6.

CYTOTOXIC DRUGS BOUND TO HUMAN IgG TO TREAT STAGE IV NEUROBLASTOMA (Nb). G. Melino, M.A. Castello, D.M. Forrest, Z.B. Tabara, A. Clerico, K.B. Cooke, J.R. Hobbs, for the 2ª Clinica Pediatrica, Università di Roma, Italia, and the Chemical Pathology Department, Westminster Medical School, London, SW1P 2AR.

12 stage IV Nb patients were treated with Chlormabucil (CHL) or daunorubicin (DNR) covalently bound to allogeneic polyclonal antibody (Ab), trying to increase the local concentration of the drug on the tumour cell. 3 stage II and III patients with lymph node deposits and residual tumour after surgery were also included. Ab were raised in well informed volunteer parents immunised with one million Nb cells irradiated with 12000 rads and sonicated with the adjuvant Dimethyl Dioctadecyl Ammonium bromide at 1 mg/ml. Immune IgG were purified from the plasma by the cold ethanol fractionation method and coupled to CHL or DNR using 1-ethyl-3(3[1]-dimethylaminopropyl) carbodiimide as condensing agent. The preparations which passed pyrogen, sterility, anti-HLA Ab, auto Ab tests were used clinically at 0.6 mg CHL and 2 mg DNR/Kg body weight. This clinical drug targeting has shown to be effective in producing measurable responses in 10 out of 12 patients with observed reduction of metastases in bones and lungs, and in 1 patient with resolution of bilateral chylothorax. 4 patients are still alive, 2 over 18/12 from starting treatment appear to be free of disease. The treatment appears to be safe, with minimal discomfort and few side effects. The need of human Ab for long term management and the preference for polyclonal Ab will be emphasised.

7.

MONOCLONAL ANTIBODY CHARACTERIZATION OF SURFACE ANTIGENS IN CHILDHOOD T-CELL MALIGNANCIES. M.Roper, W.Crist, R.Metzgar, A. Ragab, S.Smith, K.Starling, J.Pullen, B.Leventhal, A.Bartolucci, M.Cooper, for the Pediatric Oncology Group (POG). The University of Alabama in Birmingham, Alabama, USA.

Tumor cells from 42 children with T-cell acute lymphocytic leukemia (ALL) and 14 with non-Hodgkin lymphoma (NHL) were analyzed with monoclonal antibodies to determine whether the malignant cells from patients with these disorders are biologically distinguishable. T-ALL was defined as >40% E-rosette forming cells and/or >70% pT antigen positivity. Tumor cells from marrow, blood or biopsy sites were studied with OKT9,10, 6,4,8,3 and LEU-1,2,3. We found that 16/42 (38%) T-ALL patients demonstrated surface antigens consistent with the earliest stage of T-cell development (T6-/T4-/T8-/T3-). Fourteen of 42 (33%) were mid-stage (T6+, and/or simultaneous expression of T4 and T8); 12/42 (28%) expressed antigens found on mature T-cells (T3+, variable expression of other antigens). Among the 14 T-NHL patients, 7 demonstrated mid-stage and 7 late-stage T-cell types; none were identified as the early thymocyte phenotype. Of the 23 patients additionally studied with LEU-1, 21 were positive. This antibody appears to identify an antigen found in the majority of patients with T-cell disease. Clinical features (age, sex, white cell count, mediastinal mass) in this patient population did not distinguish the three T-ALL subgroups. These data demonstrate that considerable immunologic heterogeneity exists in both T-ALL and T-NHL, and that both T-ALL and T-NHL primarily involve T-cells of mid- to late maturational stages. The finding of the early thymocyte phenotype exclusively in our T-ALL patients suggests that this particular cell type preferentially spreads to marrow and blood early in the evolution of the disease.

8.

OCCURRENCE OF T-CLL IN CHILDHOOD. V.Vecchi, S.Pileri, L.Serra, A.Pession, P.Rosito, A.Vitelli, G.Paolucci, P.Paolucci, for Section of Haematology/Oncology, Department of Pediatrics and Department of Pathology, University of Bologna, Italy. Supported by CNR, PFCCN n.80.01605.96 115.12574, Rome.

The occurrence of T-CLL in a seven year-old boy represents an interesting and original observation, as T-CLL has never been observed in patients younger than 20 years. At the onset of the disease, diffuse eruptive macular-papular lesions, massive splenomegaly, liver and cervical lymph nodes enlargement were present, but no mediastinal mass and CNS involvement. The bone marrow was only partially infiltrated (30%). WBC count was 10×10^5/mmc. Atypical small lymphocytes had the same cytomorphological features in blood (60%), bone marrow and skin, i.e. a thin rim of cytoplasm and nuclei with small protuberances and clefts, which did not penetrate more than halfway into the diameter of the nucleus, without visible nucleoli. Few corse granules acid phosphatase activity was seen as a paranuclear, small roundish spot. As defined by sheep-E rosettes, heteroantisera and monoclonal antibodies, the small lymphocytes both in blood and bone marrow corresponded to a mature T-cell phenotype (E_R^+HuTLA$^+$OKT3$^+$, Tdt$^-$, OKIa-1$^-$, SmIg$^-$, c-ALLA$^-$). The ratio of OKT8$^+$ to OKT4$^+$ cells was 4:1, but circulating OKT8$^+$ cells were only 36% of nucleated cells, suggesting that this phenotype was prevalent but not representative of the whole atipical population. Small collections of atypical lymphocytes occurring at the grenz zone and within the epidermis were also observed. The modified LSA_2-L_2 protocol was employed, but the boy died for cardiopulmonary arrest during the induction phase. At autopsy, foci of infiltration could be recognized in the bone marrow, liver, spleen and lymph nodes

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9.

PANELS OF MONOCLONAL ANTIBODIES USED IN THE IMMUNOHISTOLOGICAL DIAGNOSIS OF BRAIN TUMOURS. J.A. Garson, H.B. Coakham, E.I. Harper, Betty Brownell, J.T. Kemshead, for the Brain Tumour Research Laboratory, Dept. of Neuropathology, Frenchay Hospital, Bristol, U.K.

Classification of tumours arising in the nervous system can prove to be difficult and extremely time consuming using conventional histological and cytological techniques. We have investigated whether panels of monoclonal antibodies could act as diagnostic aids in neuropathology. Monoclonal antibodies UJ13A and A2B5 (neuro-glial markers), LE61 (anti-cytokeratin), RT97 (anti-neurofilament), D19 (anti-glial fibrillary acidic protein) and a group of reagents reactive with different haemopoietic cells have been applied to frozen sections of over 100 different cerebral tumours (malignant and benign intracranial and spinal neural tumours as well as secondary deposits of carcinomas and lymphomas). Binding of monoclonal antibodies to normal and malignant tissue was assessed by indirect immunofluorescence. Gliomas, neuroblastomas, ependymomas, choroid plexus papillomas, medulloblastomas and meningiomas have been shown to bind only a proportion of the total antibodies used in the screens, indicating that each tumour can be recognised by a characteristic antigen profile. Similarly poorly differentiated metastatic carcinomas and cerebral lymphomas can be distinguished from neural tumours by their differential binding to specific groups of monoclonal antibodies. This type of immunohistological analysis of intracranial and intraspinal tumours has thus enabled diagnosis of malignant cell types to be made in situations where conventional techniques have proved unsatisfactory - examples of this will be presented.

10.

MONOCLONAL ANTIBODIES HELP IN THE DIFFERENTIAL DIAGNOSIS OF "SMALL ROUND CELL" CHILDHOOD TUMOURS. **J. Pritchard, J. S. Malpas, J. T. Kemshead. ICRF Oncology Laboratory, Institute of Child Health, Guilford Street, London W1N, England.**

The exact diagnosis of small round cell tumours of childhood still often present problems to the histopathologist. We have applied a panel of 26 monoclonal antibodies to various paediatric primary and secondary tumours, seeking patterns of antigen expression consistent enough to be of diagnostic use. The antibodies were raised by immunizing Balb/C mice with (i) human neural tissue, (ii) human lymphoid tissue, (iii) human rhabdoid tissue, (iv) several human neuroblastoma and lymphoid cell lines and (v) chick retinal tissue. Tumours were tested either in frozen section (solid samples) or as a single cell suspension (heavily infiltrated marrow samples) using indirect immunofluoresence. Distinct patterns of reactivity have emerged: lymphoid tumours and neuroblastoma can confidently be distinguished by antibody 'profiles'; Ewing's and rhabdomyosarcoma also show distinctive antigen profiles though few specimens have been studied to date. Heterogeneity of antigen expression between individual tumours of the same histological type underlines the need for the use of panels of monoclonal reagents. In 2 instances, each child presenting with diffuse bone marrow tumour infiltration, antibody studies were responsible for the correction of faulty initial diagnosis based on cytological and histochemical criteria alone.

We conclude that panels of monoclonal reagents have important potential for the differential diagnosis of small round cell tumours of childhood.
