Abstracts of Poster Papers

Characterization of biologically active ANCA induced in mice: pathogenic role in experimental vasculitis

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Objective: To induce antineutrophil cytoplasmic antibody (ANCA) in mice by idiotypic manipulation, to characterize them antigenically and biologically, to test their ability to induce WG in mice.
Methods: Twenty mice were immunized with IgG from a patient with fulminant WG; 20 mice were immunized with normal human IgG as controls. Mice were tested for production of ANCA and development of signs of lung and kidney disease. ANCA were tested for their ability to induce adhesion of neutrophils to endothelial matrix, and their ability to induce the enzyme superoxide dismutase (SOD) in neutrophils.
Results: Two months after the immunization the mice developed cANCA specific for proteinase-3 (PR3) as determined by immunofluorescence and ELISA. Beginning at 5 months after immunization the mice began to develop signs of vasculitis and focal mononuclear infiltrates in the lungs. Incubation of human neutrophils with mouse ANCA caused increased adherence of the neutrophils to extracellular matrix components such as fibroneuctin. Moreover, the mouse ANCA induced respiratory burst in human neutrophils as determined by the SOD assay.
Conclusion:Our finding may suggest a pathogenic role for cANCA on the development of WG, perhaps by inducing adhesion of neutrophils to endothelium and respiratory burst, theerby initiating the vasculitic process.

Pathogenic and non-pathogenic mouse anticardiolipin antibodies: binding properties and V gene analysis

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More people have anticardiolipin (aCL) antibodies in their blood than those suffering from the overt antiphospholipid syndrome (APLS). To better define pathogenic aCL antibodies from those non-pathogenic ones, we have analysed 3 monoclonal aCL: CAM (IgG), CAR (IgM) and CAL (IgG). The first two were shown by us to induce APLS in mice both by passive transfer and following active immunization. CAL was found to be non-pathogenic in this respect. We found that the important factors in which CAM and CAR differ from CAL were: (1) affinity to cardiolipin, (2) being also on anti-coagulant, (3) usage of specific pathogenic heavy chain nucleotide sequence (J558). CAL had low affinity, is not an anticoagulant and its heavy chain sequence is encoded by the 'sofar' known non-pathogenic group 7183. The ß2GP-I dependency of the antibody in binding to cardiolipin was not found to be so important in this respect. It can be concluded that high affinity, anticoagulant properties and special nucleotide sequences in heavy chain CDRs may contribute to their pathogenic potential and explain the enigma of subject with high titers of aCL but not overt APLS.

Contamination of antibody preparations by bacterial peptidoglycans

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Bacterial peptidoglycans (BPG) are normal constituents of the bacterial cell wall. Their soluble catabolic products are regularly formed in a surface of mucosal barriers, first of all in the intestine tract, and then as they penetrate the organism. We demonstrate here that the soluble BPG are capable of interacting with serum IgG. The above interaction results in changing the IgG molecule conformation detected by u.v. differential spectrophotometric analysis and other methods. In normal conditions, the complex of IgG with BPG is regularly observed in serum whereas in the infectious diseases caused by different viruses or bacterial agents their level rises significantly. The above complex demonstrates an ability to trap free electrons and behaves itself as antioxidant agent irrespective of the specificity of IgG as the antibody. These results are discussed in terms of a role of the BPG-antibody complexes in the immunopathological complications followed by the infectious diseases.

Characterization and labelling of hybridoma-produced MAb

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A single antigen molecule contains several characteristic antigenic determinants or epitopes. Traditional methods for preparation of antisera against determinants on antigens by immunization of animals always leads to the production of antibody molecules which are a complex mixture of different classes, specificities and affinities. More than 100 antibodies against human carcinoma have been reported in the literature, most have been characterized as unique to range of reactivity and reaction antigen. A hybridoma against Hela cells (human carcinoma cervix line) was developed and tested in relation to antigen specificity, protein content and molecular weight. It was found that anti-Hela contains 41 μg/ml of protein with molecular weight of 3×10^5. The antibody reacted with the Hela cells showing high absorbance in ELISA. The antibody was labelled with two enzymes: horseradish peroxidase and alkaline phosphatase by two methods: two-step glutaraldehyde and periodate oxidation. Each conjugate with its effective working dilutions, protein contents, molecular weight and cost was tested. Effective immunohistochemical staining was standardized by HRP and Al..Ph conjugates. The best working dilutions were obtained with Al.Ph conjugated by two-step glutaraldehyde method and also contains the maximum protein. The molecular weight as determined by the glutaraldehyde and periodate methods were 4×10^5 and 6×10^5, respectively. Reaction of conjugates prepared by Al.Ph and HRP in periodate method gave strongly positive reaction in cancer cervix tissue grade III.

Identification and reactivity of anti-Kelly monoclonal antibody

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During the last decade a growing number of monoclonal antibodies recognizing various useful cellular molecules have been produced and frequently used for immuno-histological diagnosis. Monoclonal antibody against plasma membrane of neuroblastoma cells has been developed and characterized. The identification of immunoglobulin by purification and characterization in relation to antigen specificity, isotype and chain composition has been performed.
The immunoperoxidase method has been employed successfully to study the reactivity in normal and malignant lesions of various sites. In fetal brain it showed positive reaction in some areas of round cells. Sections of retinoblastoma and neuroblastoma were positive but no reaction was observed in cases of medulloblastoma, oligodendroglioma, neurofibroma, and neurilemmoma. Mesenchymal tissues such as skeletal muscle, plain muscle, fibrous tissue and its tumors, meningioma and liver and kidney showed no binding.

Characterization of a HuMAb against oxidised low density lipoprotein (oxi-LDL) secreted by hybridoma derived from lymphocytes of a patient with CAD

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Atherosclerosis is currently considered to be an immune/inflammatory reaction of the intima of vessels to injury. Recent investigations have clearly shown that the immune system is involved in more than one way in the development of atherosclerosis. Activated T-cells and macrophages are found quite early in 'fatty' streak in the vessel wall and autoantibodies against modified LDL are readily detected in the circulation of patients with CAD. MAbs secreted by hybridomas raised from patients' lymphocytes would be quite valuable when used as specific probes to analyse in detail the Fc receptors involved in the internalization of immune (autoantibody/mLDL) complexes or in analysis of epitopes of modified LDL which are immunogenic in the patients. Peripheral blood lymphocytes from a patient with CAD were incubated in the presence of oxi-LDL and fused with P3U1 myeloma cells. A hetero-hybridoma was selected by ELISA which secreted HuMAb (MAb IvNi2D2, IgG isotype) reacting in a specific way with oxi-LDL. Isolated native LDL was oxidised *in vitro* after treatment with horseradish peroxidase in the presence of substrate H2O2 which leads to a considerable fragmentation of the LDL molecule and used as a target antigen. Absorption ELISA experiments have shown that MAb IvNi2D2 recognized an epitope specific to oxi-LDL but did not react with isolated native LDL. This MAb binds to immobilized and/or soluble lysine treated with malondialdehyde. MAb IvNi2D2 recognizes that portion of the protein modified during oxidation and that modification of the lysine residues of the protein molecule is the result of LDL oxidation *in vivo*. Currently, an *in vitro* test system is being developed to check the effect of MAb IvNi2D2 on the effectivity of oxi-LDL engulfment by HuMAb/ macrophages. Next, MAb IvNi2D2 will be applied to determine the localization of oxi-LDL in human atherogenic plaques by immunohistochemical methods.

Construction of heterohybridomas secreting human monoclonal antibodies against antigens specific to *Tr. pallidum*

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Infection with *Tr. pallidum* and development of syphilis still poses a number of questions concerning the treponemal components which are immunogenic in humans. A possible approach to this problem would be production of human monoclonal antibodies (HuMAbs) using lymphocytes from patients with syphilis. Following a stimulation of *Tr. pallidum* extract *in vitro* for 5-6 days peripheral blood lymphocytes from syphilis patients were fused with mouse myeloma cells and subsequently five hybridoma clones were selected which secreted human immunoglobulins reacting positively against *Tr. pallidum*. The specificity of each antibody was assessed by ELISA and indirect immunofluorescence and it was established that the antibodies selected did not react with rabbit testis and human organs (buccal mucosa, kidney, colon, testis, bladder). The activity of these MAbs was confirmed in serological methods routinely used in clinical laboratories, such as TPHA, treponema immobilization test (TPI), VDRL and FTA-abs. Some of the monoclonals (MAb 1C11, MAb 1D11) react with antigens specific to *Tr. pallidum* while others (MAb 2A2, MAb 2C8, MAb 2C11) bind to treponemal components which demonstrated group selectivity. In attempts to characterize the antigens recognized by the MAbs, SDS-PAGE and Western blotting were used. The electrophoretic separation of *Tr. pallidum* extract components were satisfactory and the electrotransfer onto nitrocellulose was effective. However, after immunostaining with the monoclonals as the first layer, well defined protein bands were not observed and the reaction was read as negative. Because of this treponemal extract was fractionated on FPLC column and eight protein peaks were outlined corresponding to each molarity of the buffer used. Each protein fraction was tested in ELISA against the monoclonal antibodies under investigation and two protein peaks were found to contain proteins which reacted positively. Analysis by SDS-PAGE shows that MAb 2A2, MAb 2C8 and MAb 2C11 react positive with a fraction containing at least three polypeptides with relative molecular mass between 48 and 62 kd.
Experiments are in progress to characterize biochemically the treponemal antigens recognized by human monoclonal antibodies.

Macrophage subpopulations in the thymic hyperplasia of patients with myasthenia gravis

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Macrophages in sections of the thymus from patients with myasthenia gravis (MG) were studied, using a panel of MAbs against the monocyte/macrophage lineage. The markers used were differentially expressed in the cortex and the medulla, namely CD14 and CD35 were preferentially expressed in the medulla while RM3/1 and 25F9 markers stained essentially cortical macrophages. CD11c-labelled cells were densely located throughout the thymus. The antibody 27E10 displayed a particular staining pattern characterized by clusters of cells scattered in the cortex and medulla and located around or in the perivascular areas. Macrophages in the germinal centers were stained with CD14, CD35, CD11c and 25F9 markers but not with RM3/1 and 27E10. The numbers of CD14- and CD35-positive cells were significantly increased in thymic hyperplasia in MG patients compared with those observed in control thymus. This increase was clearly due to cells in the germinal centers and in the areas surrounding the follicles. Altogether, these results show a heterogeneous monocyte/macro- phage population in MG thymus and point out an association between the morphological abnormalities seen in thymic hyperplasia and the presence of an increased number of macrophages/dendritic cells. The potential role of these cells in accessory cell function and antigen presentation could be relevant for the intra-thymic sensitization and autoantibody production that are clearly demonstrated in thymic hyperplasia from MG patients.

Anti-tyrosinase antibodies in vitiligo, melanoma, and melanoma-associated hypopigmentation (MAH)

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Tyrosinase is the enzyme responsible for melanogenesis in melanocytes and other pigmented cells. MAbs against melanoma cells recognize distinct molecular forms of tyrosinase, as well as pigment- and melanoma-associated antigens. Some of these antigens (e.g. melanoma antigens present on B16 cells) have been shown to serve as targets of immune response in patients with melanoma, vitiligo and MAH. The development of MAH in melanoma patients results from binding of anti-B16 antibodies, generated against melanoma-associated antigens, to normal melanocytes, which leads to their destruction. To assess their role in the evolution of MAH, **anti-tyrosinase antibodies** (ATA) were measured by ELISA in sera of patients with malignant melanoma (with either metastatic disease or no evidence of disease), in patients with melanoma and MAH, in patients with vitiligo and in healthy volunteers. The mean relative optical density (ROD) was calculated by dividing the optical density of each sample by the mean optical density of the control group. From this, the ROD of the control group was 1.000 (S.E. 0.08). The ROD of patients with metastatic disease (1.516; S.E. 0.22) was significantly higher ($p = 0.03$) than the ROD of the controls, but insignificantly higher than the ROD of patients with no evidence of disease, 1.216, (S.E. 0.15). Patients with no evidence of disease, in whom the primary lesion originated in the lower limb had a significantly higher ($p = 0.01$) ROD than the healthy volunteers. Those with metastatic disease showed higher ROD if their primary lesions were confined to the area of the head and neck or to the lower limb. Those with vitiligo had higher ($p < 0.0001$)ATA titers (ROD 4.536, S.E. 0.32) than any of the other groups.However, those with melanoma and MAH (vitiligo-like) had the same titer of ATA as the controls of the patients with metastatic mela-noma. This reflects the possible absorption of ATA to melanoma antigens and points to role of ATA in the destruction of normal melanocytes in patients with melanoma, as part of the immune reaction.

Human monoclonal antibodies to i blood group: EBV alters B-cell glycolipid which is recognized by cross-reactivity of these MAbs

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To study the differentiation-associated glycolipid, two anti-i MAb producers (GL-1 and GL-2) were established by the combination of Epstein-Barr virus (EBV)-induced transformation of normal peripheral lymphocytes and immune lysis of fluoresent dye-trapped liposome containing bovine i-active glycolipid. The MAb GL-1 reacted with both sialosylparagloboside and pentahexosyl ceramide and the bovine i-active glycolipid whereas MAb GL-2 reacted only with the bovine i-active glycolipid in liposome immune lysis assay (LILA). Both MAbs cold-agglutinate human cord red cells and adult i-red cells but not adult red cells.

Based on complement cytolysis with the MAb, 15 hematopoietic cell lines and normal peripheral lymphocytes were screened for susceptibility to the MAbs. EBV-negative Burkitt lymphoma cell lines, Ramos and BJA-B were most sensitive among those tested, and Daudi, Namalwa in the B-cell lines, TALL-1, Jurkatt in the T-cell lines and HL-60 in the non-lymphoid cell lines were sensitive; whereas normal lymphocytes or other 8 cell lines were not.

In addition, we found the occurrence of a surface Ag recognized by these MAbs in EBV-negative human B-cell lines, BJA-B and Ramos, but not in EBV-positive sublines, BJA-B/ B95-8, BJA-B/HR-1, Ramos/B95-8 and Ramos HR-1. Fresh EBV infection reveals that the suppressed expression of GL-1 Ag is a result of EBV infection. The Ag recognized with these MAbs is extremely minor surface Ag from binding assay of the MAb. However, unexpectedly, TLC-immunostaining using the MAb revealed that the major immuno-reactive substance in red cord cells and EBV-negative B-cell lines but not in adult red cells and EBV-positive sublines was an extremely minor glycolipid distinct from i-active glycolipid. The GL-1 antigenic substance is considered to be a glycolipid distinct from the i-active glycolipid because the immunoreactivity was abolished with endo-glycoceramidase, which cleaves a linkage between the oligosaccharide and ceramide. The possible presence of a new glycolipid Ag determined by the MAbs altered by EBV was speculated.

Antibodies specific for human thyrotropin receptor induce MHC antigen expression in thyroid cells

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Autoantibodies (AAbs) to hormone receptors are found in autoimmune diseases such as Graves' disease (GD) or myasthenia gravis. A structural link between hormone receptor and MHC genes has been documented suggesting a possible correlation of MHC and hormone receptor genes. Thus, *in vitro* experiments were designed to search for a pathological role for AAbs. In a model study, we investigated whether adding murine anti-human thyrotropin (hTSHR) MAbs would affect MHC gene expression in either cloned human thyroid epithelial cell or primary murine thyroid epithelial cell cultures. We found that two anti-hTSHR MAbs, 11E7 and 34A induced, with an intensity comparable to that of γIFN, transcription and expression of class I and class II/Ii chain proteins in human and murine thyroid epithelial cells, whereas two other anti-hTSHR MAbs, 12E3 and 243-3 were ineffective. These data suggest a new role for AAbs in the pathology of autoimmune endocrinopathies.

Human recombinant anti-Rhesus D antibodies: characterization and effector function analysis

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Aim: HuMAbs are required for therapeutic applications. But only few HuMAbs have been obtained by using conventional hybridoma technology. Recently, the development of molecular engineering has allowed the production of complete recombinant antibodies. Moreover, by such recombinant DNA techniques, the study of variable and constant parts of immunoglobulins can be performed separately. Novel antibodies could then be created by joining variable regions selected for their functional properties. Such antibodies are expected to be fully effective *in vivo.*
Methods: RNAs from hybridomas producing one anti-D IgG1 and one anti-D IgG3 were extracted. After reverse transcription, sequences coding for heavy and light chains were amplified by PCR, cloned and sequenced. Sequences were, either directly or after exchange of variable parts by recombinant PCR, introduced in a eukaryotic expression vector. After co-transformation of murine myeloma cells with expression vectors and a selective vector (conferring G418 resistance), cell lines producing whole antibodies were established. Electrophoretic characteristics, N-terminal sequences, affinity, specificity and functional properties of such recombinant antibodies were studied and compared together with the IgG of parental hybridomas.
Applications: The main objective was to produce anti-D IgG showing simultaneously strong binding to target antigen and appropriate functional properties (phagocytosis, ADCC for example). According to the same protocols, recombinant antibodies for other applications (passive immunotherapy) will be produced. Constant parts will be provided by hybridomas or peripheral blood lymphocytes. Variable parts will be obtained from hybridomas or selected by the phage display technology.

Immortalization of plasma cells by plasmid DNA

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A new immortalization scheme is reported by use of DNA transfection of simian virus 40 (SV40) DNA fragment. A plasmid containing the T-antigen region DNA of SV40 was constructed and several immortalized B-cell lines were obtained from human peripheral blood mononuclear cells by transfection using the plasmid DNA (Kanki T, *Hybridoma* 1994; **13:** 327). In these lines, CD21 minus cells were found and cloned by limited dilution method (M37#1-2, -12, -17, -18, -22). These lines were secreting IgG(*k*), defectively expressed CD21, CD23 and CD10, while strongly expressed CD20, CD38, CD40 and HLA-DR, proved to be plasma cell lines. Epstein-Barr virus-transformed B-cells are useful source of human MAbs. However, they attach to the cell surface receptor (CD21) which is present on all mature B-cells but not on actively proliferating B-lymphocytes and finally differentiated plasma cells. The present scheme will provide new cell lines covering CD21 minus plasma cells for further hybridization with preconstructed partner myeloma cell.

Selection of non-competing monoclonal antibodies for a sandwich EIA

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Sensitive and specific enzyme immunoassays (EIA) can permit accurate monitoring of biological products. These assays are best achieved using a pair of non-competing monoclonal antibodies (MAbs) in a sandwich EIA format. We have developed simple methodology for the selection of non-competing MAbs. Briefly, an initial, characterized MAb is proteolytically degraded to $F(ab)'_2$ coated to EIA plates and used to capture antigen. These EIA wells are then used to screen a range of hybridomas, using a horseradish peroxidase-labelled anti-mouse F_c (γ-chain specific).

This procedure greatly increases the probability of obtaining a non-competing MAb specific for the native antigen in the solution phase. The methodology has been used to develop assays to cytokines, enzymes, toxins, and bacterial antigens.

cDNA cloning and sequencing of variable region gene of monoclonal antibody SZ-39 specific for human brain glioma

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We have designed a set of oligonucleotide primers to amplify the cDNA of mouse immunoglobulin heavy and light chain variable region gene by PCR. The primers incorporate restriction sites that allow the cDNA of the variable domain to be force cloned for sequencing and expression. Here we have applied the technique to clone and sequence the heavy and light chain variable domain of monoclonal antibody that reacts with human brain glioma line SHG-44. Total RNA were isolated from SZ-39 hybridoma cells and NS-1 myeloma cells by extraction with guanidinium thiocyanate followed by $CsCl_2$ ultracentrifugation. The RNA were separated by formaldehyde gel electrophoresis, and Northern transferee to nitrocellulose membrane, then hybridized to murine cDNA for immunoglobulin constant IgG_{2a}-chain and *k*-chain, respectively. The results indicate that RNA preparations contain IgG_{2a} mRNA and *k*-mRNA specific for monoclonal antibody SZ-39. After cDNAs amplified by PCR, we sequenced ones by the Sanger dideoxymediated chain-termination method. The result shows that the length of heavy chain in variable region gene of this antibody is 348 base pairs and the light one is 318 base pairs. This lays solid foundations for constructed singer chain antibody and chimeric antibody.

Keywords: Reverse transcriptase-PCR; MAb; brain glioma; variable-region gene

Characterization of the epitopes recognized by human antibodies against the tumor-associated polymorphic epithelial mucin

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The polymorphic epithelial mucin (PEM) is a highly glycosylated membrane anchor glycoprotein expressed by most epithelial cells. The protein core region is composed by a variable number of tandem repeats (TR) of 20 amino acids. In tumor epithelial cells, mostly of breast and ovarian origin, PEM is overexpressed and distributed along the entire cell surface and cytoplasm. In tumor cells, aberrant glycosylation appears to be responsible for the exposition of highly immunogenic determinants of the TR region of the molecule. Human antibodies generated by EBV-immortalized B-cells from tumor-draining lymph nodes of ovarian cancer patients were screened for reactivity against synthetic peptides corresponding to the TR positive B-cells clones were obtained from 60% of the patients. The reactivity of the human antibodies was characterized by ELISA and immunohistochemistry on cell lines and tumor samples of breast and ovarian origin. Interestingly, a higher reactivity on the ovarian than on the breast samples was observed. This may suggest a specific affinity of the antibodies for the PEM glycoforms expressed by ovarian tissues. To assess the peptide sequences within the TR able to induce the humoral response in human, epitope mapping was performed using overlapping octamers corresponding to the TR region synthesized on a SPOT scan cellulose membrane tested in ELISA. The human antibodies also recognize other sequences within the TR region distinct from the APDTRP sequence, highly immunogenic in the mouse system. The study of the different epitopes recognized by human antibodies may be important for the immunological definition of the response against PEM, for the serum evaluation of this immune response, and finally for the construction of synthetic conjugates for immunotherapy.
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Natural anti-idiotypic antibodies against a tumor immunoglobulin of a mucosa-associated lymphoid tissue lymphoma (MALT-type) occur in normal and gastric patients

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To investigate the mechanisms triggering MALT-type lymphoma development, we examined the occurrence of the humoral response and normal B-cells in lymphoid tissue and chronic gastritis with the same tumor immunoglobulin as an IgA-expressing MALT lymphoma in the stomach. Lymphoma idiotype IgA was produced by human MAb technology. Against this idiotype a murine MAb 27/165 with anti-idiotypic (*a*-Id) specificities was raised, and applied to identify the non-neoplastic precursor B-cells in non-neoplastic human tissues. Crosslinking of tumor-Ig using anti-idiotypic antibodies, mimic binding of antigen by the tumor cells *in vitro*, is likely to be comparable to normal B-cells derived from tonsillar tissue or low grade follicular lymphoma. Moreover, recent data indicate that *a*-Id antibodies also occur *in vivo*. Using the IgA tumor immunoglobulin we found large amounts of heterologous IgA and IgG with *a*-Id specificities in sera of normal and gastritis patients but not in neonates. Significant levels of IgM were found only in some patients with gastric MALT. The target antigen of the lymphoma IgA has been found to be an autoantigen of MALT plasma cells and it is suggested that this MALT-type lymphoma may have arisen after triggering by an autoimmune response. Natural anti-idiotypic antibodies occur and may tune or drive the auto-immune response as well as the antigen.

Mouse/human chimeric IgE antibodies directed to the house dust mite allergen *Der p 2*

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To study crosslinking of IgE bound to IgE-receptors on basophils, mast cells and APCs, we are developing mouse/human chimeric antibodies directed to different, non-overlapping epitopes of the house dust mite allergen *Der p 2*. The chimeric antibodies are being constructed from the heavy chain variable region and light chain of murine origin, and of the heavy chain constant region of human origin. The chimerization of two monoclonal antibodies against *Der p 2* was performed: $_{c}$_DpX and 2B12B3. A heavy chain plasmid was constructed consisting of the V_H-domain of the hybridoma coupled to a human heavy chain constant region of the epsilon isotype. These heavy chain constructs were transfected to their respective 'heavy chain loss' cell lines. Both transfections gave rise to the expression of chimeric IgE antibodies, but only the 2B12B3 antibody showed reactivity with mite extract. The reason for the loss of mite reactivity of the chimeric $_{c}$_DpX antibody is being studies now. IgE antibodies of the 2B12B3 transfectant were able to sensitize the IgE receptor present on human basophilic granulocytes. These basophils could be activated by crosslinking this IgE with anti-IgE anti-bodies. [This study was financially supported by The Netherlands Asthma Foundation through grant number 91.35.]

Effect of mutations in the primer encoded FR1 and FR4 regions of V_H and V_K on the reactivity of scFv-s

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Two murine anti-hCG monoclonal antibodies coded McAB2 and McAB4 were converted into scFv-s. The molecules produced initially were not reactive against the antigen. We believed that this failure was caused by mutations located in the primer encoded regions. The entire wildtype sequences were determined. New degenerated primers were synthesized and used for the construction of small phage display libraries for both antibodies. After two rounds of panning, an enrichment was found. Individual clones were analysed in ELISA and those giving the strongest reactions were analysed on sequence and production of scFv. The isolated clones were never entirely wild type in respect to the primer encoded regions. Therefore, complete wild type clones were generated that expressed reactive scFv-s against hCG. Mutant analysis revealed that the inactivity of the first constructs was caused by a mutation on position 6 in FR1 of V_H (the wild type glutamine was substituted by glutamic acid) for McAB2 and McAB4. For McAB4 an additional residue was disturbing: position 4 in FR1 of V_K (the wild type methionine was substituted for serine). This study showed that introduced mutations within the FR- regions, that are encoded by the primers, may be the cause of the production of non-functional anti-body fragments. It also reveals that other mutations are tolerated and might improve the production levels.

Isolation of scFv-s reactive against HIV1-antigens from a random-combinatorial human library

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A phage display library containing 5 x $10^{\wedge 7}$ clones was constructed using peripheral blood lymphocytes of a HIV1 patient. At the time of harvesting of the lymphocytes, the patient was anti-p24 positive. RNA was isolated from 1 x $10^{\wedge 7}$ frozen lymphocytes and because of the poor quality of these cells a small amount of RNA was obtained. Panning was performed with the envelope precursor protein gp160 and the gag-protein p24, both immobilized on polystyrene plates. After three rounds of selection a distinct enrichment was found in the gp160 panning and four different binding clones were identified, based on BstNI fingerprints, which were sequenced, characterized on protein production and binding specificity. For p24 the enrichment was less clear but analysis of individual clones obtained after round 4 revealed that four different binding clones were present, again based on BstNI fingerprints. Two of these clones were sequenced and characterized on scFv production. In spite of the fact that low numbers of peripheral blood cells were used for the construction of the library we succeeded in the isolation of several HIV1-specific antibody fragments.

Cell cycle-independent surface labelling of differentiated glioma cells by HuMAb BT32/A6

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To be of potential clinical value in anticancer therapy, a HuMAb should ideally label the surface of live tumor cells in a cell cycle-independent fashion. Flow cytometric studies of SK-MG-1, a human malignant glioma line, indicate surface labelling of viable clonogenic tumor cells by BT32/A6, a HuMAb derived from the fusion of PBL from a patient with a pilocytic astrocytoma and TH-H2-SP2, a human myeloma-like cell line. By growing SK-MG-1 at different culture split ratios, it was possible to manipulate various parameters associated with the cell cycle such as growth rate, culture viability, and the % of cells in G_0/G_1. Surface labelling with BT32/A6 was observed to be independent of cell cycle state. When SK-MG-1 cells were forced to differentiate either by serum deprivation or growth in the presence of 2% dimethyl sulphoxide (DMSO), there was an increase in surface labelling that was independent of changes in cell volume. Earlier work demonstrated no binding of BT32/A6 to cultured normal human astrocytes. Further testing of HuMAb BT32/A6 may support its utility in the treatment of low grade gliomas - a form of brain cancer for which there is currently no satisfactory teratment - because the antibody recognizes a cell cycle-independent surface antigen that is upregulated on differentiated glioma cells.

Using differential binding to normal and tumor cell lines to screen phage antibody libraries from the lymph nodes of human cancer patients for anti-tumor cell scFvs

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A method has been developed for selecting out, from phage-antibody libraries, antibodies with specificity for antigens expressed on tumor cells. In order to develop and test the method, phage antibodies recognizing a known tumor-associated antigen were mixed, at a ratio of 1 to 10^5, with phage antibodies recognizing an irrelevant antigen. The mixed population of phage antibodies was first adsorbed to a cell line that did not express the tumor-associated antigen on its surface. This cell line was, however, closely related to the tumor cell line that was to be used for positive selection. Phage antibodies that did not bind to the antigen-negative cell line were then adsorbed to a tumor cell line that expressed the tumor-associated antigen on its cell surface. Phage antibodies that bound to the antigen-positive cell line were used to infect *E. coli*. The panning procedure was repeated with the enriched population of phage antibodies. After two or three rounds of panning, the majority of the phage antibodies in the enriched population were the original phage antibody that recognized the tumor-associated antigen. This procedure was tested using phage antibodies against two different, well-characterized tumor-associated antigens and using tumor cell lines that expressed the tumor-associated antigen at different densities. This method has now been used to screen a phage-antibody library derived from the draining lymph nodes of a colorectal cancer patient. Results will be presented.

Affinity enhancement by formation of bi- functional and multimeric complexes of single chain antibodies (scFv) fused to core-streptavidin

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Multivalent and multispecific human antibodies could provide valuable tools for biological and medical research for the diagnosis and therapy of cancer. We have therefore fused single chain antibodies (scFv) with core-streptavidin. This chimeric protein, expressed by the vector pSTE-215 (plasmid for streptavidin-tagged expression) from *E. coli*, can form tetrameric complexes, binds antigens and contains four biotin-binding sites per tetrameric complex which are almost completely accessible and may be used for further complex formation. An additional cysteine was inserted near the carboxyterminus for further stabilization. The scFv fusion protein could be enriched by affinity chromatography using biotin analogs. We have also shown that the scFv fusion protein could be used for direct detection of its antigen in ELISA and Western blots when stained with biotinylated horseradish peroxidase. The affinity of the antibody complex is increased by a factor of 35 by avidity effects due to the tetrameric structure.

Production of a recombinant anti-Rhesus MAb with insect cells: interest in public health

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At postpartum fetal red blood cells (RBC) enter the maternal circulation and induce immunization to the D-antigen in Rhesus (Rh) negative women. After further pregnancies the Rh antibodies provoke a hemolytic anemia in the fetus which needs *in utero* or *ex sanguino* transfusions in severe cases. The prevention of newborn hemolytic disease is obtained by injecting 100 µg of anti-Rh immunoglobulins in Rh negative women within 2 days postpartum or postabortion. Currently the Rh antibodies are polyclonal immunoglobulins obtained from hyperimmunized male Rhesus negative volunteers. Recombinant anti-Rh antibodies would provide a source of anti-Rh antibodies as well as diminishing the risks of contamination by virus and other pathogens which may be present in the Ig preparations made from pooled human sera. The immunization of volunteers would also be avoided. The recombinant antibody that we made was obtained from the human monoclonal line D7C2, which secretes an IgM anti-Rh antibody. The D7C2 clone was isolated after EBV immortalization of peripheral blood lymphocytes, originating in an Rh- donor, immunized several times. The culture supernatant of this immortalized cell line agglutinated all Rh+ RBCs and most of the partial and weak Rh phenotype. Nucleo-tide sequences coding for the variable parts of heavy and light chains of this antibody showed that the VH fragment belonged to the VH4 family and the VL fragment to the V(lambda)IV family.
Construction of the baculovirus expression vector was accomplished as follows: the genes coding for the two variable regions of D7C2 cell line were inserted separate-ly into the plasmids carrying a genetic cassette coding for the constant light chain and a cassette coding for the constant heavy chain IgG1. The resulting fused genes are controlled by promoters P10 and Polyedrine respectively. A baculovirus light chain was obtained after transfection of insect cells with the plasmid light chain. The double recombinant baculovirus was then obtained after co-transfection and recombination between the light chain baculo-virus genome and the heavy chain plasmid. Ig expression was studied by intra- and extra-cellular protein analysis after infection of SF9 insect cells. An evaluation of the biological activity of this recombinant antibody was made: (1) by an agglutination test in tubes, mixing the supernatant insect cell culture (30 µg/ml) and different papain-treated RBC. The test showed agglutination on all Rh+RBC; (2) ADCC test, in which the effector cells (lymphocytes), the target cells (^{51}Cr-labelled RBC) and the recombinant anti-Rh showed specific lysis of 93%; commercial polyclonal Ig anti-Rh tested in parallel showed a specific lysis of 76%; human clinical trials are in progress. Ten Rh negative women receive 2 ml of labelled ^{51}Cr Rh positive erythrocytes. The recombinant antibody (100 µg) will be injected intravenously 48 - 72 hours following the first injection. The efficacy of the antibody will be estimated by the disappearance of the labelled RBC. Recombinant antibodies could also be used for other treatment namely cancer therapy. This technology allows repeated injections in contrast to murine MAbs which induce immun-ization quickly and like this, allows extended clinical trials.