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Deviated Lysis: Generation of the Activity by Physicochemical Means

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C3-EDTA in 10 minutes at 27°C, is totally inactive when INH is added during the formation of the insoluble C3 convertase. On the contrary, normally preformed ZX is only partially inhibited.

The C3/C5 convertase of the alternative pathway fixed onto sheep red cells as an EA, 4b, 3b, Bb intermediate, shows a partial susceptibility to the inhibitor: 60 percent inhibition of lysis is obtained when the factor is present during the formation of such convertase. To the contrary, no inhibitory effect is obtained when NF is present in the mixture, nor does INH shorten the half-life of NF-stabilized C3/C5-solid phase-convertase.

The CH 50 level of human serum is reduced from 450 to 210 CH 50 units/ml by INH: this inhibitory effect is totally reverted by the addition of purified C3. The distribution of INH is not yet definitively established: it is present in other, but not all, tested patients with NF, and seems present at very low concentration in NHS. Its small molecular weight distinguishes this factor from other substance(s) isolated from grafted tissues. It is not likely to be a product of C3 catabolism as its level in the serum of the original patient is not correlated with that of C3 degradation products, C3c and C3d.

The Physiological Breakdown of C3b. R. A. Harrison and P. J. Lachmann, Department of Biochemistry, Imperial College, London, England and the MRC Group on Mechanisms in Tumour Immunity, the Medical School, Hills Road, Cambridge, England.

The requirements for and products of C3b inactivator (KAF) action on C3b have been studied. C3 was purified from human serum. Final traces of contaminating proteins were removed by passage through a Sepharose "anti-impurity antiserum" column. C3b was generated by using insolubilized cobra venom factor convertase, and separated from C3a by chromatography on hydroxyl-apatite.

The KAF-dependent cleavage of fluid phase C3b was found to have an absolute requirement for β1H (or possibly a contaminant of β1H preparations); neither KAF nor β1H alone had any discernable proteolytic action on C3b. The initial pattern of cleavage was found to be the same with purified KAF and β1H or with serum (as a source of these factors). The α-chain of C3b was cleaved into two polypeptide chains, an α^{1a} chain of about 68K and an α^{1b} chain of about 46K. The α^{1b} chain was rapidly 'trimmed' to about 43K(α^{1b1}). All of these three α-chain fragments were still covalently bonded to the β-chain. On antigenic analysis at this stage there was still a single component—presumably C3bi. More prolonged incubation with the β1H lead to cleavage of the α^{1a} polypeptide, yielding finally a product of 29K (α^{1a2}) no longer covalently bound to the β-chain. This is presumably C3d.

C3c purified from aged human serum contained three chains at 75K (β), 43K (α^{1b1}), and 29K (α^{1a2}). We therefore conclude that C3d is a breakdown product of C3c and not formed concomittant with it. This further cleavage of C3bi to C3c and C3d did not occur with DFP-treated β1H whereas DFP had no effect on the initial cleavages of C3b to C3bi.

In addition to β1H a second protein of 85K affecting the action of KAF + β1H on C3b has been identified. This protein acts as an inhibitor of C3b cleavage, and this inhibition is overcome by excess β1H. As yet it has not been possible to show any relationship between this protein and other known components of C.

During this study we have also observed two further splits of the α-chain. In the first, a fragment of about 80K was seen, apparently arising from cleavage at the KAF/β1H site of the intact α-chain. In the second, a 10K fragment was lost from the α'-chain of C3b when C3 was offered to EAC142 cells, but not when C3b was put onto erythrocytes or inulin in a bystander fashion. KAF/β1H treatment of the C3 product that decayed off the cells produced two chains at 58K and 43K (α^{1b1}). This suggests that the 10K fragment is part of the α^{1a}-chain of C3bi, and is located at one end of the α'-chain.

Deviated Lysis: Generation of the Activity by Physicochemical Means. Gertrud Hänsch, Ursula Rother, and K. Rother, Institut für Immunologie der Universität Heidelberg, Heidelberg, Germany.

The term deviated lysis (d.l.) describes a particle bound or fluid phase C function, which lyzes unsensitized cells (E) in the presence of EDTA. Its generation via the classical or the alternative pathway has been described. The activity was stable in the fluid phase. It was found in the region of 180,000–220,000 daltons or 9.7S, respectively, and consisted of C5, C6, C7, C8 and C9. Once generated, the activity could be blocked by the respective antibodies, but not by anti-C3 or anti-B.

We now report on a similar activity generated by exposing human serum either to acid pH or low ionic strength. As in C activation by inulin, the factors C5–C9 are involved. Although factor B is converted when normal human serum is brought to pH 6.4, C3 is not. C3, factor B, or properdin, respectively, are not needed. The activation takes place at 0°C within seconds.

It is proposed that physicochemical influences such as low ionic strength or low pH can facilitate the interaction of the C5 through C9 proteins in solution, thus generating a lytic potential. On a sucrose gradient, the main activity peak is found at 9.7S, again, similar to results on d.l. generated by C activation with inulin. Upon incubation with the target cells at 0°C, the factors C5–C9 were found to attach in a sequential manner. D.l. activity in the fluid phase, whether generatd by bypass activation or physicochemical means, thus does not seem to be associated with a pentamolecular complex of C5 through C9. Rather, it is a mixture of single component activities.

The Inhibition of B Lymphocyte Stimulation by C3b Inactivator. Klaus-Ulrich Hartmann, Abt. für Experimentelle Immunologie, Universität Marburg, Germany.

Purified human C3b added to mouse lymphocyte suspensions triggers stimulation of up to 50% of the B lymphocytes; the stimulation was detected by increased incorporation of thymidine, blast cell formation, and antibody secretion (K.-U. Hartmann and V. A. Bokisch, J. Exp. Med. *142,* 600, 1975). Purified human C3 does not stimulate these lymphocytes. Addition of purified C3b inactivator (C3bINA) (M. K. Pangburn, R. D. Schreiber and H. J. Müller-Eberhard, J. Exp. Med. *146,* 257, 1977) - 1 to 2 μg C3bINA to 100 μl lymphocyte cultures - strongly inhibited the stimulation of B lymphocytes induced in the presence of C3b or LPS. This inhibition was not dependent or enhanced by the addition of purified human β1H-globulin. The inhibition caused by the addition of C3b INA was reduced or prevented by the presence of FCS. Preincubation of C3b with C3bINA did not inactivate the activity, leading to stimu-