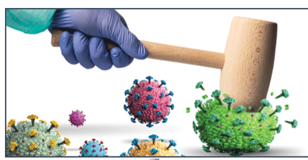
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The Capacity of Lymphoblastoid Cells to Activate the Alternative Complement Pathway Is Correlated with their Transformation by Epstein-Barr Virus

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C1s. Recently, Leibovitz (Cancer Res. *36*:4562, 1976) established and characterized several colorectal adenocarcinomas into three groups based on morphology, modal chromosome number, and ability to synthesize carcinoembryonic antigen (CEA). Analysis of two cell lines from each group for $C\bar{1}$ synthesis showed that group 1 cells, which produced the lowest level of CEA, synthesized $C\bar{1}$, whereas group 2 and group 3 cells did not.

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The Capacity of Lymphoblastoid Cells to Activate the Alternative Complement Pathway Is Correlated with their Transformation by Epstein-Barr Virus. I. McConnell, P. J. Lachmann and T. Lint, MRC Group on Mechanisms on Tumour Immunity, Cambridge, England and G. Klein, Karolinska Institute, Stockholm, Sweden.

Cultured human lymphoblastoid cells derived from patients with Burkitt's lymphoma or infectious mononucleosis have been shown to activate the alternative complement pathway by an antibody-independent mechanism. Lymphocytes from normal or leukemic patients do not have this activity.

All lymphoblastoid cells and most lymphoma lines derived from patients with Burkitt's lymphoma carry Epstein-Barr virus DNA in their genome. Recently, EBV-negative Burkitt lymphoma lines have been established and these can be converted to EBV-positive sublines by superinfection *in vitro* with EBV. The existence of these lines allows us to test the hypothesis that transformation of cells with EBV confers on them the property of activating the alternative pathway.

Two EBV-negative lines and eight EBV converted sublines were tested for their ability to activate the alternative pathway in hypogammaglobulinaemic and normal human serum. Activation was assayed by demonstrating bound C3 on the membrane by immunofluorescence; by measuring C3 conversion in the serum by the Laurell technique; and consumption of total alternative pathway activity in serum by using a hemolytic assay.

By all three criteria it was shown that activation of the alternative pathway by the EBV negative cell lines was slight and was markedly enhanced in the EBV-converted sublines. By immunofluorescence there was a striking qualitative difference in the pattern of C3 deposition between EBV-negative and EBV-positive cells. The rate at which C3 conversion occurred was also greater for the EBV-positive than for the EBV-negative lines.

The correlation of this cell-associated complement activation with EBV transformation of the cells may provide a mechanism whereby EBV-transformed cells are normally controlled or eliminated *in vivo*.

Mechanism of Endotoxin-Initiated Complement-Dependent Platelet Lysis. David C. Morrison and Zenaida G. Oades, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037.

We demonstrated earlier that bacterial endotoxins (lipopolysaccharides, LPS) have the capacity to activate both pathways of complement, with the polysaccharide portion of the molecule activating the alternative pathway and the lipid A region being responsible for classical pathway activation (Morrison, D. C. and Kline, L. F., 1977, J. Immunol. *118*:362). We further showed that only preparations of LPS which initiate alternative pathway activation are capable of LPS-initiated complement-dependent rabbit platelet lytic responses. (Morrison, D. C. *et al.*, 1976, Fed. Proc., *35*:516).

Although the lipid A region of the LPS molecule is, itself, incapable of initiating lysis, our current experiments provide strong evidence that it does, in fact, play a critical role in the rabbit platelet response to LPS. Treatment of LPS with mild alkali (using conditions known to enhance binding of LPS to both erythrocytes and lymphocytes) significantly enhanced the extent of the complement-mediated platelet lysis. Such conditions have no detectable effect on the polysaccharide region of the LPS molecule but do alter the ester-linked fatty acids associated with the lipid A. Incubation of platelets with LPS prior to the addition of complement promoted LPS binding and significantly increased the subsequent extent of lysis. Finally, preincubation of platelets with either purified lipid A or polysaccharide-free LPS isolated from the *S. minnesota* R595 mutant (neither of which can initiate complement-dependent lysis) markedly inhibited the subsequent ability of polysaccharide-containing LPS preparations to initiate lysis. Preparations of LPS which contain bacterial protein bound to the lipid A (termed LAP, lipid A-associated protein, Morrison, D. C. *et al.*, 1976, J. Exp. Med., *144*:840) are significantly enhanced in their capacity to initiate the lytic response, as compared with LAP-free LPS. Since our data have demonstrated that the LAP content of LPS does not influence its capacity to activate the alternative pathway of complement, these data would suggest that LAP facilitates the binding of LPS to the platelets.

Our data would support the hypothesis that the LAP-lipid A region of the LPS molecule is responsible for attaching the LPS to the platelet membrane and that the polysaccharide portion of the LPS molecule subsequently provides the appropriate surface for the assembly of alternative pathway components resulting in platelet bystander lysis.

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C3bINA and its Macromolecular Weight Co-factor; Purification and Characterization. Shigeharu Nagasawa (Hokkaido University, Japan) and Robert M. Stroud (University of Alabama in Birmingham).

The complement system is proteolytically modulated by the C3bINA-C4bINA system which is generally believed to cleave C3b into two fragments, C3c and C3d. However, little is known as to the exact mechanism of the proteolytic action of C3bINA on C3b and C4b. Recently, we documented the requirement of a macromolecular weight co-factor for the proteolytic function of C3bINA (*Immunochemistry*, in press). On the other hand, it was recently reported that β_1H plays a role also as a co-factor for the proteolytic activity of C3bINA.

In the present study, we have purified the macromolecular weight co-factor and characterized it as a new serum protein. Also, we have highly purified the C3bINA by affinity chromatography with C3b-Sepharose, in order to study the cleavage mechanisms.

Purification and characterization of the macromolecular weight co-factor. The co-factor was purified from human plasma by chromatography with i) QAE-Sephadex A-50 (0.2 M NaCl, pH 8.0), ii) heparin-Sepharose (0.5 M NaCl, pH 8.0), iii) Bio-Gel A-15m (0.3 M NaCl, dextrose, glycine, pH 8.0), iv) Bio-Gel HTP (0.15 M phosphate, pH 7.5), and finally with v) DE-52 (0.3 M NaCl, pH 8.0). Upon gel filtration on Bio-Gel A-15m,

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