Poster session

Generation of 1 and k chain scFv fragments against thyroid peroxidase by combinatorial libraries: Evidence for both 1 and k chain-secreting lymphocytes in the thyroid from patients with Graves' disease

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We have constructed three combinatorial libraries from unpurified, CD19+ and anti-thyroid peroxidase (TPO+) B cells extracted from thyroid tissue of Graves' disease patients. Eighty per cent of anti-TPO single-chain variable fragments (scFv) produced, used a heavy chain (VH) gene derived from the VH1-3 gene segment whatever the combinatorial library used; other gene segments used by the anti-TPO scFvs were VH1-8, VH1-69, VH3-30, VH3-64, and VH5-51. On the other hand, the light chain (VL) gene segments used by the scFvs were more heterogeneous with genes from the k locus, i.e., Vk1-39, Vk1-12, Vk1-5, and Vk3-11, and also from the l locus, i.e., Vl1-51, Vl1-44, Vl1-40, Vl2-14, and Vl2-8, with a dominance of the Vk1-39 and Vl1-51 gene segments. H/L pairing similar to that obtained from an in-cell library was obtained with our random-derived anti-TPO scFvs. In particular, one VH1-3/Vl1-51 scFv, A16, showed exactly the same nucleotide sequence as in-cell scFv ICB7, demonstrating that in vivo rearrangement can be obtained from a random combinatorial library. The majority of the TPO-specific antibody chains showed evidence of somatic hypermutation, with a number of mutations typically higher in-the VH than in the VL chain and a replacement/silent ratio greater in the complementarity determining regions (CDR) than in the framework regions (FR). Seventeen anti-TPO scFvs showed high affinities to TPO using BIACORE technology, with values between 0.77 and 12.3 nM, and defined seven antigenic regions on the TPO molecule; all but four used a VH1-3 gene pairing either with l or k genes. TPO-specific autoantibodies from the sera of 20 patients suffering from Graves' disease completely inhibited scFv binding. Reciprocally, the anti-TPO fragments, particularly random-associated scFvs B4, T13, and in-cell scFv ICA5 efficiently displaced the binding of serum autoantibodies to TPO, suggesting the predominance of these types of scFv in the serum. Our study indicates that k as well as l chain gene usage is found in the TPO antibody, repertoire of thyroid-infiltrating B cells with a VHI-3 dominance, leading to high affinity Ab fragments mimicking the binding of serum autoantibodies to TPO.

A chimeric mouse-human anti-CD4 Fab expressed in baculovirus demonstrates similar antigen binding and immunosuppressive properties as the parental 13B8.2 monoclonal antibody

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The anti-CD4 mAb 13B8.2, directed against the CDR3-like loop of the D1 domain of CD4, inhibits signal transduction pathways leading to both T cell activation and HIV replication. The VH9/DSP2/JH2 and Vk12-13/Jk2 rearrangements, corresponding to genes encoding the heavy and light chain variable regions of the 13B8.2 mAb, were inserted into baculovirus cassettes upstream from pre-installed human CH1g1 and Ck genes respectively. After expression in insect cells, a complete correctly.-processed Fab was secreted in the culture medium and protein G-immunopurified with a yield of 5–10 mg/L. The chimeric Fab 13B8.2 showed anti-CD4 activity with an affinity value of 5 nM and recognized the same region on the CDR3-like loop as the parental mAb. The mouse-human Fab inhibited IL2 secretion following antigen presentation and displayed a strong capacity to-prevent HIV-1 promoter activation. Taken together, these results indicate that the chimeric Fab has retained a major part of the parental

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	First treatment		4 months	Second treatment	
	Before cA2	After cA2		Before cA2	After cA2
LymphocytesTNF-α	9.8%	0.6%	4.2%	13.1%	4.6%
Monocytes-TNFα	25.5%	0.0%	52.4%	94.5%	30.0%

13B8.2 mAb properties and, suggests that it might be a valuable therapeutic tool.

Chimeric monoclonal antibody (cA2) to TNFα remove in vivo TNFα-producing cells in Crohn's disease

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Crohn's disease is a chronic inflammatory disorder of the bowel characterized by granulomatosus inflammation of the gastrointestinal tract. Tumor necrosis factor alpha (TNFα) is a key mediator of mucosal inflammation in all inflammatory bowel diseases. An overproduction of TNFα in the affected intestinal mucosa has been documented in animal models and in human patients with active Crohn's disease. Antibodies to TNFα prevent or reduce inflammation. A single infusion of the chimeric mouse/human monoclonal antibody cA2 to TNFα has been established as a new terapeutical procedure of moderate-severe Crohn's disease refractory to corticosteroids. The cA2 antibody neutralizes TNFα with high affinity, and also binds transmembrane expressing cells. The aim of the study was to determine the cA2 effect on TNFα-producing cells. Twenty healthy subjects, as control, and one Crohn's disease patient refractory to corticosteroids, were analyzed. We studied the TNFα expression by flow cytometry, before and after cA2 treatment, in peripheral blood monocytes and lymphocytes. The cA2 antibody, that binds transmembrane TNFα, and FITC-anti human IgG were used in flow cytometry. The patient, with severe Crohn's disease (Crohn's disease activity index CDAI > 350), had a higher number of TNFα-producing cells than controls before the treatment: Lymphocytes 9.8% vs 4.2% and Monocytes 25.5% vs 10.9%. Two weeks after the cA2 treatment (5 mg/Kg of body weight) had a dramatic decrease in TNFα-producing cells with a clinical remission (CDAI < 150): Lymphocytes 0.6% and Monocytes 0.0%. After four months the patient had a clinical response with a large number of TNFα-producing cells: Lymphocytes 4.2% and Monocytes 52.4%. One year after the first treatment, a second cA2 treatment was averaged due to the severe activity of the disease (CDAI > 350). Before the treatment the patient had a large number of TNFα-producing cells: Lymphocytes 13.1% and monocytes 94.5%. One week after the treatment there was a dramatic reduction in the number of TNFα-producing cells, with an important clinical improvement: Lymphocytes 4.6% and Monocytes 30%.

These results suggest that cA2 antibody, as well as of neutralizing soluble TNFα, also removes TNFα-producing cells, which may collaborate with the anti-TNFα activity of the antibody treatment.

Different aspects of mumps mucosal (intranasal) immunization

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Serological results of adult volunteers intranasal mumps vaccination were investigated. In adult volunteers mumps intranasal vaccination induced as intensive increase of circulating antibodies level, as subcutaneous mumps vaccination did. Kinetics of their titers was nearly the same in groups immunized with both methods. Mumps antibodies were determined in HAI test and with virus neutralization method. The first method allowed us to determine the more intensive antibodies level increase after intranasal mumps vaccination. In volunteers nasal washings specific antibodies were determined more regularly after intranasal than after subcutaneous mumps vaccination; mumps IgA were determined early in seven days after immunization with the first method. It seems that the double vaccine dose, which was tested in intranasal vaccination, was not optimal: antibodies level increased, rather slowly after its use. Blast transformation indexes of volunteers blood lymphocytes were investigated to test immunosuppressive activity of the vaccine in both methods of its utilization. 7–14 days after subcutaneous mumps vaccine introduction real decrease of the index was noted. After intranasal mumps vaccination the effect was not so strong. So intranasal mumps revaccination is the safe and effective method, which could be recommended for practice.

Po protein/myelin-specific human cell line. Some characteristics

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A myelin specific B cell clone from peripheral blood of patient with polyneuropathy associated with monoclonal gammopathy of uncertain significance (PNMGUS) with M component was established. B cells were isolated from blood using magnetic beads (Dynal, Oslo, Norway) coated by Po protein. Selected cells were transformed by Epstein-Barr virus and cultivated in Iscove-OptiMem medium with 10% of fetal calf serum and all necessary additives. Specificity of antibodies in the wells with growing clones was tested by ELISA. One of the most positive clones producing autoantibodies (TJ99D) was selected and expanded. Up to 35% of expanded cells were CDS positive. During 4-month cultivation, however, cells gradually lost CDS expression although continued to secrete anti-Po/myelin antibodies. Antibodies were of IgM(λ) isotype. Earlier 2 human B cell clones (M2D6 and E2C5) were obtained by the same approach (Sidorova et al., *Human Antibodies* **8**(2), 1997, 65). Both cell clones producing IgM antibodies to synthetic viral peptides were initially DS5 positive and gradually lost CD5 expression during cultivation also. The transitional appearance of CD5 antigen on the cell surface points out that it is rather activation than lineage marker. Antibodies produced by M2D6 and E2C5 clones were shown to be polyreactive. To check the specificity of anti-Po/myelin autoantibodies different antigens were used, and it was established that antibodies were monospecific. it was shown that TJ99D antibodies were coded by VH3 gene. Myelin-specific clone from the patient with PNMGUS is obtained for the first time. Biological functions of autoantibodies to Po/myelin are now under investigation.

Selection of single chain antibodies to vaccinia virus from combinatorial phage library

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Vaccinia virus (VACV) was used in the past as an effective vaccine against smallpox. Eradication of smallpox stopped vaccination by VACV. But using the VACV as a vector for vaccination against different diseases, and existing of one more major human diseases – monkeypox, have fuelled renewed interest in VACV as a vaccine and as a diagnostic tool for other poxvirus infections. Although VACV is a generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such infections can be treated by therapeutic administrations of human VACV immune globulin (VIG). Human monoclonal antibodies offer an obvious alternative to VIG. Common techniques used for making human Mab, are labor intensive and often result in cell lines that are unstable or produce low levels of antibody. To circumvent these problems combinatorial phage libraries of human antibodies have been developed. The library of human scFv antibodies displayed on the surface of bacteriophage was provided us by the Medical Research Council Centre, Cambridge, England. Library (Griffin.1 library) was panned against VACV, strain Elstree, variant L-IVP, purified by sucrose gradient method. Approximately 10^{12} pfu of bacteriophage were used in each of three subsequent rounds of binding on immunotubes, coated with antigen (VACV). We carried out two variants of panning procedure using coating with VACV in PBS-buffer (pH 7.2) and carbonate buffer, pH 9.6. As a result we've got two enriched libraries: variant 1 – selected in PBS (pH 7.2) and variant 2 – selected in carbonate buffer (pH 9.6). Pools of phages after each round of selection were tested in ELISA for binding with VACV. The increasing optical density in ELISA with VACV confirm enrichment of the library with antibodies against this virus. Individual clones from both enriched libraries were screened in ELISA for binding with Vaccinia virus.96 clones from library 1 (pH 7.2) and 68 clones from library 2 (pH 9.6) were tested for their binding with VACV. 56% clones from library 1 and 75% clones from library 2 were positive in ELISA. Some of them were selected for further analysis. Clones, selected from second library (pH 9.6) showed higher affinity than clones from library 1 (pH 7.2). Further analysis includes plaque-reduction neutralization test, which is carried out at pH 7.2. So, we have tested clones from library 2

(pH 9.6) for binding with VACV at pH 7.2; majority of clones save their high affinity to VACV at these conditions. A standard assay of virus neutralization as the ability of the phage antibodies to inhibit plaque formation by Vaccinia virus, was performed with selected clones. Phage antibodies and VACV were mixed and incubated, then the mixtures were added to VeroE6 cells monolayer. 5 phage antibodies from library 1 and 3 antibodies from library 2 showed neutralizing activity. Two clones were able to neutralize Vaccinia virus in dilution 1/64, when the initial amount of phages taken for neutralization was approximately 10^{10} phages per 120 pfu of Vaccinia virus. So, recombinant human antibodies to Vaccinia virus were selected from combinatorial phage library and some of them possessing neutralizing activity against Vaccinia virus.

Latent maternal infection as trigger (etiology factor) for immune-related neurology dysfunction in newborns and children

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Latent maternal infection (especially viral) is a factor of risk for development of fetus/newborn neuropathology. In a most cases not microorganisms per se, but infection-induced immune deviations, such as changes in natural autoantibodies (a-Ab) production in pregnant women are the ground for the nervous system pathology. Natural "neurotropic" a-Ab1 and their anti-idiotypic "countervallances" (a-Ab2) supposedly are important participants of neural-immune interactions and steady deviations in Ab1/Ab2 contents (negligible for the health state of adult woman) may be hazard for unmature nervous system. Accordingly abnormal trans-placental transfer of "neurotropic" Ab1 and Ab2 of IgG class from mother to fetus may lead to misdevelopments of the fetal nervous system and influence the fetus general state. Besides direct action upon fetus, anomalies in transplacentally transferred Ab1/Ab2 repertoires may be a reason for abnormal "tuning" of the fetal immune system by mechanisms of epigenetic immune imprinting (leads to long-lasting similarity between child and his/her mother but not father in repertoires of circulating a-Ab). Deviations in the contents of Ab1 against brain proteins S100, GFAP, MP-65, NGF and/or counterbalancing Ab2 we observed in the most (68%) of newborn's umbilical blood samples from mothers who suffered with latent (without clinical manifestations) herpetic, cytomegalovirus, or Coksakie virus infection. Early adaptation period of such newborns was complicated, and children' general morbidity level was 3–5 times above average. Fortunately most observed newborns revealed tendency to normalization of deviated Ab1/Ab2 serum contents during first 1–3 weeks of life, and consequent development of such babies usually is nearly normal. However if Ab1/Ab2 deviations were conserved for months, it was unfavorable indication, and the serious neurology and/or mental problems were revealed latter in 65–70% of such children. Most of them developed clinical signs of mental retardation, autism, epileptiform syndrome, hallucinatory syndrome, etc., during few consequent months/years of life.

Conclusion: (1) Long-lasting persistence of infection agents in woman organism may lead to subclinical immune deviations; the latter are usually insignificant for adult organism health state, but may be crucial for developing fetus. (2) Infection-related subclinical immune deviations in pregnant woman, may accomplished by changes in serum contents of natural "neurotropic" Abs of IgG class and according anti-idiotypic Abs; the latter could be an important pathogenic factor for neuropathology development in newborn and/or child during first months/years of life. (3) Treatment strategy for women with infection-induced immune deviations before planned pregnancy and during pregnancy should be directed at the one hand – to suppression of infection microorganisms, and at the other hand – to normalization of her immune state including serum levels of natural antibodies.

Immunoprevention and immunocorrection of diabetic fetopathy

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Characteristic features of patients who suffered with insulin-dependent diabetes mellitus (IDDM) are steady or periodic abnormal rise of serum contents of antibodies against insulin (Ab1) and antiiditypic antibodies (Ab2) which bind Ab1 as well as membrane insulin receptors. Nearly 30% of polyclonal Ab1 of IgG class in IDDM-patients may cross-react and bind to nerve growth factor (NGF) because of insulin/NGF common epitop. NGF is neurotrophin which is needed for maintenance of mature neurons functioning and especially for surviving and differentiation of unmature neuroblasts during early ontogenesys. Our observations indicate both Ab1 and Ab2 are important pathogenic factors for development of diabetic fetopathy (DF) in fetuses and newborns from IDDM-suffered women and severity of DF is roughly proportional to excess of maternal Ab1 and Ab2. Transplacentally transferred Ab1 of IgG class influence fetal/newborn nervous system development and may lead to coarse neuropathology. Excessive transfer of Ab2 of IgG class to fetus usually leads to general metabolic dysfunction and may be the most often cause of fetal intrauterine/antenatal death cases in diabetic pregnant women. Preclinical trials were conducted in MONIIAG Specialized Department in 18 IDDM-suffered women of a fertile age. The preceded pregnancies of all of them were terminated by miscarriages or intrauterine or antenetal fetal death because of severe DF. All women were characterized by abnormally high Ab1 and Ab2 serum contents. Trials were aimed to prevent DF (during future planned pregnancy) by treatment procedures directed to Ab1 and Ab2 levels decrease before pregnancy and during course of pregnancy. Heparin-therapy, wobenzym-therapy (peroral using of proteolitic enzymes in high dosages), and specific desensibilisation (prolonged regular mucosal applications of low dosages of human insulin derivatives) were used. All variants of treatments were accompanied by significant decreasing of Ab1 and Ab2 serum contents. But the most effective results (nearly steady state normalization of Ab1/Ab2) were obtained in women treated by specific desensibilisation procedures. Women of this subgroup successfully became pregnant after treatment. Their pregnancy courses were nearly normal, and all cases were completed by births of newborns with minimal dysfunction (without pronounced marks of DF).

Conclusion: We suppose, antigen-specific immunocorrection of deviated humoral immunity in IDDM-suffered women of fertile age may be the most effective approach for prevention of severe diabetic fetopathy during future pregnancy.

The specific role of anti-microvascular endothelial cell (EC) and anti-macrovascular EC antibodies in atherogenesis of vasculitis and other autoimmune diseases

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Affinity-purified AECA F(ab)2 from four patients with TTP or anti-PF4/heparin, from patients with HIT (heparin induced thrombocytopenia), found to bind and differentially activate only microvascular endothelial cells (EC) and not large vessel EC (HUVEC). The activation was expressed by enhanced thrombomodulin, IL-6 and vWF release, raised levels of adhesion molecules (P-selectin, E-selectin, VCAM-1) and CD36 expressed on the EC, followed by an increase in monocyte adhesion to ECs. Interestingly, specific activation of large vessels ECs (HUVEC) was demonstrated by polyclonal and monoclonal AECA from patients with antiphospholipid syndrome, Wegener's granulomatosus, Kawasaki vasculitis and Takayasu arteritis (TA). In sum, AECA which targets either macrovascular or microvascular can activate specifically microvascular or macrovascular EC via elevation of thrombomodulin, NFkB, adhesion molecules expression associated with monocyte adhesion to EC or induce apoptosis. These biological functions of AECA might therefore play a pathogenic role in the development of the vasculopathy.

Apoptosis of B cell secreting anti-β2GPI derived from patients with APS by synthetic peptides

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We present herein effort to develop a model for B cell peptide therapy for APS. Anti-β2GPI correspond-

ing synthetic peptides were used as monovalent, divalent and tetravalent on Fmoc backbone. The peptides as divalent and mostly in tetravalent form, inhibited significantly the secretion of anti-β2GPI by B cells 4 APS patients. The peptides as tetravalent caused apoptosis of the specific B cells. The apoptosis was determined by DNA fragmentation, and could be prevented by caspase $3 + 8$ or transfection with bcl-2. Regulation of anti-β2GPI secretion by the specific B cells upon exposure to the divalent and tetravalent peptides by cytokines was analyzed. The apoptosis was abrogated by IL-10 and IL-15 while TGFβ enhanced the process. IL-2 and IFNβ had no effect on the studied B cell function. We propose a new attitude for treating B cells secreting pathogenic anti-β2GPI in APS or generally pathogenic autoantibodies.

β2-glycoprotein I (apolipoprotein-H) as an influential determinant in atherosclerosis

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We have recently suggested that immune response against β2GPI is involved in enhanced atherosclerosis. These data derives from studies in transgenic LDL-receptor and apoE knockout mice showing that induction of an anti-β2GPI immune response by immunisation with the respective glycoprotein is followed by accelerated atherosclerosis associated with enhanced fatty streak formation, and CD4+ cells in the atherogenic lesions. We have also shown that β2GPI is abundantly present in the subendothelial regions of atherosclerotic lesions of humans. This observation suggests a local immune response to β2GPI may take place within atherosclerotic lesions, potentially influencing its progression. Moreover, oxidized LDL specifically competes with radiolabeled β2GPI on the incorporation into human umbilical vein endothelial cells and into a myelomoncytic human cell line (U937). Thus, β2GPI is present in atherosclerotic lesions, may be uptaken by its cellular constituents and consequently could culminate in enhancement of lesion progression.

Anti-ICAM therapy improves neurologic manifestation of experimental antiphospholipid syndrom – The role of inflammation in APS

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Antiphospholipid syndrome (APS) is associated with various neurological complications, but the exact etiopathology is not well defined. Antiphospholipid antibodies (aPL) can induce a procoagulant and proinflammatory endothelial phenotype with enhanced expression of adhesion molecules. In this study we evaluate the anti-inflammatory effect of anti-ICAM monoclonal antibody on the neurologic dysfunction of mice with experimental APS. Experimental APS was induced in BALB/c mice ($n = 45$) by β2-GPI immunization (10 μg/mouse in CFA). Three weeks after immunization, the mice were treated with 5 weekly i.p. injections of 500 μg of anti-ICAM antibody. Five month after immunization β2-GPI- immunized mice displayed impaired motor coordination on rotating bar ($p < 0.01$), cognitive dysfunction in T-maze ($p = 0.01$) and showed a tendency for hyperactivity in staircase system. Anti-ICAM treatment of β2-GPI immunized mice resulted in a significant improvement of motor coordination, in hyperactivity, but did not change their cognitive abilities. We assume that ICAM-mediated process account for neurological manifestation in APS and might provide a novel target for managing APS.

Review of 1HNMR ganglioside resonances aassociated with brain gliomas after the discovery of monstruous aberrant spectra in the low field

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Our method producing homogenates (Bratislava, Firenze and Bari, 1993) and the high resolution 1HNMR spectrometers of Prague (500 MHz), Firenze (600 MHz) and Bremen (600 MHz) made possible the discovery of the CERAMIDE (sphingosine-unsaturated fatty acid) broad resonance at 5.3–5.4 ppm of the aberrant GANGLIOSIDE in high malignant brain gliomas. The 5.3–5.4 ppm CERAMIDE spectra of low field corresponded to the crosspeack 2.0 ppm spectra of high field. Of $N = 3$ cases of normal human brain, $N = 12$ cases of astrocytomas (gr.II-III), $N = 20$ case of glioblastoma multiforme, $N = 2$ cases

of brain metastasis and $N = 4$ cases of meningioma, an extensive series of 1HNMR spectra will be reviewed.

Serological techniques system for tularemia diagnosis

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Tularemia infection as well as vaccination with live tularemia vaccine is known to induce the long-lasting cell-mediated immune response and humoral immune response to *F. tularensis* bacterium accompanied by specific antibodies synthesis. Humoral response is an indicator for testifying of human (or animal) organism immune status. Tularemia diagnosis is performed by means of immunological methods based on stereocomplementary reactions, using of immunoglobulins marked fluorochroms, ferments etc. This immunodiagnostic tests complex is used both for specific antibodies revealing and tularemia pathogen rapid identification. A hibridoma technique is tested in tularemia investigations. However, the monoclonal antibodies were applied mainly for scientific investigations (Kchlebnikov et al., 1991). The purpose of this research was to elaborate tularemia immunological diagnosis system by means of simple and available methods for epidemiological and epizootological investigations on tularemia. 379 serum samples from 330 individuals (patients and persons vaccinated for different periods after infection or vaccination) were tested for tularemia antibodies presence. The complex of immunological methods was used: microvariants of the agglutination reaction (AR), different modification of the passive hemagglutination test (PHAT), ELISA and the immunofluorescent assay (IFA). Patient antibodies were revealed by comparative analysis of serological methods in 1 week later after the disease onset by ELISA (diagnostic titers $\geqslant 400$) while they could be detected only in 2–3 weeks by means of AR, PHAT, IFA ($\geqslant 100$). The maximum level of antibodies titers determined in 4–7 weeks by ELISA was higher (in 4–20 times) as compared with AR, PHAT, IFA, and the specific antibodies were found to persist in high titers ($l : 1600$–$1 : 6400$) for several years ($\geqslant 10$ years). Class-specific antibodies (Ig A, lg M, Ig G) by ELISA was revealed in 1 week while the titers have became diagnostic (> 400) just in 2 weeks. Higher level of Ig M and Ig A testified about acute tularemia. The correlation Ig G/Ig M > 2 was evaluated as the indicator of ananmestic reaction. ELISA advantage was also established especially for early determination of humoral immunity in vaccinated individuals (in 2 weeks after vaccination day). The revealing of Ig M-levels for several years after infection (or immunization) may attest to the tenacity of the intracellular microorganism presence. The tularemia infection was produced at high-sensitive experimental animals which were injected subcutaneously (white mice) or intraperitoneally (guinea pigs) with 10–20 DCL virulent strains (two subspecies of *F. tularensis*). The pathological material from animals (tissue from the inoculation place, spleen, liver, lungs, blood, urine) was collected and studied during the infection process. The following sensitivity indices were established: IFA to reveal 10^4–10^6, PHAT – 10^5–5×10^5, inhibition of PHAT – 10^6, ELISA – 10^4–5×10^4 cells/ml. *F. tularensis* was detected by ELISA in the organs of infected animals (spleen, liver, lmgs) from 2–3 days, by IFA and inhibition of PHAT – from 2–4 and 5–6 days respectively. IFA advantage was found in a rapid answer. The inhibition of PHAT possessing relatively low sensitivity is express method for rapid revealing of *F. tularensis* (or antigen) from different objects of the environment. Thus the parameters of immunological methods (sensitivity, specificity, time of analysis) were determined and their estimation was taken as well, the role and place of every test in the common system of tularemia immunological diagnosis was established.

Antegenic analysis of Rift valley fever virus strains using monoclonal antibodies

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Rift valley fever virus (RVFV), member of the a *phlebovirus* genus of the bunyavirideae family, is an arthropod-borne virus which emerges periodically throughout Africa where it is a major cause of endemic and epidemic disease of human and domestic livestock. Several Rift valley fever virus isolates from diverse localities of Africa and from various host were analysed to assess strains variation using monoclonal antibodies prepared against the RVF reference strain Ar B 1976. By the indirect immunofluorescence assay, all the monoclonal antibodies reacted with the different strains. The isolates appear to be antigenically identical to the Ar B 1976 strains.

Antinuclear antibodies in the sera of patients with Systemic Lupus Erythematosus (SLE)

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Objective: There is variability in the frequency of autoantibodies in the serum of patients with connective tissue diseases. In most studies the prevalence of the antinuclear antibodies (ANA) have been found. The investigation of these autoantibodies, concerned with clinically significant immunological methods, contribute to differential diagnosis of disease. The objective of work was assessment the possible relation of ANA, antibodies to double-stranded DNA (d-DNA), total activity of complement (CHSO) and some clinical features of disease.

Methods: The group of 46 patients was investigated. ANA were determined by the method of indirect immunofluorescence, d-DNA – immunoassay (ELISA), activity of CH50 – evaluated according to 50% lysis of sensitized sheep erythrocytes.

Results: The investigations carried out revealed that the occurrence of ANA in the serum of patients with SLE was in the range of 48–80%, antibodies to d-DNA – 60.8%, while the activity of CHSO was lowered in 47.8% of cases. The presence of ANA was not always related to the stage of disease or other clinical features. Moreover investigated indices of the disease activity were significantly dependent on the disease activity stage. The correlation of disease activity indices to the clinical courses of disease and damage of some internal organs was frequent. There was no relation of ANA occurrence and the alteration of other investigated indices.

Conclusion: The investigations revealed significant relation of disease activity indices to the disease stage in the course of SLE, though the correlation of ANA to other immunological indices was not found.

Monoclonal antibodies against a Staphylococcus aureus surface protein protect against bacteremia induced mortality

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Background: S. aureus is a feared pathogen and a common cause of nosocomial and community acquired infections. The continued emergence of antibiotic resistant strains require the development of novel therapies to prevent and treat staphylococcal infections. McGavin et al. (McGavin et al., *Infect. Immun.*, 1993, 2479–2485) identified a 72 kDa surface protein, from *S. aureus* strain FDA 574, that binds a variety of host proteins including BSP, fibrinogen, fibronectin, vitronectin, and thrombospondin. The gene, designated *map*, was cloned and sequenced and found to contain 6 repeated units each subdomain (110 amino acids) displaying similarity to the peptide binding groove of MHC class II DRβ molecules from mammalian species. We now report that monoclonal antibodies against MAP, a highly conserved cell surface localized protein expressed by virtually all *S. aureus* strains, are protective in a murine model of bacteremia.

Methods: Mice ($n = 15$/group) were treated by a single IP injection with mAbs (36 mg/kg) H07, H10 or PBS. Nineteen hours after mAb administration, the mice were challenged with an IV injection of *S. aureus* strains Barnett, ATCC 25923, or ATCC 49230. The mice were then followed for 6 days and the survival data of each group of mice was analyzed by the Mantel-Cox test.

Results: mAbs H07 and H10 provided superior protection against all 3 strains of *S. aureus* compared to control. This is the first scientific report of monoclonal antibodies, against a staphylococcal protein, that can protect against a lethal infection.

Test agents	S. aureus Bacterial Strain	*Statistic*
H07 vs. PBS	ATCC 25923	$p = 0.0001$
H10 vs. PBS	ATCC 25923	$p = 0.0078$
H07 vs. PBS	ATCC 49230	$p = 0.0008$
H10 vs. PBS	ATCC 49230	$p = 0.0062$
H07 vs. PBS	Barnett	$p = 0.0173$
H10 vs. PBS	Barnett	$p = 0.0396$

Conclusions: These data clearly demonstrate that a single infusion of a MAP monoclonal antibody can significantly prevent sepsis mediated death against multiple strains of *S. aureus* in a relevant in vivo model.

Plantibodies$^{\mathrm{TM}}$ as mucosal protectants

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Previously we have shown that vaginal delivery of monoclonal antibodies (MAbs) can prevent genital herpes and *Chlamydia trachomatis* infections in the mouse, and pregnancy in the rabbit. Similar studies by other investigators have shown that mucosally applied antibodies can prevent infection by a wide variety of pathogens. MAbs produced by conventional methods have been too expensive for human use as mucosal protectants, but MAbs can now be produced in plants (Plantibodies$^{\mathrm{TM}}$) at a fraction of the cost. Here we compare human anti-herpes simplex virus IgG, IgA, and secretory IgA (SIgA) MAbs produced in plants (soybean and rice) with conventionally gen-

erated MAbs. MAbs were evaluated for neutralization activity in vitro, stability in human reproductive tract fluids, ability to diffuse in human cervical mucus, and for protective efficacy in a mouse model of vaginal transmission. In all of these assays, MAbs produced in plant were indistinguishable from conventionally produced MAbs. Studies looking at complement mediated activity as well as antibody dependent cellular cytotoxicity by Plantibodies^{TM} are currently underway. With recently obtained clinical data on the vaginal residence time of antibodies in humans, we expect that several days of protection might be provided with a single dose. Furthermore, additional experiments of ours suggest that antibodies can be incorporated into sustained release devices for months of protection. Based on these results and those of other investigators, we believe that Plantibodies^{TM} are specific, flexible, potent and cost-effective agents that may prove clinically useful for a wide variety of health applications.

β-Globin gene clusters haplotypes associated with sickle cell mutation among Egyptian patients

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This work was designed to study the sickle cell chromosomes (B^s) in order to detect the Egyptian haplotypes and genotypes. It has been carried out on 36 patients, out of them, 26 were diagnosed as sickle cell anemia (SCA) and 10 as sickle thalassemia. Their origin was more in upper Egypt than lower Egypt. The consanguinous mating was found to be 73% in SCA and 40% in sickle thalassemia. The patients were exposed to clinical examination and investigations with emphasis on blood counts and Hb electrophoresis. DNA was extracted from lymphocytes, quantitation, then PCR amplification was done using primer pairs specific to 5 regions of the beta globin cluster followed by restriction enzyme analysis. These included B (Ddel and Ava II), Ay (Hind III), Gy (Hind III), II B (Hind II) and II B 3' region (Hind II). Restriction enzyme Ddel revealed homozygosity in 26 subjects while 10 subjects were sickle thalassemia. We found six haplotypes. Out of the 62 B^s chromosomes studied, 39 (62.9%) were of the Benin haplotype, 17 (27.4%) were of the central African haplotype. This information may be used as one of the methods reported for prenatal diagnosis and for gene therapy in the future.

mRNA of circulating cytokines in juvenile rheumatoid arthritis

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The study comprised patients with JRA and control. Patients were enrolled according to their final diagnosis into three groups: G1 included patients with pauciarticular onset, G2 included patients with polyarticular onset, G3 included patients with systemic onset. All patients were in activity. For all the individuals the following have been done: (1) history and clinical examination and investigations for diagnosis, (2) detection of the following cytokines in the serum; IL-2, IL-4, IL-6, IL-10, TNFα, Ifγ, (3) detection of mRNA by reverse transcription polymerase chain reaction for the previously mentioned cytokines. The results could be summarized as follows: (1) All cytokines were significantly increased in patients than control, (2) TNFα and Ifγ more in polyarticular, IL-2 more in pauciarticular, and IL-4, IL-6, IL-10, more significantly increased in systemic, (3) mRNA expression revealed the same previous results, (4) stepwise discriminant analysis showed that TNFα, IL-2 and Ifγ are the most important cytokines in the general pathogenesis. In conclusion, the cytokines studied play important role in the pathogenesis of JRA and that macrophage and Thl response may be the dominating process. Based on the transcription of mRNA, there is no defect in gene expression, effective and safe therapy should be based on its specific effect on the transcription of the mRNA and hence inhibiting the production of the cytokine more responsible to the pathogenesis of certain type of JRA.

Study of the 563 and 1311 mutations of the G6PD gene among Egyptian children with favism

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The objective of this work is to study the 563 and 1311 mutations among Egyptian children presenting with acute hemolysis due to favism (i.e giving history

of intake of fava beans). The work included 75 male children aged up to 15. In addition 20 normal matched controls. Cases were collected from the Hematology Clinic of Mansoura City University Children Hospital (MUCH), Mansoura, Egypt. Their paternal and maternal origins were from different provinces of Egypt. Qualitative and quantitative estimation of the G6PD enzyme activity had been done. Molecular detection of the Mediterranean mutation 563 C-T and the silent mutation 131 C-T of the gene were also attempted through PCR amplification of the region of interest followed by restriction enzyme analysis, Mbo II and Bcl I respectively. The enzyme was found deficient in the 75 subjects both by decoloration test and spectrophotometric assay. Out of the 75 cases 45 subjects showed G6PD enzyme activity below 10%. Mediterranean mutation 563 were found in 45 (60%) of the 75 deficient cases but in none of the normal 20 controls whereas the silent 1311 mutation were present in the same 45 cases and in 2 of the controls. All the 45 cases were those having the activity less thyan 10%. Studies of other G6PD gene mutations in the other 30 cases that had no G6PD-Mediterramean mutations are still underway in our Unit.

Soluble tumour necrosis factors and interleukin-2 receptors in juvenile rheumatoid arthritis

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The objective of this work is to assess whether circulating concentrations of soluble necrosis factor receptors (sTNFR; p55 and p75) and soluble interleukin-2-receptors (sIL-2R) reflect clinical response. These soluble receptors were determined in control children and before treatment, and 48 weeks after treatment by immunoassays in patients with active JRA. The patients entered prospective and randomized double blind trial of corticosteroids and NSARD as the more used therapy. The results could be summarised as follows: (1) concentrations of p55, p75 and sIL-2R were significantly higher in JRA than in controls, (2) the concentrations significantly decrease with treatment and the decrease is significantly more in p55 in patients with complete improvement compared to those with incomplete improvement, (3) the response to the two groups of drugs showed insignificant difference in the pauciarticular, polyasticular and systemic types. In conclusion, measurement of TNF p55 may be useful in the evaluation of JRA activity and response to therapy, and the effect of both corticosteroid and NSAID on the production of the cytokines is the same.

Relation between apoptosis and HLA-DR alleles in juvenile rheumatoid arthritis

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This work was planned to study if there is relation between apoptosis and HLA-DR alleles in JRA. Sixty patients with JRA and 60 normals as control were typed first by serology (microcytotoxicity test) for DR-1, 2, 4, 5, 6 & 8 and then subtypes were analysed by sequence specific primers polymerase chain reaction (SSP-PCR). Apoptosis was measured by preparation and culture of blood neutrophils. The cells were incubated for 24, 48 and 72 hours in tissue culture medium. Both morphology and DNA fragmentation assessed the percentage of neutrophil apoptosis in each culture. The results revealed a significant and strong correlation between inhibition of apoptosis and the alleles DR4 *0404 ($P < 0.01$). Furthermore, both the frequency of DR4 and the inhibition of apoptosis were significantly changed in patients compared to control. Based on these results, we conclude that the mechanism of cell death in JRA may be some what related to the immunogenetic make up of the patients, and more risky in those having HLA DR4 *0404.

Correlation between tumour markers and immune profiles in children with acute lymphoblastic leukemia

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The objective of this work is to study tumour markers in diagnosis and follow up of acute lymphoblastic leukemia (ALL) in correlation to apoptosis, natural killer (NK) cells and T cell receptors. The participants were 26 children diagnosed to have ALL sand 26 samples from normal mononuclear cells. Tumour mark-

ers namely, activated protein kinase receptor (PKRm RNA), telomerase activity, ALL 1 gene and ETV 6 (TEL) gene were detected in blood sample of Alt and control using reverse transcription PCR. In the meanwhile, apoptosis (as assessed by morphologic and quantitative DNA fragmentation), NK cell, Il4 and INF gamma were studies using flowcytometer. The result showed that the mean PKRm RNA levels were increased in ALL compared to normal MNCs. Also compared to the age-matched normal levels of telomerase activity in the peripheral blood cells, we determined that 19 (69.6%) of 26 acute lymphoid leukemia patients had elevated telomerase activity. The levels of telomerase activity significantly decreased in patients in complete remission. Most of the patients in complete remission showed a normal level of telomerase activity; however, two of them had low to moderate telomerase activity, and they relapsed shortly after entering complete remission. In relapsed patients, there is a general trend for increased telomerase levels, and 2 of the 13 patients retained high telomerase activity, whereas the other 11 had normal to moderate telomerase activity. These findings provide no evidence to support the hypothesis that PKR acts as a tumour suppressor in human leukemic cells and suggest that telomerase activity may he a useful additional method for monitoring the disease condition in acute leukemia patients. ALL-1 gene have been observed in patients with specific acute lymphoblastic leukemias. Here a novel structural alteration of the ALL-1 gene was observed in three patients presenting with acute lymphoblastic leukemia (ALL) without chromosomal translocations or self-fusion of ALL-1 gene. Of 26 children with newly diagnosed ALL, 9 (11.5%) had a total 12p abnormalities. Loss of genetic material was observed in 17 (64%) of these abnormalities. Cases with 12p alteration had a much lower frequency of hyperdiploidy greater than 50 (7%) than did the ALL population in general, but these cases had a similar distribution of immunophenotype. Rearrangement of the ETV6 gene was identified in 13 (56%) of 26 cases evaluated. The ETV6 (TEL gene) fusion transcript was found in 15 (66%) of 26 cases evaluated, and all but one of these showed ETV6 rearrangement. Importantly, ETV6 rearrangement was associated with a favourable prognosis. We conclude that most but not alt 12p abnormalities in childhood ALL involve ETV6, and that rearrangement of ETV6 is associated with a favourable treatment outcome. Consistent low levels of spontaneous apoptosis were observed in lymphoblastie cells from normal peripheral blood samples, while untreated cells collected from 26 de novo ALL patients showed variable apoptosis. Also the expression of CD7, CD33, CD34, CD56, and frequently HLA-DR but not other NK, T-cell, and B-cell markers was observed. Cytoplasmic CD3 was detected in 9 of the cases. Significantly decreased CD7, IFN-γ and IL-4 expression was observed in the patients with ALL ($P < 0.001$). While CD7 negativity and IL-4 expression were more frequent in the later stages of the disease, this did not attain statistical significance. These results suggest a possible explanation for the reduced cellular and humoral immunity in ALL. From this study we can conclude the important role of tumour markers in early diagnosis and follow up of ALL. Also, these have a significant cnelation with apoptosis, NK, TH_1, and TH_2.

Apoptosis, natural killers cell and antineoplaston A10 in normal individuals and breast cancer patients

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Antineoplastons are naturally occurring peptides and amino acid derivatives which control the neoplastic growth. A10 in the urine of 42 patients with a histologically confirmed diagnosis of breast cancer (who were free from other cancers and without previous treatment for breast cancer) and 42 age matched nomal women was measured using high performance thin layer chromatography (HPLC). BL samples were obtained and apoptosis in neutrophils was assisted both morphologically and by DNA fragmentation and natural killer (NK) cells were assisted using HNK-1. A_{10} was prepared, chemically and direct effect on neutrophil apoptosis was detected in vitro after adding A_{10} in concentration of 10 ng/ml of culture. Significantly lower A_{10} level was detected in patients with breast cancer ($P < 0.001$). A significant higher neutrophil apoptosis level and high NK counts were detected in breast cancer. In vitro A_{10} was found to inhibit significantly the neutrophil apoptosis. These finding suggest a strong inverse association of urinary A10 level with breast cancer and this test may be an excellent predictive for women who are at risk of developing breast cancer. Also our data confirm the presence of immune defect

in breast cancer and that such findings should stimulate the development of new strategies to induce and augment immunity for treatment of breast cancer and that AO may be used as adjuvant therapy in breast cancer patients.

Apoptosis and autoantibodies in relation to activity and severity in juvenile rheumatoid arthritis

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This work was planned to study the relation between apoptosis and autoantibodies production on one side and between them and activity and severity on the other side in juvenile rheumatoid arthritis (JRA). Sixty children with JRA and 20 control children were included in the study. The following have been carried out: (1) clinical examination and assessment of activity and severity, (2) assessment of apoptosis, (3) autoantibodies assay of APF, AKA and AFA. The results have been exposed to statistical analysis and interpretations and revealed the following: (1) significantly higher mean percent apoptosis (MPAP) in the control than in the JRA patients, (2) no significant relations between the type of onset and either the MPAP or the antibodies titers, while the relation was significant between them and disease activity, (3) significant negative correlation between MPAP and the increase in the three antibodies titers during activity, (4) significant negative correlation between apoptosis and autoantibodies in the whole group of patients and in the three subtypes (pauciarticuolar, polyar-ticular and systemic), (5) significant negative and positive correlation between spread severity index (SSI) and apoptosis and antibodies respective, (6) multiple stepwise regression revealed high correlation to severity accounted by APF and AP(0) in pauciarticular, AFA and AP (48 hs) in polyarticular, and APF in systemic JRA. In conclusion, there is a clear relation between apoptosis, autoantibodies production, activity and severity in JRA. Prediction of severity could be done by certain equations.

Genes expression and circulating cytokines in juvenile rheumatoid arthritis

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The objective of this work is to investigate the production of the proinflamatory and antiinflamatory cytokines to evaluate their possible importance in the development of JRA by estimation of genes expression through transcription to mRNA and then translation to circulating cytokines. The study comprised 60 children with JRA and 20 healthy control. A detailed history and clinical examination was completed for every individual. All patients had active disease defined by the presence of arthritis, CRP and, ESR levels. In addition complete blood picture, ANA and CAU status. Patients were enrolled according to their final diagnosis into three groups: (1) G1: 27 patients with polyarticular onset; (2) G2: 24 with pauciarticular onset; (3) G3: 9 with systemic onset. For all individuals the estimation of the following cytokines and their mRNAs have been done; Th1 cytokines (IL-2 and IFNγ), Th2 cytokines (IL-4 and IL-10) and monokines (TNFγ and IL-6). The results could be summarized as follows: (1) All the investigated cytokines were significantly elevated. Comparison between the levels of each cytokine in the three types revealed that: TNFγ is higher in polyarticular, IL-2 in pauciarticular and IL-4, IL-6, IL-10 in systemi. IFNγ showed no difference. (2) mRNA expression revealed parallel results (with the exception that IFNγ is higher in polyarticular) to their circulating cytokines (3) stepwise regression showed that TNFα, IL-2 and IFNγ are the most important in the pathophysiology of the disease. In conclusion, there is an upregulated genes expression through unaltered transcription of mRNAs and translation to proinflammatory and antiinflammatory cytokines to initiate the autoimmune damage. The monokine TNFα and Th1 IL-2 and IFNγ may be essential in the induction and maintenance of JRA, while Th2 cytokines may be involved in the progression of the disease. This may be important for monitoring the activity and response to therapy and provide knowledge for future immunotherapy.

Genomic detection of hepatitis C by RT-PCR

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Studies aimed at correlating the intrahepatic hepatitis C virus (HCV)-RNA level and anatomo-clinical

features have been difficult because of sensitivity and specificity shortcomings of available techniques. We titered the HCV RNAs by a semiquantitative reverse-transcriptase polimerase chain reaction (RT-PCR) in the liver tissue of 73 patients with chronic hepatitis C. Findings were correlated with the levels of HCV RNA in the serum, and the response to interferon alfa (IFN-α) treatment. The HCV-RNA level in the serum correlated with the genomic-strand. The response to IFN-α treatment could be predicted by the serum HCV-RNA level. These results suggest that, although the detection of the HCV RNA identifies the presence of replicating HCV in the liver the quantitative measurement of viremia mains the clinically meaningful "golden standard" for sessing the level of HCV replication.

Histological evaluation of the effect of a new schistosomicidal drug (Mirazid)

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Schistomiasis is adebilating and sometimes deadly parasitic infection that afflicts hundreds of millions of people in developing countries. Great efforts have been devoted to develop safe and effective schistomicidal agent. Myrrh is an oleo gum resin obtained from the stem of a plant Commiphora molmol. An alcholoc extract of this plant followed by steam distillation produced resin and volatile oil. In this work a special formulation of Myrrh, Mirazid was prepared and its efficacy was evaluated histologically in comparison to praziquantel. Mirazid was given orally in two doses (250&500 mg per kg.bodyweight) to Swiss mice 45 days after infection with 200 Schistosoma mansoni cercaria. Liver sections were stained with Hx&E, mallory trichrome (for collagen fibers) Gorden&Sweet (for reticular fibers). Histological examination of the livers revealed a decrease in the number and size of granulomas in mice treated with Mirazid at dose of 500 mg. Also there was marked decrease in the intensity of collagen and reticular fibers in granulomas and decrease of liver fibrosis. From this work we can concluded that Mirazid has a schistosomicidal and antifibrotic effect as fact that excessive deposition of collagen in several types of lesions is known to undergo resorption when provocative causes were removed.

Histological and immunohistochemical study of the effect of immunization with a purified 74 KDa antigen recognized by protective monoclonal antibody in schistosoma mansoni infection

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Chemotherapy of schistosome infections remain the cornerstone of intervention but rapid reinfection demand frequent retreatment and emphasize the need for a more long term approach. So the need for vaccination against schistosomiasis is an achievable goal. In this work the protective and antifibrotic effect of 74 KDa antigen reactive with the BRL4 monoclonal antibody was evaluated histologically and immunohistochemically. Swiss mice were immunized with different doses of purified 74 KDa antigen with and without Freund's adjuvant. Then mice were infected with 200 S. mansoni cercaria three days after final immunization. The results revealed that the highest protection level (76.6% and 80%) was obtained using the 50 μg antigen dose with and without Freund's adjuvant. Histological examination revealed marked decrease of the granuloma number and size. Also, marked reduction of both collagen and reticular fibers was observed around granulomas of mice liver that immunized with 74 KDa antigen immunohistochemical study revealed decrease of the intensity or even absent of the schistosomal antigen in immunized liver compared to that infected and not immunized. From this work we concluded that in addition of the ability of the 74 KDa antigen to mediate protection against schistosoma mansoni infection. It. can reduce the granulomatous reaction around eggs lodged in the liver by its ability to modulate the immune system of the infected host. So the protection conferred by the 74 KDa enhanced additional experiments that may lead to improvement of its protective capacity as a candidate vaccine.

A possible antibody evolution attributes the B cells accumulating – supposedly driven by antigen – in medullary breast carcinoma

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Introduction: Our current knowledge of the human immunoglobulin (Ig) repertoire is derived from three main sources: fetal B lymphocyte repertoire, B cell

malignancies and autoantibodies stemming from a variety of autoimmune diseases. Up to now no detailed immunoglobulin repertoire analysis has been reported from B lymphocytes that accumulate in any solid tumors.

Study design: A model system was set up in order to reveal the potential antibody evolution of B lymphocytes infiltrating solid tumors (TIL-B). Comparative data analysis of TIL-B Ig heavy and light chain variable region sequences were performed by IMGT/DNAPLOT database in terms of closest germline sequences.

Results and conclusions: Based on the search above, several families and clusters could be distinguished. The number of investigated cluster members varied, some were overrepresented. Among the clusters a preferential Ig V, D J gene usage could be determined. Few mutations were found in the light chain V region sequences located randomly, but the V and J joining was often involved. A close homology to germline sequences could be also stated in the case of heavy chains. There were TIL-B heavy chain clusters with no additive nucleotides at all, while others showed variability in this respect. Based on our data a clonal proliferation was constructed, that gives information about the antibody evolution of the TIL-B.

Natural anti-FcεR1α autoantibodies isolated from the repertoire of healthy donors show a conditional autoreactivity on human basophils

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We are investigating the role of natural autoantibodies (Abs) to the α-chain of the high affinity receptor for IgE (FcεRIα). It has been suggested that triggering of this receptor by anti-α-chain Abs may be involved in the pathogenesis of autoimmune urticaria. However, our previous results have shown that these antibodies are widespread, apparently non-pathogenic and belong to the natural antibody repertoire. To investigate the role of these natural anti-α-chain Abs we applied repertoire cloning to antibody IgM libraries constructed from children's tonsils. Two anti-α-chain Abs have been isolated by selecting on recombinant human α-chain produced in CHO cells. Both antibodies recognised cell bound α-chain and were able to trigger histamine release from freshly isolated blood basophils. However the histamine release was dependent on removal of IgE from the FcεRIα. Similar results were seen using purified anti-α-chain antibodies from multi-donor IgG preparations. Sera from individual normal donors also showed the same pattern of reactivity. Recent results have shown that upregulation of the FcεRI occurs upon antigen challenge and could thus become accessible to the natural anti-FcεRIα antibodies. Based on these results we propose a new model of conditional autoimmunity whereby the degree of occupancy of the FcεRIα determines the access of the anti-α-chain antibodies to their binding site and their eventual patho-physiological effect. This model may provide a unifying hypothesis for the many different forms of urticaria.

C-erbB-2 oncogene as a potential target for the study of breast and ovarian cancer

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Monoclonal antibodies against C-erbB-2 oncoprotein has been generated in our laboratory using MDAMB361, a human breast cancer cell line which overexpresses the oncoprotein gp185 (a glycoprotein of MW 185 KD) as immunogen by conventional Hybridoma technology. The Hybrids were cultured at 5–7% CO_2 in HAT medium. The culture supernatants of clones were screened for the presence of specific antibody by ELISA using plates coated with 2 μg/well of the protein isolated by Triton X 100 extraction of MDAMB361 cells, ultracentrifugation and HPLC purification. Of the several positive clones identified, 4 clones A8, B6, H3, D1 were found to exhibit specific reactivity with MDAMB 361 cells by Immunoperoxidase test. The specificity of these antibodies has also been evaluated by Immunoprecipitation, Western blot and Flowcytometric analysis. These antibodies stained specifically the tumor cell membrane in Frozen tissue sections of Breast and Ovarian tumors overexpressing the C-erbB-2. The diagnostic and therapeutic potential of these antibodies will be discussed.

Immunogenicity of therapeutic proteins – Natural and induced antibody responses

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Clinical and preclinical development of biological therapeutics, including monoclonal antibodies, recom-

binant cytokines, soluble receptors and fusion constructs, require evaluation of their immunogenic potential. Immunogenicity is inherent to a molecule, but host factors, including pre-existing natural antibodies, can influence occurrence of antibody response to a recombinant protein. The presence of a broad range of natural antibodies has been detected during the development of a number of biopharmaceuticals. Typically, a search for such antibodies does not occur outside the scope of development of recombinant proteins. Uniquely engineered biopharmaceuticals demand a development of custom immunoassays to measure anti-drug antibodies and their natural counterparts. In addition to detecting antibodies, an essential part of the evaluation of immunogenicity is related to consequences of antibody formation. Characterization of antibody response includes specificity, epitope mapping and neutralizing potential. In this paper, immunogenicity of three biopharmaceuticals, a monoclonal antibody, a recombinant human cytokine and a recombinant human growth factor, will be discussed. These constructs induced immune responses in animals, however, the existence of natural antibodies in both animals and humans was also discovered. Incidence and nature (binding specificity, neutralization) of the natural antibodies cross-reacting with the recombinant proteins have been determined in human and non-human primates.

Novel sperm antigens recognized by polyclonal antisera from prepubertal boys and generated in Scid mice system

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We have studied sera samples ($n = 26$) from infertile adults and prepubertal boys ($n = 93$) with testicular failures (cryptorchidism, mobile testis, torsion of testis) as well as control healthy and fertile population ($n = 7$) for antisperm antibodies reactivity (ASA). We have applied immunobead test (IBT) and ELISA to preliminary evaluate positive reactions. We have found approx. 12% of cases among the population of control prepubertal boys ($n = 17$), positive for ASA when using both methods of detection. In boys with testicular failures, we have revealed 14% of positive individuals by IBT and 34% by ELISA. For biochemical characterization of recognized by these antibodies sperm antigens, we have applied Western immunoblotting (64% of positive reactions in a group of boys with testicular failures, 55% of infertile females and 65% of infertile males) after extraction of sperm with Triton-deoxycholate lysis buffer. It was about 40 reactive bands representing broad range of sperm antigens with molecular weights from 21–80 kDa. The most frequent antigen in women group appeared in 63 kDa, in male group – 67 kDa while sera from prepubertal boys most frequently recognized entities of 58, 62 and 63 kDa. In immunoprecipitation studies, we have revealed over 70 sperm antigenic entities of molecular weight ranging from 15–157 kDa. Majority of these determinants overlapped with antigens on somatic cells (erythrocytes and lymphocytes), however some of them were sperm-specific and not referred until now in existing literature (15 to 115 kDa). Partial deglycosylation of sperm antigenic extract (N-linked sugar moieties) have revealed a new pattern of reactions performed by both applied biochemical tests.

Interestingly, N-deglycosylated antigenic extract, when administered to Scid mice previously deposited with human lymphocytes from vasectomized males, induced different type of polyclonal antibodies than glycosylated, 'native' extracts. Antisperm antibody levels were on quite high level (as evaluated by ELISA) when induced by glycosylated sperm, however when sperm agglutination activity was checked the function of these antibodies fell down shortly. On the contrary, antibodies induced by deglycosylated sperm maintained high agglutinating activity after all three sensitizations. However, using immunofluorescence, we have observed better reactions (21% of reactive sperm) by polyclonal antibodies induced by glycosylated sperm extract than by deglycosylated one (5% of reactive sperm). Thus immunomodulatory activity of obtained ASA was strictly dependent on partial sperm deglycosylation. Human monoclonal antisperm antibodies, obtained from hu-Scid system undergo biochemical characterization.

Diagnosis of diffuse alveolar hemorrhage associated with Wegener's granulomatosis using flowcytometric analysis of broncho alveolar lavage fluid – Case report

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Although the lung is the most common organ system involved in Wegener's granulomatosis (WG), diffuse alveolar hemorrhage (DAH) is uncommon. The clini-

cal preservation of those patients with DAH often differs from that of other patients with WG. Furthermore, in patients with WG who develop DAH, the DAH often could be the initial presentation of their disease. A 46-year-old woman presented with rapidly progressive dyspnea, mono-arthritis (right knee), chest pain and necrotizing skin ulcers was admitted with hypoxemia, anemia, bilateral pulmonary infiltrates on chest radiograph. Broad-spectrum antibiotics were administered. Several days after admission she developed progressive renal insufficiency despite therapy with corticosteroids cyclophosphamide and IVIG and died. The immunological data revealed: ANA (−); RF (+); pANCA (−); ACA (−); cANCA (+); elevated serum total IgE, e.c. Bronchoscopy revealed blood throughout the airways of the upper and lower respiratory tract. Flowcytometric evaluation of Broncho Alveolar Lavage Fluid (BALF) with a broad panel of monoclonal antibodies showed hemorragic sample with elevated activated neutrophils (CD11b++/CD62L−), decreased lymphocytes especially CD4+, normal Th/Ts ratio together with a peripheral blood lymphopenia and esinophilia. Using the flowcytometric conclusion of BALF the DAH was diagnosed prior to sera immunological data and additionally was confirmed with the clinical, immunological and biopsy (autopsy) results. On the basis of the reported case and our clinical approach to flowcytometric analysis, the role of an early BALF evaluation in cases with DAH will be discussed.

Humanisation of murine anti-human IgE antibody, BSW17, by phage display

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It is generally accepted that the specific IgE response has to be down regulated in order to treat allergic disease. Anti-IgE antibodies that block binding of IgE to high affinity receptors on mast cells and basophils and inhibit IgE synthesis have been shown to be of therapeutic value to treat atopic individuals. A monoclonal murine anti-human IgE antibody called BSW17 has been shown to possess all these characteristics. The development of a human like BSW17 anti-IgE antibody that would represent similar and improved characteristic as the current antibody used in clinical trial (E25). As rodent antibodies are highly immunogenic in humans we used a procedure to convert the murine antibody, BSW17, into a human antibody with similar binding characteristics. In this procedure, the murine V domains are sequentially replaced by human V domains, using two consecutive rounds of variable domain shuffling and phage selection on human IgE. In a first step the murine anti-IgE antibody, BSW17, was cloned into the phagemid vector, pComb3H. A mouse Fab clone was isolated that recognised the same human IgE binding site as the whole BSW17 IgG molecule. This was demonstrated in an inhibition assay where the binding of the BSW17 Fab clone on IgE was inhibited by using the full length BSW17 IgG molecule. The murine Fd fragment was then substituted by a chimeric Fd fragment composed of mouse VH linked to the human CH1 domain. A chimeric Fd clone was obtained and then paired with a human VLCL gene library. Work is now in progress to screen on human IgE the resulting phage library displaying hybrid Fabs in order to isolate a chimeric antibody with the best affinity for IgE. Further steps involve pairing the human light chain obtained from the best chimeric antibody with a human VH gene library in order to isolate a human antibody with similar binding characteristics as our original murine anti-IgE antibody, BSW17.

“Troy-bodies”: recombinant Abs that deliver T cell epitopes to APC

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$CD4^+$ T cells are central in most adaptive immune responses since they can help B cells and $CD8^+$ T cells, and because they secrete cytokines with important immunoregulatory roles. A major objective in vaccine development is therefore the design of reagents that give strong specific T cell responses. $CD4^+$ T cells are activated when they recognize antigenic peptides presented on MHC class II molecules on professional antigen presenting cells (APC). To increase T cell activation, we have taken an approach by which we target T cell epitopes to antigen presenting cells. The strategy is based on recombinant antibodies (Abs) called Troy-bodies. These have APC-specific V regions and C regions with T cell epitope inserts. Such Abs are expected to target the epitopes to APC, thereby increasing the number being presented on MHC class II molecules. Four different T cell epitopes, $\lambda 2^{315}$(91–101), OVA(323–339), HA(110–120), and HEL(46–61) have been introduced genetically into the first constant domain of a NIP-specific human IgG3. Since V domains have been shown to accept large sequence variations in their CDR loops, the CH1 domain loop that corresponds to CDR3 was chosen as site for the epi-

topes. We find that the Abs are secreted, thus the C domain can accept loops elongations and variations. Furthermore, when the Abs are added to cultures of APC and T cells, they are able to induce T cell stimulation. Hence, the inserted peptides are properly excised from their new positions and presented on MHC class II molecules. Next, targeting to professional APC has been obtained by adding APC-specific V regions. Two specificities have been tested so far: IgD and MHC class II. When IgD and MHC class II-specific Abs are compared to non-targeting control Abs, a 1000-fold increase in T cell stimulation is observed. The Abs have also been injected into mice, where both IgD- and class II-specific Abs are at least 100-fold more efficient at loading spleen APC with Ag. Thus, both in vitro and in vivo, targeting to APC lead to an increase in $CD4^+$ T cell activation. The work is supported by grants from the Norwegian Cancer Society and the Norwegian Research Council.

Pepbodies – **Novel antibody fragments with natural effector functions**

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Antibodies bind antigens and eliminate them via immunoglobulin (Ig) effector functions such as activation of the complement system and interaction with cellular receptors. All effector functions are associated with the Fc-region. Fully functional Igs must be glycosylated and thus be produced in eukaryotic expression systems. Fv, scFv or Fab fragments can be successfully expressed in *E. coli*, but lack the Fc-region and thus also the effector functions. We have developed small bacterially produced antibody fragments that can crosslink antigen and antibody effector molecules. We call these molecules *Pepbodies*. Pepbodies are defined as binding molecules comprising antigen- binding sites genetically fused to peptides that display one or more of the effector functions associated with the Fc-region. Phage peptide libraries were constructed and used for the isolation of peptides that bind specifically to different antibody effector molecules including C1q, FcγR1 and poly IgR. Systems for easy exchange of peptides between the phage format and ScFv- and Fab-fragments were established. The genetic fusion of C1q binding peptides to ScFv fragments resulted in the production of fragments capable of antigen binding, C1q binding and complement activation. Peptides binding other effector molecules will be analyzed for effector function in the *Pepbody* format.

Conclusions: Pepbodies can recruit natural antibody effector functions without the need of an antibody Fc-region or additional antigen binding domains.

Future prospects: Pepbodies can be constructed to combine more than one effector function by displaying different peptides as fusions to the antibody fragment. Pepbodies can be used as antibody therapeutics.

Subtractive selection using phage antibody display on human healthy and diseased tissue to identify novel targets and antibodies

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Crucial elements of pre-clinical research involve the identification of novel targets, and the ability to rapidly develop therapeutics that bind to these targets and that are engineered for optimal clinical efficacy. Through employing scFv phage display libraries in subtractive selection, we have developed a method for the simultaneous detection of novel targets and antibodies. These antibodies can then be rapidly engineered into fully-human antibodies. Crucell's subtractive phage selection technology, MAbstract, is designed to obtain antibodies against a wide variety of cell surface antigens, and to discover drug targets not identifiable in genomics or proteomics. In MAbstract, antibodies are selected for specific binding to cells of interest while an absorber cell population removes undesired specificities. This approach yields human antibodies against subtle variations of cell surface molecules, such as changes in glycosylation or conformation that are often associated with disease processes. Antibodies obtained through MAbstract are validated by immunohistochemistry to assess expression of the target antigen in our extensive collection of healthy and diseased human tissue. Antibodies with desirable specificities are further analyzed via in vitro and in vivo functional experiments, followed by the assembly of packages of preclinical data for IND filing.