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Influence of Lymphokine on Complement Components Synthesis by Guinea Pig Spleen Cells *In Vitro*

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(CA limiting), PNH III E and two normal E showed a) EM lesions in proportion ot ^{125}I-C5 binding; and b) lysis of normal E was associated with 4-19 times as many EM lesions as found on PNH E with equivalent lysis. With equal input of CA and C, PNH E (both types II and III) exhibited 2-3 times more C5 binding and 5-10 times more EM lesions than normal E.

The differences in C5 binding/lysis ratios between PNH and normal E were less prominent when lysis was initiated by rabbit Ab to human E, particularly in an Ab limited/C excess system. With *equal* input of both rabbit Ab and human C, C5 binding was similar for PNH and normal E, but with greater lysis of PNH E.

In other studies in which C5 convertase was presented on a surrogate cell (sheep EAC423^{hu}) in the presence of C5-C9, ^{51}Cr release from "bystander" PNH II and III E was comparable to that from normal E. In later studies, using greater input of C8 and C9, lysis of PNH III E could exceed normal E lysis by 1.2-2.5 fold, depending on the normal E donor.

These experiments raise the possibility that there may be 2 lytic defects in type III PNH E: 1) a mildly increased sensitivity to the terminal C5-C9 complex, as shown by the bystander studies (not sufficient to account for the 20-25-fold increased lysis induced by Ab); 2) an abnormality in the assembly, stability, or inhibitability of C3 or C5 convertase complexes on PNH E.

Molecular Interactions of the Control Proteins C3b Inactivator and β1H with Various Complement Intermediates. Pangburn, M. K., Schreiber, R. D., and Müller-Eberhard, H. J. Research Institute of Scripps Clinic, La Jolla, Calif. 92037

Radioiodinated β1H was demonstrated to bind to sheep EAC4b, EAC4,2,3b and EC3b, but not to E, EA, EAC4,2 or to C3bINA and β1H treated EC3b. This binding was dependent upon the C3b concentration on the surface of the cells and could be inhibited by unlabeled β1H, free C3b or high concentrations of Factor B. The presence of activated properdin on EC3b cells did not affect β1H binding. Binding of radioiodinated C3bINA was demonstrated by stabilizing the enzyme substrate complex EC3b,βIH,C3bINA at 0°C. Binding of ^{125}I-C3bINA to EC3b in the presence of β1H was 30-fold greater than in the absence of this cofactor. C3bINA binding was approximately stoichiometric with respect to bound β1H and this interaction had an apparent binding constant at 0°C of 10^8 M^{-1}.

Fearon and Austen (J. Exp. Med. *146:* 22, 1977) have reported that C3b on rabbit erythrocytes is protected from C3bINA and β1H action. We have examined the binding of ^{125}I-β1H to rabbit and sheep cells and found that E_RC3b bind only 10% as many β1H molecules per C3b as E_SC3b. The binding of C3bINA was directly proportional to the amount of β1H bound on both cell types. E_RC3b and E_SC3b demonstrated identical Factor B, $\bar{P}$ and anti-C3 Fab binding properties. These results suggest that while the alternative pathway components have unhindered access to C3b on rabbit erythrocytes, β1H (and consequently C3bINA) is only 10% as effective on these cells. A similar protective effect could be generated on the surface of sheep erythrocytes by treatment with neuraminidase. β1H binding was reduced to 30% of that of untreated sheep cells whether the neuraminidase treatment was performed before or after C3b was attached to the cells. Furthermore, neuraminidase treated sheep E were lysed by C4 depleted human serum, untreated sheep E were not. Thus, neuraminidase treatment results in the simultaneous generation of a surface that protects from β1H control and one which activates the alternative pathway in serum.

When C3 and Factors B and D were incubated together at serum concentrations with E_R, C3b deposition via the amplification pathway occurred only at C3bINA and β1H concentrations which controlled fluid phase C3 consumption. While the mechanism of initial C3b deposition in this system is not clear, the role of C3bINA and β1H appears to be to confine C3b amplification to a surface which protects C3b.

Interaction Between Lymphocytes and Fluid Phase Murine and Human C3b. M. Papamichail and M. B. Pepys. Dept. of Immunology, Royal Free Hospital, London NW3 2QG and Dept. of Medicine, Royal Postgraduate Medical School, London W12 OHS

C3 participates in lymphocyte cooperation leading to antibody formation and in antigen-localization by germinal centers. These functions probably involve the adherence of fixed C3b to complement receptors on lymphocytes and possibly macrophages. During investigation of the role of C3 in antibody formation in human and murine *in vitro* culture systems, the interaction of purified fluid phase C3b with lymphocyte complement receptors was studied by immunofluorescence. Human peripheral blood and tonsil complement receptor lymphocytes (CRL) were stained by incubation with human or murine C3b followed by specific $F(ab)_2$ anti C3-FITC. Murine CRL failed to stain with these same reagents, despite the fact that murine C3b blocked formation of complement-dependent rosettes. Binding of mouse C3b to murine CRL was directly demonstrated using C3b itself labeled with fluorescein and could be blocked by unlabeled mouse C3b. It affected the same cells as formed C3-dependent rosettes, none of which were T lymphocytes. Initial "ring" fluorescence progressed rapidly at 37° to patching, capping, and endocytosis, all of which was inhibited at 4° or by the presence of azide, cytochalasin B, chlorpromazine, or lignocaine. Prefixation of the cells with paraformaldehyde also yielded preparations showing only ring fluorescence. The failure to detect mouse C3b binding by indirect fluorescence was not due to endocytosis or other membrane modulation after initial adherence since it was unaffected by prefixation of cells or the presence of cytochalasin B or chlorpromazine throughout. These observations suggest that the interaction of mouse C3 with mouse cells differs from that with human cells so that the relevant antigenic determinant on the C3b are rendered inaccessible to antibody. Further evidence of difference between the C3b receptors on the two cells was provided by the fact that human C3b labeled with rhodamine stained human CRL very clearly but mouse CRL not at all. Human C3b-Rho stained the same CRL as made rosettes with EAC^{hu} or EACmo and there was cross-inhibition between human C3b-Rho and mouse C3b-FITC, indicating that they both reacted with the same receptor.

Influence of Lymphokine on Complement Components Synthesis by Guinea Pig Spleen Cells *In Vitro*. A. P. Peltier and A. Cambier, Hôpital Lariboisière, Paris, France.

A large part of complement components are synthetized by cells of the macrophage-monocyte type which are under narrow control by lymphokines. Preliminary results obtained in our laboratory have shown that addition of phytohemagglutinin M to a culture of whole normal guinea pig spleen cells decreased

the amount of C2 detectable in the culture supernatant at the same time where it strongly stimulated lymphocyte proliferation, as shown by a high rate of cellular 3H-thymidine incorporation. One possible explanation for this phenomenon would be the inhibition by some lymphokine of C2 synthesis by macrophages.

In order to substantiate this hypothesis, a two step experiment was designed. Whole normal guinea pig spleen cells (0.5 ml of 20×10^6/ml suspension) were first cultivated for 24 hours in the presence of an optimal 50 μg dose of insoluble concanavalin A. The supernatant obtained was assayed for its lymphokine activity by a classical Macrophage Inhibition Test. In the second step of the experiment, increasing volumes of this lymphokine preparation (0.1 to 2 ml) were added to a culture of adherent guinea pig spleen cells (3 ml of a 25×10^6/ml preparation). Supernatants of this second culture were collected at day 8 and 15, and assayed for their haemolytic C2 and C1 concentrations.

The results obtained show that, after addition of lymphokine at day 0 of the second culture, the C2 levels at day 8 and 15 decrease regularly and significantly, while the C1 levels increase or remain unchanged. A dose-effect relationship is observed between the decrease of C2 detectable and the amount of lymphokine used. Further experiments indicate that this decrease of C2 is due to a diminished cellular synthesis of this component and not to its inactivation by the C1 present or by any product of the cultivated adherent cells.

Stable Evolutionary Conservation of Complement-Activating and Related Pentagonal Serum Proteins: C-reactive Protein and Protein SAP. M. B. Pepys, A. C. Dash, Dept. of Medicine, RPMS, London. N. Richardson, E. A. Munn, A. Feinstein, Dept. of Immunology, ARC, Babraham. Thelma C. Fletcher, Institute of Marine Biochemistry, Aberdeen.

Human CRP efficiently activates the classical complement pathway via C1q. Protein SAP (amyloid P-component) is a normal plasma protein which resembles CRP structurally, but differs in not being an acute phase reactant. Both proteins can interact with cell membranes but their *in vivo* functions are not known; they are presumably important since, as we now report for the first time, very similar molecules are also present in the sera of diverse species including elasmobranchs, teleosts, amphibia, birds and non-primate mammals. CRP and protein SAP were isolated by calcium-dependent affinity chromatography on insolubilised pneumococcal C-polysaccharide and agarose respectively, followed by gel filtration. The different proteins all had pentagonal disc-like molecules in the electron microscope and there were marked similarities between the CRP and SAP of such diverse species as the plaice and man. The pure proteins each yielded homogeneous subunits in SDS-PAGE with molecular weights of between 20–30,000 in different species.

The existence of this family of proteins, some of which activate complement, stably conserved in such a wide range of species is of considerable interest and is the subject of continuing investigation particularly with regard to whether SAP as well as CRP interacts with components of the complement system.

A New "Inhibitor" of Complement (C5)-Derived Chemotactic Activity. H. Daniel Perez, Donna Manning, Mark Lipton, and Ira M. Goldstein, N.Y.U. Medical Center, New York, N.Y.

In the course of examining polymorphonuclear leukocyte (PMN) chemotaxis in patients with systemic lupus erythematosus (SLE), we have found a previously undescribed serum "inhibitor" of complement (C5)-derived chemotactic activity. Serum from a 25-year-old Black female with untreated SLE, when activated with zymosan, failed completely to attract either her own or normal PMN (measured by the "leading front" method of Zigmond and Hirsch). Incubation of normal PMN with the patient's serum (PS) did not affect random motility or chemotaxis towards normal zymosan-treated serum (ZTS). Whereas levels of C3 were modestly low in PS, no gross abnormalities involving alternative complement pathway activation could be detected. Furthermore, treatment of PS with zymosan resulted in the generation of normal amounts of C5-derived PMN lysosomal enzyme releasing activity (attributable to "C5a"). Incubation of normal ZTS or column (Sephadex G-75)-purified "C5a" with PS did not affect lysosomal enzyme releasing activity but did result in a significant dose-dependent diminution of chemotactic activity. PS, however, did not influence the chemotactic activity of either casein or bacterial chemotactic factor (from *E. coli*). Chromatography of PS (65% $(NH_4)_2SO_4$ pellet) on Sephadex G-200 yielded three distinct peaks of "inhibitory" activity. Two were heat-labile (as previously described) whereas the third and most active peak (in the molecular weight range of 40-50,000 daltons) resisted heating at 56°C for 30 minutes. Similarly treated normal serum yielded only the two heat-labile peaks of "inhibitory" activity. Thus, we have found a new, heat-stable serum "inhibitor" of complement (C5)-derived chemotactic activity which does not affect at least one other biologic activity of "C5a".

SC5b-9: Regeneration of the Ability to Interact with Lipid by Selective Removal of the S-Protein. Podack, E. R., Halverson, C., Esser, A. F., Kolb, W. P. and Müller-Eberhard, H. J. Research Institute of Scripps Clinic, La Jolla, Calif. 92037.

The various complexes were prepared by mixing isolated C5b-6 in the presence of detergents or phospholipids with equimolar amounts of C7, C7 and C8, or with C7, C8 and C9 in molar ratios of 1:1:1:3. C5b-9 was also prepared by treating the SC5b-9 complex purified from inulin activated serum with 6 mM Na deoxycholate (DOC) at pH 8.1 and subjecting it to gel filtration on Sepharose 6B in the presence of DOC. This treatment resulted in selective dissociation of the S-protein, in exchange for binding of DOC by C5b-9.

Detergent-binding studies showed that C5b-6, C5b-7 and C5b-8 bind 26, 28 and 65 mole ^{14}C-DOC per mole of complex, respectively, as measured by equilibrium dialysis. C5b-9 (prepared from SC5b-9) binds 86 mole DOC per mole of complex, measured by gel filtration in the presence of ^{3}H-DOC. DOC binding as well as cetyl trimethyl ammonium bromide (CTAB) binding of all complexes could also be demonstrated by charge shift electrophoresis. Non-ionic detergents do not bind to C5b-6, C5b-7 or C5b-9.