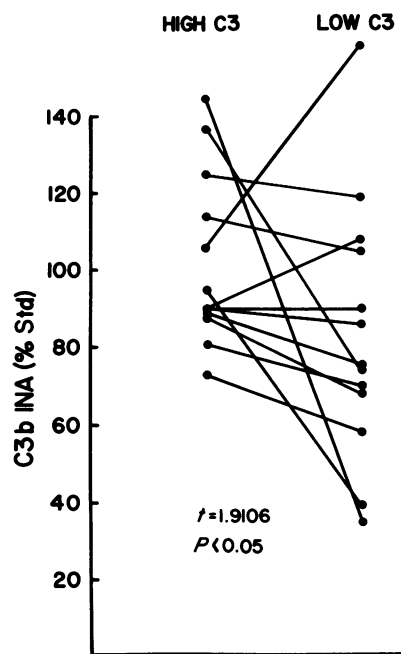


FIGURE 4 C3bINA concentrations in SLE. Serum samples taken from 13 patients during remission are shown on the left, and samples taken from the same patients during disease exacerbation are shown on the right.

obtained between the immunochemical concentration and the logarithm of the functional activity ($r = 0.860$). Since the activity of C3bINA in serum progressively diminished with repeated freezing and thawing (approximately 50% reduction after four cycles), and since the immunochemical assay was stable and more convenient, clinical studies were conducted using radial immunodiffusion.

Serum levels in SLE. Serum samples from 13 patients with SLE were studied. One sample was obtained during a period when the disease was clinically inactive and serum C3 concentration normal and a second obtained during an exacerbation when the C3 was subnormal. Of the 13 patients, C3bINA levels in 10 fell during disease exacerbation, 1 did not change, and 2 rose (Fig. 4). Three patients showed marked fall of C3bINA concentration during disease exacerbation, but there were no obvious differences in either the clinical course or the magnitude of the reductions of factor B, properdin, and C3 concentrations, between these three and the remaining patients. Using a sign test, the probability of the differences between C3bINA concentrations during exacerbation and remission occurring by chance was less than 0.05; by a t test, the difference in the means was of borderline significance. Serum concentrations of C3bINA, C4, C3, factor B, and properdin were measured in serial samples obtained during 2- to 3-yr periods in four patients with SLE. In general,

changes in C3bINA tended to parallel changes in the other proteins. The results of one such serial study are illustrated in Fig. 5. Patient V. B., a white female, was diagnosed as having SLE in 1957 at the age of 11 yr. She had evidence of skin, hair, joint, pleural, hematologic, and renal involvement between 1957 and 1972. From February, 1972, through September, 1974, there were two occasions on which C3bINA levels were found to be subnormal: late 1973 and August, 1974. The patient was in clinical remission until May, 1973, when she complained of a sore throat and developed some alopecia; urinalysis was normal and no other stigmata of SLE were present. At this time she had a marginally reduced C4, a normal C3, but a markedly reduced C3bINA and a reduced factor B concentration. By September, 1973, there was 4+ proteinuria and some erythrocytes in the urinary sediments. Serum C4 and C3 levels were normal, but C3bINA, factor B, and properdin were all subnormal. By December, she was frankly nephrotic. At that point, C4 and C3 levels were markedly subnormal, whereas C3bINA had risen to 50% and factor B and properdin were within the normal range. With high dose corticosteroid therapy, a remission was obtained which lasted until August, 1974, when she again became

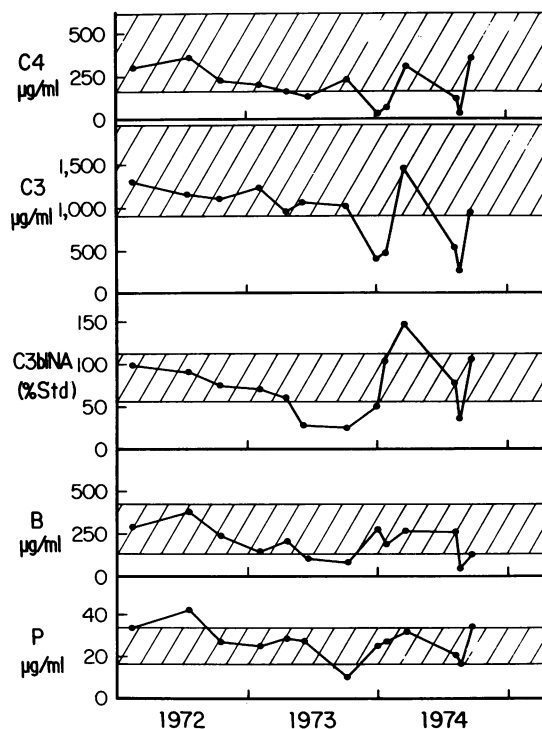


FIGURE 5 A serial study from patient V. B. having a diagnosis of SLE. Variations in serum concentrations of C4, C3, C3bINA, factor B, and properdin over a 3-yr period are shown. The hatched areas represent the normal ranges (mean \pm SD) for each protein.

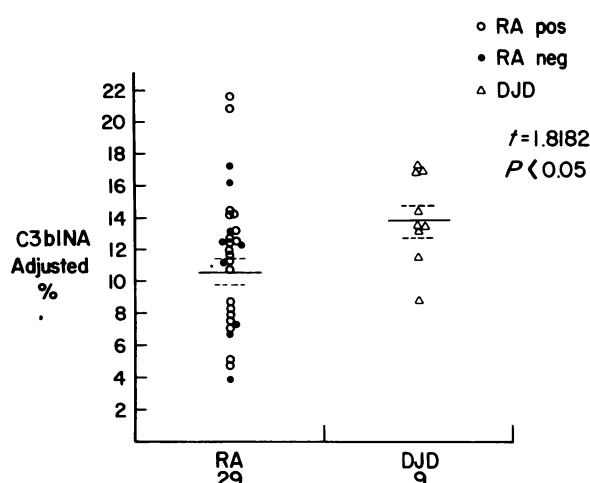


FIGURE 6 Synovial fluid concentrations of C3bINA in rheumatoid arthritis (RA) and degenerative joint disease (DJD). The heavy lines represent the mean for each group, and the interrupted lines, the standard error of the mean.

edematous, had proteinuria, and an abnormal urinary sediment. At this time, C4, C3, C3bINA, and factor B were subnormal, and properdin was at the lower limit of normal. Once again, high dose corticosteroid therapy was associated with improvement in renal function and return of C4, C3, C3bINA, factor B, and properdin to within the normal range.

Serum and synovial fluid levels in arthritis. Serum C3bINA levels in 7 patients with degenerative joint disease were within the normal range, whereas 14 of 20 patients with seropositive rheumatoid arthritis had supranormal values. The mean concentration for the seropositive rheumatoid arthritis group (119.8%) was significantly higher than the mean for the degenerative joint disease group (97.3%) ($P < 0.025$), whereas the mean for the seronegative group (115.2%) did not differ significantly from either of the others. Concentrations of C3bINA were adjusted for total synovial fluid protein and serum C3bINA concentrations (44) by the formula: corrected synovial fluid component concentration = Synovial fluid concentration (% Std.) \times 100 [Serum concentration (% Std.) \times total synovial fluid protein concentration (g/100 ml)]. This correction is applied to compensate for leakage of plasma proteins into the synovial space consequent upon inflammation, and for alterations in the serum level of the protein in question. The results are shown in Fig. 6. The mean among 29 patients with rheumatoid arthritis was significantly lower than among samples from 7 patients with degenerative joint disease ($t = 1.81$, $P < 0.05$).

Correlation between C3bINA and C3, C4 factor B, or properdin in serum or synovial fluid. When all 88

serum samples obtained from patients with rheumatoid arthritis, SLE, or degenerative joint disease were examined for correlations between C3bINA and other complement or properdin proteins, high degrees of correlation were observed for C3 ($r = 0.70$, $P < 0.0005$) and factor B ($r = 0.77$, $P < 0.0005$). A significant but lesser correlation between C3bINA and properdin was also found ($r = 0.37$, $P < 0.0005$) but no correlation between C4 and C3bINA existed ($r = 0.09$). These strong correlations were mostly attributable to covariation in the sera of patients with rheumatoid arthritis, as when the SLE patients were examined separately the only correlation found was between C3bINA and factor B ($r = 0.32$, $P < 0.025$). As in serum of patients with rheumatoid arthritis, a good correlation between the concentrations of synovial fluid C3bINA, and C3 ($r = 0.72$, $P < 0.0005$), factor B ($r = 0.68$, $P < 0.0005$) and properdin ($r = 0.70$, $P < 0.0005$) were observed. No correlation was observed between C3bINA and C4 ($r = 0.09$).

DISCUSSION

Two different assays for the enzyme C3bINA in biologic fluids have been employed in this study. The immunochemical concentration of the enzyme has been measured by radial immunodiffusion using a monospecific antiserum, and the function has been measured hemolytically by the reduction in C3b on EAC43 cells after exposure to the enzyme.

Evidence for the monospecificity of the antiserum is based upon a single precipitin line against normal human serum on immunoelectrophoresis and double diffusion in agarose gel, and a single ring on radial immunodiffusion of serum. That the specificity is directed against C3bINA is indicated by its reaction with a highly purified C3bINA preparation, neutralization of C3bINA activity in the fluid phase (55), and the failure of serum from a patient congenitally deficient in the enzyme to absorb out the antibody activity.

The functional assay measures the capacity of the biologic fluid to inactivate cell-bound C3b. C3b on EAC43 cells has the ability to complex with factor B in the presence of Mg^{++} to form C3bB, the alternative pathway convertase (53). On the exposure to a source of C3-C9, in this case rat complement, diluted in EDTA, cleavage of C3 and activation of the terminal sequence results, leading to cell lysis. When the number of C3b sites is limited and factor B and rat complement are present in excess, then the degree of lysis depends upon the number of C3b sites. If sufficient excess of B is supplied, then neither factor D nor properdin are required. The ability of cell-bound C3b to form C3bB on exposure to factor B provides a simple method for its quantification.

Since the components capable of forming both classical and alternative pathway convertases, and thus additional C3 sites, were present in the biologic fluids examined, it was necessary to prevent their formation by the chelation of divalent cations with EDTA. Previous studies have demonstrated that C3bINA activity does not require metallic cations (7). In addition to EDTA in the assay buffer, it was found that a relatively low pH and low ionicity produced a 10- to 20-fold enhancement of C3bINA activity (Fig. 2) in both serum and purified C3bINA preparations. After the exposure of EAC43 to the test samples, extensive washing was required to remove fluid phase complement components contained in the reaction mixture. To compensate for variable cell loss during the washing procedure, 100% values were obtained for each cell sample. Since C3b inactivation continued to progress for up to 2 h at 37°C, a kinetic assay based on the rate of inactivation by a single dilution of test sample was used. Using EAC43 bearing between 0.5 and 2.0 hemolytic C3b sites, the extent of inactivation, expressed as Z' units, is a linear function of time (Fig. 3A). At any given time, the number of Z' observed is linearly related to the input of C3bINA (Fig. 3B), either as the purified enzyme or in serum.

The lability of C3bINA activity in the hemolytic assay to repeated cycles of freezing and thawing, compared with the stability of the immunochemical measurement in the radial immunodiffusion assay, and the good correlation between both assays ($r=0.86$) led us to use the radial immunodiffusion assay for all clinical measurements.

Among 50 normal individuals, serum C3bINA concentrations were distributed in a log-normal fashion. This appears to be the case for many plasma proteins including immunoglobulins and complement components (59). The mean serum concentration was 81.2% with ± 2 SD range of 57.9–116.5%. The latter is considerably narrower than has been observed for most other complement proteins (59). Results were expressed as per cent of a normal human serum pool because absolute quantification of the concentration has not yet been achieved. Preliminary data indicate that the mean value is in the range of 50–100 $\mu\text{g/ml}$.

In rheumatoid arthritis, subnormal serum concentrations did not occur, but supranormal values were found in 14 seropositive and 4 seronegative patients. This suggests that like C3, C4, and factor B, C3bINA is an acute phase reactant, concentrations increasing during chronic inflammatory conditions (21, 60).

Although the differences are for the most part small, the reduction in C3bINA levels in serum samples taken from SLE patients during exacerbation, and reduced concentrations in the synovial fluid from patients with rheumatoid arthritis compared with synovial fluid from

patients with degenerative joint disease, suggests that under certain circumstances, reductions in the concentrations of C3bINA occur during activation of the complement system.

Complete absence of C3bINA in vivo, as shown by a patient congenitally deficient in the enzyme (61) and in vitro, after immunochemical depletion (62), results in spontaneous activation of the alternative pathway. These findings suggest that perturbations in C3bINA concentrations may modulate the degree of alternative pathway activation. To test this hypothesis, we examined the correlation coefficients between concentrations of C3bINA and those of C3, factor B, and properdin and found good correlations in rheumatoid sera and synovial fluids. The correlation between C3bINA and factor B concentrations in synovial fluid may be explained on the basis that patients having low C3bINA concentrations do not inactivate C3b rapidly, thus allowing it to complex with factor B to form the alternative pathway C3 convertase, C3bB . This would also account for the correlation between C3bINA and properdin, as properdin is responsible for the stability of the C3bB enzyme (63), and, therefore, patients with increased formation of C3bB , i.e., those with low C3bINA concentrations, could theoretically utilize more properdin. Likewise, the correlation between C3bINA and C3 levels could be explained by increased cleavage of C3 by C3bB in patients with low concentrations. The lack of correlation between C3bINA and C4 concentrations is not unexpected as C4 does not appear to play a role in the properdin amplification loop.

In contrast to the situation in rheumatoid arthritis, in SLE, C3bINA concentrations did not correlate with C3 or properdin concentrations, and the correlation coefficient between C3bINA and factor B levels was only of borderline significance. Serial studies in a patient with SLE showed that at times reduction in serum C3bINA concentrations was related to reductions in the alternate pathway proteins, properdin, and factor B, and dissociated from C3 and C4 concentrations whereas at other times reduction in C3bINA was closely correlated with reductions in all four complement proteins. From the serial study shown in Fig. 5, it appears that glomerulonephritis may occur when serum C3 and C4 levels are normal, but when C3bINA, factor B, and properdin are low. This suggests that alternative pathway complement activation may exacerbate lupus nephritis in the absence of classic pathway activation. The failure of C3 or properdin levels to correlate with C3bINA concentrations in SLE also supports the notion that primary activation of the alternative pathway may occur in this disease. Such a conclusion is based on static measurements of the serum concentrations of C3 and C4, and in the absence of kinetic data, it is impossible to say that

classic pathway activation was not occurring. However, immunofluorescence studies of the kidney in glomerulonephritis have shown that certain glomeruli contain immune complexes, and both classic and alternative pathway complement components, whereas others show only properdin, C3-C9, but no immunoglobulin, C1q, or C4 (32).

The explanation for the lowered C3bINA concentrations could be due to decreased synthesis or increased catabolism of the enzyme, or both. In vitro studies in which functionally purified C3bINA was incubated with EAC43 cells showed that depletion of the enzyme did not occur (7). Similarly we have found that incubation of 10 mg of zymosan with 1 ml of normal human serum for 1 h at 37°C did not result in immunochemical or functional depletion of C3bINA, despite the complete cleavage of C3 and factor B. It is, therefore, unlikely that increased catabolism of C3bINA accounts for its low concentrations in biologic fluids, and decreased synthesis is probably a more important factor. However, this point can only be answered by studying turnover rates of purified radiolabeled protein. The control of the synthesis and catabolism of this essential homeostatic protein are of considerable importance in view of the evidence presented here that reductions in its concentrations may be important in modulating recruitment of the properdin amplification loop.

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