A FUNCTIONAL DEFICIENCY OF C1q IN 3 FAMILY MEMBERS. *A.J. Hannema, H.C. Kluin-Nelemans, C.E. Hack*, C. Mallée, H.P.T. van Helden, A.J.M. Eerenberg-Belmer;* Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam and St. Antonius Hospital, Utrecht, The Netherlands.

Two sisters and a brother from one family are described with a funtionally deficient C1q. All 3 had suffered from glomerulonephritis during childhood. The 2 sisters had recently a SLE-like disease (butterfly erythema, oral ulceration, arthralgia, alopecia, grand mal seizure in one, and leukopenia in the other.) The brother is apparently healthy although a microscopic hematuria is present.

By radial immunodiffusion (RID) with anti-C1q a faint but measurable precipitation ring was found only after staining the plate. By double immunodiffusion a partial identity of this C1q with normal C1q was found. The abnormal C1q gave a line of complete identity with reduced and alkylated C1q. This abnormal C1q was found in all 3 patients. The sera from the sisters had no hemolytic activity, however the brother had a CH50 titer of 24% of normal serum. The hemolytic defect was restored by the addition of purified C1q to the serum. Less than 0.1%, of C1q was present in the sera from the sisters, the brother had 0.1%, as measured by hemolytic assay. C1r, C1s and $\overline{C1}$-inhibitor were normal or increased as measured by RID. Aggregated IgG (AHG) did not activate C1 in any of the sera, as was demonstrated by the $\overline{C1}$-inhibitor complex assay. Also, no C4 or C3 were fixed to AHG, though the abnormal C1q showed some affinity for AHG. Both defects were restored by the addition of C1q to the sera. The abnormal C1q sedimented in the 4.6 S position. This pattern was not changed in the presence of Ca-ions. The abnormal C1q probably represents the 3 chains A, B and C of C1q.

A $\overline{C1}$-INHIBITOR-COMPLEX ASSAY (INCA): A METHOD TO DETECT C1 ACTIVATION IN VITRO AND IN VIVO *C.E. Hack*, A.J. Hannema, A.J.M. Eerenberg-Belmer, T.A. Out and R.C. Aalberse.*

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We developed a radioimmunoassay (the $\overline{C1}$-inhibitor-complex assay, INCA) for the detection of complexes that are composed of at least $\overline{C1}$s and $\overline{C1}$-inhibitor. This INCA is based on demonstrating that C1s and $\overline{C1}$-inhibitor ($\overline{C1}$-In) are linked: after an incubation with anti-C1s-Sepharose, bound $\overline{C1}s\overline{C1}$-In complexes are detected by ^{125}I-anti-$\overline{C1}$-In. $\overline{C1}s\overline{C1}$-In complexes were prepared by the addition of a slight excess of $\overline{C1}$s to normal human serum (NHS). As little as 2 ng $\overline{C1}$-In bound to $\overline{C1}$s was detected. Additional free C1s in serum hardly influenced the detection of $\overline{C1}s\overline{C1}$-In complexes.

Complexes presumably composed of $\overline{C1}rC\overline{1}s(\overline{C1}\text{-In})_2$, were generated by the addition of aggregated IgG to NHS. This generation was inhibited by lowering the temperature to 0°C, and by EDTA, and depended on the concentration aggregated IgG. These complexes had a sedimentation value of approximately 9S. Complexes of $\overline{C1}$s and $\overline{C1}$-In were also generated in NHS by the addition of DNP-albumin and protein A, but not by zymosan.

The INCA was aplied to blood samples from normal donors and patients. Sixteen out of 19 samples from patients with acute glomerulonephritis contained increased amounts of $\overline{C1}rC\overline{1}s(\overline{C1}\text{-In})_2$ complexes as compared with the amounts in blood samples from normal donors. Results obtained from a longitudinal study of a SLE patient indicate that the half-life time of $\overline{C1}rC\overline{1}s(\overline{C1}\text{-In})_2$ complexes is at most 24 hrs.

The INCA provides a useful tool to assess the activation of $\overline{C1}$ in the presence of $\overline{C1}$-In, both in vitro and in vivo.

C3b INDUCES THE RELEASE OF PGE AND TXB_2 FROM PERITONEAL MACROPHAGES *U. Hadding*, H.P. Hartung, H. Rasokat, D. Gemsa* Inst. of Med. Microbiology, Univ. Mainz and Inst. of Immunology, Univ. Heidelberg, FRG.

A range of inflammatory stimuli have been shown to cause an increase in sythesis and relaease of protaglandins by macrophages. In order to elucidate interactions of C3b with macrophages, we investigated its role in inducing the release of arachidonic acid oxygenation products. From 10-80 μ g/ml homologous C3b was added to 1.5 x 10^6 albumin-elicited guinea pig peritoneal macrophages cultured in 1.5 ml medium in the absence of serum. After 18 hrs Prostaglandin E (PgE) and Thromboxane B_2 (TxB_2) release into culture supernatant was determined by radioimmunoassy. C3b dose-dependently stimulated the release of PgE. Its effect was comparable to that of zymosan, a well-known inducer of prostaglandin synthesis (75 μg/ml C3b: 6.5 ng PgE/ml vs. 50 μg/ml zymosan: 5 ng PgE/ml). C3b also stimulated macrophages to release significant amounts of TxB_2 e.g. 80 μg/ml C3b caused release of 16.5 ng TxB_2/ml. Kinetic studies determining TxB_2 levels in supernatants at timed intervals after stimulation with C3b indicate that release to most part is completed within 12 hrs (2 hrs: 2.23; 6 hrs: 10.76; 12 hrs: 17.97; 18 hrs: 19.81 ng/ml). Release of PgE and TxB_2 was almost abolished if the C3b preparation was passed over an anti-C3 immunosorbent column or if 0.5 μg/ml indomethacin was added to cultures simultaneously with C3b. Addition of the Fab fragment of anti-C3 IgG did not diminish the stimulatroy action of C3b nor did Fab of unrelated IgG. In conclusion, C3b is a stimulator of the snythesis and release of arachidonic acid oxygenation products in peritoneal macrophages. Our findings lend further support to the view that C3b is a factor capable of modulating macrophage functions and thereby affecting activities of cooperating and bystander cells.