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Modulation of the Alternative Pathway in Dilute Whole Serum by Introduction of Purified Components and Control Proteins FREE

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the co-factor activity was eluted as a peak with a m.w. of 1.5 $\times 10^6$ and separated from the protein peak of β_1H. On SDS-PAGE, a nonreduced sample does not enter the gel, however, a reduced sample gives a single band of approximately 80,000, suggesting that the co-factor is a large disulfide-linked polymer. The co-factor migrates in the β-globulin region on Pevikon blocks. The macromolecular weight co-factor acts as a co-factor for cleavage of both C4b and C3d by C3bINA, whereas β_1H acts as a co-factor for cleavage of only C3b, not for C4b, by C3bINA.

Affinity chromatography of C3bINA with C3b-Sepharose. Although C3bINA alone is incapable of cleaving C3b, it appeared to loosely interact with C3b, so we used C3b-Sepharose as a biospecific adsorbent. A partially purified C3bINA was prepared from human plasma. When the C3bINA fraction was applied to a column of C3b-Sepharose (0.5 M NaCl, pH 7.0), most of the proteins were recovered in the pass-through fraction, whereas the C3bINA was bound and eluted with 0.15 M NaCl. The C3bINA thus obtained gave a single band on Disc-PAGE as well as SDS-PAGE. The reduced sample gave two protein bands with m.w. of about 52,000 and 40,000, respectively, on SDS-PAGE.

During the course of this study, we found a new plasma protein, which forms a complex with C4b and inhibited the cleavage of C4b by the C3bINA macromolecular weight co-factor. The protein is a β-globulin with a m.w. of 92,000 to 100,000. The protein differed immunochemically from C2 and β_1H. This protein was also found to form a complex with C3b and to inhibit the cleavage of C3b by C3bINA-β_1H.

Analysis of the Peptide Maps of Human C2 and Factor B: Evidence for Structural Homology and Common Ancestry. Bo-Yee Ngan and J. O. Minta. Departments of Pathology, University of Toronto and the Toronto Western Hospital, Toronto, Ontario, Canada M5S 1A8.

C2 and Factor B are heat-labile β-globulins in normal human serum and are the precursors of the C3 and C5 convertase enzymes of the classical and alternative complement pathways, respectively. Genes controlling C2-deficiency and polymorphism and Factor B polymorphism have been linked to the HLA locus. In this study, proteolysis of C2 and Factor B have been compared by a peptide mapping technique on SDS polyacrylamide gel electrophoresis. C2 and Factor B were purified from the 20% Na_2SO_4 supernatant of human plasma by sequential column chromatography and by preparative gel electrophoresis in barbital-glycine buffer, pH 8.8. The highly purified C2 and Factor B sedimented at 5.2S and 5.0S, respectively, and were hemolytically and esterolytically active. The m.w. for C2 and Factor B estimated by SDS polyacrylamide gel electrophoresis in the presence or absence of reducing agents were 117,000 and 93,000 daltons, respectively, suggesting that each protein is comprised of a single polypeptide chain. Treatment of C2 with $\overline{C1s}$ resulted in the cleavage of the polypeptide chain into two fragments, C2a (86,000) and C2b (38,000). Factor B, however, was not cleaved by $\overline{C1s}$. Incubation of Factor B or C2 with trypsin or *Staphylococcus aureus* protease followed by peptide-mapping analyses in SDS polyacrylamide gel electrophoresis revealed that the two substrates were cleaved in a similar pattern. C2 was initially cleaved into two fragments, "C2a" (80,000) and "C2b" (37,000), and the major fragment was then further cleaved with the release of a small peptide (m.w. 15,000) from a residual fragment (m.w. 65,000). Factor B was also cleaved initially into two fragments "Bb" (72,000) and "Ba" (34,000). The major fragment was further cleaved to give two fragments (m.w. 60,000 and 16,000). The similarities in the cleavage pattern of C2 and Factor B by two enzymes of entirely different specificities may be indicative of some common structural homologies and raises the possibility that the two proteins may have evolved from a common ancestral origin.

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A Role for IgG in the Activation of the Human Alternative Pathway by Rabbit Erythrocytes. Ben Nelson and Shaun Ruddy, Medical College of Virginia, Richmond, Virginia.

A serum factor which participates in the early steps of the lysis of rabbit erythrocytes (E) by human serum in Mg-EDTA has been partially characterized. Normal human serum (NHS) when adsorbed with rabbit E or stromata at 0°C in the absence of divalent cations, lost 35% of its hemolytic activity against fresh rabbit E. The feedback loop activity (B, D, P) of the adsorbed serum was intact. The lost hemolytic activity was restored with a pH 3.4 eluate from the adsorbed stromata; the activity in the eluate has been termed "0° factor E."

When 0° factor E was added to serum depleted of 0° factor E by prior absorption with rabbit E, a linear dose response was obtained which was concave to the abscissa, indicating that a single activity was being added. The activity was unaffected by heating to 56°C for 30 min and had an apparent molecular weight of 700,000 on Sepharose 4B gel filtration. An eluate from zymosan which had been similarly adsorbed with NHS at 0°C did not reconstitute rabbit E-adsorbed NHS, indicating a degree of specificity in the factor's function in the alternative pathway.

An assay for 0° factor E in whole serum was used to measure levels in NHS and three hypogammaglobulinemic sera. The assay detected levels of 0° factor E which correlated closely with the IgG levels of the corresponding sera. The major protein in preparations of 0° factor E was IgG. The specificity, heat stability, lack of ion requirements and correlation in serum with IgG levels all suggest that 0° factor E is either IgG or a complex thereof.

Modulation of the Alternative Pathway in Dilute Whole Serum by Introduction of Purified Components and Control Proteins. U. Nydegger, D. T. Fearon, and K. F. Austen. Departments of Medicine, Harvard Medical School and Robert B. Brigham Hospital, Boston, Massachusetts.

Activators of the alternative pathway of complement such as zymosan, rabbit erythrocytes, and heat-killed *E. coli* protect bound C3b from C3bINA and P, C3b, Bb from decay-dissociation by β1H, thereby amplifying C3b generation and deposition. The capacity of purified components and control proteins to modulate alternative pathway activation by zymosan and rabbit erythrocytes (E^r) was assessed in dilute C2-deficient human serum.

Zymosan-induced inactivation of C3 and B was determined at different intervals after 100 μg of zymosan were added to 150 μl of serum diluted 1:6 inVBS^{++}. The mean inactivation of C3 and B at 45 minutes was 61 ± 5.4% (±1 SD) and 62 ± 5.7%, respectively. Various amounts of the control proteins were added to the serum to determine the increase over endogenous concentrations necessary to inhibit 50% of the zymosan effect. Increases of C3bINA by 43% and of β1H by 25% inhibited 50%

of zymosan-induced inactivation of C3. The corresponding increases for 50% inhibition of B inactivation were 70% in C3bINA and 43% in β1H. The 50% inhibitory effect of the combined additions of C3bINA and β1H was completely reversed by increasing either C3 by 60% or B by 50% above endogenous concentrations. Since a 1:7 dilution of C2-deficient serum in the presence of 1×10^8 E^r resulted in 50% lysis after 28 ± 1.2 minutes, the 30 minute time point in this system was chosen to determine the increment of control protein necessary to inhibit 50% of lysis. Under these conditions, a 40% increase of C3bINA and a 22% increase of β1H over endogenous concentrations were sufficient for 50% suppression of lysis. When these amounts of the control proteins were added simultaneously there was complete inhibition at 30 minutes. This effect was fully reversed and lysis even increased to 90% when the B concentration was augmented by 50%.

These experiments suggest that the component proteins C3 and B, because of the normal presence of $\bar{D}$ in serum, form an activation system that is equilibrated with the control proteins C3bINA and β1H. This equilibrium is sensitive to modest alterations in the concentrations of individual components. The increments in component and regulatory proteins utilized in these experiments are well within the ranges observed in patients with infections and other inflammatory conditions.

Studies of the Alternative Complement Pathway (AP) in Normal Children. Michael E. Norman, Arlene Taylor, Paul Green, Larry Laster, and Ulf R. Nilsson, University of Pennsylvania School of Dental Medicine, Phila., Pa.

Increasing evidence suggests that activation of complement by AP contributes an important protective mechanism in early defense against bacterial invasion, before effective antibody levels are established. Since this is particularly important in children, we undertook a survey of AP in 83 normal children, ages 3 days to 15 years ($\bar{x}$ = 54 months). There were approximately equal numbers of males and females, blacks and whites, and all children were free of fever and intercurrent infection. C3, Factor B, properdin,[1] and β_1H were quantitated by radial immunodiffusion with monospecific antisera; hemolytic function of AP was monitored by lysis of glutathione-treated human erythrocytes with inulin activated serum (N. Engl. J. Med. 286:180, 1973). Data were compared to normal adults:

	C3 (mg/dl)	Factor B (mg/dl)	Properdin (%)[a]	β_1H (mg/dl)	AP Lysis (%)[a]
Children	124 ± 24[b] (72–183)	19 ± 7 (6–39)	80 ± 30 (28–170)	129 ± 44 (30–248)	72 ± 33 (0–121)
Adults	119 ± 17 (73–145)	18 (12–30)	95 (67–190)	171 ± 23 (148–234)	91 ± 14 (64–109)

[a] Percentage of normal reference serum pool; [b] mean values ± 1 S.D.

There was a wide range of values when compared to adults except for C3. An intercorrelation matrix revealed that each parameter of AP in normal children was highly related to every other parameter ($p < .001$) and to age, as we had previously reported for C4 and C5 (J. Pediat. 87:912, 1975). Data were further analyzed statistically by a principal factor analysis in order to examine the underlying relationships among the variables. Results of these studies will be presented.

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[1] Anti-properdin antiserum was the generous gift of Dr. Roger Spitzer.

Antibody Responses to a T Cell Dependent Antigen in C4 Deficiency. Hans D. Ochs, Charles G. Jackson, Michael M. Frank, Stephen W. Hosea, Ralph J. Wedgwood. Department of Pediatrics RD 20, University of Washington School of Medicine, Seattle, Washington 98195 and the Clinical Immunology Section, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

Complete absence of the fourth component of complement has been described in man and in guinea pigs. Recently, a close linkage between the gene(s) controlling synthesis of C4 and the major histocompatibility complex has been demonstrated in man as well as in the guinea pig.

Because of a possible relationship between immune responses and the complement system, we have studied antibody responses to a T cell dependent antigen in a patient and in guinea pigs with C4 deficiency. Bacteriophage $\Phi\chi$ 174, given intravenously, was cleared within one week after primary injection, similar to normal controls. The primary antibody response was markedly suppressed and shortened: in normal controls, antibody titers (mean Kv > 8) persisted for over 4 weeks; in C4 deficiency, antibody (peak titer < 1) disappeared 2–3 weeks following immunization. In the normal control population, a second injection of antigen resulted in a rapid rise of antibody (Kv > 100) and transition from IgM to IgG. In contrast, both the C4-deficient patient and the C4-deficient guinea pigs lacked amplification of antibody production (Kv < 1) and failed to switch from IgM to IgG antibody. Again, the antibody in the C4-deficient population disappeared rapidly. Bacteriophage $\Phi\chi$ 174, mixed in complete Freund's adjuvant and injected into the footpad resulted in a continuous antibody rise for over 7 weeks in the normal guinea pigs (peak Kv > 1000). The antibody response in the C4-deficient guinea pigs was again depressed (mean Kv = 120) although the titer was much higher than in the nonadjuvant treated guinea pigs.

These experiments suggest that C4 may play an important role in antibody production and that a functioning classical complement pathway may be necessary for antigen trapping and processing.

The C2 Polymorphism: Its Genetics and Presentation of Typing Technique Modifications. B. Olaisen, P. Teisberg, E. Thorsby, and T. Gedde-Dahl Jr., Institute of Forensic Medicine, Rikshospitalet, Oslo; Medical Department 7, Ullevaal Hospital, Oslo; Tissue Typing Laboratory, Rikshospitalet, Oslo; Genetics Laboratory, Radiumhospitalet, Oslo.

The originally described C2 typing technique has been modified to allow typing on ordinary electrofocusing equipment, without the use of specific C2-deficient serum. The modifications include prolonged electrofocusing time, iodine treatment of gels after focusing, and the use of low concentration normal human complement as "C2-lacking complement."

In a Norwegian population sample C2 gene frequencies were: $C2^1$: = 0.97, $C2^2$: = 0.03, these frequencies fit well with those reported in other Caucasian populations.

C2-linkage relations and haplotype associations have been examined in an extensive Norwegian family material. No recombinations were found in 22 informative *C2/HLA-B* meioses and the rare $C2^2$ allele was accompanied by the *HLA-Bw15* allele in 6 out of 7 instances. Our data therefore confirm the linkage of *C2* to the HLA region loci, and we conclude that *C2* is situated in close proximity to the *HLA-B* locus.