11.

PRODUCTION AND CHARACTERIZATION OF ANTIBODIES TO SURFACE ANTIGENS OF HUMAN NEUROBLASTOMAS. S.Schönmann, J.Iyer, K.Blaser, A.Morell and H.Käser. Institute of Clinical and Experimental Cancer Research,University of Berne,Switzerland

Neuroblastomas are the most frequent solid tumors in children. In contrast to other cancers, spontaneous regression can be observed in some cases, while only little progress in the therapy of this tumor was made up to now. In order to characterize the molecular structures displayed by the surface of neuroblastoma cell membranes, we produced monoclonal (Mabs) and polyclonal (Pabs) antibodies which react with neuroblastoma surface membranes. A number of Mabs against the human neuroblastoma line IMR-32 was obtained. The specificity of these antibodies was tested by a solid-phase ELISA using cells of other neuroblastoma lines, of several solid tumors and various cells of different origin. In addition, the reactivity of Mabs and Pabs directed towards different membrane preparations of IMR-32 and SK-N-SH neuroblastoma cells was studied. For this purpose, cells were mechanically broken. After removal of the cellular contaminants by centrifugation at low speed and the nucleic acids by enzymatic digestion with DNase, five fractions were obtained by centrifugation at 70,000 x g in a sucrose-density gradient. Again, the reactivity of antibodies was estimated in a solid-phase ELISA. Furthermore, the membrane fractions were subjected to electrophoresis in SDS-polyacrylamide gel. After electrophoretic transfer of separated glycoproteins and glycolipids onto a nitro-cellulose plate, the membrane components were incubated first with antibody and then with 125 I labeled staphylococcal protein A. The reaction was visualized by autoradiography. Further characterization of the available antibodies and attempts to isolate the relevant glycoproteins and glycolipids by immunoadsorption are under progress.

12.

SELECTIVE LOCALISATION OF RADIOLABELLED MONOCLONAL ANTIBODIES TO HUMAN NEUROBLASTOMA XENOGRAFTS IN A NUDE MOUSE MODEL. A. Goldman, J. Pritchard and J.T. Kemshead, for the ICRF Oncology Laboratory, The Institute of Child Health, Guilford Street, London WC1N 1EH.

To determine whether monoclonal antibodies coupled to radioisotopes could be employed for the identification of metastatic disease and occult primary tumours in patients with neuroblastoma a mouse model system has been investigated. The TR14 cell line, characterised as a human neuroblastoma, has been established as a xenograft subcutaneously in nude mice. Following immunisation of mice with either human foetal brain or purified neural antigen a panel of monoclonal antibodies with specificity to neuroectodermal tissue has been produced (UJ13A, 223.8, anti-human Thy-1). These antibodies have been labelled with ^{125}Iodine by a modified chloramine T technique and injected intravenously into mice bearing TR14 tumours. The distribution of the antibody in vivo, assessed by measuring the level of radioactivity in the tumour and a range of mouse organs, shows selective uptake by the tumour. Autoradiography of the tumours and mouse tissues confirms the localisation of ^{125}Iodine labelled antibodies to the membranes of the tumour cells. Mice with radiolabelled tumours are being scanned using a gamma camera to determine the size of tumour that can be delineated by this technique. In view of the heterogeneity of the surface antigens on neuroblastoma tumours a panel of monoclonal antibodies may be needed in the clinical situation. The use of a panel of antibodies compared with individual monoclonals for localisation is currently being investigated using the mouse model. When the optimal conditions for localisation have been determined in the mouse model we plan to extend the technique to patients.

13.

DIAGNOSTIC AND THERAPEUTIC APPLICATIONS OF A MONOCLONAL ANTIBODY AGAINST HUMAN OSTEOGENIC SARCOMA. R.W. Baldwin, M.J. Embleton, G.R. Flannery, J.M. Pelham, M.V. Pimm, R.A. Robins Cancer Research Campaign Laboratories, University of Nottingham, Nottingham, U.K.

A murine monoclonal antibody (791T/36) reacting with antigens expressed upon human osteogenic sarcoma cells was evaluated for the detection of human tumour xenografts in immunodeprived mice. ^{131}I-labelled antibody was shown to localize preferentially in tumours. Also tumours could be detected by whole body gamma scintigraphy when ^{131}I-labelled antibody was used in conjunction with ^{113m}In indium chloride for blood pool image enhancement. The monoclonal antibody has also been evaluated for targetting anti-tumour agents. Vindesine conjugates have been prepared and these show selective toxicity in vitro for osteogenic sarcoma cells. This selective toxicity is dependent upon conjugates binding to tumour cell surface antigens. Monoclonal antibody has also been conjugated to human lymphoblastoid interferon to provide a reagent for targetting interferon to the tumour cell surface. These conjugates retain anti-tumour antibody activity determined in a number of ways including complement dependent lysis of tumour cells and binding to immobilized tumour cells. Interferon activity assayed by the augmentation of NK cell activity in peripheral blood lymphocytes was also retained. These studies establish the validity of using anti-tumour monoclonal antibodies for targetting anti-tumour agents such as interferon

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14.

COMPARISON OF TWO PREPARATIVE REGIMENS FOR BONE MARROW TRANSPLANTATION (BMT) IN THE TREATMENT OF CHILDREN AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). Mark Nesbit, William Woods, John Kersey, William Krivit, Tae Kim, Philip McGlave and Norma Ramsay for the University of Minnesota BMT Team, Minneapolis, MN 55455.

Twenty-seven patients (pts.) with ALL in remission have been transplanted using a HLA matched sibling at the University of Minnesota since August 1978. Twenty-two pts. were in their second marrow remission and five pts. were in their third or greater remission. Fifteen pts. were prepared with cyclophosphamide 60 mg/kg/day x 2 followed by total body irradiation (TBI) (750 rads at 26 rad/min) from August 1978 to January 1981 (Regimen 1) and twelve pts. from January 1981 to present (Regimen 2) were prepared with Cytosine Arabinoside (ARA-C) 300 mg/m^2/day IV and VM-26 165 mg/m^2/IV on days -12, -9, and -6 plus cyclophosphamide and TBI as in Regimen 1. Pts. in both regimens were comparable as to age, sex, prior marrow remissions and prior central nervous system disease. Following transplantation, three methods were used for prevention of graft-versus-host disease (GVHD): 1) Methotrexate (MTX), 2) MTX plus prednisone plus antithymocyte globulin or 3) OKT3 treatment of marrow plus MTX. Life table estimates 1 year following BMT is as follows:

	REGIMEN 1	REGIMEN 2	P-VALUE
Bone Marrow Remission	60%	70%	0.69
Disease-Free Survival	47%	58%	0.75
Survival	47%	56%	0.87

In Regimen 1, eight patients have died (relapse 4, infection 4) and in Regimen 2 five pts. have died (relapse 2, GVHD 1, infection 2). Thus the addition of ARA-C and VM-26 to cyclophosphamide and TBI as a preparative regimen for BMT of ALL does not appear to improve significantly to the pts.' outcome or relapse rate.

15.

ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ACUTE NON-LYMPHOBLASTIC LEUKEMIA IN PEDIATRIC PATIENTS. D.KIRKPATRICK, R.DINSMORE, N.KAPOOR, B.SHANK, R.J.O'REILLY, and the MSKCC MARROW TRANSPLANT TEAM. Memorial Sloan-Kettering Cancer Center,New York, N.Y.,10021. U.S.A.

34 patients (pts) between 1½ and 17years of age were transplanted for acute non-lymphoblastic leukemia (ANLL) utilizing a pre-transplant conditioning regimen of cyclophosphamide 120mg/kg and either 1000 rads single dose or 1320 rads hyperfractionated total body irradiation (TBI) given in 11 doses of 120 rads within 4 days. 21 pts (61%) are surviving without any evidence of disease from 3 to 36 months post-transplant when evaluated without regards to remission status at the time of bone marrow transplant (BMT). If this group is divided according to remission status,the survival of 1st remission ANLL pts is 77% (14 out of 18), pts were from 3 to 36 months post-BMT, median follow-up was 19 months.There were 4 deaths in the 1st remission pts;3 due to pulmonary complications,and 1 due to leukemic relapse. Relapse occurred in a pt with a biphenotypic leukemia, with blasts of the lymphoid phenotype present at relapse. In those pts transplanted for ANLL in 2nd remission, 6 out of 8 pts (75%) are surviving from 3 to 27 months post-BMT, median follow-up 12 months.There were 2 pulmonary deaths in 2nd remission.There were 8 pts transplanted either in relapse or a tertiary remission,only 1 pt is alive (12%) at 30 months post-BMT. There were 7 death in this group(88%), and all were the consequences of leukemic relapse. Considering the poor results, transplant in relapse or third remission is not recommended for children with ANLL.2nd remission ANLL pts are,at present doing as well as 1st remission cases; however,the number of pts in this group is small. The excellent survival in 2nd remission cases may reflect the low toxicity of the 1320 rads hyperfractionated protocol.
