371 ORAL

Peptide loaded human autologous dendritic cells (DC's) as a potential anti-cancer vaccine

M. Adams[1], H. Navabi[1], B. Jasani[2], A. Evans[3], T. Crosby[1], M. Mason[1], L. Borysiewicz[4]. [1]Velindre NHS Trust; [2]Department of Pathology; [3]Department of Gynaecology; [4]Department of Medicine, University Hospital of Wales Healthcare NHS Trust, Cardiff, UK

Rationale: DCs are powerful antigen presenting cells which, when loaded with tumour associated antigen peptides (TAAPs) can prime specific cytotoxic T cells (CTLs) to exert powerful anti tumour effects against murine tumours expressing the antigen (Mayodoma et al Nature Med 1: 1297, 1996). Such DCs may be prepared from human peripheral blood mononuclear cells (PBMCs) using GMCSF & IL4, and when antigen primed represent a potential specific anti-cancer vaccine.

Aims of Study: To characterise DCs prepared from PBMCs of normal volunteers and cancer patients for their ability to induce in vitro autologous CTL response against AAPs derived from HPV16E7 and HER-2 antigens, as a feasibility study prior to clinical application.

Methods: Autologous DCs were cultured using method of Romani et al (J Exp Med 180: 183, 1995) from 8 human volunteers and 4 cervical cancer patients and their yield and morphological and FACS characteristics determined at 8 days. These were loaded with haplotype specific TAAPs – HPV16E7 (YMLDLOPETT) and HER-2/neu (KIFGSLAFL) respectively. The flu peptide (SRYWAIRTR) was used as a positive control. PBMCs were primed with these DCs and tested for their CTL activity against peptide loaded autologous EBV transformed B cells.

Results: Cells with morphological and immunophenotypic features of DCs were grown from all individuals with yields ranging between 4.0–10.8 $\times$ 10^5/20 mls blood. All DC preparations also showed uniformly strong positivity for Class II, CD80 (B7.1) C54 (ICAM1) & CD86 (B7.2). CDla was variable expressed in the volunteers (22–25% cells) but consistently negative in the cancer patients. Specific MHC class 1 restricted CTL responses have been demonstrated in 3 normal HLA-2 volunteers to all 3 peptides and to HPV16E7 in 1 cervical cancer patient tested to date.

Conclusion: It is feasible to prepare functionally active DCs from cervical cancer patients with the same efficiency as the normal volunteers and induce specific MHC class 1 restricted responses to TAAPs. DCs primed with TAAPs therefore have a potential in anti-cancer immunotherapy.

372 ORAL

Immunization of melanoma patients with peptide-pulsed dendritic cells

D. Schadendorf[1], S. Aljagic[1], M. Gilliet[2], S. Grabbe[3], Y.-S. Sun[1], R. Dummer[2], F.O. Nestle[2]. [1]Clinical Cooperation Unit for Dermatooncology, Mannheim; [3]Depart. of Dermatology, University Münster, Germany; [2]Depart. of Dermatology, University Hospital Zurich, Switzerland

Purpose: Cytotoxic T lymphocytes (CTLs) attack melanoma cells in a MHC restricted and tumor antigen specific manner. Several melanoma-associated antigens were identified recently. Such antigens are ideal candidates for any kind of vaccination approach in melanoma. Dendritic cells (DC) are effective in the induction of primary T cell responses making DC an ideal candidate for the induction of anti-tumor immunity in melanoma.

Methods: DC were generated from peripheral blood using GM-CSF and IL-4 over 8 days and pulsed with a cocktail of peptides known to be recognized by CTLs depending on the patient's HLA haplotype. 5 $\times$ 10^6 DC were weekly injected into inguinal lymph nodes over 1 months. Immunizations were repeaed in monthly intervals up to 10 injections. In parallel, clinical response was monitored as well as DTH reactivity, T-cell reactivity to peptides by ELISPOT assay and immunhistological expression of tumor antigens.

Results: Vaccination was well tolerated by all patients. Some patients developed a positive DTH reactivity to peptide- pulsed DC. So far, clinical responses were evident in 4 out of 9 evaluable patients (2CR, 2PR). Further immunological evaluation is in progress.

Conclusion: Vaccination with autologous DC generated from peripheral blood seems to be a new, safe and promising approach in the treatment of metastatic melanoma.

373 ORAL

Six years experience using inhalatory interleukin-2 in pulmonary metastatic renal cell carcinoma

E. Huland, H. Heinzer, H. Huland. Department of Urology, University of Hamburg, University Clinic Eppendorf, Germany

Purpose: Evaluate effective immunotherapy with high quality of life

Methods: 116 patients use inhalatory IL-2 application, a non-toxic, effective treatment for patients with progressive pulmonary metastases of renal cell carcinoma (RCC). In different protocols three different IL-2 preparations of IL-2 were used. All protocols had in common a high-dose inhalatory IL-2 application either exclusive (11%), in addition with low dose systemic IL-2 (33%), or low dose systemic IL-2 and interferon-a (56%).

Results: Maximum toxicity per total treatment time (median treatment time 7.2 months) was mild (WHO grade III 16%), mainly cough. Explicitly, pulmonary disease was influenced. Progressive pulmonary metastases responded in 15% for a median of 15.5 months (4.1–33) and were stabilised in 55% for a median of 6.6 months (3–51.7). Overall response rate was 15%, stable disease 50% and progressive disease 35%. Median overall response duration was 9.6 months. Median achieved survival was 11.8 months (1.7–68.8) while expected survival according to risk analysis was 5.3 months.

Conclusion: Inhalatory IL-2 prevents progress of pulmonary metastases effectively in 70% of patients. Low toxicity allows outpatient therapy, employment and good quality of life.

374 POSTER

E1B gene defective adenoviruses as an antitumor treatment in human cancer

P. Martín Duque, C. Alonso[1], R. Sánchez-Prieto, M. LLeonart, S. Ramón y Cajal. Pathology Dept. Clín, Puerta de Hierro; [1]INIA-CISA, Valdeolmo, Madrid, Spain

Purpose: The E1A gene of adenovirus 5 induces marked sensitivity to chemotherapy and radiotherapy and exerts an antitumor effect *in vivo* that is counteracted by the E1B gene. The purpose of this study is to determine the therapeutic potential of E1B-defective adenoviral vectors, owing to the effect of the E1A gene.

Methods: The human tumor cell lines used were: HeLa, Saos-2, A431, HT29 and MCF7; a group of uveal melanomas including MKTBR, SP6 and OCM1 tumor cell lines; and a nontumoral melanocytic cell line, UW3. Wild-type (WT) 300, adenovirus type 5 and two deleted mutants (from Dr. Ginsberg and Dr. Shenk) were employed: Ad dl 118 (with the E1B region deleted but E1A intact) and Ad dl 312 (with E1A deleted but E1B intact). For drug treatment, cisplatin (Bristol-Myers) was used at a dose of 1 μg/ml. In irradiation assays, cells were gamma-irradiated with a Co-60 source. Cell density was evaluated by the crystal violet method.

Results: The Ad dl 118 mutant produced marked but variable lethality in all infected cells. Viability at 70 h postinfection was 10%–110% for the Ad dl 118 mutant vs 100–389% for the WT form, 97%–619% for the Ad dl312 mutant and 180%–528% for uninfected controls. In melanic cell lines, after infection at 200 PFU/cell and chemotherapy or radiotherapy, cells infected previously with Ad dl 118 mutant were more sensitive to treatment.

Conclusions: The results suggest that infection with E1B-defective adenoviruses expressing E1A produces a marked cytotoxic and antitumor effect. Thus, the use of these vectors is proposed as a novel antitumor approach in human anticancer therapy.

375 POSTER

The E1a Gene as antitumor agent: Trials in murine experimental models

R. Sánchez[1], M. Quintanilla[1], A. Moreno, A. Cano[1], M. LLeonart, P. Martín, S. Ramón y Cajal. Pathology Dept. Clínica Puerta de Hierro; [1]IIB (CSIC) Madrid, Spain

Purpose: The adenovirus E1a gene is a potent inducer of chemosensitivity and radiosensitivity by p53-independent mechanisms in different cell models. We evaluate the *in vitro* and *in vivo* effects of the E1a gene.

Methods: HaCa4 and MSC11 A5 (moderately differentiated and sarcomatoid epidermoid carcinomas, respectively) were used. These cell lines present several genetic alterations including H-ras or p53 mutations. The expression vector chosen was the MD-E1, a retrovirus that encodes for the 13S transcript of E1a. The results of dose/response trials with cisplatin (at doses of 1 to 10 mg/ml) and radiation trials (from 1 to 8 Gy) were quantified