L(HBD) vaccine on wound healing, reproduction and embryonic development were performed in mice. Vaccination with the L(HBD) vaccine did not result in alterations in mean time to wound healing when compared to unvaccinated animals or those treated with a liposome control. In addition, L(HBD) vaccinated female mice were not impaired in their ability to become pregnant, support the growth and development of embryos, or deliver viable offspring. Furthermore, these offspring did not demonstrate any alterations in organogenesis when compared with pups born to untreated mothers or those treated with liposome control preparations. Thus, while vaccination against FGF-2 inhibited angiogenesis and tumor development, it did not appear to adversely alter wound healing or reproduction. Taken together, the generation of an active immune response that targets FGF-2 to block angiogenesis and tumor development is a unique approach to vaccine development that warrants clinical investigation.

458

MSI derived frameshift mutations represent novel tumor specific antigens

M. Linnebacher[1,2], Y. Schwitalle[3], E. Ripberger[2], J. Gebert[2], S. Woerner[2], Y. Yuan[4], P. Bork[4,5], M. von Knebel Doeberitz[2]. [1]*Institut fuer Immunologie, Abteilung fuer Molekulare Pathologie, Heidelberg, Germany;* [2]*Institut fuer Pathologie, Abteilung fuer Molekulare Pathologie, Heidelberg, Germany;* [3]*Chirurgische Klinik, Abteilung Urologie, Heidelberg, Germany;* [4]*EMBL, Heidelberg, Germany;* [5]*Max Delbrueck Zentrum fuer Molekulare Medizin, Berlin, Germany*

Microsatellite instability (MSI) caused by defective DNA mismatch repair (MMR) is a hallmark of hereditary non-polyposis colorectal cancers (HNPCC) but also occurs in about 15% of sporadic tumors. If instability affects microsatellites in coding regions, translational frameshifts lead to truncated proteins often marked by unique frameshift peptide sequences at their C-terminus. Since MSI tumors show enhanced lymphocytic infiltration and our previous analysis identified numerous coding mono- and dinucleotide repeat bearing candidate genes as targets of genetic instability, we examined the role of frameshift peptides in triggering cellular immune responses. Using peptide pulsed autologous CD40-activated B cells we have generated cytotoxic T lymphocytes (CTLs) that specifically recognize HLA-A2.1-restricted peptides derived from frameshift sequences. Among 33 frameshift peptides predicted from mutations in 12 different genes, 9 peptides conferred specific lysis of target cells exogenously loaded with cognate peptide. Four peptides derived from a (-1) frameshift mutation in genes coding for TGF-betaIIR, OGT, MSH-3 and Caspase-5 gave rise to CTL capable to lyse MSI cancer cell lines, carrying this frameshift mutations. Given the huge number of human coding microsatellites and assuming only a fraction being mutated and encoding immunologically relevant peptides in MSI tumors, frameshift protein sequences represent a novel subclass of tumor specific antigens. It is tempting to speculate that a frameshift peptide directed vaccination approach not only could offer new treatment modalities for existing MSI tumors but also might benefit asymptomatic at-risk individuals in HNPCC families by a prophylactic vaccination strategy.

459

Active and protective immunity induced by a protein based vaccine targeting the HER2/neu oncogenic protein

C. Gerard[1], T. Foy[2], N. Baudson[1], C. Genart[1], T. Ory[1], G. Fanger[2], M. Cheever[3], C. Bruck[1]. [1]*GlaxoSmithKline Biologicals, Cancer Vaccine Program, R&D;* [2]*CORIXA, Immunology, Seattle, USA;* [3]*CORIXA, Medical Affairs, Seattle, USA*

More than 185 000 new cases of breast cancer are diagnosed each year. Among them, 30% are shown to overexpress the HER2/neu oncogene which plays a crucial role in the pathogenesis and contributes to a poor clinical outcome. The HER2/neu protein is an attractive therapeutic and immunogenic target. The existence of antibody, helper T cells and cytotoxic T cell immunity to HER2/neu have been demonstrated in patients with cancer. Moreover, passive transfer of HER2/neu specific monoclonal antibodies such as Trastuzumab, have been shown to be of clinical benefit in patients with HER2/neu overexpressing tumors. A vaccine targeting Her2/neu should be effective if serum antibody responses are elicited that mimic trastuzumab and is anticipated to show increased efficacy if T cell responses are elicited in addition to serum antibody responses. Approaches to elicit immunity to HER2/neu currently tested include peptide-based, DNA-based and protein-based vaccines. Our group (GSK/ Corixa) has developed a protein-based vaccine composed of both the extracellular domain (ECD) and the carboxyl terminal portion of the intracellular domain (ICD), formulated in a strong adjuvant. We show that after several vaccinations, mice develop both humoral and cellular responses to HER2/neu. This Her2/ neu specific immune response is able to protect mice against a tumor challenge with an HER2/neu expressing mouse tumor and implies both CD4 and CD8 T cells. HER2/neu specific antibodies were induced in rabbits and monkeys. The presence of functional antibodies that inhibit the *in vitro* growth of the human breast cancer cell line SKBR3 and the *in vivo* growth of human ovarian SKOV3 tumor xenograft was demonstrated in sera form vaccinated animals. These studies demonstrate that a vaccine based on a purified dHER2 protein formulated in a strong adjuvant can induce a systemic antitumor immune response with both humoral and cellular components directed against the extracellular and intracellular domains of the HER2/neu oncogene.

460

Development of a WT1 protein vaccine

A. Gaiger[1,2], S. Mossman[1], V. Reese[1], M. Smithgall[1], P. McNeill[1], L. Ewans[1], N. Ordonez[1], M. Cheever[1]. [1]*Corixa Corporation, Seattle, USA and 1st Dept of I;* [2]*First Dept of Internal Medicine, University of Vienna, Div. of Hematology, Vienna, Austria*

The Wilms' tumor (WT1) gene is overexpressed in most human leukemias as well as in several solid tumors. There is substantial evidence that WT1 is immunogenic in humans and in mice. The current study (1) assessed the optimal WT1 protein formulation in terms of immunogenicity and toxicity in C57/Bl6 mice and (2) tested *in vivo* efficacy of this formulation in HLA-A2/Kb transgenic mice. Two immunological adjuvants (MPL-SE, ENHANZYN) were compared at 2 different dose levels (10ug, 25ug) with WT1 protein vaccine in C57/Bl6 mice. 10ug MPL-SE stood out as being especially effective for induction of IgG2a antibodies and potent IFN-gamma responses against WT1. A multiple dose titration study (doses ranging from 25ug, 100ug to 1000ug WT1 protein) in female C57/B6 demonstrated induction of antibody and T-cell immunity against WT1, without any signs of toxicity. To assess *in vivo* efficacy of the WT1 protein vaccine formulation a WT1 tumor model was established in the HLA A2 transgenic mouse strain. Mice were immunized with either saline (Gp 1, control) or 100ug WT1 protein using 10ug MPL-SE as adjuvant (Gp 2). Three weeks after the last immunization mice were inoculated with 2 million WT1 positive tumor cells. Histopathological analysis demonstrated that 10/10 (100%) animals in the control group developed tumors. In marked contrast only 4/9 (45%) animals in the WT1 protein immunized group showed tumor take. Given that 1) existent immunity to WT1 is present in some patients with leukemia 2) vaccination to WT1 protein elicits WT1 specific Ab, and T-cell responses in mice without toxicity to normal tissues and 3) WT1 protein immunization shows *in vivo* efficacy in the A2/Kb transgenic mouse model, human phase I trials are being contemplated testing WT1 protein with MPL-SE as adjuvants in patients with AML and MDS.

461

Inhibition of tumor cell growth by antibodies induced after vaccination with peptides derived from the extracellular domain of Her-2/neu

J. Jasinska[1,2], U. Wiedermann[3], S. Wagner[1,3], R. Sedivi[4], O. Scheiner[1,3], C.C. Zielinski[1,2]. [1]*BioLife Science Inc., Department of Medicine I, Vienna, Austria;* [2]*University Hospital, Department of Medicine I, Vienna, Austria;* [3]*University of Vienna, Dept. of Pathophysiology, Vienna, Austria;* [4]*University Hospital, Dept. of Clin. Pathology, Vienna, Austria*

Monoclonal antibodies directed against Her-2/neu have inhibiting effects on tumor growth. The humanized murine anti Her-2/neu antibody Trastuzumab is successfully used in the clinical routine. However, an active vaccination inducing a long-term immunity to Her-2/neu is still a desirable goal. The aim of the presented study was to induce production of specific Her-2/neu antibodies and to test their efficacy to inhibit tumor cell growth. BALB/c were immunized with peptides derived from the extracellular domain of the human Her-2/neu, coupled to tetanus toxoid (TT). Seven days after the last immunization animals were sacrificed and antigen-specific antibody levels were measured. The IgG fractions from these sera were isolated and used for *in vitro* proliferation assay performed with the breast cancer cell line SK-BR-3. Moreover, hearts, lungs, livers, and kidneys were histopathologically screened for inflammatory infiltrations. Immunization with the peptides led to induction of anti-Her-2/neu antibodies - in particular IgG_1 – which were able to precipitate human Her-2/neu from cell lysates of SKBR-3. Incubation of the SKBR-3 cells with the IgG fractions from the mice sera led to a significant reduction of the cell growth, indicating the anti-tumor activity

of these antibodies. Concernining histopathological examinations no evidence of organ damages were detected. We conclude from our data that immunization with Her-2/neu peptides successfully induced anti-tumoural immune responses, which is the basis for further development of peptide-based cancer vaccines.

462

Mimotopes for high molecular weight - melanoma-associated antigen fused to albumin binding protein elicit anti-melanoma antibodies in balb/c mice

A. Riemer[1,2], B. Hantusch[2], G. Kraml[5], B. Sponer[2], C. Hafner[1,3], H. Breiteneder[1,2], C. Zielinski[1,4], O. Scheiner[1,2], E. Jensen-Jarolim[1,2], H. Pehamberger[1,3]. *[1]BioLife Science, Vienna, Austria; [2]University of Vienna, Dept. of Pathophysiology, Vienna, Austria; [3]University of Vienna, Dept. of Dermatology, Vienna, Austria; [4]University of Vienna, Dept. of Internal Medicine, Vienna, Austria; [5]Vienna University of Technology, Dept. of Algorithms and Data Structures, Vienna, Austria*

Background: Tolerance phenomena make it difficult to elicit anti-cancer immune responses. The aim of novel cancer vaccines is to break tolerance by immunization with structures mimicking the original antigen, but being not completely identical to it, thereby stimulating low affinity B cell clones which have not been negatively selected. Appropriate mimicking structures - mimotopes - might be generated by the phage display technology.
Methods and Results: In this study we performed biopannings of phage display random peptide libraries with the anti-melanoma antibody 225.28S. It is directed against high molecular weight - melanoma-associated antigen (HMW-MAA), an antigen carried almost exclusively by melanoma and nevus cells. One selected nonapeptide mimotope was termed MelMim1 and chosen to be fused to streptococcal albumin binding protein (ABP), an immunogenic carrier molecule. The resulting fusion protein MelMim1-ABP was recognized by moAb 225.28S in ELISA and immunoblot, indicating that the fused mimotope retained its structural equivalence to the 225.28S epitope of HMW-MAA. Subsequently, groups of BALB/c mice were immunized with MelMim1-ABP, or ABP alone as a negative control. The induced humoral immune response in the MelMim1-ABP group contained antibodies against the carrier protein and against the mimotope. Importantly, the latter antibodies recognized the natural antigen HMW-MAA on 518A2 melanoma cells.
Conclusion: Our data demonstrate that peptide mimotopes fused to an immunogenic carrier protein are novel tools to induce anti-melanoma antibodies with possible functions in anti-tumor defense and are therefore candidates for the generation of epitope-specific cancer vaccines.

Cellular therapies

463

Interleukin-8 (IL-8) promotes the growth of metastatic prostate cancer cells

M. Hamill, J. Messenger, M. Monaghan, J. Draffin, P. Johnston, D. Waugh. *Queens University Belfast, Cancer Research Centre, Belfast, United Kingdom*

Prostate cancer is currently the most prevalent cancer in men. Over-expression of the chemokine IL-8 has been reported in the sera of patients with localized and metastatic cancer of the prostate. Animal models have also positively correlated the expression of IL-8 with the development of metastasis in prostate cancer. However, the mechanism by which IL-8 appears to promote disease progression remains poorly understood. We wished to determine whether IL-8 acts as a growth factor in prostate cancer cells using two prostate cell line models, the metastatic PC3 cell line and the transformed PNT1A epithelial cell line. We initially characterised IL-8 receptor (CXCR1 and CXCR2) expression on each cell line using RT-PCR, IP westerns, immunocytochemistry and flow cytometry. IP western analysis and flow cytometry illustrated a higher expression of CXCR1 than CXCR2 in both cell lines. Cell surface expression of CXCR1 was detectable in the PC3 cell line only but saponin permeabilised flow cytometry analysis of both cell lines demonstrated a high degree of intracellular receptor expression, which was later confirmed by immunocytochemistry. ELISA analysis revealed that both cell lines exhibit endogenous IL-8 secretion supporting the prevalence of receptor desensitisation. In growth assays conducted on PC3 cells, stimulation with exogenous IL-8 produced a consistent, concentration-dependent increase as assessed by cell count analysis. The response had an apparent EC50 of 1nM and a mean increase of 45% in cell number compared to controls. The PNT1A cell line demonstrated negligible response to exogenous IL-8. Further studies using the PC3 cell line attempted to determine the signalling pathways that underpin the IL-8 induced proliferation of these cells. In growth assays, co-incubation with the specific pathway inhibitors, U0126, SB203580 and LY294002 established the involvement of ERK1/2, p38 MAPK and PI3K signalling cascades. Western blot analysis of the phosphorylation status of ERK1/2 and p38 by exogenous IL-8 stimulation showed activation of both pathways in the metastatic PC3 cell line. Our work to date has demonstrated the role of IL-8 as a potential growth factor in the PC3 metastatic prostate cancer cell line. Ongoing experiments are focused on using specific neutralising antibodies to CXCR1 and CXCR2 to determine which IL-8 receptor is coupled to the growth-promoting pathway.

464

Analysis of the expression of 90K/Mac-2 binding protein (M2BP) in lung cancer and generation of cytotoxic T lymphocytes that recognize M2BP with an HLA-A2 restriction

O. Yoshitomo, Y. Ozaki, K. Kontani, K. Teramoto, N. Tezuka, S. Sawai, S. Fujino. *Shiga University of Medical Science, Department of Surgery, Otsu, Japan*

Purpose: 90K/Mac-2 binding protein (M2BP) has been reported to be highly expressed in patients with various types of cancer and to modulate the expression of surface molecules involved in immune responses on cultured cancer cells. We examine the expression of M2BP in lung cancer cells and attempt to generate M2BP-specific cytotoxic T lymphocytes (CTLs) using synthetic peptide derived from M2BP.
Methods: Eight cultured lung cancer cell lines and 28 tumor tissues from patients with lung cancer were examined for the expression of M2BP mRNA and protein. Using six peptides (9-mer or 10-mer) derived from M2BP with the HLA-A2 binding motif, we induced M2BP-specific CTLs from peripheral blood lymphocytes (PBLs) of HLA-A2-positive healthy donors by multiple stimulations of CD8-positive T lymphocytes with M2BP peptides. Antigen-specific responses of the induced CTLs were examined by the interferon-gamma production assay.
Results: Seven of the 8 (87.5%) lung cancer cell lines and 17 of the 28 (60.7%) tumor tissues were shown to express high levels of M2BP mRNA by Northern hybridization. Eleven of the 27 tissues (40.7%) were positive for M2BP expression immunohistochemically. CTLs stimulated with two M2BP-derived peptides (M2BP238-246, M2BP274-283) recognized peptide pulsed-autologous peripheral blood mononuclear cells (PBMCs) and -T2 cells. These CTLs also recognized a lung cancer cell line, A549 cells with both HLA-A2 and M2BP expressions. The cytokine production by these CTLs were blocked by monoclonal antibody against HLA-A2.
Conclusions: M2BP is abundantly expressed in lung cancer and sufficiently immunogenic to elicit M2BP-specific CTLs. This molecule is expected to be useful as a target antigen in cancer immunotherapy.

465

Abrogation of IRF-1 response by high-risk HPV E7 protein *in vivo*

J. Park[1], C. Kim[1], J. Rhyu[2], E. Kim[1], J. Myeong[1], E. Hwang[3], S. Namkoong[1], S. Um[4]. *[1]The Catholic University of Korea, Medical College, Dept. of Obstetrics and Gynecologya; [2]The Catholic University of Korea, Medical College, Dept. of Animal Experiment; [3]University of Seoul, Dept. of Life Science, Seoul, Korea; [4]Sejong University, Dept. of Bioscience and Biotechnology, Seoul, Korea*

We have previously reported that human papillomavirus (HPV) E7 interacts with IRF-1, a key regulator of cellular immune response, and abrogates its transactivation function at the molecular level *in vitro*. To confirm our previous data, we investigated *in vivo* the E7-mediated down-regulation of IRF-1 using HPV E7-inducible cells and transgenic mice expressing HPV-18 E6/E7. When E7 was induced in the absence of tetracycline, the expression of target genes of IRF-1 (TAP-1, IFN-β MCP-1 that are important for cellular immunity) was clearly reduced as determined by RT-PCR. In addition, the IRF-1 activity was down-regulated in E7-expressing cells into which INF-β-CAT reporter plasmid was transfected. To further investigate the E7-mediated immune regulation *in vivo*, we constructed transgenic mice expressing E6 and E7 genes of HPV-18 under the control of HPV-18 promoter (URR). From several rounds of breeding, we obtained from a transgenic line that developed a cervical dysplasia and expressed E6/E7 as determined by histological examination and RT-PCR, respectively. Subsequent RT-PCR analysis indicated that TAP-1, IFN-β, and MCP-1 genes were less