The Journal of Immunology

RESEARCH ARTICLE | MAY 01 1978

The C5 Chemotactic Fragment Isolated from Activated Human Serum FREE

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J Immunol (1978) 120 (5): 1764.
https://doi.org/10.4049/jimmunol.120.5.1764.b

and B and depletes C3; but C5 through C9 are spared. CVF^h and CVF^n were found to be equally resistant to $C3bINA^{hu}$.

When CVF^n was incubated with normal human or guinea pig serum, an activity was generated which was detectable as a zone of hemolysis in agarose plates containing guinea pig erythrocytes and EDTA, when placed near a well containing human or rat, but not guinea pig, CEDTA. Experiments with partially purified components showed that this activity corresponded to the CVF^n-Bb complex, in that it could be generated from B, $\bar{D}$, CVF^n and Mg^{++} and required all of these. CVF^h did not generate such an activity, showing that CVF^h-B, if formed, is not a C5 convertase. Incubation mixtures of factors, B, $\bar{D}$, and CVF destroyed $C5^{hu}$ and generated $C\bar{5}6$ from $C5^{hu}$ + $C6^{hu}$ only if formed from CVF^n and not if formed from CVF^h, whether or not additional C3 was present.

The differences between CVF^n and CVF^h are best explained by the failure of CVF^h-B to exhibit C5 convertase activity.

***In Vivo* and *in Vitro* Inhibition of Complement by Chlorazol Fast Pink.** N. Bauman and J. A. Brockman, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York 10965

Chlorazol Fast Pink (CFP) is a sulfonic acid azo dye, first described as an *in vitro* complement inhibitor in 1952 (Lambert & Richley, B. J. Exp. Path. *33*, 327, 1952). We have examined CFP *in vivo* and *in vitro* and found that it inhibits complement dependent lesions in laboratory rodents, and that these effects are correlated with depression of serum complement. In some cases, CFP has been compared with pentosan polysulfate (PPS), another anionic complement inhibitor.

CFP and PPS, injected intraperitoneally, block Forssman shock and Forssman cutaneous vasculitis in guinea pigs and the reverse passive Arthus reaction in guinea pigs and rats. Inhibition of these reactions is accompanied by an inhibition of serum complement, demonstrable by an assay of "undiluted" serum. In this assay, serum is laid over EA embedded in an agarose matrix in a capillary tube, and the zone of lysis measured with an optical micrometer. The geometric constraints result in an assay of complement with minimal dilution of any inhibitory compound in serum.

In vitro, CFP and PPS both potentiate the reaction of $C1^{hu}$ and C1-INH in a manner analogous to that of heparin. However, neither heparin nor PPS potentiates this reaction with guinea pig components, so the inhibition in guinea pig serum must be by some other mechanism.

The C5 Chemotactic Fragment Isolated from Activated Human Serum. D. Beebe, S. Goralnick, C. Gerard, J. Ozols and P. A. Ward. Departments of Pathology and Biochemistry, University of Connecticut Health Center, Farmington, CT 06032

A chemotactic factor for rabbit and human polymorphonuclear leukocytes antigenically related to C5 has been isolated from human serum activated by zymosan in the presence of ϵ-amino caproic acid (EACA). Antiserum to the purified chemotactic factor as well as antiserum to human C5, inihibits the chemotactic activity in human serum activated by zymosan, aggregated IgG, immune complexes, or bacterial lipopolysaccharide. The chemotactic activity of bacterial (*E. coli*) factor is not inhibited by either antiserum. The C5 chemotactic factor is purified by a combination of gel filtration and ion exchange gel chromatography. Analysis for anaphylatoxin activity in fractions from each step of the procedure indicates that the muscle-contracting activity is separated from the bulk of the chemotactic activity in the anion exchange step. The purified chemotactic factor migrates as an α globulin as assessed by elution activity in agarose. These results suggest that two separate biologically active peptides are produced from C5 by activation of the complement system: C5a which possesses anaphylatoxin and chemotactic activity and a second, nonanaphylatoxin, C5 fragment that accounts for the majority of the chemotactic activity.

Cooperation of Complement (C) and Polymorphonuclear Leucocytes (PMN) in the ingestion, killing and destruction of C-resistant *E. coli*. R. Berto and J. Menzel*, Institut für Immunologie und Serologie, Universität Heidelberg, Germany

Attachment of bacteria to phagocytic cells is augmented by opsonins due to Fc and C3 receptors. It has also been shown, that intracellular killing even of C-resistant bacteria is increased following opsonization by C, but not by IgG. The mechanism of this C activity is yet unknown. C activation could lead either to an increased ingestion or increased discharge of lysosomal enzymes into the phagosome. The contribution of C activation to ingestion, killing and destruction of *E. coli* and to the secretion of lysosomal enzymes into the phagosome was therefore the subject of this investigation.

To a monolayer of human PMN radiolabelled *E. coli* were added following opsonization with IgG, C or IgG and C. After various time intervals, cells and nonattached bacteria were separated, cells were destroyed and the cell sap was separated by centrifugation into three fractions: 1) membrane (and nuclei), 2) granula, and 3) cytoplasma. The granula fraction was further separated into lysosomes and phagosomes by zonal sedimentation on a sucrose gradient. Radioactivity (total bacteria) and viable bacteria as well as lysosomal enzymes were determined in all fractions.

Ingestion, i.e. transport of membrane-associated bacteria into the phagosome was independent of opsonins; however, the destruction of *E. coli* was more rapid when C was used for opsonization compared to IgG alone. But no corresponding increase of lysosomal enzymes in the phagosome was observed.

The killing of *E. coli* in the fractions was determined by the ratio of total/viable bacteria. The ratio was smaller in the membrane fraction as compared to the combined granular and cytoplasmatic fraction. However the ratio was much greater than one in all fractions, indicating that killing was performed both at the cell surface and in the cell. Only killing at the cell surface was increased upon activation of C.

It was concluded that the bactericidal system of PMN consists of a membrane-associated and an intracellular part. C and membrane bound enzymes or lysosomal enzymes discharged during attachment and ingestion may cooperate at the cellular surface leading to increased intracellular destruction in spite of a decreased ratio enzyme/bacterium in the phagosome.

(Supported by the Deutsche Forschungsgemeinschaft ME 336/4.)