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.

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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

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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.

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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.

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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