47

RETROVIRUS-MEDIATED GENE TRANSFER OF B7.1 INTO PRIMARY ACUTE MYELOID LEUKAEMIA BLASTS. W.J.R.Hirst[1], A.S. Buggins[1], D.Darling[2], J.Gäken[2], S.J. Hollingsworth[1], F. Farzaneh[2], G.J.Mufti[1].

Expression of immunomodulatory genes by tumour cells has been shown in several models to induce a tumour-specific immune response and has provided the basis of clinical trials. A major obstacle to the application of this technology is gene-modification of primary human tissue. Replication-disabled retroviral vectors have been used in the majority of trials to date, as they have a good safety profile and integrate into the host genome but only into proliferating cells. We have studied retrovirus-mediated gene transfer into AML blasts, including the effect of cell cycling, clonogenicity and growth factors on transduction efficiency. Leukaemic cells were obtained from 6 patients with AML (M2=3, M4=2, CML-blast crisis=1) by leukapheresis or density-gradient separation of bone marrow. Transduction was by 48 hour co-culture on irradiated (100Gy) packaging cells producing a replication-disabled M_3P-SVHygro retroviral vector encoding human B7.1(CD80) and hygromycin resistance genes. Cells were grown in either no growth factors, stem cell factor (SCF) alone or SCF, IL3 and GM-CSF. Transduction efficiency judged by B7.1 expression increased in the presence of growth factors; no growth factors = 1.5% (0.3-6.0%), SCF alone = 2.5% (0.4 - 5.9%), combination = 4.5% (0.6-10.7%). This could only be explained in part by increase in proliferation assessed by bromodeoxyuridine (BrDU) /anti-BrDU and propidium iodide labelling. In CFU-AL assays using transduced cells in the absence or presence of hygromycin (300μg/ml), 18-57% of colonies were hygromycin resistant suggesting a much higher level of transduction of clonogenic cells, although this was not affected by the addition of growth factors. CD34 enrichment did not increase the transduction efficiency but the cells could be maintained in culture longer allowing better selection by culture in hygromycin (up to 64% B7.1 expression). Transduction efficiency correlated with proliferation and clonogenicity except for the case with the highest proliferation and second highest frequency of clonogenic cells which had the lowest transduction efficiency. In this case 26% CFU-AL were hygromycin resistant and detection of viral integration by PCR points to the absence of B7.1 gene expression rather than a failure of viral integration. Simultaneous immunophenotyping and FISH has demonstrated a 25% higher frequency of viral integration than was suggested by immunophenotyping alone which supports this concept. Immunoselection with magnetic beads enabled separation of transduced cells with 34-69% expressing B7.1. This provides a means to produce gene-modified cells expressing immunomodulatory genes for immunotherapy even with low transfection efficiencies. This method also allows for double and triple expression vectors using surface expression as a means of selection. Efficiency of retrovirus-mediated gene transfer into AML blasts is determined by proliferative capacity and is increased by growth factors but not through effects on proliferation alone. Inhibition of transgene expression may occur in some cell populations although the mechanism is unclear.

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48

IMMUNE RECONSTITUTION OF SEVERE COMBINED IMMUNE DEFICIENT (SCID) MICE: A POTENTIAL MODEL FOR TESTING IMMUNE GENE THERAPY FOR ACUTE MYELOID LEUKAEMIA.

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The transfer of a number of immune modulatory genes (*eg.* B7, interleukins, γ-IFN, GM-CSF) into murine cell lines has demonstrated the potential for immune gene therapy of cancer. Furthermore, recent studies have shown the transfer of such genes into human acute myeloid leukaemia (AML) blasts. However, our ability to test the efficacy of such gene modifications of human blast cells has been limited to *in vitro* assays which, apart from inherent practical problems and production of misleading results, might not adequately reflect the human situation. Production of SCID-human leukaemic chimeras, if immunologically competent and with a stable leukaemic engraftment, potentially provide a model to test the efficacy of such gene modifications for the immune therapy of AML. Other studies have demonstrated human AML engraftment in SCID mice. However, in this study we have examined the immune reconstitution of SCID mice receiving human leukaemic and normal donor grafts. SCID mice (♀, C.B-17IcruCru-SCID, 6-8 wks) were irradiated (3-4 Gy, ^{137}Cs) and reconstituted *i.p.* with PBMCs from patients with overt AML (3 x 10^7;>95% blast cells), or human 'normal' donors (2 x 10^7). All animals received tetanus toxoid (2 iu) and tuberculin PPD (5,000 iu) boosters (0, 3 and 6 wks), and animals with AML grafts also received human growth factors (IL-3, GM-CSF, SCF). Immune reconstitution was analysed by the production of human IgG (huIgG) in the peripheral blood and a delayed hypersensitivity to tuberculin PPD (Mantoux skin test; *s.c.* 40 μl of 100,000 iu ml^{-1}). Both AML and normal grafts showed an immunological reconstitution as measured by the production of huIgG; AML grafts, 198 (160 - 260) μg ml^{-1} huIgG (median and interquartile range, n = 9); normal grafts, 260 (105 - 1020) μg ml^{-1} huIgG (n = 6). Total number of reconstitutions to date with > 50 μg ml^{-1} (baseline) huIgG; 9/11 AML, and 22/24 normal. Furthermore, we have shown both huIgG (B-cell engraftment), and passive transfer of delayed hypersensitivity to tuberculin PPD (T-cell engraftment) in 9/12 'normal' reconstitutions, and 7/12 AML reconstitutions despite the AML graft being at least 95% blast cells. However, little or no AML blast cell engraftment was seen, perhaps indicating the different *ex vivo* proliferative capacity of myeloid and lymphoid cells. This model is being further investigated to develop both AML blast engraftment and immunological competence.

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49

EFFICIENT COSTIMULATORY SIGNAL FOR T CELL PROLIFERATION INDUCED BY HUMAN LYMPHOMA CELLS TRANSDUCED WITH B7-1 AND B7-2 MOLECULES BY RECOMBINANT ADENO-ASSOCIATED VIRUS (AAV) VECTORS

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Immunotherapy of malignant disease has recently focused on the gene transfer of surface antigens of the B7 family which induce proliferation and lymphokine production of T cells after binding to their counter receptor CD28/ CTLA-4. So far, two costimulatory molecules are characterized, i.e. B7-1 (CD80) and B7-2 (CD86). In preclinical models, it could be shown that these molecules can be utilized to induce antigen-specific immunostimulation and protection from different tumors. Non-Hodgkin's lymphomas, which partly loose expression of B7 antigens during progression of disease, are an attractive target for gene transduction of these molecules in order to create tumor specific vaccines. The human B7 genes, driven by a CMV promoter and linked to a neomycin resistance gene cassette, were subcloned into recombinant adeno-associated virus vectors (pAAV/B7-1; pAAV/B7-2) and packaged in COS7 cells, yielding viral titers in the range of 10^{10} particles/ml. These particles were used to transduce different human lymphoma cell lines (LP-1, RPMI 8226). Expression of the B7 molecules was controlled by flow cytometry. Functional analysis of B7-1 and B7-2 transduced tumor cells was assessed by measuring the proliferation of prestimulated allogeneic human T cells in response to tumor cells by [^{3}H] thymidine incorporation. For this purpose, T cells were incubated with suboptimal concentrations of concanavalin A and subsequently stimulated with γ-irradiated lymphoma cells. Stimulation of T cells with B7-1 or B7-2 transduced lymphoma cells resulted in a significant higher proliferative response in comparison to tumor cells transduced with genes coding for neomycin resistance or β-galactosidase. The proliferative effect could be specifically blocked by monoclonal antibodies against the B7-1 or B7-2 epitope. These experiments show that AAV vectors can be used for the efficient gene transfer of T cell costimulatory genes into lymphoma cells. Future experiments will test whether additional molecules, costimulators or cytokines, can further augment T cell stimulation and whether B7 positive tumor vaccines can induce tumor regression in murine lymphoma models.