Metabolic Studies of the Third Component of Complement and the Glycine-Rich Beta Glycoprotein in Patients with Hypocomplementemia

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Abstract Metabolic studies using radiiodine-labeled third component of complement (C3) and the glycine-rich β glycoprotein (GBG), a major component of the C3b-feedback pathway, were undertaken in normal subjects, in 22 patients with evidence of complement activation, and in 11 patients with various renal diseases without evidence of complement activation. In seven normal subjects GBG was found to be a rapidly metabolized protein with catabolic rates ranging from 1.7% to 2.2% of the plasma pool/h, synthesis rates from 0.14 to 0.21 mg/kg per h, and extravascular/intravascular distribution ratios from 0.81 to 1.31. In patients with reduced plasma C3, both increased C3 fractional catabolic rates and reduced C3 synthesis rates were observed, and in some patients there was evidence of increased extravascular distribution of the protein. GBG catabolism was usually increased when there was evidence of C3 activation, presumably reflecting activation of the C3b-feedback; but GBG turnover was normal or only slightly accelerated in some patients with accelerated C3 catabolism and profound hypocomplementemia, suggesting that reduced C3 synthesis had limited activation of the C3b-feedback.

Introduction

Reduced plasma concentrations of the third component of complement (C3) are a feature of certain forms of glomerular disease, including acute post-streptococcal glomerulonephritis (AGN),1 nephritis complicating systemic lupus erythematosus (SLE), and mesangiopapillary glomerulonephritis (MCGN). Such hypocomplementemia may be accompanied by evidence of activation of C1, C4, and C2, indicating involvement of the classical pathway (as in SLE) or of an alternative pathway, where C3 activation is independent of these components. Of special interest has been the finding of a circulating C3 activator—the so-called C3 nephritic factor (C3NeF)—in the serum of patients with MCGN (1-3). More recently hypocomplementemia and a factor closely resembling C3NeF have been demonstrated in the serum of patients with partial lipodystrophy (PLD) (4, 5).

We have demonstrated that C3NeF causes C3 breakdown by activation of the C3b-feedback cycle, a mechanism independent of C4 and C2 (6). A major constituent of the C3b-feedback is the glycine-rich β-glycoprotein (GBG), now known to be identical with the C3 proactivator of Götze and Muller-Eberhard (7) and factor B of the properdin system (8). Activation of GBG results in the production of the glycine-rich γ-glycoprotein (GGG), which is a C3-convertase. This activation of GBG is brought about by a GBG-ase, a necessary component of which is C3b (9, 10). Activation of the C3b-feedback is therefore dependent upon the equilibrium between C3b generation on the one hand and C3b inactivation by the C3b inactivator (KAF) on the other. In the patient T. J., described by Alper, Abramson, Johnston, Jandl, and Rosen (11), it has been demonstrated that hypocomplementemia is due to persistent activation of the C3b-feedback, due to congenital absence of KAF (12); and a similar activation of the C3b-feedback can be produced in vitro by specific immunochemically depleted GBG; SLE, systemic lupus erythematosus.

Abbreviations used in this paper: AGN, acute post-streptococcal glomerulonephritis; C3NeF, C3 nephritic factor; CoF, purified cobra factor; C3PA, C3 proactivator; EV/IV, extravascular/intravascular distribution ratio; FB, fractional breakdown rate; R(GBG), reagent; J. A. CHARLESWORTH, D. GWYN WILLIAMS, E. SHERINGTON, P. J. LACHMANN, and D. K. PETERS

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Received for publication 18 October 1973 and in revised form 8 February 1974.
munochemical removal of KAF from normal human serum (9).

To examine the relationship between C3 activation and activation of the C3b-feedback cycle, we have studied the metabolism of radioactive labeled C3 and GBG in a variety of patients with hypocomplementemia. No studies of GBG metabolism in health or disease have yet been reported but previous studies of C3 metabolism in patients with hypocomplementemic MCGN have given variable results. Some workers (13, 3) have found reduced C3 synthesis, while others (14, 15) have found accelerated C3 catabolism to be the major immediate cause of hypocomplementemia.

METHODS

Subjects

Normal controls. 18 healthy members of the medical and technical staff received one or both labeled proteins.

Patients. Two groups of patients were studied. The first (22 patients) had diseases associated with hypocomplementemia. These were: MCGN, six patients; MCGN plus PLD, four patients; PLD without renal disease, two patients; SLE, five patients; AGN, three patients; one patient with recurrent urticaria of the type recently reported by McDuffie, et al. (16); and a patient with mixed cryoglobulinemia and glomerulonephritis, probably induced by repeated self-administration of a vaccine containing diphtheria and tetanus toxoids, and Hemophilus pertussis (17). The second group (11 patients) had various renal diseases with normal serum complement. The diagnoses were: chronic glomerulonephritis, six patients; acute tubular necrosis, two patients; focal glomerulonephritis, one patient; Goodpasture's syndrome, one patient; and membranous nephropathy associated with carcinoma of the bronchus, one patient.

Purification of complement components

C3, GBG, and KAF were prepared from hepatitis B-antigen-free serum obtained from healthy volunteers. A full description of preparative techniques is given by Lachmann, Hobart, and Aston (18). The purity of C3 and GBG was tested by immuno-electrophoresis and Ouchterlony gel analysis with antibodies specific for C3, GBG, and whole human serum. KAF was functionally tested by the method of Lachmann et al. (18).

Radio-iodination

Proteins were labeled with 125I or 131I by a modification of the chloramine-T method (19, 20). Human serum was added to the labeled proteins to reduce self-irradiation and the preparations were sterilized by Millipore filtration and pyrogen-tested as described elsewhere (20). Autoradiography of immunoelectrophoresis against antibody to whole serum revealed insignificant amounts of labeled contaminants, and precipitation with mono-specific antiserum removed 90% of radioactivity of labeled C3 and 94% of GBG.

Assessment of biological activity of labeled preparations

C3. Radiolabeled C3 was tested for hemolytic and conglutination activities (18) and for binding to EAC 142.

None of these functions was significantly altered by iodination.

GBG. This was tested with a reagent immunochemically depleted of GBG [R(GBG)] by precipitation of normal human serum with a F(ab)'s anti-human GBG, as described by Lachmann (21) and Nicol and Lachmann (9). The capacity of labeled GBG to produce a cobra-factor-dependent C3-convertase was then tested by incubation with purified cobra factor (CoF) and R(GBG). The activity of the C3-convertase generated was measured by the lysis of guinea pig erythrocytes in the presence of human EDTA serum (18). Addition of radio-labeled GBG restored the capacity of the R(GBG) to generate a CoF-dependent C3-convertase. When the labeled protein was administered intravenously to a rabbit, which was subsequently given a decomplementing dose of CoF, conversion of GBG to GGG in vivo could be demonstrated by autoradiography of immunoelectrophoresis of plasma samples.6

KAF-treatment of [125I]C3

To investigate the possibility that labeled preparations of C3 contain sufficient C3b to activate the C3b-feedback cycle in vivo, one preparation of [125I]C3 was incubated with purified KAF at a concentration of 0.05 mg/ml for 3 h at 37°C before injection.

Serum complement assays

Serum concentrations of C3 and GBG were measured by radial immunodiffusion and expressed in milligrams per 100 milliliter, having been standardized against purified preparations of these proteins. KAF and properdin were measured by radial immunodiffusion and the results expressed as percentage of reference pooled normal serum.

Detection of C3NeF was performed by crossed immunoelectrophoresis (22) as described by Peters et al. (3). Circulating breakdown products. Fresh EDTA plasma was examined by immuno-electrophoresis in 1.5% agarose, containing 0.02 M EDTA in sodium barbitural buffer at pH 8.6, with antibodies specific to C3 and GBG.

Metabolic studies

11 normal subjects and 30 patients received [125I]C3, of whom 17 (13 patients and 4 normal subjects) simultaneously received [131I]GBG. Three patients and three normal subjects were studied with [125I]GBG alone. Informed consent was obtained from each person before commencement of the study and the doses of radioactivity (20-30 μCi) were approved by the Medical Research Council. Thyroidal uptake of iodide was blocked by oral potassium iodide, 180 mg daily for 3 days, before radioactivity was administered. Each study was continued for 5-7 days. Plasma and urine specimens were processed as described by Charlesworth, Williams, Sherington, and Peters (20).

KAF-treated [125I]C3. One patient, who had previously been shown to have accelerated C3 turnover, received [125I]-C3 preincubated with KAF. The same [125I]C3 was also incubated for 3 h at 37°C, in the absence of KAF, and given to a healthy volunteer.

Assessment of red-cell-bound radioactivity. In seven subjects, red-cell-bound radioactivity was measured. This group included two normal volunteers, two patients with PLD,


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TABLE I

Metabolic Data for C3 and GBG in Normal Subjects

<table>
<thead>
<tr>
<th>Protein</th>
<th>Subjects</th>
<th>Half-life</th>
<th>FCR</th>
<th>EV/IV distribution ratio</th>
<th>Synthesis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 range</td>
<td>11</td>
<td>64–81</td>
<td>1.36–1.95</td>
<td>0.19–0.55</td>
<td>0.57–0.98</td>
</tr>
<tr>
<td>mean</td>
<td>71</td>
<td>1.66</td>
<td>0.35</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>GBG range</td>
<td>7</td>
<td>61–74</td>
<td>1.7–2.2</td>
<td>0.81–1.31</td>
<td>0.18–0.21</td>
</tr>
<tr>
<td>mean</td>
<td>66</td>
<td>1.98</td>
<td>1.07</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

one patient with PLD and MCGN, one patient with AGN, and one patient with SLE. Red cells were washed four times in complement fixation test diluent (Oxoid, Oxoid Ltd., London, U.K.) after separation from plasma. 2 ml of packed cells were then counted.

Methods of analysis of turnover data

Only data from patients judged to be in a steady state, i.e. in whom the serum concentration of GBG or C3 varied by less than 20% during the turnover studies, were included for analysis. The turnover data were analyzed by the following methods: urine/plasma ratios (23); exponential analysis (24); and integrated rate analysis (22), as previously applied (20).

Statistical analysis. Regression analyses were carried out by the method of least squares, and a nonparametric regression analysis used Spearman’s ranked correlation coefficient.

RESULTS

Normal subjects

Table I summarizes metabolic data for C3 and GBG. The values for t1 and fractional catabolic rate (FCR)

![Figure 1](image1.png)

**Figure 1** C3 concentration vs. C3 synthesis. The lines indicate the normal values for plasma C3 concentration (mean ±2 SD) and the normal range C3 synthesis rates. r = 0.643; P < 0.001.

![Figure 2](image2.png)

**Figure 2** Serum GBG concentration vs. FCR GBG. All values for GBG concentration fall in the normal range (10–50 mg/100 ml). r = 0.519; P < 0.01.

of GBG were similar to those for C3; however, extravascular/intravascular distribution ratios (EV/IV) for GBG were higher. Nossal’s analysis of the regression equation for FCR of GBG showed the value for the rate constant for extravascular catabolism must approximate to zero (25).

Patients

**Serum complement.** Reduction in serum C3 (less than 84 mg/100 ml) was found in 17 patients (Fig. 1). The diagnoses and numbers in each group were: MCGN, four; MCGN plus PLD, four; PLD alone, two; SLE, three; AGN, two; and the patients with urticaria and mixed cryoglobulinemia. C3d was detected in the plasma of 10 patients: MCGN, 1; MCGN plus PLD, 3; PLD alone, 2; AGN, 2; and the patients with urticaria and mixed cryoglobulinemia.

Sera of all patients were tested for C3NeF, this activity was detected in three patients with MCGN plus PLD, and two with PLD alone. Values for GBG (Fig. 2) fell within the normal range (10–50 mg/100 ml). GGG was never detected in fresh plasma. KAF levels were normal in all patients. Significant reduction in serum properdin (less than 40% of reference serum) occurred in four patients: two MCGN, one SLE, and one AGN (Fig. 3).

**Metabolic Data**

**C3**

Types of disappearance curve and their analysis. In patients with renal failure and iodide retention it was necessary to apply Matthew’s analysis (24) to the plasma curves. It became evident that in patients with hypercatabolism, two types of plasma curve occurred (Fig. 4).
In the first there was a linear disappearance curve, similar to normal subjects but with a steeper final exponential slope. In these patients when analysis of daily FCRs was possible by Nosslin's method, or by daily measurements of urine/plasma ratios, the FCR remained high throughout the study. However, in the second type the plasma disappearance curve was strikingly different, with early rapid clearance and a slow late phase (Fig. 4). In this type, daily measurements of FCR showed early rapid catabolism, slowing towards normal by the third or fourth day (Fig. 5). This group (nine patients)
plasma C3. In Fig. 6 an interrupted line has been drawn indicating the calculated relationship between C3 concentration and FCR C3, if synthesis rates and distribution had remained constant. At high values for FCR, C3 concentrations fall below this line. Fig. 1 shows the good correlation between calculated synthesis rates and plasma C3, and Fig. 7 that there is, in general, an inverse relationship between FCR-C3 and C3 synthesis, especially at high catabolic rates. In Figs. 1, 6, 7 the data from patient T. J. of Alper et al. (11) are shown: in T. J., C3 synthesis rates do not fall in the face of marked hypercatabolism. EV/IV ratios (Fig. 8) were increased in six patients, five of whom had marked hypocomplementemia and circulating C3d.

Red cell-bound radioactivity. This was measured in seven patients; in six of these, red cell radioactivity constituted less than 3% of the activity found in equivalent volumes of plasma. However in the seventh patient, a 26-yr-old nurse with SLE, auto-immune hemolytic

all had C3d in the circulation. For reasons which will be elaborated later, we believe that the slowing of catabolic rate is related to C3d becoming the predominant labeled protein. Accordingly, Matthew’s analysis could not be applied to this type of curve. In such patients, when renal function was normal, Nosslin’s analysis, using values obtained 24–48 h after injection of the labeled protein, was therefore used to determine FCR. However, in two patients with renal failure (one with AGN and one with MCGN and PLD) neither Matthew’s nor Nosslin’s method could be applied and a precise quantitative assessment of their obvious hypercatabolism of C3 could not be made.

Serum C3, C3 catabolism and synthesis, EV/IV distribution. Fractional catabolic rates for C3 greater than 2% h were found in 19 patients. The greatest increase in FCR was seen in patients with marked hypocomplementemia, and, as shown in Fig. 6, there was a good inverse correlation between log C3 concentration and FCR C3 ($r = 0.96$, $P < 0.001$). However, the increase in C3 catabolism alone was insufficient to account for the fall in
anemia, and normal renal function, significant binding of $[^{125}]$C3 was found. At 22 h 22% of the blood radioactivity was bound to erythrocytes.

KAF-treated $[^{125}]$C3. A patient with MCGN plus PLD, previously shown to have accelerated C3 turnover, was given KAF-treated $[^{125}]$C3. No reduction in turnover was observed.

**GBG metabolism**

10 out of 23 patients had FCR GBG greater than 2.2%/h. There was a significant inverse correlation between GBG concentration and FCR GBG (Fig. 2) but no relationship between GBG synthesis and GBG levels (Fig. 9). Synthesis values varied widely, being slightly reduced in one patient had normal or elevated in the remainder.

**Interrelationships between C3 and GBG metabolism**

C3 concentration vs. FCR GBG (Fig. 10). No correlation was observed ($r = 0.428, P > 0.05$). However, exclusion of patients with C3d in plasma left a group with a significant inverse relationship between serum C3 and FCR GBG ($r = 0.636, P < 0.01$).

FCR C3 vs. FCR GBG (Fig. 11). Inclusion of all subjects (13 patients and 4 normals) produced no significant relationship between catabolic rates of C3 and GBG ($r = 0.27, P > 0.1$). However, exclusion of the six patients with C3d in plasma again left a residual group with a significant correlation ($r = 0.83, P < 0.01$).

Serum properdin and C3 and GBG catabolism (Figs. 3, 12)

No correlation was observed, even when the comparison was limited to patients with PLD and MCGN plus PLD.

**C3NeF**

All patients with C3NeF had fast turnover, but accelerated turnover was observed in MCGN in the absence of detectable C3NeF.

**DISCUSSION**

The purpose of these experiments was to investigate the interrelationships of C3 and GBG metabolism in patients with hypocomplementemia. GBG is now known to be a major component of the C3b-feedback cycle and its rate of catabolism should therefore reflect the degree of activation of the feedback by C3b. No studies of the metabolism of GBG in health or disease have previously been reported.

Variable conclusions regarding the relative roles of hypercatabolism and reduced synthesis in the immediate causation of hypocomplementemia have been drawn in previous work on C3 turnover in MCGN (3, 13, 15). We have suggested (3) that the coexistence of C3 activation and depressed C3 synthesis is related to a sec-
**FIGURE 10** Serum C3 concentration vs. FCR GBG.

**FIGURE 11** Relationship between FCR GBG and FCR C3. Overall there is no significant correlation; however when patients with C3d in plasma are excluded, there is a good correlation between the catabolic rate of C3 and GBG ($r = 0.83; P > 0.01$).

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ondary reduction in synthesis resulting from in vivo complement activation. Our results show that hypercatabolism and depressed C3 synthesis occur together in patients with hypocomplementemia, either when complement is activated by the classical pathway (involving C1, C4, and C2) or when activation is known to be independent of these components, as occurs in MCGN (6) and in PLD (5). Depressed C3 synthesis has also been reported in hypocomplementemic SLE by Sliwinski and Zwaifler (26).

More detailed scrutiny of plasma protein radioactivity curves showed a complex pattern in some patients; this pattern is characterized by a rapid early clearance of radioactivity from the plasma, followed by a slowing of the disappearance rate, so that in some instances the slope finally reached a half-life greater than normal. In our patients with normal renal function, daily estimates of FCR showed high initial rates of catabolism, which fell into the normal range after 3-4 days. Analysis of total body radioactivity curves also indicated an abnormally high proportion of the C3 to be extravascular, suggesting extravascular sequestration of the label. We have found similar patterns of C3 turnover in rabbits in which C3 activation was caused by administration of CoF (27). In these animals, it was possible to demonstrate hypercatabolism and extravascular sequestration of C3 and to show that the slow phase of the plasma curve was associated with circulating labeled C3d, as shown by autoradiography of immunoelectrophoresis of fresh plasma. The doses of radioactivity used in our human studies were insufficient to permit such identification of labeled proteins, but all patients showing this pattern of early rapid catabolism had C3d detectable in fresh plasma samples. The complexity of C3 catabolism in such patients means that measurement of catabolic rates must be based on observations obtained during the first or second day of the turnover study, before C3d becomes the predominant labeled protein. This is undesirable, since denaturation of the protein may introduce errors, but there is no alternative method of avoiding the problem. Errors due to denaturation were minimized by the use of one highly purified C3 preparation and every patient was studied simultaneously with a healthy volunteer.

Our findings show that in addition to hypercatabolism, reduction in C3 synthesis is a major determinant of hypocomplementemia. This conclusion is supported by the in vitro observations of Colten, Levy, Rosen, and Alper (28) on short-term cultures of fresh liver biopsy material from patients with hypocomplementemic diseases. In our patients the reduction in synthesis was roughly proportional to the FCR’s of C3, and the greatest depression of C3 synthesis was observed in patients with circulating C3d. By contrast, in the KAF-deficient patient T. J. (11), who cannot generate C3d, C3 synthesis did not fall in response to hypercatabolism and the plasma radioactivity curves do not show a later slow phase. We have previously postulated (3) that C3 breakdown products may be responsible for reduced C3 synthesis in MCGN. Our latest findings support the suggestion by Alper, Bloch, and Rosen (4) that the generation of C3d, or at least the breakdown of C3b by KAF, may be the cause of the reduction of C3 synthesis.

Although the majority of hypocomplementemic patients showed elevated FCRs for C3, the net breakdown (i.e. the absolute catabolic rate) of C3 was reduced. However, this reduction in overall catabolism was associated with other unequivocal evidence of in vivo C3 activation (such as the finding of C3d in plasma). These observations indicate that C3 activation creates an abnormal pathway of C3 catabolism, since the same absolute amounts of C3 are catabolized under normal circumstances, without generating detectable quantities of C3d. It therefore seems unlikely that normal C3 turnover can be explained by the continued activation of the complement system by minor allergic reactions. Since the normal site of C3 catabolism is in close equilibrium with the extravascular compartment (13, 20), it is possible that the abnormal intravascular/extravascular distribution observed in some patients, together with the

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reduced C3 concentrations in plasma, results in a reduced proportion of the total C3 being catabolized by physiological mechanisms. Thus, although the overall rate of C3 breakdown might be reduced, a high proportion of C3 would be catabolized by a nonphysiological process, i.e., C3 activation, and reduced amounts by the normal mechanism. This interpretation would reconcile the apparently paradoxical observations of a reduction in the absolute rate of C3 catabolism on the one hand and the finding of C3 breakdown products in plasma on the other.

Our studies on the catabolism of GBG showed that this was a rapidly metabolized protein with fractional rates of catabolism comparable to C3. Unlike C3, no significant alterations in GBG synthesis rates were observed in patients with complement activation. The concentrations of GBG in the serum of healthy subjects may vary widely (29), but in the seven normal subjects in whom its turnover was studied, the GBG concentrations fell within a close range. We therefore do not know if the variation in serum concentrations amongst healthy subjects is due to variation in synthesis or catabolism.

The rate of turnover of GBG was used as a measure of activation of the C3b-feedback cycle. Stimulation of this cycle by C3b must depend on C3 synthesis, C3b generation by C3 activation, and C3b inactivation by KAF. In no patient was there significant lowering of KAF concentrations in serum; it therefore seems likely that C3b-feedback activity in this group of patients is principally related to C3 synthesis and C3 activation. On theoretical grounds, it could be anticipated that a moderate degree of C3 activation, associated with relatively little reduction in C3 synthesis, would generate more C3b and cause a faster turnover of GBG than would more marked degrees of C3 activation, where reduction in C3 synthesis could limit C3b generation. When patients with C3d (who had the most marked reduction in C3 synthesis) were excluded, there was a significant correlation between the FCRs of C3 and GBG, but this correlation was lost when patients with circulating C3d were included; thus one patient with MCGN and PLD (Fig. 11) showed markedly increased C3 catabolic rates and C3d in plasma, normal GBG concentrations, and no detectable increased GBG turnover. This patient, who had greatly reduced C3 synthesis, did not activate the feedback sufficiently to cause a detectable increase in GBG turnover, presumably because of failure to generate sufficient amounts of C3b.

One interesting feature to emerge from these studies is our failure to relate C3 or GBG turnover to plasma properdin concentrations. In MCGN, particularly the demonstration that C3NeF activates the C3b-feedback (6) and the finding of low plasma properdin and properdin in an altered electrophoretic form (30), together with its deposition in diseased glomeruli, (31) have suggested its involvement in the disease. However, our observations accord with our recent failure to demonstrate a requirement for properdin in C3 activation in vitro by C3NeF (32). The C3 turnover studies also show that C3 metabolism may be accelerated in MCGN, even when C3NeF cannot be detected in the circulation.

During the course of this study, the question arose whether C3b, if present in labeled C3 preparations, would be capable of initiating temporary activation of the C3b feedback cycle; KAF treatment of one labeled C3 caused no significant slowing in C3 turnover in a patient with hypercatabolism of C3, negating this possibility.

We do not know how much of the normal turnover of GBG is related to activation of the C3b-feedback; and, as with C3, there may be at least two separate mechanisms resulting in GBG catabolism, one from activation of the molecule by GBG-ase and another by a physiological process of catabolism independent of activation of the C3b-feedback.

ACKNOWLEDGMENTS

We thank Drs. J. S. Cameron, June Lloyd, J. Scopes, Professeur G. Richet, Liliane Morel-Maroger, and O. Kourilsky for allowing us to study patients under their care. We thank Miss Jane Fallows for expert technical help.

We would like to thank the Wellcome Trust for generous support.

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