

Session 8: Therapeutic antibodies: Infection

Thursday 13th November 2008. Moderator: Alois Lang

[16.00–16.30]

The therapeutic potential of monoclonal anti-human endogenous retrovirus W (HERV-W) envelope protein antibody in multiple sclerosis: Results from a new EAE-animal model

Alois B. Lang, Corinne Bernard and Hervé Perron
GeNeuro SA, CH-1228 Plan-les-Ouates, Geneva, Switzerland

Human endogenous retroviral family W (HERV-W) RNA in circulating virion particles (Multiple Sclerosis associated RetroViral element, MSR) is associated with the evolution and prognosis of Multiple Sclerosis (MS). HERV-W encodes an envelope protein (ENV) that is a potent agonist for Toll-Like Receptor 4 (TLR4) on antigen-presenting cells. It activates a pro-inflammatory and autoimmune cascade, triggering superantigen-like dysregulation of T-lymphocytes, which may underlie inflammation and demyelination in this disease. As part of a programme to develop monoclonal antibodies (mAb) with therapeutic application in MS, we have been seeking evidence that MSR involvement is detectable *ex vivo* in a significant proportion of MS patients' blood through the circulating ENV antigen expressed by the HERV-W family, and not only via MSR RNA or as a protein in brain lesions *post-mortem*. We have tested 103 patients with MS, 14 with Clinically Isolated Syndromes (CIS) prodromal to MS and 88 with other neurological and non-neurological diseases, using a specific ELISA immunoassay for HERV-W/ENV protein, comparing serum levels with those in 76 normal controls. The prevalence of HERV-W/ENV in MS patients was high (about 75%). Antigenaemia also occurred in 5 of 8 cases of Chronic Inflammatory Demyelinating Polyneuropathy, but not in other neurological and non-neurological diseases tested. No significant difference in antigenaemia was seen between either possible (CIS) or definite MS, or between different stages of MS.

We assessed the effects of HERV-W/ENV injected with myelin basic protein (MBP) antigen in a mouse

model similar to classical Experimental Allergic Encephalomyelitis (EAE): severe combined immunodeficient (SCID) mice grafted with human peripheral blood mononuclear cells providing a functional human lymphoid immune system. We found that ENV can trigger a CNS inflammatory response reproducing MS features. In contrast to inactive controls, ENV induced paresis, weight loss, and central nervous system inflammation and demyelination visualised on MRI scanning and confirmed by terminal immunohistology. Splenocytes harvested from the mice on day 28 after two injections of MBP and ENV responded to MBP challenge by significant IFN- γ secretion. Next, we selected and tested in this model, mAbs capable of inhibiting immunopathogenic effects induced by HERV-W/ENV protein, in the hope that this will open perspectives for targeted serotherapy. A specific anti-ENV mAb selected for its inhibiting effects on ENV interaction with TLR4 in human lymphoid cell culture reversed the clinical, MRI and histological changes in comparison with untreated EAE controls. It had no apparent adverse effects in non-EAE mice. A therapeutic strategy using chimaeric / humanised therapeutic antibody against ENV protein has also been evaluated.

In view of the association of HERV-W with multiple sclerosis, the prevalence of its pro-inflammatory antigen in affected patients and the finding that this protein can induce MS-like inflammation and demyelination in an animal model, prevented by a specific mAb, suggests this is an appropriate therapeutic target.

[16.30–17.00]

Development of a HuCAL GOLD® human monoclonal antibody for the therapeutic treatment of inflammatory diseases

Matthew Downham
MorphoSys AG, Lena-Christ-Str. 48, D-82152 Martinsried, Germany
Tel.: +49 (0)89 899 27 176; Fax: +49 (0)89 899 27 5176;
E-mail: matthew.downham@morphoys.com

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is a haematopoietic growth factor that functions as a proinflammatory cytokine activating a number of different cell types i.e. macrophages, monocytes, neutrophils and eosinophils. Inflammatory diseases have been ameliorated by the blocking of GM-CSF activity by subsequent reduction in the harmful activities of GM-CSF responsive cell populations in disease models. Using a process of affinity maturation via CDR diversification an anti-human GM-CSF HuCAL GOLD® human monoclonal antibody (anti-hGM-CSF hMAb) has been generated and selected for development into the clinic. The selected anti-hGM-CSF hMAb candidate has recently completed preclinical development and has shown promising results in established rheumatoid arthritis disease rat models. A Phase I clinical trial to assess the safety, tolerability and pharmacokinetic characteristics of the anti-hGM-CSF hMAb is currently on-going. Principally presented will be development of the lead anti-hGM-CSF hMAb candidate from selection through to the clinical trial. This will cover aspects of lead candidate characterisation, efficacy, safety and tolerability for development of the anti-hGM-CSF hMAb. Described will be a human antibody having pro-inflammatory cytokine targeting therapeutic capability for the potential treatment of inflammatory diseases such as rheumatoid arthritis.

[17.00–17.30]

Creation of hybridoma populations from post-germinal center B-cells and their use to clone human antibodies specific for botulinum neurotoxins

Sharad P. Adekar*, R. Mark Jones, M. D. Elias, Tsuyoshi Takahashi, Fetweh H. Al-Saleem, Michael J. Root, B.P. Kapadnis, Lance L. Simpson and Scott K. Dessain

Lankenau Institute for Medical Research, 100 Lancaster Ave., Wynnewood, PA 19096, USA
Presenting author: Sharad P. Adekar, E-mail: sharadadekar30@gmail.com

The affinity-matured human antibody repertoire may be ideal as a source for antibody therapeutics against infectious diseases and bioterror agents. Hybridoma methods have the advantage that they can directly access these antibodies in their native configurations. However, hybridoma methods are hampered by the limited number of post-germinal cells that can be routinely obtained from peripheral blood. To overcome this limitation, we have created an efficient, three-step method

that uses human peripheral blood B-cells to produce stable hybridoma populations that are highly enriched for affinity-matured human IgG antibodies. Peripheral blood mononuclear cells (PBMCs) are (a) selected for expression of CD27, a marker of post-germinal center B-cells, (b) cultured *in vitro* to promote B-cell proliferation and class-switching, and (c) fused to a genetically modified heteromyeloma cell line. Using this strategy, we cloned a panel of high affinity antibodies that bind botulinum neurotoxins (BoNT), Category A Select Bioterror agents and the causes of the food-borne paralytic illness, botulism. One of these antibodies is the first to bind to the BoNT light chain, which is the catalytic domain of the toxin involved in the intracellular cleavage of the synaptic vesicle fusion protein, SNAP-25. Studies of the *in vitro* and *in vivo* neutralization activity of these and other BoNT-specific native human monoclonal antibodies will be presented.

[17.30–17.50]

Targeting viral antigens with radiolabeled antibodies as a novel approach to treatment of viral infections and virus-associated cancers

E. Dadachova, X.G. Wang, M.C. Patel, E. Revskaya, R. A. Bryan, A. Morgenstern, C. Apostolidis, H. Goldstein, M. Gorny, S. Zolla-Pazner and A. Casadevall

Albert Einstein College of Medicine, Bronx, NY, USA
European Commission, Joint Research Centre, Institute for Transuranium Elements, Karlsruhe, Germany
New York University School of Medicine, New York, NY, USA
Veterans Affairs New York Harbor Healthcare System, New York, NY, USA

Radioimmunotherapy (RIT) utilizes antigen-antibody binding to deliver cytotoxic doses of alpha- or beta radiation to tumor cells. RIT has been used to successfully treat refractory and recurrent lymphomas, with two radiolabeled monoclonal antibodies (mAb) targeted against CD20 (Zevalin® and Bexxar®) having received FDA approval for this purpose. This "traditional" cancer RIT targets "self" antigens. We have demonstrated that RIT has also broad potential for the treatment of fungal and bacterial infections through targeting microbial antigens with radiolabeled mAbs in experimental models of fungal and bacterial infections. Later we found that HIV-1 infected cells could be eliminated *in vitro* and *in vivo* by targeting gp120 and gp41 viral glycoproteins expressed on the surface of infect-

ed cells with radiolabeled viral protein-specific human mAb.

Virus-associated cancers have major impact on human health worldwide - for example, hepatocellular carcinoma (HCC) is the 5th most common cancer and the 3rd most frequent cause of cancer death worldwide, causing an estimated 550,000 deaths per year with chronic HBV infection being the major risk for development of HCC. Another example of the scope of the virus-associated cancers problem is cervical cancer (CC) which is caused by HPV. According to the World Health Organization (WHO) cervical cancer is the second most common cause of female cancer mortality in the world. WHO estimates the number of cervical cancer deaths to be 250,000 in 2006.

We have recently suggested that RIT targeted against viral antigens could be used in the treatment of a broad range of virus-associated tumors. Many virus-associated cancers express viral antigens either internally or on their surfaces. It is important to note that even viral antigens expressed intracellularly are potential targets for RIT, since tumor cell turnover is likely to result in the release of these proteins into the interstitial space of the tumor. We hypothesized that it was possible to treat experimental HPV16-associated CC and Hepatitis B-associated HCC by targeting viral antigens with radiolabeled antibodies to viral antigens.

We performed evaluation of the HPV-16 associated oncoprotein E6 and Hepatitis B-associated HBx viral protein (both intranuclear antigens) as potential targets for RIT of CC and HCC, respectively, by performing Western blot and immuno-fluorescence of CasKi human CC cells and Hep 3B2.1-7 human HCC cells. We radiolabeled E6 and HBx-specific antibodies with ^{188}Re - a radionuclide with 17 hr physical half-life which emits tumoricidal beta-radiation and is capable of "cross-fire" effect in tissue. The ability of ^{188}Re -labeled mAbs to E6 and HBx viral proteins to target viral proteins in vivo for RIT purposes was evaluated in experimental CC and HCC tumors in nude mice. Treatment of experimental CC and HCC tumors with ^{188}Re -labeled mAbs to E6 and HBx viral proteins, respectively, resulted in significant and dose-dependent retardation of tumor growth in comparison with untreated mice or mice treated with unlabeled antibodies with CC being much more sensitive to RIT than HCC. The strategy of targeting viral antigens on cancers with radiolabeled antibodies is fundamentally different from the prior uses of RIT in oncology and promises exclusive specificity and minimal toxicity of treatment because it targets foreign viral antigens and not "self" human antigens which are found throughout the body.