Abstracts of Poster Papers

Generically-specific common antigenic epitopes on Salmonella flagellins and applications of their monoclonal antibodies

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The multiple epitopes of common antigen on Salmonella flagellins were defined by a panel of monoclonal antibodies in Immunogold and Indirect Immunofluorescent test. This result was further verified by detecting these epitopes on the expression product of flagellin gene $fliC^i$ in E. coli Lc-2a. These common antigenic epitopes were different from that of serotypic H antigens, and they presented on flagellins of both phase I and phase II. The properties of these epitopes were assayed in SDS-PAGE, Western blotting, and ELISAs. The distribution of these epitopes was assessed by examining the binding patterns of each MAb to a set of 219 Salmonella strains covering A through O-67 serogroups and 96 other enteric bacteria including E. coli, Shigella, Citrobacter, Klebsiella, Proteus, Yersinia, Enterobacter and Serratia. It was found that these common epitopes showed the generic specificity of Salmonella.Furthermore, an EIA method was developed based on two MAbs CB8,de7 for detecting these epitopes on Salmonellae.The results of identification of 892 Salmonella isolates and detection of Salmonella contamination from 10785 samples of food,feed and clinical specimens were coincided well with conventional culture method. This showed that these common epitopes on Salmonella flagellin molecules could be used as a new recognized marker for the genus of Salmonella.

Key words: Salmonella; flagellin; common epitope; monoclonal antibodies

* Supported by National Natural Science Foundation of P. R. China.

Monoclonal antibodies against human adenovirus type 4 proteins

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Adenoviruses are causative agents of acute respiratory infections as well as conjunctivitis and gastroentheritis. In addition, adenoviruses have provided an excellent model system for the study of fundamental processes in eucaryotic cells and being useful vectors for gene therapy. Monoclonal antibodies (Mabs) are very powerful tool for this research. We used human adenovirus type 4 (Ad4) as a model. It should note that this virus is of clinical significance being the cause of epidemic conjunctivitis. We obtained 23 hybridomas after fusion myeloma cells Sp2/o with spleen cells from mice immunized with Ad4. Finally, I4 from them were selected for amplification as ascitic fluids. This ones were tested by dot immunobinding, immunofluorescence, radioimmunoprecipitation, ECL Western blotting, neutralization and passive haemagglutination. Our special interest was Mabs against capsid and core proteins.So, we obtained some Mabs which had strong binding with major capside protein, hexon. They shown diffuse nuclear fluorescence in Ad4 infected cells as well as in cells infected with Ad2 or Ad7. So, this ones have group cross-reactivity and it was also confirmed by immune precipitation with labelled cells infected with Ad4, Ad2, Ad7. The information on epitope mapping of the Ad4 hexon were received from Mabs competitive binding assay. For this purpose we developed the simple, sensitive and rapid "a competitive avidin-biotin ECL dot-blot assay". As a result we determined that Ad4 hexon molecule contain at least 2 different non-overlapping epitopes with group-specificity, and each contain 2 overlapping epitopes. We obtained also Mabs against internal core protein VII. This ones reacted with protein VII of the human adenoviruses type 2, type 5 (subgroup C), type 7 (subgroup B), type IO (subgroup D) and type 4 (subgroup E) of course but was not binding with Ad40 (subgroup F) and AdI2 (subgroup A). We used Mabs for examination of synthesis of the Ad4 virus polypeptides and epitope mapping. The last one for protein VII was realized partly by selection with Mabs of the active peptides from phage peptide library. The potential consensus sequence was $RX_1YX_2PX_3$ which possible correspond sequence in Ad2 protein VII RNYTPT. The corresponding peptide was synthesized and it was active in direct and competitive immunoassays. Thus this peptide represents the linear epitope in composition of the protein VII with group specificity. Functions of this one as well as other epitopes are under investigation now.

Acknowledgements. We wish to thank Dr. P.Szawlowski for work with phage peptide library as well as his advice.

Antitumor Activity of Monoclonal Antibody CIBCNSH3 Generated to the Human EGF Receptor

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The Overexpression of the human epidermal growth factor receptor (EGFR) has been demonstrated in many human malignancies like squamous cell carcinoma of the head and neck, cervix, breast etc. which are most prevalent in India. This is often associated with poor prognosis and high mortality in these patients. It has been reported that Monoclonal antibodies generated against EGFR which have the capacity to exhibit binding of ligands like EGF, TGF etc to their receptor have antitumor activity and hence great therapeutic application in the management of patients who do not respond to other treatment modalities like chemotherapy and endocrine therapy. In the case of breast tumors, Estrogen Receptor negative tumors have been found to be mostly EGFR positive. For these patients immunotherapy using specific antibodies directed to EGFR might prove to be of clinical value. One such Monoclonal antibody designated CIBCNSH3 has been generated in our laboratory which has been found to recognize an epitope in the extracellular domain of EGFR by immunoprecipitation and Western blot. By immunoperoxidase test, this antibody was found to exhibit strong reactivity to EGFR in head and neck cancers and breast cancers studied whereas with lymphoma cell lines like SUD6 and human lymphoma tissues, no staining was observed. This antibody inhibited the binding of EGF to its receptor on MDAMB 468 breast cancer cells rich in EGFR as revealed by competitive binding assay using 125_I EGF indicating its antitumor activity.

In vitro studies performed using cell lines in culture like MDAMB 468, HN5 etc with overexpression of EGFR revealed 90% cell death when incubated with the antibody. The in vivo therapeutic efficacy has been demonstrated by performing Tc99m immunoscintigraphic studies on mice bearing these tumor xenografts before and after treatment with the monoclonal antibody. A total dose of 1.5 mg effected complete tumor regression which was also confirmed by histopathological studies. This monoclonal seems to have promising future application as therapeutic agent for tumors which overexpress EGFR.

IMMUNOCHEMICAL APPROACH TO STUDY THE ROLE OF THE HIGHLY CONSERVATIVE EPITOPE OF E1 IN THE FUNCTION OF THE PYRUVATE DEHYDROGENASE COMPLEX

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The multienzyme pyruvate dehydrogenase complex (PDC) catalyzing the oxidative decarboxylation of pyruvate involves three different enzymes, i.e., EC 1.2.4.1 (E1), EC 1.8.1.4 (E2) and EC 2.3.1.12 (E3) Little is known about the mechanism of self-assembly of the multienzyme system, in particular, of the role of E1α and E1β subunits in this processe. For the first time monoclonal antibody (mAb) F7F10 against E1 component of PDC from pigeon breast muscle has been produced. The dissociation constant of the E1-mAb complex was determined to be 59.3 nM. It was shown that mAb F7F10 cross reacted with E1 components of PDC from various species (including Escherichia coli and human) and did not react with other thiamine diphosphate dependent enzymes. The existance of highly conserved epitope common for pro- and eucaryotes indicates the essential role of the antigenic amino acid sequence which is unique for PDC. The immunoblotting data showed that the mAb F7F10 interacted with both α and β subunits of E1. It suggests that amino acid residues of both subunits contribute to the antigenic determinant. This supposition is sustained by the fact that each isolated subunit also interacts with the mAb. It was shown that mAb F7F10 has no influence on E1 activity measured in model reaction with artificial electron acceptor. However it inhibited the full NAD and coenzyme A dependent activity of the whole PDC. The competition of the F7F10 antibody with the E2 component of PDC for the binding with E1 was revealed by immunoenzymatic and kinetic analysis. Thus mAb F7F10 is assumed to interact with an antigenic determinant, located in the immediate vicinity of or overlapping with the E1 region, responsible for E1-E2 interaction within the PDC. The data obtained suggest that while assembling native PDC it may be the E1β subunit that binds to the E2 component.The formation of the antigen-antibody complex disturbs the E1-E2 interaction thus blocking the coordinated mechanism of catalysis. A number of severe human pathlogies are known to be due to congenital defect of E1 component of PDC. In this work mAb F7F10 was used to devise a model solid-phase immunoenzyme method for quantitative assay of E1 and PDC. The detection limit of the method is 3.2 ng/ml for E1 and 79 ng/ml for PDC. Thus mAb F7F10 obtained can be used at least twofold: i) to locate and examine the contribution of various fragments of E1α and E1β subunits to the formation of functional domains and to intersubunit interaction areas, underlying the multienzyme complex formation; ii) on the other hand mAb obtained are useful when creating express-diagnostics for estimating concentrations of the complex components.

In vitro production of human antisperm antibodies as probe of autoimmune response associated with immunological infertility

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A questions concerning the etiology of human infertility associated with antisperm antibody (As-Ab) production have been discussed for many years. The most important result from the development of isoimmune or autoimmune response against sperm antigens, due to a breakdown of sperm immune tolerance by different mechanisms, is manifested by As-Ab production. For these reasons, identification and analysis of antigens, recognized by As-Abs which interfere with fertilization, is the most important object in the study of the immunological infertility.

A strategy for obtaining human monoclonal antisperm antibodies using the method of Epstein-Barr Virus (EBV) transformation of peripheral blood lymphocytes (PBLs) from infertile women possessing high serum titers of sperm immobilizing antibodies (SI-Abs) , combined with 'pick-up' cloning method has been developed to investigate immunological infertility associated with sperm isoantigens. Using PBLs from 16 infertile patients with SI-Abs, we succeeded to obtain three stable cell populations (designated B1, B2, D5) of transformed PBLs originated from three different patients. They produced IgM SI-Abs directed against antigens expressed on the tail of live, methanol fixed and $NaIO_4$-treated human spermatozoa.

The established As-Abs recognized noncarbohydrate sperm membrane antigens with different specificity and distribution in male reproductive system. As-Ab B2 corresponding antigen seems to be specific for the male reproductive system. This antigen is excreted from the epithelial cells of ductus epididymidis and binds to the spermatozoa in the lumen of the ductus. As-Abs B1 and D5 corresponding antigens were expressed on the spermatozoa in the seminiferous tubules and were common to the secretions of ductus epididymidis, prostate and some other somatic organs.

We are now attempting to obtain an information concerning the DNA sequence of the variable region of human Ig heavy chain of the established As-Abs B1, B2 and D5. This analysis will contribute to study the generation and diversity of As-Abs associated with human immunological infertility.

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The new conceptual model of immune response of the lymphocytes

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The new conceptual model of immune response (Titova 1996 in press) is presented. It includes basic propositions, part of which was known earlier but did not take into account, the other are postulated here: 1. Lymphocytes have information nesessary for a synthesis of antibodies to a majority of natural antigens, which induce derepression of cell genome and blasttransformation of cells. 2. According to the theory of polyvariance matrix processes in the cells the realization of genetic information may be different and dependent on the conditions. That's why we assume that the quality and the rate of the blasttransformation depend on the dose of antigen included by each single cell (limited by the optimal range of doses). Large doses induce rapid formation of large blastcells with the corresponding phenotype and function, middle and small doses induce formation of middle and small blastcells, which appear after the large cells and have distinguishable phenotype and function. We suppose also that the blasttransformation is the reversible process during which lymphocytes become the memory-cells. 3.The suspensions of antigens (before and after the processing by the macrophages) are heterogeneous by the size of particles and consequently by the doses perceived by the cells. It results in different cells reactions and different rates of there exposion, in exponential increase of the number of small blastcells during immune response, since small doses usually dominate. We think, that the increase of the number of the cells is not linked with cell division and the maturation of cells during immune response does not take place. 4. We suppose, that mechanisms of the contact antigen-dependend interaction cells during secondary response, restricted by MHC I or MHC II antigens, are identical in the main. The difference between them is determined by functional state of the cells and their phenotype. The former leads to the lysis of the cells by the T_c , the last , as we assume, results in the fusion of T_h with B cells or macrophages and following division of the polyploid forms, which leads to a formation the short-life end-plasmacells or the macrophages, which acquire new properties and morphology (granulomas cells). This hypothesis may become the key for the understanding of development mechanisms of immunodeficit state and pathogenesis of AIDS, for the new understanding of mechanisms of the immunity. It is known, that the fusion is the property of the immunocompetent cells.
Many data,which contradict the clonal selection theory,not only not contradict our model, but support it: 1) it may be an activation without proliferation,2) conception of immature large blastcells inside germinal centres into less immature small cells is not evident,3)plasma cells form a minority among antibody-secreting cells,4) hypermutations are not linked intimately with cell division and are prior to the entry that cell into a germinal centre, 5) the increase of inclusion H^3 thymidine is not compelling evidence of the proliferation of lymphocytes, 6) the phenotype T_h and T_s is not constant property of the specialized cells, it may change.

IMMUNOGLOBULIN SWITCHING OF HYBRIDOMA CELLS *IN VITRO*.

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Hybridoma technology has been most valuable in providing monoclonal antibodies to a variety of antigens, however the monoclonal antibodies generated are frequently of a class or subclass that is not optimal for the task to be performed. The type of monoclonal antibodies produced may vary according to the immunization protocol or to whether the antigen is T dependent or T independent. Antibody response to most T independent antigens consists largely of IgM antibodies of low affinity and does not show significant switching to other isotypes. When the isotype of the antibody is not suitable, immunoglobulin switch variants may be isolated. Using the ELISA spot assay we have been able to identify hybridoma cells that have switched their isotype from one class to another. These rare isotype switch variants can be isolated using the sib selection approach described by Cavalli-Sforza and Lederberg for the isolation of bacterial mutants. The likelihood of recovery of spontaneous isotype switch variants is dependent on the frequency of switching events *in vitro*. Unlike spleen cells where switching can be enhanced and targeted to certain isotypes by cytokines, the fate of hybridoma cells depends on the spontaneous rate of switching of each clone. Frequencies of 1-5 per 10^5 cells or 1-10 per 10^7 are common among IgG1 and IgM secreting hybridoma cells respectively. These low frequencies require repeated steps of enrichment before final isolation can be achieved, or may fail to yield switch variants, either because switched cells are overgrown by the rest of the cells or die. Furthermore, when isotype switch variants are identified and isolated, they usually are of a certain isotype only. Attempts to enhance the frequency of switching *in vitro* by cytokines such as IL-4, IFN-γ or TGF-β have been occasionally successful. A few hybridoma lines have switched at 35-50 fold higher frequency, while others were not affected. Due to the high cost of recombinant interleukins and the fact that the conditions required to stimulate hybridoma lines varies so much, we have tested the effect of a number of mutagens on the frequency of switching. Among the four mutagens tested, melphalan, mitomycin, ethylmethansulfonate and acridine orange (ICR), the last was the most consistently effective, increasing the frequency of switching by 5 to 50 fold. Antigen binding and fine specificity analysis as well as biological assays suggest that these antibodies maintain their binding and constant region sites intact.

INVESTIGATION OF HEPATITIS B VIRUS (HBV) MARKERS IN EYE TISSUES.

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Recently there were published some data, which allow to suggest an inducting or aggravating role of hepatitis B virus during some forms of eye diseases (V.K.Singh et al., 1990; S.G. Robbins et al., 1990, 1991, 1992; V.N. Kushnir, 1992; R. Achiron, 1994; O.S. Slepova, V.N. Kushnir et al., 1995).

However, in connection with the wide spread of HBV-infection, traditional serodiagnostics is not informative enough when discovering HBV-associated ophthalmopathology. Discoveries of HBV-markers in eye structures can be considered more convincing.

Using immunoferment analysis (ELISA), we examined HBV-markers (HBsAg and HBeAg; anti-HBs, anti-HBe, anti-HBc) in water-salt extracts of lens masses (n=66) and corneas (n=21). These samples were taken during the operation of cataract extraction from the following patients: 22 children aged from 6 months to 4 years with congenital cataracts; 23 adults aged up to 40 with complicated cataracts of unknown etiology; 11 persons aged from 60 to 72 years with senile cataracts; or during the keratoplastics operation (21patients with corneal leucoma aged from 16 to 57). All the operated patients were residents of Moldova Republic, epidemiologically-unfavourable region in terms of HBV-infection.

Examination of lens masses showed the presence of HBe-Ag marker of active viral replication in 45% of samples. It was discovered mostly in children with congenital cataracts (62.5%) and in patients with complicated cataracts (75%). This marker was discovered seldom during senile cataracts (12.5%). Australian antigen - HBsAg-marker of active and chronic hepatitis B - was discovered in 34% of cases. It was mostly discovered in lens of patients with senile cataracts (87.5%); significantly less often it was discovered in young adults with complicated cataracts (35%) and in children with congenital cataracts. Antiviral antibodies (anti-HBc, anti-HBe and anti-HBs) characterizing different fases of infections process (including the postinfection immunity stage) were discovered in 33%, 55%, 34% of samples correspondingly. Friquency of their discoveries variated from 18% to 100% during different cataract forms.

HBsAg was discovered in 8 from 21 (38%) corneal extract samples from patients with corneal leukomas. HBeAg was discovered in 2 samples, which fact testifies an active virus replication.

We consider the discoveries of HBV- markers (especially of acute stage) in lenses and corneas of patients with cataracts and leucomas in testimony of a possible role of HBV-virus as the reason of these eye diseases.

EXAMINATION OF ANTIBODIES TO RETINAL S-ANTIGEN AND TNF-α IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM).

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S-antigen (S-Ag; 48 kDa) is the tissue-specific retinal protein with ability to induce the autoimmune reactions in human and animals (C. Pfister et al., 1985; Y. De Kozak et al., 1987; R. Nussenblatt et al., 1989; O.S. Slepova, 1991).

We examined serum antibodies (ab) to S-Ag (S-IgM and S-IgG) in patients with IDDM without any of eye pathology and with different stages of diabetic retinopathy (DR). At the same time we examined concentration of TNF-α in serum. We also took in the account data about possible role of TNF-α in the mechanism of the autoimmune reactions development during IDDM (C.O. Jacob, 1992). We used enzyme linked immunosorbent assay (ELISA).

We examined 9 patients with primary IDDM without DR, 23 patients with early stages of DR (DR0 - 11, DR1 - 12) and 58 patients with developed DR (DR2 - 12, DR3 - 46). The control group consisted of 16 practically healthy donors.

In control group TNF-α variated from 820 to 2100 pg/ml; mean levels of S-IgM was 0.75±0.58 , S-IgG 1.09±0.24. We found that patients with primary IDDM had tendency to increase of TNF-α (1200 - 3800 pg/ml) and S-IgM level (1.33± 0.61) in comparison with the controls. Appearance of retinal changes (DR0 and DR1) was associated with tendency to accumulation of S-IgG (1.24± 0.50) with simultaneous decrease of S-IgM level (0.99 ± 0.28) . During this stage there was discovered also a significant decrease of TNF-α in comparison with primary IDDM and with controls ($p<0.02$). In 80% of patients of those groups it was not higher than 140 - 600 pg/ml. Development of pathologic process in retina (DR2 and DR3) was associated with backward increase of TNF-α in comparison with DR0 and DR1stages ($p<0.05$). In 60% of patients its concentration variated from 540 to 6400 pg/ml and had the tendency to exceed control parameters ($p\leq 0.05$). In DR2 stage there was a tendency to backward increase of S-IgM level (1.3 ±0.7), the level of S-IgG remained comparatively high (1.25±0.5). Proliferative stage (DR3) was characterized by maximum levels of S-IgM (1.42±0.8) and S-IgG (1.42±0.3) in comparison with controls ($p<0.02$).

Therefore, we observed maximal increase of TNF-α on the stages of primary IDDM and DR2 - DR3. In both cases the TNF-α level increase was combined with accumulation of serum S-IgM, characterizing the primary immune response, and preceded of the increase of S-IgG level which reflected the development of autoimmune reactions to S-Ag. It is characteristic that these peaks of the "immune activity" were noticed in periods antecedent to the appearence and aggravation of changes on the eye bottom.

These data allow to suppose that the increase of TNF-α concentration in serum is a factor contributing to enhancing humoral autoimmune response induced by retinal S-Ag. Differentiated examination of serum S-IgM and S-IgG may be used in forecasting the development of proliferative DR.

Prevalence of CMV-M and CMV-Total antibodies in children with inflammatory eye disease (uveitis)

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383 children with various uveitis have been examined for a decade. The children age varied from a few months to 15 years. The marcers of latent (Ig G antiviral antibodies) and acute (Ig M antiviral antibodies) cytomegalovirus (CMV) infection were investigated. ABBOTT CMV Total diagnostic kits and ABBOTT CMV-M EIA diagnostic kits were used for detection of total and Ig M antibodies to CMV in the serum. CMV Total antibodies have been detected in 300 out of 383 children (78,5%). The rate of antibody detection increases with age. Antibody level to CMV among seropositive children varied at wide range (absorbance value appears to be from 0.099 up to 1.268). CMV-M antibodies, found at primary CMV infection or in acute reccurence state, have been detected only in 8 children (2%) who showed active uveitis process (recurence or postoperative complication). Such a rare detection of CMV-M antibodies emphasizes the autonomy of the inflammatory condition in eye.

Atlas of markers for cellular membranes' status. (Immunophenotypical atlas of cell's membranes)

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Intensity and distribution of immunohistochemical staining of molecular cell membrane markers depend on localization and the feature forming of tumor structure. So there is actual task to create the database about the results of immunological reactions of different reagents with the structure components of the tumor cells. We suggest that for each type of tissues or cells it should be determinated sufficient group of markers and part of cellular surface covered by these markers. On the base of these data it is possible in the following to develop the classification characteristics of tumors.

In particular the offered approach should expose quite difinitely percent of "blank spaces" of membrane surface, which is occupied by not identified marker molecules. It can be "minor" or "major" markers, but a researcher should, at least, to have a opportunity to evaluate the areas, occupied by these markers.

We have shown the correctitude of this task on the example of experimental data. We have examined immunological reaction of mAbs ICO-25 with the epithelium of different localization tumors. We have found there are very much similar interaction of mAbs ICO-25 with epithelial cells of embrion and adult human, but between malignant and normal epithelium the considerable variations exist in the distribution of the antigen. Three types of reactions with the different intensity and distribution of the label are distinctly manifasted.

Every type is characteristic of the definite group of tumors. It would follow from the above that the principle of oncodiagnosis must be based on the possibly largest number of onconosological units and the attention should be drawn to the distribution and intensity of the label. We believe that lectins on the surface tumor cells can find application as diagnostic markers.

In perspective we believe to develop of diagnostic and classification methods, giving opportunities for the systematic costruction of the offered atlas. Each section of the atlas, connected with the cell and tissue discription for one or another desease, can include as a different microphotos, so sets of profiles and histogrames, obtained by flow cytometry.

AUTIIMMUNE INFLAMMATION IN THE PATHOGENESIS OF POSTTRAUMATIC VITRORETINOPATHY.

(Immunohistochemical investigation)

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The goal of this investigation is to study the pathogenesis of progressive proliferative syndrome after repeated eye trauma-proliferative vitreoretonapathy (PVR). We used with immunohistochemical methods, monoclonal antibodies to Ig A,G,M; C1,C3 complement component, S- retinal antigen, HLA-DR antigen, adgesive glycoproteide- fibronectine. There were determined also T and B lymphocites. Fragmentes of epiretinal membranes from posttraumatic enucleated eyes (20) were matherial for the investigation.

RESULTS. There were revealed the accumulatiom of immune deposites of Ig A,G; C1,C3 complement components in the epiretinal membranes. There were determined T and B lymphocites among the cellular infiltration. We observed the expression of HLA-DR antigen by pigment epithelial cells, the S-retinal antigen and fibronectine were discovered in the epiretinal membranes.

CONCLUSIONS. The data obtained confirmed the role of autoimmune disorders in posttraumatic PVR. It is possibl that the first perforative trauma of the eye accompanied with the autosensibilisation by ocular tissues. Due to this sensibilisation, the repeated surgical trauma, such as extraction of posttraumatic cataract, could lead to the development of autoimmune inflammation which plaied the key role in the pathogenesis of PVR.

The Growth of Hybridoma Cells in Serum-free Medium

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During the last time the interest to the using of monoclonal antibodies (McAb) as the instrument in diagnostic, therapy and immunoaffinity of different medical substances like digoxin, α-interferon and others raised up. But the application of McAb from mouse ascit is limited because of the immunological reaction of human organism followed by the possible containing of mouse immuniglobulins and other proteins in preparats. So, one of the perspective way is seems to be the production of McAb in vitro, that allows to standardize and to control the production conditions. The necessary component of the media for cultivation of hybridoma is the fetal serum, which is become one of the limited factor in scale-up production of McAb because of the their high-costing and deficiency. So, it seems to us very actual the problem of the developing of serum-free media, as well as also the using of the complex of growth factors from sera and other sources instead of the fetal serum. This article is about the solving of these problems.

Using the hybridoma, producing McAb to digoxin, tumor necrose factor and several phytoviruses, we show the possibility and perspectivity of using of the medium with the supernatant of mieloma cells Ag 8.653.X63 for the changing of medium with fetal serum. The exchanges of 10% of fetal sera on 1% of the last with 20% of supernatant media component allows to produce the same cell growth as in control. The total cell concentration was $(1.8\text{-}2.5)*10^6$ cell per milliliter. But the surviving of the cells in the novel medium was lower, like as 70-80%. Also, the possibility of serum-free cultivation of hybridoma, producing McAb to digoxin and α-TNF, was shown. The serum-free media contains of the media IDMEM with the addition of specific components. The results of growth parameters of the suggested medium during 6 passages were the similar with the control and the antibody producing activity was more effective in serum-free variants.

INCREASED INTERLEUKIN-1 AND INTERLEUKIN-3 LA IN SCHIZOPHRENIA

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The interleukins play an important role in the development and maintenance of the immune system. Decreased cell mediated immunity measures were found in schizophrenic patients. The purpose of the present study was to investigate the spontaneous production of interleukin 1 (IL-3) and interleukin-3-like activity (IL-3-LA) by human mononuclear cells from schizophrenic patients compared to healthy individuals. IL-1 was significantly increased in schizophrenic patients as compared to controls. IL-3-LA was slightly elevated in schizophrenic patients as compared to controls. These findings support the hypothesis of an autoimmune dysfunction in some schizophrenic patients.

NON PATHOGENICITY OF ANTI-DNA AND ANTI-PHOSPHOLIPID ANTIBODIES IN IVIG PREPARATIONS

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Intravenous immunoglobulins (IVIG) are therapeutic preparations of pooled normal polyspecific immunoglobulin G. We investigated the presence as well as the *in vivo* pathogenic potential of autoantibodies against phospholipids and DNA in several commercial IVIG preparations. The presence of autoantibodies and their anti-idiotypic antibodies in the IVIG preparations was detected by ELISA. Naive mice were actively immunized with either IVIG preparations or pathogenic monoclonal antibodies against cardiolipin or DNA. Following boost injection the mice were tested for the presence of mouse autoantibodies, and for clinical parameters of the autoimmune condition (erythrocyte sedimentation rate, prolonged aPTT, platelets and white blood cell counts, fetal resorption rate and urinary protein excretion). We found high levels of autoantibodies against a panel of phospholipids and DNA, including pathogenic idiotypes, as well as their anti-idiotypic activity, in all the IVIG preparations. Following immunization with those IVIG batches, the mice developed high levels of autoantibodies against phospholipids and DNA, similar to mice immunized with pathogenic anti-DNA or anti-cardiolipin Abs. However mice which were immunized with pathogenic anti-cardiolipin (H_3 Id+) monoclonal Ab had thrombocytopenia, prolonged aPTT and increased fetal resorption rate, while mice immunized with pathogenic anti-DNA (16/6 Id+) monoclonal Ab had high erythrocyte sedimentation rate, leukopenia, and significant proteinuria. In contrast, mice immunized with several commercial IVIG preparations did not develop any of these manifestations. We conclude that commercial IVIG preparations contain high levels of anti-phospholipids and anti-DNA autoantibodies, as well as their anti-idiotypic antibodies. Although these antibodies can induced the generation of mouse autoantibodies, they did not prove to be pathogenic *in vivo*

ANTIGEN BINDING, AND INDUCTION OF EXPERIMENTAL APS BY ANTI-CARDIOLIPIN CORRESPONDING SINGLE CHAIN Fv DOMAINS

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Single chain Fv was prepared from two anti-cardiolipin and one anti-cardiolipin/anti-DNA mouse monoclonal antibodies:1) CAM (IgG) binds to CL , has lupus anti coagulant (LAC) activity and induces experimental APS in naive mice. 2) 2C4C2 (IgM) binds to CL, DNA, reacts as LAC, and induces experimental APS associated with SLE. 3) CAL (IgG) binds to CL and is non pathogenic in-vivo. The CAM and 2C4C2 uses the VH gene of the J558 family, while CAL uses the 7183 VH family. All the three anti-cardiolipin monoclonal antibodies were converted into single chain Fv-s (scFv-s) and showed the same antigen bindings properties as the original monoclonal antibodies. Replacement of the CAM VH domain with CAL VH decreases the binding avidity of the scFv to cardiolipin and completely abrogates the lupus anticoagulant activity (did not prolong the APTT- activated thromboplastin time). Replacement of the pathogenic CAM VL with the non pathogenic CAL VL, did not affect the avidity for cardiolipin or the lupus anticoagulant activity.

BALB/c mice were immunized with the scFv domains of the three anti-cardiolipin antibodies and the scFv-s resulting from the replacement of the heavy and light chains. The mice which were immunized with CAM, 2C4C2 and CAL scFv-s, developed the same clinical manifestations, as the original mAbs (e.g. elevated titers of mouse aCL and anti-phosphatydilserine antibodies followed by lupus anticoagulant activity, thrombocytopenia, elevated APTT and high percentage of resorptions in the CAM group. High titers of aCL, anti-ss/dsDNA were observed in the sera of the 2C4C2 scFv immunized mice and the APS picture was associated with lupus findings: leukopenia , prolonged erythrocyte sedimentation rate and immunoglobulin deposition in the kidneys. CAL scFv did not cause any clinical findings). The mice immunized with the scFv following the heavy/light chains replacements showed the follow: 1) Mice immunized with CAM(VH)+CAL(VL)scFv develop experimental APS. 2) Mice immunized with CAL(VH)+CAM(VL)scFv did not develop any clinical manifestations of APS.

The current study shows that scFv of pathogenic antibodies are capable of inducing the manifestations of the whole antibody molecule and points to the importance of the heavy chain variable domain in the pathogenic potential of anti-cardiolipin antibodies.

MONOCLONAL ANTI-ENDOTHELIAL CELL ANTIBODIES (AECA), CHARACTERIZATION AND BINDING PROPERTIES

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Mouse anti-endothelial cell mAb were raised by fusion of splenocytes separated from mice immuized with human AECA IgG fraction and mouse non-secreting myeloma cells (NSO) using polyethylene glycol (PEG). Supernatants from growing hybridomas were screened for specific antibody production by cyto-ELISA and FACS analysis using human or mouse endothelial cells. Following the two fusions twelve clones were identified as positive by initial screening. Antibody formation was stabilized following limiting dultion cloning in three of these clones (BGM, 3C8 and 7G2).
The immunoreactivity of mAbs against panel of different antigens is shown in Table .

Table 1. Analysis of reactivity of mouse monoclonal antibodies against different antigens by ELISA
(Mean absorbance at 405 nm ± SD)

	Clones			
Antigens	BGM	3C8	7G2	S2.9
HUVEC	1.720±0.101	0.952±0.070	0.843±0.112	0.060±0.003
Hep 2	0.094±0.009	0.072±0.013	0.101±0.020	0.082±0.030
H5V	0.852±0.050	1.350±0.150	1.020±0.095	0.070±0.013
Matrix	0.073±0.008	0.095±0.014	0.084±0.012	0.051±0.004
CL	0.098±0.023	0.113±0.025	0.124±0.035	0.094±0.012
PS	0.107±0.031	0.115±0.030	0.130±0.038	0.103±0.024
PR-3	0.074±0.011	0.082±0.009	0.079±0.010	0.052±0.001
Gelatin	0.062±0.013	0.085±0.010	0.079±0.010	0.080±0.013
BSA	0.073±0.011	0.063±0.020	0.071±0.015	0.067±0.008

The mAbs showed restricted reactivity with components associated with endothelial cells (HUVEC, H5V) and no reactivity with either Hep 2 cells, extracellular matrix or other antignes tested. The FACS analysis confirmed membrane specific binding of mAbs to endothelial cells.

PATHOGENIC ROLE OF ANTIENDOTHELIAL CELL ANTIBODIES (AECA) IN VASCULITIS: AN IDIOTYPIC EXPERIMENTAL MODEL

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Idiotypic manipulation of naive mice has previously been used for induction of systemic autoimmune diseases (eg. anti phospholipid syndrome, systemic lupus erythematosus, Wegener's granulomatosis). The aim of this study focused on the utilization of this technique to induce the production of anti-endothelial cells antibodies (AECA) and autoimmune vasculitis in a murine model. AECA were derived from a Wegener's granulomatosis patient plasma. IgG was purified by absorption on a proteinase-3 affinity column resulting in the depletion of anti-neutrophil cytoplasmic antibody activity. The absorbed IgG fraction displayed a high titer of AECA as evidenced by a cyto-ELISA against unfixed HUVEC. BALB/c mice were actively immunized with the purified AECA. Three months after a boost injection with the human AECA, mice developed endogenous AECA (Ab3) but not antibodies to proteinase-3, cardiolipin or DNA. Histological examination of lungs and kidneys revealed both lymphoid cell infiltration surrounding arterioles and venules as well as deposition of immunoglobulins at the outer part of blood vessel walls. This experimental animal model of vasculitis, a product of our method of idiotypic manipulation, has provided the first direct proof for the pathogenicity of AECA.

The monoclonal antibodies against K-and O- antigens of Salmonella typhimurium

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K-antigen (Ag) is a surface-somatic glycoprotein (M.w. 55 kDa) which concentration correlates with the virulence of Salmonellas. We obtained 16 clones of hybrid cells secreting the monoclonal antibodies (MAb) to Salmonella typhimurium. MAb had different specifity, one of the clones showed high specifity to K-Ag in indirect Enzyme Linked Immunosorbent Assay ((ELISA). The MAb specific for K-Ag of S.thyphimurium were IgG1"cappa". MAb specifically reacted with whole bacterial cells of S.typhi-murium, S. abortus bovi, S.stanley, S.choleraesuis, S.mission, S.london, S.cottbus, S. infantis, S. dublin, S. newport, S. gallinarum-pullorum, S. moscow, S.anatum and hadn't the cross-reactivity with antigenic structures of Escherichia coli, Proteus vulgaris, Citrobacter frandines, Enterobacter cloacae, Hafnia alvey, Shigella sonnei, Morganella morganii, Y. pseudotuberculosis in ELISA. Another clone had high specifity to purified O-Ag of S.typhimurium in ELISA. The MAb were IgG2a 'cappa', specifically reacted with whole bacterial cells of S. typhimurium and showed no activity to cells of Escherichia coli. According to the epitope analysis binding epitope of O-Ag contains the saccharine sequence abequosa-mannosa-rhamnosa. K-Ag determinants differed from O-Ag determinants. We can recommend the characterizated MAb for further study of antigenic structure and pathogenic functions of Salmonellas.

IMMUNOPHENOTYPE OF PERIPHERAL BLOOD LYMPHOCYTES IN THE PATIENTS WITH OPHTHALMOTOXOPLASMOSIS

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Purpose. To study the role of immunocompetent cells in pathogenesis of ophthalmotoxoplasmosis.
Methods. Indirect immunofluorescence reaction on poly-L-lysin with MKA ICO 1, 11, 12, 15, 20, 36, 40, 52, OKT8 for the determination of antigens Ia-, CD 11a, CD22, LFA-3, HNK-1, CD 38, RFB-1, CD1c, β_2-microglobulin, HLA-ABC, CD8.
Results. 40 patients with toxoplasmosis uveitis were examined in acute (14), in subacute stage (15) and in remission (11). The studies showed that acute stage of uveitis had developed in deficiency of the cells, expressing Ia-, LFA-1, CD38 antigens, β_2-microglobuline in 42,9%, 57,1%, 42,9%,21,4% of the patients accordingly. The number of CD22+ cells was increased in 35,7% of the patients, HNK-1+ was increased in 46,2% and CD1c - in 23,1%. The deficiency of CD8+cells was established in 42,9% of the patients and it was analogously to the changes in the blood of the patients with systemic autoimmune diseases. The picture of correlative connections in acute stage of uveitis was mediated by supressive-autotoxic, natural killer subpopulations of T- and B- cells. A state of remission was determined by increasing of Ia-+ in 36,4% of the patients, LFA-1+ in 60%, CD8+ in 55,6%, CD38+ in 50% of the patients and by decreasing of CD22+ cells in 20%, HNK-1 in 44,4% of the patients. The increase of CD1c+ cells was observed in 41,2% of the patients and didn't depend of the stage of uveitis and characterized B-cells activity. The correlation between specific antibodies formation and the number of B-cells ($p<0,05$), Ia-+ ($p<0,05$), CD8+ cells ($p<0,01$) was established and pointed to cooperative participation of different cells subpopulations in a forming of specific response. The increase of B-cells was observed in initial disease in 58,3% of the patients, the increase of HNK-1+ was in 66,7% and CD38+ cells in 69,2% of the patients. On the whole the immunophenotype of lymphocytes in the patients with ophthalmotoxoplasmosis was the following: LFA-3+, RFB-1+, CD1c+, CD22+ and it indicated the predominance of cytotoxic, killer and B-cells populations.
Conclusions. The increasing activity of B-cells, cytotoxic and killer populations was established in pathogenesis of acute ophthalmotoxoplasmosis as a response on antigen stimulation. The revealed perculiarities in expression of membrane antigens of peripheral blood lymphocytes can be as additional marker for etiologic and differential diagnostics of ophthalmotoxoplasmosis.

SPECIFIC TRIATS OF IMMUNOPATOGENESIS IN OPTIC NEURITIS OF VARIOS ORIGINS.

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Purpose. We have study the immonologic characteristics of optic neuritis (ON) originating from multiple sclerosis (MS) and of ON of other ttiology.
Metods. Metod of immunofluorescens utilizing from cytometry on Epix Profile 11 La.zer Cytometer with LT3, LT4, LT8, IC072, IC016, IC01.
Results. We styded 43 patiens with MS-caused during active phase (22) and remission (21) and 37 patients with ON other etiology during active phase (18) and remission (19). In active phase of ON during MS the numbers of CD3+- and CD4+-cells remained unchanged. In 22,2% of patients CD8+-cells were deficiets. CD4+/CD8+coefficient was elevated in 36.4% of patients, possubly due to autoimmune component. Numbers of CD16+-cells were unchanged. Main changes were found in B-cells population: D-lymphocytes and DR+-cells were deficient in 59% and 40,9% respectively. Remission was characterized by normalization of CD4+- and CD4+/CD8+ coefficient. Number of CD16+-cells remaind unchanged. In 47,6% of patients the diciency of B-lymphocytes persisted. During remission the numbers of CD4+-cells were restored, but the CD4+/CD8+ coefficient was decreased in 31,6% of patients. Contrary to patients with ON during MS, the patients with ON of other etiology during active phase or reccurence had the deficiency of CD4+-cells in 22,2% of cases. CD4+/CD8+ coefficient was increased in 22,7% of patients. Defficiency of CD16+-cells and B-lemphocytes was noted in 44 and 33.3% of patients respectively. During remission the number of CD4+-cells was restored, but the decrease of CD4+/CD8+ coefficient was noted in 31,6% of patients. Deficiency of CD16+-cells persisted in 31;6% of patients and the number of B-lymphocytes was lowered in 23,6% of patients. Lowering the number of DR+-cells was present in 31,6% of patients.
Discussion. The obtaind results have shown, that the main trait in pathogenesis of ON during MS is the condaction of immune deficiency of T-suppressor population, which is characteristic for autoimmune diseases, as well as deficiency of B-cells. Contrary to ON during MS, the ON of other etiology was accompaind predominatly by the decrease in CD16+-cells and B-lymphocytes numbers.

USE OF MONOCLONAL ANTIBODIES FOR THE EVALUATION OF THE QUALITY OF ACELLULAR PERTUSSIS VACCINE

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A collection of monoclonal antibodies (MaAb) to different *Bordetella pertussis* antigens was obtained. A complex of antigens was isolated from *B. pertussis* cultivation medium by acidic precipitation. FHA, 92, 69, 65 kD proteins, pertussis toxin subunits and LPS were detected in this complex by electrophoresis and immunoblotting with the use McAb. After detoxication the complex exhibited high immunogenic and protective activity and very low acute and chronic toxicity for mice. The monopreparations of acellular pertussis vaccine, made from the detoxified complex, as well as their mixture with diphtheria and tetanus toxoids, were adsorbed on aluminium hydroxide gel and lyophilized. The completeness and stability of the adsorption of antigens in the finished dried preparations were evaluated after adding water for injections by the analysis of supernatants for the presence of free desorbed antigens in ELISA and electrophoresis with subsequent immunoblotting with the use of McAb to *B. pertussis* antigens and diphtheria toxoid. The conditions of antigen adsorption and lyophilization of acellular DTP vaccine, used in this process, ensured the firm and stable binding of all antigens contained in this vaccine with its protective activity being preserved for at least 3 years. The sensitivity of the antigen determination with the use of McAb was 0.002 Lf/ml for diphtheria toxoid, 5-15 ng for *B. pertussis* proteins in immunoblotting and 8 ng/ml for LPS. The values of international protective units for the pertussis component of lyophilized acellular DTP vaccine were higher than those of the pertussis component of commercial whole-cell DTP vaccine.

INDUCTION OF CHANGES IN THE COMPOSITION OF CELL POPULATION WITH *PSEUDOMONAS PSEUDOMALLEI* ANTIGEN IN THE COURSE OF IN VITRO IMMUNE RESPONSE

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The study of mechanisms of *in vitro* immune response, the choice of optimal conditions for its stimulation with antigens of different nature present good prospects for obtaining immune lymphocytes for use in hybridoma technology, and particularly for obtaining cells producing human immunoglobulins. We failed to induce *in vitro* antibody synthesis in the nonfractionated mixture of spleen cells with an indefinite number of macrophages (MP) of BALB/c mice. The antigen in combination with some polyclonal activators was found to alter the composition of the responding cell population. After adding the definite number of MP, previously incubated for 24 hours with different doses of killed *P. pseudomallei* cells, we found out that at the presence of the maximal dose of the antigen in the MP culture (2 IOU/ml) and in the mixture with B cells (0.64 IOU/ml) resulted in the death of the cells and the absence antibodies by day 6 of cultivation. With the dose of *P. pseudomallei* cells equal to 0.05 IOU/ml in the MP culture and 0.016 IOU/ml in the B cell culture after the addition of MP, the highest total survival rate of cells (64%), the least total amount of MP (2.8%) and the most intensive synthesis of anti-*Pseudomonas* Ab (1:64 in ELISA) were noted by day 6 of cultivation. At moderate doses of the antigen the number of cells increased with MP constituting 30% of the number of live cells; antibody synthesis was twice as low. Polyclonal activators, when added on day 2 of cultivation at the presence of the antigen, produced the following changes: The highest Ab synthesis the minimal increase of the total number of cells (1.7-fold) and MP (4-fold) were noted in the culture with PHA added. The lowest Ab synthesis (1:2 in ELISA), the increase of the total number of cells (3.8-fold) and the number of MP (29-fold) were noted after the addition of ConA. Thus under certain conditions *P. pseudomallei* antigen induced the proliferation of MP, leading to their domination, and the suppression of Ab synthesis. Some mitogens enhanced this effect. At the minimal doses of the antigen and the moderate content of MP we could induce primary immune response to *P. pseudomallei* cells *in vitro*. These data are of interest for the development of schemes of *in vitro* immunization. They may also be useful for the study of the pathogenesis of melioidosis, often leading to the development of granulomas which consist, to a great extent, of macrophagal elements.

Immunotoxins based on antimelanoma monoclonal antibodies B3F7 and ricin A-chain or ricinus agglutinin A-chain.

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Murine monoclonal antibodies (mAbs) B3F7 have been obtained. These mAbs react with melanoma cell lines, with freshly isolated melanoma cells and stain melanoma parafin tissue section. The antibodies do not react with PBL, tested normal adult and fetal tissues (10 specimens) and with tumours (lung adenocarcinoma, breast cancer, sarcomas). It was shown also by immunofluorescent method, that antigen, identified by monoclonal antibodies B3F7, localized on cell membrane. The Mr of antigen recognized by mAbs B3F7 is about 35 kDa.

Monoclonal antibodies B3F7 have been chemically conjugated to ricin toxin A-chain (RTA) and to ricinus agglutinin A-chain (AGA). Data from indirect immunofluorescence assay on cells MeWo and A9 demonstrated specificity and immunoreactivity of the RTA/B3F7 and AGA/B3F7 immunotoxins, which was identical to that of native antibodies B3F7.

Antigen-negative A9 (murine fibroblasts) cells incubated for 72 hours with immunotoxins showed no increased cytotoxicity compared with A9 cells exposed to A-chains alone. However, the immunotoxins was toxic to antigen-positive MeWo and MS cells. RTA/B3F7 and AGA/B3F7) immunoconjugates killed MeWo melanoma cells (LD50 2*10(-9) M and 10(-8) M). Various lysosomotropic agents augmented immunotoxins cytotoxicity. Monensin and NH4Cl, when combined with both immunotoxins, augmented their cytotoxicity more than 10-fold.

The antigen recognized by mAb B3F7 may be used as a target for immunotherapy of human melanoma.

ENHANCEMENT OF MONOCLONAL ANTIBODIES REACTIVITY TO SPECIFIC ANTIGEN BY ELIMINATING ADMIXTURE OF METAL-BINDING BACTERIAL PRODUCTS

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The commercial preparations of tissue culture media were found to be contaminated with bacterial catabolic products (BCP) with metal-binding capacity. According to data presented elsewhere BCP are ready of binding to the proteins produced by the methods of cell biotechnology including the hybridomas deriving monoclonal antibodies - MAb (A.J.Kulberg et al., 1994-95).

Here we describe a general principle for elimination of BCP admixture from MAb by using insolubilized natural metal-scavengering substance with well established origin and structure (termed AV-23). Passage of the commercial MAb preparations through a column with insolubilized AV-23 resulted in purification MAb from BCP, as tested with specific anti-BCP reagent, followed by a significant increase in MAb reactivity to specific antigens showed in indirect ELISA.

Data obtained may imply that BCP induce the conformational rearrangement within the antibody molecule with at least partial blocking the antibody active sites. Therefore, the technology offered can be useful in manufacturing MAb preparations applied for different diagnostic assays.