these methods CSF cells and peripheral blood lymphocytes from patients with either bacterial meningitis, aseptic meningitis, encephalitis or multiple sclerosis were investigated. Intracytoplasmatic IgG, IgM and IgA as well as Pan T-cells, helper and suppressor subsets and natural killer cells were determined. Results and correlations between these parameters will be discussed.

University of Konstanz, Faculty of Biology, 7750 Konstanz, FRG

## I.39 Covalent binding of insulin to alpha-2-macroglobulin

K. Keck, D. Auer, H. Gröger, A. Meichle, and A. Eckert

We intended to isolate «processed antigen» by incubating antigen presenting cells with radiolabeled insulin and fractionating the culture supernatant on sephadex G200. A small fraction of labeled material was eluted with the void volume of the column. Surprisingly a similar fraction was obtained if insulin was incubated with medium alone or with fetal calf or human serum. This fraction contained the insulin covalently bound to a high molecular weight serum protein. SDS-electrophoresis and binding to specific antibody demonstrated that the insulin binding protein is the protease inhibitor alpha-2-macroglobulin (a2M). The insulin is bound to this protein by two types of stable bounds. Most of the insulin could be split off by treatment with dithiothreithol, indicating a disulfide bound. A small part of the insulin is stable to this treatment and may stem from reaction with the a2M's activated thioester. Non denaturing polyacrylamide gel electrophoresis demonstrated, that similar to protease/a2M complexes, insulin/a2M complexes migrate as the electrophoretic fast form. It has been shown by other authors, that the conformational change from the slow (native a2M) to the fast form is accompanied by the exposure of a receptor recognition site, by which macrophages can bind the a2M. Some experiments were done in order to find out, wether or not reaction with insulin could influence the binding of a2M to the macrophage receptors in a similar way as reaction with proteases. Clearing experiments with mice demonstrated, that a2M/insulin complexes were rapidly cleared with a half life of about 5 minutes, they are bound by the same receptors, which bind a2M/protease complexes. We conclude, that a2M may participate in two biological functions: clearing the insulin from the circulation and concentrating it on macrophages.

[1]I. Medizinische Universitätsklinik Kiel, [2]Abteilung Immunologie der Universität Kiel, FRG

## I.40 Determination of total immunoreactive rat insulin (IRI) in culture supernatants of rat islets by an enzyme linked immunosorbent assay (ELISA) as a routine method to assess the viability of rat islets prior to their use in islet transplantation

J. Kekow[1] and K. Ulrichs[2]

Several radioimmunoassays (RIA) are used as routine methods for the determination of IRI in human sera. Antibodies recognizing human insulin are easily provided by guinea pigs immunized with porcine insulin. The application of RIA or conventional ELISA is limited when rat insulin has to be determined. This results mainly from the low affinity of available

anti-insulin antibodies to rat insulin in comparison with porcine or human insulin. In order to easily screen large quantities of cultivated rat islets for their viability, especially their capacity for insulin secretion after stimulation with glucose, a new ELISA method was developed. This is an important tool to assess the value of islets prior to their use in islet transplantation. The ELISA is based on the principle of sequential saturation of an anti insulin antibody with preoxidase-labeled/unlabeled insulin. First, microtitre plates were coated with a 1:1000 dilution of a goat anti guinea pig serum (coating buffer pH 9.6). Then the anti insulin antibody (M8309, Novo, DK) was added after extensive washings. This antibody was used in a 1:200 dilution made by an isotonic incubation buffer at pH 7.4 and with 6 % serum albumin. This coating procedure was necessary to obtain sufficient insulin binding. After repeated washings standards of rat insulin or dilutions of rat islet culture supernatants were transferred into the microtitre plates and incubated overnight at 4 °C. The next day, a peroxidase-labeled porcine insulin (Sigma) was added for another incubation period of 4 h at 4 °C. Then the microtitre plates were washed again and orthophenylenediaminedihydrochloride (OPD) as substrate was added. The extinction was measured at 489 nm in a microtitre plate reader. The standard curve ranged from 0.8 ng/ml to 100 ng/ml rat insulin. Statistical analysis showed an acceptable accuracy characterized by a standard deviation in the variation within/between an assay of 10–15 %. In summary, the advantages of this ELISA are a high capacity, the determination of rat insulin from 0.8 ng/ml–100 ng/ml (RIA 0.5 ng/ml–10 ng/ml), less consumption of anti insulin antibodies in comparison with RIA, and no use of radionucleotides whose application is limited by their stability.

Institut für Immunologie und Genetik, DKFZ, Heidelberg, FRG

## I.41 Expression of cDNAs of the Ia associated invariant chain, HLA-DR beta chain and T cell receptor alpha chain in E. coli

SUSANNE KOCH, A. SCHULZ, and N. KOCH

There is a number of immunologically relevant molecules, against which antibodies with a desired specificity are lacking. There are no antibodies against constant parts of the antigen receptor on T cells (TCR) available. All reagents described so far are directed against clonotypic determinants or recognize only small subpopulations of T cells. In case of the HLA-DR antigens antibodies against allotypic determinants as well as against constant parts of the molecules would be desirable. We used a molecular genetic approach to construct recombinant expression plasmids, containing entire cDNAs or parts of them which permit expression of the respective proteins in *E. coli* as fusion proteins with MS2 polymerase. Using this method we obtained large amounts of the constant region of the TCR alpha chain, of the entire HLA-DRw6 beta chain, of tis N-terminal variable part, of its C-terminal constant part and of the Ia-associated invariant chain. Immunization of mice with the N-terminal variable portion of the HLA-DR beta chain will be used to obtain monoclonal antibodies against polymorphic determinants of this molecule. All of the genetically engineered molecules were used to immunize rabbits. The specificity of the antisera obtained so far will be shown.

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