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ANTIGENS RECOGNIZED ON HUMAN TUMORS BY CYTOLYTIC T LYMPHOCYTES.

Pierre Coulie, Pierre van der Bruggen, Benoit Van Den Eynde, Vincent Brichard, Aline Van Pel, Etienne De Plaen, Thierry Boon.

We have used gene transfection approaches to identify genes that code for antigens recognized by cytolytic T lymphocytes (CTL) on human tumors. Human melanoma cell line MZ2-MEL presents at least six different antigens. The gene coding for one of these antigens has been isolated. This gene, named MAGE-1, belongs to a family of at least 12 closely related genes located on chromosome X. The MAGE genes are not expressed in normal tissues with the exception of testis. But several members of the family, namely MAGE-1, 2, 3, 4, 6, and 12, are expressed by a significant proportion of tumors of different histological types. Gene MAGE-1, for example, is expressed in 40% of melanoma tumors, 20% of breast tumors, or 30% of non small cell bronchial tumors. Antigen MZ2-E consists of a nonapeptide encoded by MAGE-1 and presented by HLA-A1. Gene MAGE-3 encodes nonapeptides that are presented on HLA-A1 and -A2 and are recognized by anti-tumor CTL. We have recently characterized two new genes, named BAGE and GAGE, that code for antigens recognized on melanoma by CTL restricted by HLA-C molecules. Like the MAGE genes, these genes are not expressed by normal tissues with the exception of testis, but they are expressed by several types of tumors. We have also identified two genes that code for differentiation antigens recognized by CTL on most melanomas of HLA-A2 patients. The first gene codes for tyrosinase, the enzyme that synthesizes DOPA in the melanin pathway. This gene is expressed in melanoma and, among normal tissues, only in melanocytes. Two different peptides encoded by the tyrosinase gene have been identified that are presented by HLA-A2 and recognized by CTL clones obtained from the blood of melanoma patients. The second gene, named Melan-A, is unrelated to presently known sequences. Its expression is also restricted to melanoma and melanocytes. We found that an antigen recognized on a human melanoma by autologous CTL clones was encoded by a mutated gene. The gene is unrelated to known sequences. It is expressed ubiquitously. The sequence of the gene in the DNA of the tumor cells differed from that of the gene in normal cells by a point mutation. This mutation modifies one aminoacid in the antigenic peptide. The normal peptide is not recognized by the anti-tumor CTL. The mutation could not be found in the DNA of other tumors, indicating that this antigen is restricted to this particular melanoma.

Ludwig Institute for Cancer Research, 74 avenue Hippocrate, UCL 7459, B 1200 Brussels, Belgium.

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RETROVIRUS MEDIATED GENE TRANSFER FOR GENE THERAPY

WALTER H. GÜNZBURG and BRIAN SALMONS

Retroviral vectors are presently being used as gene transfer vehicles for the majority of clinical gene therapy trials, including those designed to treat tumours. Retroviral vectors offer a number of advantages including efficient gene transfer, and the stable integration of transmitted DNA in low copy numbers. Currently used retroviral vectors are derived from murine leukaemia virus (MLV) and can infect a variety of different cells types as long as they are dividing. This requirement for dividing cells offers a means of preferentially infecting rapidly dividing cells including some types of tumour cells. Retroviral vectors are also being constructed based upon other retroviruses. These include human immunodeficiency virus (HIV), which is able to infect nondividing cells and mouse mammary tumour virus (MMTV) which may be useful for targeting mammary tumour cells.

Future facile gene therapy protocols will require *in vivo* delivery of therapeutic genes in which virus or virus producing cells are directly introduced into the patient. In such patients it is of utmost importance to ensure that replication competent retrovirus is not produced as a result of recombination, since such virus is associated with tumorigenesis and immune dysfunction. A second prerequisite for the use of retroviral vectors for the *in vivo* delivery of such genes is the ability to limit their delivery and expression to predetermined cell types. Strategies for the construction of safe, tissue targeted vectors will be discussed.

Institute of Molecular Virology, GSF-Research Centre for Environmental and Health Research, Ingolstädter Landstraße 1, D-85758 Oberschleißheim, Germany

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CYTOKINE GENE THERAPY WITH GENE TRANSFECTED CELLS: LONG TERM EXPRESSION IN VIVO AND THERAPEUTIC POTENTIAL

F.M. Rosenthal*, R. Früh+, G. Köhler§, P. Kulmburg*, H. Veelken*, A. Mackensen*, A. Lindemann*, R. Mertelsmann*
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The development of cell based delivery systems that can release therapeutic levels of hematopoietic growth factors into the systemic circulation would facilitate treatment of patients requiring cytokine therapy. We have investigated the potential of GM-CSF and G-CSF secreting, autologous or allogeneic murine cells to accelerate hematopoietic recovery after cytotoxic chemotherapy. Murine BALB/c 3T3 and NIH 3T3 fibroblasts as well as murine CMS-5 fibrosarcoma cells, were transduced with a retroviral vector containing the murine GM-CSF cDNA or lipofected with an expression construct containing the human G-CSF cDNA. Clones secreting high levels of cytokine were selected. After irradiation, in vitro cytokine production initially increased up to 5-fold and was measurable for about 2 weeks. BALB/c or SCID mice were injected with irradiated or unirradiated cytokine secreting autologous and allogenic fibroblasts or tumor cells. Cytokine serum levels were measured and survival of transfected cells was determined by histological and immunohistochemical analyses. In a therapeutic model of cyclophosphamide treated mice a single injection of irradiated G-CSF or GM-CSF secreting CMS5 cells was as effective in accelerating neutrophil recovery as twice daily s.c. injections of rmGM-CSF or G-CSF, respectively. These data suggest that hematopoietic growth factor secreting cells might offer an alternative to parenteral injections of recombinant cytokines in the treatment of neutropenic patients.

Med. Univ. Klinik, Abt. Innere Medizin I*, Chir. Univ. Klinik+, und Pathologisches Institut§, 79106 Freiburg, Germany

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CYTOKINE GENE TRANSFER: FIRST CLINICAL TRIALS

B. Gansbacher
Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York

Human effector cells are capable of killing tumor cells in vitro under appropiate conditions. Several of those killer cells have been identified as NK cells, LAK cells, macrophages, and T cells. These effector cells have in common that they respond to interleukin-2 (IL-2). The most powerful effector cell populations are the T cells. It is the only cell population characterized by memory and specificity and the capability to migrate from one tumor deposit to the next one destroying tumor cells until the last cancer cells are gone. CD 8+ cells play the predominant role for lysing tumor cells. CD 8+ T cells are usually MHC class I restricted and recognize octamers ir nonamers assembled in the groove of the MHC class I molecule. Interferon-gamma (IFN-g) upregulates MHC and adhesion molecules on the cell surface and makes tumor cells more visible to T cells. Intracellular tumor associated antigens (TAA) must be processed before being presented by MHC class I molecules to CD 8+ T cells. Frequently tumor cells have defects in antigen presentation. In several instances these defects were reversible by IFNg. The observation that IL-2 stimulates CD 8 cells and IFNg upregulates MHC molecules and reverses defects in antigen presentation makes a combination of these molecules very attractive for gene therapy approaches to cancer.
We are planning to study LNCAP/IL-2/IFNg, a prostate carcinoma cell line transduced with a retroviral vector carrying both the humen IFNg and IL-2 cDNA. This cell line will be characterized and then used as a vaccine in patients with prostate carcinoma as an allogeneic HLA class I matched vaccine.