ABSTRACTS

ORAL PRESENTATIONS

ABSTRACTS - Second International Conference on Malignant Lymphoma, Lugano

1 Cellular oncogenes have been identified by the biological activity of tumor DNAs in transfection assays and/or by homology to the transforming genes of retroviruses. In some tumors, the biological activity, organization or expression of these genes is altered, suggesting that such alterations contribute to the development of neoplastic disease. I will review experiments leading to the identification of cellular oncogenes and discuss our current understanding of the mechanisms by which they induce transformation of cells in culture and may contribute to the pathogenesis of neoplasms in vivo.

Geoffrey M. Cooper, Dana-Farber Cancer Institute, Boston, USA

2 POTENTIAL OF MONOCLONAL ANTIBODIES IN ONCOLOGY.

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The description by Köhler and Milstein of a reliable method for producing monoclonal antibodies has created a new era in the use of antibodies as research and diagnostic tools. The production of monoclonal antibodies is based on the fusion (or hybridization) in vitro of myeloma cells with antibody-producing lymphoid cells. While selected myeloma cell lines can be grown permanently in culture, antibody-producing cells usually undergo terminal differentiation after a few cell divisions and die. In the fusion product ("hybridoma"), the myeloma cell confers permanent growth, whereas the lymphoid cell contributes the capacity to produce specific antibody. Since a given antibody-producing cell is committed to the production of only one type of antibody molecule, the hybrid cell line obtained after fusion of this particular antibody-producing cell and a myeloma cell produces homogeneous antibodies of unique specificity. Thus, once a hybridoma has been developed, it is immortal and provides unlimited amounts of specific antibody.

While the use of monoclonal antibodies has already been extremely rewarding in basic research, there is increasing evidence that these reagents will have a profound impact on clinical medicine in the near future. In the field of oncology, monoclonal antibodies can be used to differentiate between normal and neoplastic cells and may be exploited for diagnostic and, ultimately, therapeutic gain. There is already evidence that monoclonal antibodies can facilitate accurate pathological diagnosis, classification of malignancies and early detection of micrometastases. Studies are in progress to determine their potential use in tumor localization (by immunoscintigraphy) and therapy.

3 OVERVIEW ON CURRENT TOPICS IN CLINICAL RESEARCH.

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A historical review of the evaluation of treatment concepts will be presented.

4 HODGKIN'S DISEASE: FURTHER INFORMATION DERIVED FROM CELL-LINES.

Since 1978 we have established 4 cell-lines (L 428, L 538, L 540, L 591) from patients with Hodgkin's disease. The cell-lines are of malignant origin, as shown by several chromosomal aberrations. Morphological, cytochemical and immunological assays demonstrated the identity of the in vitro cells with Hodgkin (H)- and Sternberg-Reed (SR)-cells in vivo. Functional properties and surface characteristics are not in line with any known cell type of hematopoiesis or lymphoid tissue. The cell-lines produce factors involved in hematopoiesis and immunological response (CSF, IL 1, MIF). The in vitro Hodgkin-cells (L 428) are capable of presenting soluble antigen to lymphocytes.

A monoclonal antibody (Ki 1) produced against one of the cell-lines (L 428) reacts with H and SR cells in frozen sections of lymphoid tissue and with a so far unidentified cell-population in normal lymphnodes. Furthermore it binds to a minority of mononuclear cells in the peripheral blood of healthy individuals.

The established Hodgkin cell-lines may be of value in identifying the cell-markers of H and SR cells in vivo and may be helpful in eludicating the puzzle concerning the normal counterpart of H- and SR-cells.

The monoclonal antibody Ki 1 produced against L 428 is already being used in diagnosis of lymphomas in frozen sections of lymphoid tissue. It may be helpful for in vivo diagnosis of HD in man, since radiolabelled Ki 1 detects HD-tumors transplanted on nude mice.

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ABSTRACTS - Second International Conference on Malignant Lymphoma, Lugano

5 ORIGIN AND BIOLOGIC FUNCTION OF REED-STERNBERG CELLS. Richard I. Fisher, Volker Diehl, Susan E. Bates, David J. Volkman, Toby T. Hecht, and Dan L. Longo. National Cancer Institute, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20205, and Medizinische Hochshule Hanover, Hanover, Germany.

Reed-Sternberg cells in biopsies from patients with Hodgkin's disease express Ia antigens yet lack other cell surface markers associated with mature B cells, T cells, or monocytes. We have utilized the L428 cell line to test the thesis that Hodgkin's disease is a tumor of antigen presenting cells. The L428 cells are potent stimulators of the human primary mixed lymphocyte cultures (J. Immunol. 6/83). Significant proliferation occurred when mononuclear leukocytes obtained from normal donors were stimulated with radiated L428 cells at responder:stimulator ratios varying from 200:1 to 20:1. Maximal proliferation occurred on day 5. These proliferative responses can be blocked by anti-Ia antibody. Antigen processing by responder monocytes was not required. The cells that proliferated were T cells, primarily of the helper subset. Under certain conditions the L428 cells are capable of producing IL-1. The L428 cells also function as accessory cells for mitogen-induced human T cell proliferative responses (J. Immunol. 5/84). Purified human T cells that are depleted of Ia-bearing cells and adherent cells do not proliferate in response to concanavalin A. The addition of as few as 1% radiated L428 cells restores the proliferative capacity of the T cells. The tumor cells were 30 times more potent than allogeneic mononuclear leukocytes as accessory cells. The T cells from patients with advanced stages of Hodgkin's disease have impared mitogen responses even in the presence of these potent accessory cells. The L428 cells are also capable of presenting soluble antigen to T cells in a genetically restricted fashion (AACR, 1984). T cell lines from HLA-DR5 normal donors, who had been immunized with tetanus toxoid, generated tetanus specific proliferative responses in the presence of the L428 cells. T cell lines from individuals with other DR phenotypes (DR 1, 2, 4, or 7) did not generate responses in the presence of tetanus and L428 cells. Thus the L428 cells possess all the characteristics of antigen presenting cells.

A murine monoclonal antibody termed HeFi-1 that selectively binds Reed-Sternberg cells in 18/18 tissue biopsies has been produced following immunization with the L428 cells. The antigen recognized by HeFi-1 is a 120 KD glycoprotein that does not modulate in vitro. HeFi-1 does not block the ability to the L428 cells to stimulate a mixed lymphocyte culture or function as an accessory cell. This antibody should prove useful not only for the diagnosis and treatment of Hodgkin's disease but also for determining the normal cell from which Hodgkin's disease originates.

6 RADIOIMMUNODETECTION OF HUMAN B-CELL LYMPHOMAS WITH A RADIOLABELED TUMOR-SPECIFIC MONOCLONAL ANTIBODY (Lym-1). A. L. Epstein, A.M. Zimmer, S.M. Spies, D. Mills, G. DeNardo, and S. DeNardo. Northwestern University, Chicago, Il 60611 and University of California at Davis, Sacremento, CA. 95817, USA.

A new monoclonal antibody, Lym-1, has been produced to a cell surface antigen expressed in normal lymph node B-cells and a subset of B-cell derived human lymphomas and leukemias. Specificity screens using a panel of human lymphoma and leukemia cell lines and biopsies have shown that Lym-1 is positive on a subset of diffuse histiocytic and Burkitt's lymphomas and B-cell CLL. On a panel of 18 normal human organs, Lym-1 was positive with B-cell zones of lymph nodes, a low % of peripheral blood B-cells, tissue macrophages, and colonic surface epithelium. Immunoprecipitation studies revealed that Lym-1 recognizes 4 polypeptides in the range of 31-35 kd. Because of its cell surface reactivity with a subset of B-cell tumors and its low reactivity with normal organs, Lym-1 was tested in a nude mouse animal system and in volunteer cancer patients for its radioimaging capabilities. Lym-1 (IgG2a) was purified from ascites fluid by ammonium sulfate precipitation and Protein-A affinity chromatography. Radiolabeling of whole antibody, $F(ab')_2$, and F(ab) fragments was achieved with I-131 using solid phase DPG, with I-123 using chloramine-T, and with Cu-67 using benzl EDTA bifunctional chelation. Athymic nude mice bearing right thigh Raji tumors were injected with 150-300 uCi of radiolabeled Lym-1 and imaged up to 7 days after injection at which time the animals were sacrificed and organ distribution performed. Highest tumor uptake was observed for radiolabeled whole antibody followed by $F(ab')_2$, and F(ab) fragments. These studies showed that specific and significant tumor uptake of radiolabeled Lym-1 could be achieved since 4-8% and 15-26% of the injected dose of I-131 and Cu-67 labeled Lym-1, respectively, localized to the tumor. With I-131, optimal tumor visualization for radioiodinated $F(ab')_2$ fragments and whole antibody was observed at 3 and 7 days after injection. At the present time, 5 volunteer breast cancer patients have been imaged with 1-5 mCi of I-123-Lym-1 in order to obtain biodistribution data in patients bearing Lym-1 negative tumors. Quantitative tomographic imaging using single photon emission computerized tomography revealed no abnormal uptake of radiolabeled antibody in the organs or tumors of these patients. These preliminary studies confirm the low reactivity of Lym-1 with normal tissues and opens the way for future studies with patients bearing antigen positive lymphomas. Therapeutic trials with I-131 and Cu-67 radiolabeled Lym-1 are anticipated upon the successful completion of these investigations.

7

THERAPEUTIC USE OF MONOCLONAL ANTI-IDIOTYPE ANTIBODIES AGAINST B-CELL LYMPHOMA. Annemarie Hekman, Elaine M. Rankin, Reinier Somers and Wim ten Bokkel Huinink, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam.

Five monoclonal antibodies have been made against the idiotype of the membrane immunoglobulin of the malignant lymphocytes of 4 patients with B-cell non-Hodgkin lymphoma. Two have been used for therapy in patients with advanced centrocytic lymphoma, antibody T2 for patient Top, and antibody K1 for patient Klos. Both antibodies were of the $IgG2_a$ subclass, were cytotoxic with rabbit but not with human complement, and did not modulate the antigen.

Patient Top had 10 x 10^9 malignant lymphocytes in the blood and these were used to determine the treatment schedule. There was a negligible amount of free idiotype. 3 different regimens were tried: escalation by doubling the dose daily from 5 mg to 160 mg, rapid escalation by doubling the dose hourly from 10 mg to 160 mg, and bolus dose 150 mg over 2 hrs followed by 20 mg/hr over 28 hours. There was a temporary fall in the lymphocytes after each treatment. At the end of the continous infusion, free antibody was detectable in the serum (10 µg/ml) and cells in the blood, bone marrow, lymph node and ascites were coated with T2. Two further infusions of T2, each lasting 42 hrs were given. The patient received a total of 3800 mg T2. ^{111}Indium oxine labelled lymphocytes[§] were used to demonstrate that malignant cells were rapidly cleared from the circulation which was repopulated with unlabelled cells. The S-phase cells in blood remained <2%. There was no alteration in spontaneous tritiated thymidine uptake in blood lymphocytes. Serial punch biopsies of lymph nodes showed an increase in the number of lytic cells, a sign of necrosis, during treatment from 0 to 25%. Monocyte activity as measured by chemiluminescence showed some improvement but remained below normal levels. Antibody dependent cellular cytotoxicity, negligible before treatment began, reached normal levels after the last infusion.

Patient Klos had a high level of free antigen before treatment. The dose was escalated until 400 mg over 2 hr which removed all free idiotype. This returned at a lower level the next day. 1200 mg K1 saturated the cells in the blood and lymph node with antibody and reached the cells in the ascites; excess K1 was detectable in the serum at 20 µg/ml. 5.9 gms K1 have been given, the lymph nodes are decreasing in size.

Neither patient has shown any toxicity; liver and renal function and complement status are unaltered. Neither patient has made antibodies to the mouse immunoglobulin. No modulation of the antigen was seen in either case. It is too early to assess tumour response, treatment stopped three weeks (patient Top) and one week (patient Klos) ago.

[§]see abstract number T-41

8

THE IMMUNOBIOLOGY OF B CELL LYMPHOMA, Ronald Levy, Medicine/Oncology, Stanford University, Stanford, CA, 94305

Human B cell lymphomas are considered to be monoclonal cell populations, i.e., derived from a single original transformed cell. This notion is based on analyses of karyotypes, X chromosome-linked enzymes, and immunoglobulin proteins. The cell surface immunoglobulin of each B cell tumor is idiotypically distinct. We have produced anti-idiotype antibodies for a series of 25 patients with B cell lymphomas. Each antibody is specifically reactive with the cell surface immunoglobulin of only one patient. These antibodies have been used as diagnostic monitoring reagents, as therapeutic agents, and as probes for the biology of the disease. Immunoassays have been performed on serum from a series of B lymphoma patients using the anti-idiotype antibodies. We find idiotypic protein in the serum with a wide variation in levels between patients but characteristic of each patient tumor. Within a patient, serial determinations of serum idiotype correlate well with tumor burden. The serum idiotype can be lowered to approximately 10% of initial level by plasmapheresis. Therapeutic trials are underway with anti-idiotype antibodies and these will be discussed. Obstacles to therapeutic effect have been identified. These include serum idiotype, anti-mouse immune response, antigenic modulation, and tumor heterogeneity. Recently, we have determined that some B cell lymphomas are composed of two clones differing in cell surface idiotype and in immunoglobulin gene rearrangements. The incidence of this phenomenon may be as high as 10% of cases of follicular lymphomas.

9 T-CELL DIFFERENTIATION: IMPLICATIONS FOR THE ONCOLOGIST AND BIOLOGIST. S. F. Schlossman. Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Recent advances in both the cloning of antigen specific human T lymphocytes and the production of monoclonal antibodies against cell surface structures has permitted the identification of cell surface glycoproteins involved in antigen-recognition. Analysis of the functional role of both polymorphic or clonotypic structures on human T cells as well as nonpolymorphic structures has allowed the construction of a model for the human T cell antigen receptor. This receptor is a cell surface complex comprised of a clonotypic (Ti) 90 KD heterodimer and the monomorphic 20/25 KD T3 molecule. Approximately 30-40 x 10^3 Ti and T3 molecules exist on the surface of the human T lymphocyte and these cell surface glycoproteins are fully expressed during late thymic ontogeny at the time of development of immunocompetence. The Ti antigen is made up of α and β chains both containing variable and constant regions. It is now clear that the β chain of the T cell receptor has distant homology to immunoglobulins as defined by protein structure and molecular probes. Triggering of the T3/Ti molecular complex results in clonal T cell proliferation utilizing an IL2 dependent autocrine pathway. The associative recognition structures defined by the 76 KD T8 and 62 KD T4 glycoproteins clearly allows for the subsetting of human T lymphocyte. More importantly, from a functional point of view, the T8 lymphocyte and the T8 glycoprotein itself appears to restrict the response of these cells to antigens presented in association with HLA-A, B or C antigens (Class I) whereas T4 glycoprotein and its corresponding subset views antigen in association with HLA-Dr, DS or SB (Class II). The precise role of the T4 and T8 antigens in imposing MHC restrictions on T cells is still not entirely clear. Nevertheless, T4 and T8 in association with the T3/Ti complex appear to provide a critical set of structures which can account for both T cell specificity and MHC restriction. The applications of this new technology of cellular characterization is still in its infancy but is expected to have a profound impact on our understanding of clinical diseases. It is believed that the structures involved in cell-cell interactions and triggering the human T cell should provide the strategies with which to manipulate the immune response for the benefit of the host.

10 IMMUNOLOGIC PHENOTYPES OF NON-HODGKIN'S LYMPHOMAS: CORRELATION WITH MORPHOLOGY AND FUNCTION. E.S. Jaffe, M.D., J. Cossman, M.D., L.M. Neckers, Ph.D., R.M. Braziel, M.D., and C. Simrell, M.D. NCI, Bethesda Md., USA

Modern immunology has been instrumental in the delineation of distinct clinicopathologic entities within the heterogenous non-Hodgkin's lymphomas. The low grade B cell lymphomas; i.e. follicular lymphomas (FL), intermediately differentiated lymphocytic lymphomas (IDL), and well-differentiated lymphocytic lymphomas (WDL), each have a unique immunologic phenotype which may represent specific and possibly sequential stages of B cell differentiation (1). All cases expressed monoclonal surface immunoglobulin (SIg) and HLA-DR and stained with B1 and BA-1, but differed in reactivity with Leu 1 (p65), BA-2 (p24), and J5 (CALLA). All FL were Leu 1-, BA-2 -, and were + with J5 in $\sim$ 50% of cases. IDL were + with all three of the above reagents, whereas WDL were Leu 1+, BA-2 - and variably J5 +. Fluorescence intensities observed for SIg, BA-1 and B-1 showed a sequential decrease; i.e., FL>IDL>WDL.

Correlative studies were also performed to study the interrelationship of function, i.e., immunoglobulin secretion, with morphologic and immunophenotypic characteristics. WDL were readily induced to secrete monoclonal Ig after exposure to the phorbol ester TPA. Ig secretion did not require the addition of allogeneic T lymphocytes (2). In contrast, most FL did not readily secrete Ig, either in the presence or absence of TPA. Depletion of autologous T-lymphocytes and the addition of allogeneic T-cells produced maximal Ig secretion, but the readdition of autologous T-cells reduced levels of Ig secretion (3). These findings suggest a suppressor function for the T-cells found in FL. No correlation with helper: suppressor ratios was observed.

Six FL that histologically progressed to diffuse lymphomas were found to contain a predominance of T-lymphocytes (mean 69%). However, residual monoclonal B-cells could be identified in 4/6 cases by SIg staining and in one case by Southern blot analysis which demonstrated clonally rearranged heavy and light chain genes (4). The T cells, although numerically predominant, were phenotypically normal, and may represent a beneficial host response, since an indolent clinical course was maintained in 5/6 patients despite histologic conversion (5).

Post thymic T-cell malignancies are heterogenous clinically and morphologically and represent a spectrum from low grade (T-CLL) to high grade (large cell, immunoblastic) malignancies. Most post-thymic lymphomas express a helper antigenic phenotype, but correlations between phenotype and function are not consistently observed (6). Cells from angiocentric immunoproliferative lesions (lymphomatoid granulomatosis) and the T-cell angiocentric lymphomas that supervene secrete a phagocytosis-inducing lymphokine, which may lead to an erythrophagocytic syndrome mimicking malignant histiocytosis clinically and pathologically (7,8).

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11 FUNCTIONAL INTERACTIONS OF MALIGNANT CELLS DURING B CELL LYMPHOMAGENESIS. Carol L. Reinisch, Dept. of Comparative Medicine, Tufts University School of Veterinary Medicine, 136 Harrison Avenue, Boston, Massachusetts, U.S.A.

Immune regulation of B cell lymphomagenesis is not well understood, primarily because few predictive animal models exist. Recently we have developed a murine model of Waldenströms macroglobulinemia induced by the retrovirus MSV-MuLV-M. In these mice, plasmacytoid-lymphocytic tumors develop in the mesenteric lymph node two or more years following infection with virus.[1]

Cells isolated from the tumor cell population, which consists primarily of mu^+ B cells, have been repeatedly cloned in vitro. These cloned cells express the lymphocyte differentiation antigens Thy1.2, Ly1 and Qa1 and are T cells. Functionally these T cells 1) promote the differentiation of granulocytes and erythrocytes and 2) enhance antigen-independent and dependent lymphocyte differentiation and function.[2] When injected into (syngeneic) B6 mice, the T cells induce rapidly proliferating immunoblastic sarcomas which kill the recipient in 7-10 days.

These results show that there is an intimate association between mu^+ B cells and $Ly1^+$ $Qa1^+$ T cells during B cell lymphomagenesis, and suggest that there may be two malignant cells which interact during tumorigenesis. Given these data, we would emphasize that understanding the pathogenesis of non Hodgkins lymphomas in either the animal or human model necessitates the isolation and functional characterization of all the subpopulations within a tumor cell population.

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12 BLOOD LYMPHOCYTE SUBPOPULATIONS AND MITOGEN RESPONSIVENESS IN RELATION TO PROGNOSIS IN PATIENTS WITH NON-HODGKIN LYMPHOMA.

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Non-Hodgkin lymphomas (NHL) are mostly of B cell type. Non-leukemic NHL patients often have blood lymphocytes with an abnormal ratio between κ- and λ-bearing lymphocytes (normal range 1.0-3.3) with an increase in lymphocytes with the same light chain phenotype as lymph node tumour cells. Lymphocytes having specificity for the same antigenic determinant carrying the same light chain isotype with identical antigenic combining sites belong to the same clone. Thus, a blood κ:λ ratio outside the normal range with a selective increase of lymphocytes of the same light chain type as in tumour lymph nodes may suggest a "leukemic" spread of the disease. Lymphocytes from untreated NHL patients are poorly stimulated to DNA-synthesis by mitogens but the mitogenic proliferative response of the lymphocytes is restored in complete remission (CR) (Eur. J. Cancer 19, 747, 1983; Scand J Hematol 30, 68, 1983). To evaluate the prognostic information of blood lymphocyte subpopulations at diagnosis and the lymphocyte DNA-synthesis after stimulation with mitogens in CR 127 untreated and 58 CR patients were studied. Blood T lymphocyte subpopulations were identified by monoclonal OKT antibodies. B ($smIg^+$) lymphocytes were stained by direct IFL using $F(ab')_2$ fragments of antibodies. Lymphocyte response to ConA and PWM stimulation was measured after incubation with C^{14}-thymidine.

Forty per cent of the untreated patients had a ratio between κ- and λ-bearing blood B lymphocytes outside the normal range. Most of the patients were in clinical stage III-IV (76%) and had low grade malignant lymphomas identified as B-CLL, IC, CB/CC and CC (68%). Patients with CB/CC lymphomas and normal κ:λ ratios survived significantly longer than those with abnormal ratios ($p < 0.01$). The mean total number of $OKT3^+$ (PAN-T) and $OKT4^+$ (helper/inducer) T lymphocytes were significantly reduced in patients compared to controls ($p < 0.001$). The reduction was not related to clinical stage or histopathology. $OKT8^+$ (suppressor/cytotoxic) T lymphocytes were not significantly different from controls.

The majority of patients in CR were tested 6 months or more following termination of radio/chemotherapy. Patients with a normal response (= median value - 1 SD of healthy controls) to ConA 20 μg/ml had a significantly longer duration of first clinical remission time than those with a subnormal response.

It is concluded that an abnormal lymphocyte κ:λ ratio at diagnosis is a predictor of poor prognosis and the reduced blood lymphocyte response to ConA in clinical remission is associated with early relapse.

13 CELLULAR INTERACTIONS REGULATING T-CELL COLONY FORMATION IN THE ABSENCE OF ADDED GROWTH FACTORS IN PATIENTS WITH T-CELL MALIGNANCIES.

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Peripheral blood T-cell colony forming cells(T-CFC) from patients with T-cell malignancies can generate T-cell colonies in methylcellulose in the absence of added growth factors.In 13 out of 25 patients,less spontaneous colonies were obtained from $E^-OKT_3^-$ cells than from unseparated peripheral blood lymphocytes(PBL).Irradiated autologous but not E^+cells from normal subjects enhanced the plating efficiency of $E^-OKT_3^-$ precursors in co-culture experiments either in methylcellulose or in separate agar/methylcellulose.Conditioned media prepared from leukemic blasts(98%E^+ cells) was able to induce T-cell colony growth from normal mature(E^+) and immature($E^-OKT_3^-$) T-CFC.Depletion of PBL by plastic adherence resulted in a decrese of colony number in 4 out of 4 patients.Accessory adherent cells were $HLA\text{-}DR^-$(as determined by treatment with a pool of 4 anti-DR monoclonal antibodies and complement).Irradiated adherent cells enhanced the plating efficiency from adherent-depleted PBL in co-culture experiments in methylcellulose but not in the two layers system.Media conditioned by adherent cells alone or supplemented either with IL1 or IL2 did not enhance colony growth from patients' PBL,A^- cells.These results demonstrate that E^+ and adherent cells have an accessory role for the spontaneous T-cell colony formation which is mediated both by diffusible factors and cellular contact.

14

TREATMENT OF NATURAL MURINE NON-HODGKIN'S LYMPHOMA USING IMMUNOREGULATORY CELL TYPES AND IL2. R.H. Keller, S. Swartz, C.W. Patrick, G. Steven, N. Torke, The Wood VAMC Marcus Center, Medical College of Wisconsin, 5000 West National Avenue, Milwaukee, Wisconsin, USA 53193.

We have previously reported the natural development of nodular (Blood 60:114A, 1982) and diffuse (Blood 58:313A, 1981) non-Hodgkin's Lymphoma in Aged Balb C Mice. We elected to examine the effect of adoptive transfer of syngeneic normal T cells, activated macrophages and natural killer cells and purified lymphokines on the evolution of the disease process in murine NHL. One hundred Balb C mice between the ages of 16-18 months were divided into three groups. All animals were hemisplenectomized on day -1, given either 1 x 10^8 enriched T cells (T), 1 x 10^8 enriched activated peritoneal macrophages (M); or .4 x 10^7 enriched natural killer cells (NK) once, or 10 units IL2 every 3 days IP; or saline (controls). The mice monitored weekly for Kappa/Lambda ratios, PBT cell % and automated CBCs and sacrificed at 36 days. Histologic evaluation was performed at the time of hemisplenectomy and sacrifice. 40% of the M C group and 70% of the M group demonstrated NHL on initial evaluation. All of the C group and 42% of the M group demonstrated progression of disease activity (SCCL+WDLL). Similar results occurred in short term experiments employing purified IL2. The results of these studies suggest that T cell imbalances are associated with alterations of disease activity in NHL and suggest that immunoregulatory T cells and/or immunochemically purified lymphokines from these cells may prove beneficial in the treatment of NHL.

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15

HTLV IN LYMPHOID LEUKEMIAS AND LYMPHOMAS: R. C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

As discussed elsewhere at these meetings HTLV is a generic name for what are now known to be a wide family of closely and distantly related human and primate exogenous retroviruses which have the following properties in common: (1) T-lymphotropic, (2) are usually specifically T4 lymphotropic, (3) contain a reverse transcriptase of 100,000 Daltons which is Mg^{++} preferring for its catalytic activity, (4) have unusually large long terminal repeat sequences (LTRs), (5) abrogate normal T-cell functions, (6) sometimes can transform normal primary T cells *in vitro* into cells with neoplastic properties, and (7) have cytopathogenic effects including the ability to produce T-cell death after infection. As of this writing there are more than 40 isolates in our laboratory and more than 70 in the world.

There are different major subgroups. Most belong to the group we have termed HTLV-I. A few are less than 10% homologous to HTLV-I and are called HTLV-II. They were obtained from a hairy cell leukemia and an AIDS patient. Numerous additional isolates have been obtained from HTLV-I and HTLV-II patients with AIDS. The analyses of them is in progress.

HTLV-I is the subgroup closely linked to the cause of a certain T-cell malignancy. This disease covers a spectrum of histopathologically defined lymphoreticular neoplasms which HTLV-I now defines as a distinct clinical entity, usually of $T4^+$, $T8^-$, Ia^+, TAC^+, mature T-cells. The patients usually have an aggressive disease usually exhibiting systemic manifestations, often (50%) skin involvement and frequent hypercalcemia (50%). The disease clusters in various parts of the world, and where it clusters HTLV-I is prevalent. The virus is apparently transmitted only by close contact or by blood products. The epidemiological data, the *in vitro* transformation, the numerous animal models of leukemias and lymphomas caused by retroviruses, the presence of integrated HTLV nucleic acid sequences in the DNA of the neoplastic T-cells, and several other results from molecular biological experiments make HTLV, in my view, the best example of a virus caused human malignancy, and perhaps the clearest example we have of the cause of any human cancer.

HTLV-I may also be involved in the cause of a fraction of mycosis fungoided and Sezary cases and *indirectly* in some B-cell neoplasias as will be discussed. Finally, variants of these retroviruses may be important in AIDS.

16 THE CURRENT STATUS OF NCI TRIALS IN HODGKIN'S DISEASE.

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The development of effective therapies for all stages of Hodgkin's disease represents one of the most remarkable achievements of modern cancer treatment. Despite these achievements, there remain a number of areas where improvements in the management of Hodgkin's disease are needed and three of these areas have been the central focus of the ongoing clinical trials at the National Cancer Institute (USA). In early stage disease as many as 25% of patients relapse from radiation-induced complete remissions and although many can be salvaged by chemotherapy, this is accomplished at some risk of induced second malignancy. Furthermore, successful radiotherapeutic management of early stage disease demands considerable technical expertise and access to sophisticated equipment not always widely available to all patients. Because combination chemotherapy is curative in advanced disease and can salvage many patients who relapse after radiation therapy and because trials with MOPP in early stage Hodgkin's disease in Uganda showed considerable promise, we are comparing MOPP alone to radiation therapy as initial treatment of early stage disease. Important parameters for comparison include not only complete remission frequency, survival, and disease-free survival but also acute and chronic toxicities and effectiveness of salvage.

One subset of patients which has consistently had a substantial relapse rate regardless of initial stage are those patients with massive mediastinal disease at presentation. We are treating such patients with alternating monthly cycles of MOPP-ABVD after radiation ports are designed by simulation to include the entire original extent of disease. After six cycles of chemotherapy, patients receive 1050 rads to the original extent of disease, followed by another 2500-3500 r to a reduced mediastinal volume. The rationale for such an approach for mantle irradiation is to minimize the marginal and pulmonary relapses so frequent in this subset of patients.

Although the treatment of advanced Hodgkin's disease with MOPP has dramatically altered the prognosis for these patients, further progress is still needed. Nearly half of patients with advanced disease still die prematurely. Salvage therapy for those patients who fail initial induction or relapse within one year of intial treatment continues to be suboptimal. The current NCI trial for advanced disease patients (Stages IIIA, IIIB and IV) is aimed at testing the Goldie-Coldman hypothesis that early exposure of the tumor to two combinations of non-cross resistant drugs is more likely to result in cure than conventional cyclic four-drug treatment. The study compares MOPP to MOPP alternating with CABS (CCNU, Adriamycin, Bleomycin, Streptozotocin) chemotherapy. Seventy-nine patients have been randomized and complete remission rates are similar for both regimens at this point with median survivals in both groups exceeding 80% at 4 yrs for either treatment.

17 CURRENT STATUS OF STANFORD HODGKIN'S DISEASE TRIALS.

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Randomized clinical trials designed to evaluate various treatment programs for patients with Hodgkin's disease were initiated at Stanford University in 1962. These continuous studies involving 838 patients, as of March 1, 1984, have undergone four major revisions during the past two decades.

Between 1962-1967, 132 patients with CSI, II and III were enrolled on various radiation trials. Patients with CSIII disease were treated for the first time with total lymphoid irradiation (TLI) with approximately 40 % of these patients remaining continuously free of disease. Between 1968-1974, 367 patients were enrolled on studies primarily evaluating the role of adjuvant MOPP chemotherapy. Laparotomy and splenectomy was used routinely and patients with all stages of the disease were included. Adjuvant MOPP resulted in significant improvement in disease free survival for some stages of the disease, but the survival advantage was minimal, except for patients with PSIIIA disease. Between 1974-1980, 237 patients were enrolled on studies evaluating an alternative to the MOPP adjuvant, PAVe chemotherapy, and variations of the combined modality treatment plans. PAVe has proved to be as effective as MOPP in these studies without producing acute leukemia to date. An alternating treatment plan, beginning with chemotherapy, appears superior to previous programs, for patients with advanced disease (IIIB and IV).

Current studies, initiated in 1980, have enrolled 102 patients to date. A relatively mild adjuvant, VBM (vinblastine, bleomycin and methotrexate) is being studied for favorable patients, staged with laparotomy. The ABVD regimen is being evaluated in combined modality and alternating chemotherapy regimens. The major emphasis of current protocols is to reduce acute and long term morbidities, without compromising excellent survival results and to further improve the results in patients with the poorest prognoses.

The rationale, design and results of these trials will be presented.

18

ALTERNATING CHEMOTHERAPY WITH MOPP/ABVD IN HODGKIN'S DISEASE: UPDATED RESULTS. G. Bonadonna. Istituto Nazionale Tumori, Milano 20133, Italy.

From 1974 to 1982, 88 patients with PS IV were randomized to receive either MOPP for 12 cycles or MOPP/ABVD for 12 cycles. Details on this study were previously reported (New Engl. J. Med. 306: 770, 1982). The updated 7-year results with a median follow up in excess of 60 mos are as follows:

	MOPP (%)	MOPP/ABVD (%)	P
Progression	20.9	4.4	<0.05
Freedom from progression	35.1	68.1	0.001
Complete response	74.4	88.9	0.14
Bulky disease	57.1	88.9	0.10
Non bulky	82.8	88.9	0.78
"A" symptoms	76.9	85.7	0.92
"B" symptoms	73.3	90.3	0.16
NS histology	76.0	87.5	0.50
Others	72.2	90.5	0.20
≤ 40 yrs	78.6	82.8	0.94
> 40 yrs	66.7	100	0.04
Relapse-free survival	44.4	76.8	0.004
Bulky disease	37.5	75.7	0.04
Non bulky	48.7	76.6	0.04
"A" symptoms	50.0	65.5	0.36
"B" symptoms	44.5	79.5	0.006
NS histology	55.9	88.9	0.01
Others	30.8	59.9	0.10
≤ 40 yrs	43.5	75.9	0.004
> 40 yrs	51.5	70.2	0.50
Survival of CR_s	73.2	90.3	0.038
Overall survival	61.1	82.3	0.043

MOPP/ABVD was superior in all proghostic subgroups and less myelotoxic than MOPP. Acute leukemia was observed in 1 patient in each treatment group. Both patients had received chemotherapy after relapse from prior RT. In August 1982, a new randomized study was started in PS IIA (bulky), IIB, III and IV comparing MOPP/ABVD (MM/AA) vs half cycle of either regimen within a month period (MA/MA). Low dose RT (2500 rads) was limited to the site(s) of previous bulky disease. With a minimum follow up of 6 mos, the preliminary results are as follows:

	MM/AA	MA/MA
Complete response	88.9% (32/36)	92.5% (37/40)
Still in complete remission	84.4% (27/32)	91.9% (34/37)

Present data confirm the favorable impact of alternating chemotherapy vs MOPP alone. However, the optimal sequence remains to be clarified by the ongoing study.

19

COMBINED MODALITY THERAPY FOR HODGKIN'S DISEASE.
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The integration of intensive multi-agent chemotherapy and radiotherapy for the management of Hodgkins disease is predicated on a major appeal, i.e., patients who failed chemotherapy are most likely to fail in sites of overt involvement, whereas patients who fail radiotherapy are most likely to fail outside the field of radiation. When these modalities are put together for the management of Hodgkins disease, it is unclear how much of either is truly required, in contrast to the use of either modality alone. Optimal timing in integrating these two modalities also remains to be defined. In addition, the actual technique of treatment remains very important.

This presentation will review the results of combined modality approaches published in various medical centers with special emphasis on the long term outcome. In addition, it will review the available information on secondary oncogenesis. At the present time, the results of combined modality therapy in terms of sterilizing Hodgkins disease appear to be excellent. However, the long range toxicity is such that its use should probably be restricted to those patients who have an expected cure rate less than 65 % with either modality alone. This arbitrary figure reflects a) the detectable risk of leukemia related to treatment with the combined modality approach and b) the "salvage" achieved with combination chemotherapy in patients who relapse following radiotherapy.

ABSTRACTS - Second International Conference on Malignant Lymphoma, Lugano

20 CURRENT STATUS OF THE CURABILITY OF CHILDREN WITH HODGKIN'S DISEASE (HD): AN ASSESSMENT OF THE RISK:BENEFIT RATIO OF MODERN THERAPY. S.B. Murphy, St. Jude Children's Research Hospital, Memphis, TN USA.

Major improvement in the disease-free survival (DFS) rates of children with HD has occurred in the last 10-15 years. Because of appropriate concern over growth disturbances in irradiated areas, treatment policies at most large centers and cooperative groups treating children have shown a trend away from high-dose, large volume radiotherapy. In view of the effectiveness of chemotherapy in controlling advanced stages of HD, combined modality approaches, incorporating four-drug chemotherapy combinations (e.g., MOPP, CVPP, COPP, OPPA, ABVD) plus low-dose (2000-2500 rads) involved or extended field irradiation, have now become the standard treatment policy for most children (except favorable CS or PSIA). As a result of these trends, reported overall 5-year-DFS rates for children with HD are currently 85-90%. The implications of these data are profound. Since 9 out of 10 children with HD carefully staged and treated with a modern approach will become long-term survivors, there is an imperative need to better define curative therapy associated with minimal acute and long-term morbidity (infectious deaths in remission, sterility, hypothyroidism, growth failure, non-lymphocytic leukemias and other second malignant neoplasms). Further improvements in the therapeutic index of combined modality approaches for children with HD will require large, well-planned and controlled clinical trials,incorporating pretreatment stratification according to a precise estimation of the relapse hazard, testing further reductions in the dose and volume of radiotherapy (? elimination altogether) and further improvements in drug doses, scheduling, and combinations, coupled with extended follow-up, to include long-term observations on the quality of life of survivors. Due to the relative rarity of pediatric cases of HD, such studies obviously require coordination of national effort, and will take years, even decades, to complete. It is likely that there will be no threshold below which therapy can be reduced to achieve complete freedom from side effects while maintaining high rates of curability (90%). Recognizing this reality, the primary therapy for each child with HD must currently be based on curative intent, eschewing any reliance on later salvage approaches.

21 LATE EFFECTS OF CHEMOTHERAPY IN HODGKIN'S DISEASE

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It has now been clearly demonstrated that approximately fifty per cent of patients treated for advanced Hodgkin's Disease with combination chemotherapy will be free of recurrence ten years later. There are two major organic long-term side effects of the treatment most frequently used (cyclical therapy with Mustine, Vinblastine, Prednisolone and Procarbazine or variants), infertility and the development of second malignancy. Azoospermia is invariable following the first two cycles of therapy and recovery is very rare, although has been observed. A premature menopause occurs in more than half the women treated, increasing in frequency with age. The gonadal damage in both men and women has been shown to be due to 'End organ failure'. Second malignancy has been reported as occurring with a considerably greater frequency than is expected in the normal population, with acute myelogenous leukaemia being the commonest.

22 SECONDARY ACUTE NONLYMPHOCYTIC LEUKEMIA (ANLL) AND DYSMYELOPOIETIC SYNDROME (DMPS): A MODEL OF LEUKEMOGENESIS WITH IMPLICATIONS IN THE PRIMARY TREATMENT OF LYMPHOMAS.

K.S. Albain, M. M. Le Beau, J. W. Vardiman, J. D. Rowley, H.M. Golomb, and J. E. Ultmann. University of Chicago, IL.

Patients (pts) receiving chemotherapy (CT), radiotherapy (RT), or both are at an increased risk for developing DMPS or ANLL, either of which is associated with consistent chromosome abnormalities (chr abn) of No. 5 and/or 7. We describe clinical and cytogenetic analyses of 63 pts with previously treated diseases: 23, Hodgkin's disease; 10, non-Hodgkin's lymphoma; 6, other lymphoproliferative; 21, solid tumors; and 3, renal transplant. The median age was 55 years. Eleven pts had RT only, all but 2 with pelvic ports; 21 had CT only, with either prolonged alkylating agent therapy (alk ag tx) or methyl CCNU. The other 31 pts had both CT and RT, usually with standard doses. Nineteen pts developed DMPS; 15, ANLL; and 29, DMPS followed by ANLL. The median time to bone marrow dysfunction (BMD) was 56 mo, with no difference by type of primary tx. Mean time from DMPS to ANLL was 5 mo. Clonal chr abn were identified in 61/63 pts (97%), compared to 75/140 pts (54%) with ANLL de novo. Abn of No. 5 and/or 7 were seen in 55/61 (90%) secondary DMPS/ANLL pts, a higher frequency than in ANLL de novo (35%). Only 1 pt had 5q- as the only abn. Deletion analyses of 5 and 7 showed that (7)(q34→35) and (5)(q31→32) were consistently lost. The oncogene c-fms has been localized to 5q34. Chr abn were unrelated to age, sex, initial disease, or mode of primary tx. However, abn of 5 or 7 were associated with DMPS whether or not followed by ANLL, rather than with the occurrence of ANLL without DMPS (45/48 or 94% DMPS ± ANLL v. 10/15 or 67% ANLL; p=.02). Median survival from BMD was 7.8 mo and was unrelated to initial disease, treatment, or chr abn. Most pts with DMPS died of infectious or hemorrhagic complications. Eleven pts with ANLL died shortly after diagnosis without therapy. Of the 29 pts who received a standard Ara-C-based induction regimen, only 6 pts achieved a CR (4/6 did not have prior DMPS; none had loss of No. 5); 4 are alive in remission at 6-43 mo follow-up. Two pts received low dose subcutaneous Ara-C and 2 pts, retinoic acid, during the DMPS phase without response. There were no CRs in 4 pts treated with high dose Ara-C. From this analysis we suggest the following conclusions: 1) pelvic portals are causatively implicated in the pts who only had primary RT; 2) all but 1 CT only pt had high dose alk ag tx or MeCCNU given over a prolonged period; 3) the presence of DMPS, not ANLL, was most strongly associated with abn of 5 or 7; 4) the critical region of 5 and 7 were defined for the first time in this group of pts; 5) the response rate to modern induction regimens was lower than in pts with de novo ANLL, even with high dose Ara-C, in our group of pts with mostly complex abn of 5 and 7. The implications of this data for designing future up-front lymphoma treatment programs will be discussed.

23 SECOND CANCERS AFTER TREATMENT IN TWO SUCCESSIVE COHORTS OF PATIENTS WITH EARLY STAGES OF HODGKIN'S DISEASE

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Two successive cohorts of patients with HD clinical stages I-II (H1 trial : 1964-71, 334 pts ; H2 trial : 1972-76, 300 pts) were prospectively followed. Thirty-four second cancers (SC) were registered, 21 in the H1 trial and 13 in the H2 trial, including 6 leukemias (4+2), 4 non-Hodgkin lymphomas (3+1) and 24 solid tumors (14+10). Initial treatments were : a) in the H1 trial : regional radiotherapy (RT) at 40 Gy with or without vinblastine (VLB) for two years ; b) in the H2 trial : regional RT at 40 Gy plus paraaortic and spleen RT at 40 Gy or the same RT after laparotomy+splenectomy. Moreover, patients with mixed cellularity or lymphocytic depletion histological types were randomised between VLB or a combination of VLB + procarbazine (PCZ) for two years. For the present study, three treatment groups were distinguished : patients without relapse (No Rel.), relapsing patients treated without combination chemotherapy (No poly-CT) and those treated by a combination chemotherapy (Poly-CT) for relapse. In both trials, in the "No Rel." groups, the occurence of SC did not differ regardless of whether the patients received CT or not, and, in the H2 trial, whether or not they received VLB or VLB+PCZ. A time-dependant covariate analysis was used to assess the contribution of each type of therapy on occurence of SC. Time lapse to SC ranged from 2 to 16 years (H1) and from 3 to 9 years (H2) after initial treatment, and from 0 to 12 years (H1) and from 2 to 6 years (H2) after retreatment for relapse. The relative risk (RR) of leukemia in the "Poly-CT" groups was 300 in the H1 trial ($p<0.001$) relative to the general population incidence rates and 200 in the H2 trial ($p<0.001$) while it was not significantly increased in the "No Rel." groups. In the H1 trial, RR of solid tumors was 26 ($p<0.001$) in the "Poly-CT" group, 3.67 ($p=0.027$) in the "No Poly-CT" group and not significantly increased in the "No Rel." group. RR of secondary solid tumors in the H2 "No Rel." group, was 3.14 ($p<0.001$). Comparison of occurence of solid tumors between the two H1 and H2 trials showed, in the "No Rel." group, that the difference observed was due to a shorter delay between initial treatment and secondary solid tumor in the H2 trial. At 7 years, the cumulative proportion of all SC in the "No Rel. group was less than 1% in the H1 trial, while it was greater than 3% in the H2 trial ($p=0.016$). In the H2 trial, extensive RT to paraaortic and spleen regions may be responsible for the excess of solid tumors (bowel and kidney) observed. The most important factors for developping an SC were combination chemotherapy and age over 40 years. The data suggest that combination chemotherapy may be responsible for leukemias in the two cohorts, and for other second tumors only in the H1 trial.

24 LACK OF CORRELATION OF ANN ARBOR CLINICAL PARAMETERS AND BIOPSY DETERMINATION OF LIVER INVOLVEMENT IN STAGE III AND IV HODGKIN'S DISEASE: A SOUTHWEST ONCOLOGY GROUP (SWOG) STUDY.

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We reviewed the clinical and pathological staging of 273 eligible patients (pts) with pathologic stage III or IV Hodgkin's disease receiving induction chemotherapy +/- involved field radiotherapy consolidation as part of SWOG 7808. Pre-study forms plus the actual pathology and radiology reports were reviewed. Of 197 pts that had liver biopsies (BX), 24% (47) were positive (pos) by percutaneous (percut) BX or by BX obtained at laparotomy (lap), of those 150 determined negative (neg), 37% (55) had only a percut BX while 63% (95) had a liver BX at lap +/- a percut BX. The Ann Arbor (AA) clinical criteria for liver involvement, i.e. 1) hepatomegaly + ↑ alk phos; and/or 2) ↑ 2 different liver function tests (SGOT and alk phos); and/or 3) abnormal liver scan and 1 abnormal liver function test, were correlated with BX results.

45% (21/47) patients with a pos liver BX had negative AA clinical criteria for liver involvement. Conversely 20% of pts with lap neg liver BX had pos AA criteria for liver involvement. 21% (10/47) pts with a pos liver BX were clinical stage IIIA or less, whereas 11% (7/66) of all pts stage IIIA or less eligible for the study had a pos percut or lap liver BX. None of the pts with clinical stage IIIA or less rendered pathologic stage IV by liver BX had pos bone marrows or any other pathologic evidence of stage IV disease. 64% (26/41) of pts with a pos liver BX had a neg liver scan. 80% (16/20) of pts with a pos liver BX had a neg CT of the liver. 26 pts had a lap liver BX following a neg percut BX. The false neg rate was 31%.

155/197 pts were response evaluable (32 too early, 10 not evaluable). The CR rate for 23 pts who were AA clinical criteria neg but liver BX pos (AA-BX+) was not different from the 95 pts who were criteria and BX neg (AA^-BX^-) (65 vs 70%). The CR rates were similar despite the fact that only 15% of the AA^-BX^- group as opposed to 100% of the AA^-BX^+ group were pathologic stage IV.

We conclude that (1) AA clinical criteria are not useful in predicting liver involvement; (2) percut liver BX is not useful when neg; (3) pts with clinical stage IIIA or less should have an open liver BX before receiving non-systemic therapy; and (4) pathologic liver involvement without clinical signs of liver involvement is not a poor prognostic variable.

25 LONG TERM FOLLOW-UP OF PATIENTS WITH CLINICAL STAGES I.II HODGKIN'S DISEASE : COMPARISON OF INITIAL SPLENECTOMY AND SPLEEN IRRADIATION.

A controlled clinical trial (H_2 trial) was carried out in patients with clinical stages I and II Hodgkin's disease (H.D.) by the EORTC Radiotherapy-Chemotherapy group from 1972 to 1976. The aim of this H_2 trial was (a) to compare the efficacy of spleen irradiation and of splenectomy; (b) to assess the prognostic significance of the information provided by laparotomy. For this purpose, all patients with pathological stages (PS) I, II and III received the same radiotherapy (mantle field and para-aortic lymph nodes). (c) to compare for these patients with poor histological subtypes [mixed cellularity, (MC) and lymphoid depletion, (LD)] two schedules of long term chemotherapy : either Vinblastine (VLB) alone or VLB + Procarbazine (PCZ). The results of the trial including 300 patients with at least 8 years follow-up for the last patients randomised are presented.

Fourty-eight patients (30%) relapsed out of 156 patients registered in the group treated by splenic irradiation, and 35 patients (24%) relapsed out of 144 in the group treated by splenectomy. The difference is not statistically significant. The overal survival at 10 years is 84% and there is no difference in the two groups. It is noticeable that among the 53 deaths observed only 26 (49%) were due to H.D. The main other causes of death were sequellae of treatment (5 cases), intercurrent disease (12 cases) and secondary cancer or leukemia (6 cases). Non-lethal complications of the treatment were mainly complications of the digestive tract. In the group treated by laparotomy and radiotherapy (144 patients) we observed 25 digestive complications (9 small bowel obstructions, 16 ulcers) i.e. 17% versus only 4 ulcers in the group of 156 patients treated by radiotherapy alone (2,5%). The difference is highly significant ($p < 0.001$).

The patients who received chemotherapy had a significant higher disease free survival (DFS) even after adjustment for initial treatment : splenectomy or spleen irradiation.

A multivariate analysis was performed to study the prognostic factors and showed that high-risk parameters are : spleen involvement at laparotomy; erythrocyte sedimentation rate (ESR) higher than 50 mm; presence of systemic symptoms, bulky mediastinal involvement and a higher number of lymph nodes areas involved (more than 2). The delineation of clinical stages I and II H.D. between "high risk" and "low risk" group is now possible without laparotomy. For the former group, chemotherapy is the main treatment while a lighter treatment with radiotherapy alone may be adequate in "low risk patients".

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26 STAGING LAPAROTOMY WITH SPLENECTOMY IN STAGE I AND II HODGKIN'S DISEASE. NO THERAPEUTIC BENEFIT.

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In a prospective randomized study of treatment for early stage Hodgkin's disease, of 104 patients with presentation above the diaphragm, 76 patients had staging by exploratory laparotomy with splenectomy and 28 had staging by closed techniques. Treatment consisted of involved field radiation alone (44 patients), involved field radiation followed by chemotherapy (38 patients), total nodal radiation alone (15 patients) and total nodal radiation followed by chemotherapy (7 patients). Both groups had similar clinical features on presentation, and both had similar treatment distribution.

With similar median followup (87 months) a trend for longer remission and survival was observed in the group of patients staged by closed staging (68% and 92% respectively) compared to the group staged by exploratory laparotomy with splenectomy (59% and 74% respectively). These differences however were not statistically significant (p 0.27 and 0.09 respectively). There were more patients presenting multiple areas of relapse among patient staged by exploratory laparotomy with splenectomy compared to the group staged by closed techniques (11/32 relapses vs. 0/9 relapses, respectively p 0.082). Relapse in the abdomen alone or as part of disseminated relapse was observed in 12% (9 patients) in the group of patients who had staging by exploratory laparotomy as compared to 3% (1 patient) in the group staged by closed techniques (p 0.28). Two patients (7%) staged by closed techniques died with Hodgkin's disease. Thirteen patients (17%) staged by laparotomy died: 7 of Hodgkin's disease, the other 6 died in complete remission of: non-Hodgkin's lymphoma (1 patient), leukoencephalopathy (1 patient), sepsis during chemotherapy (2 patients), myocardial infarction (1 patient) and cerebrovascular accident (1 patient). Three other patients in this Group had other secondary malignancies successfully controlled: histiocytic lymphoma, squamous cell carcinoma of cervix and malignant schwanoma. No secondary malignancies were observed in the group staged by closed techniques.

Staging laparotomy with splenectomy in early stage Hodgkin's disease did not improve the duration of remission or survival or decrease the number of relapses in the abdomen as compared to closed staging.

27 SURGICAL RESTAGING AFTER 3 OR 6 COURSES OF MOPP CHEMOTHERAPY IN HODGKIN'S DISEASE (HD). UPDATED RESULTS.

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121 patients (pts) with HD, clinical staged, were treated by two different, successive protocols. 68 pts, I B 7 pts, II A 17 pts, II B 27 pts, III 17 pts, were treated from 4.72 to 12.76. 66 pts underwent surgical restaging (SR) with splenectomy after 6 courses of MOPP and Vinblastine monthly x 4. 53 pts, I B 1 pt, II A 10 pts, II B 26 pts, III 16 pts, were treated from 3.77 to 9.79. 52 pts underwent SR after 3 courses of MOPP. All pts received mantle irradiation (RT). CS III and CS I-II with persistent disease at SR received additional inverted Y or para aortic field irradiation. The clinical complete remission rate (CR), partial response (PR) and failure was respectively 80 %, 14 %, 6 % after 6 MOPP and 83 %, 15 %, 2 % after 3 MOPP. Upon SR, 83 % of pts (55/66) after 6 MOPP, 92 % of pts (48/52) after 3 MOPP, were free of residual disease. Persistant splenic disease at laparotomy in pts clinically restaged as CR was not significantly different after 6 MOPP (2/54) and after 3 MOPP (1/44). The incidence of false negative clinical restaging (clinical CR with pathologic SR) was respectively 3.5 % and 2 %. CR rate was 94 % (64/68) after 6 MOPP and RT, 96 % (51/53) after 3 MOPP and RT. The actuarial survival at 66 months is respectively 94 % and 86 % after 6 or 3 courses of MOPP followed by RT. The relapse free survival at 66 months is respectively 96.6 % and 88 %. We conclude that 3 courses of MOPP are as efficient as 6 to treate splenic occult disease in CS II A, I B, II B with supradiaphragmatic presentation and to achieved CR when combined with RT.

28 THE MANAGEMENT OF LOCALISED, INFRADIAPHRAGMATIC HODGKIN'S DISEASE: EXPERIENCE OF A RARE CLINICAL PRESENTATION AT ST. BARTHOLOMEW'S HOSPITAL.

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Between 1969 and 1982, 23 previously untreated patients with Hodgkin's disease (HD) confined to infradiaphragmatic sites were treated at St. Bartholomew's Hospital. The distinguishing clinical characteristics of the patient population were a male:female ratio of 20:3 and a mean age of 39 years which was significantly (p 0.05) older than the mean age, 32 years, of patients with supradiaphragmatic HD, referred during the same time period. Sixteen patients were surgically staged and the final pretreatment stages were PS +A: 5; PS IIA: 11; CS IIA: 1; PS IIB: 1; CS IIB: 5. Splenic involvement correlated closely with the number of lymph node sites involved, being detected in 8/9 (89%) CS IIA and 1/7 (14%) CS IA patients (p 0.001).

Complete remission (CR) was achieved in 21 (91%) patients: 13/13 following 'inverted Y' radiotherapy and 8/10 following combination chemotherapy. Twenty patients remain alive and 18 continue without recurrence of HD between 15 months and 12 years. All patients who failed to enter CR or who relapsed had presented with 3 or more sites of involvement or with constitutional ('B') symptoms. These results confirm the generally good prognosis of this uncommon presentation of HD and also suggest that prognosis is determined by the bulk of disease rather than its precise anatomical localisation, provided that appropriate therapy is administered.

29 STRATEGIES FOR MANAGEMENT OF CHILDHOOD NON-HODGKIN'S LYMPHOMAS (NHL) BASED UPON STAGE AND IMMUNOPATHOLOGIC SUBTYPE: RATIONALE AND CURRENT RESULTS.

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Childhood NHL is heterogenous in its clinical presentation and relapse hazard with modern therapy. It follows that not all children are equally benefited by a uniform treatment policy. Instead, alternative treatment strategies are appropriate for subgroups of patients, based upon pretreatment staging and determination of immunopathologic subtype. Using a simple clinical staging system, we and others have shown that children with Stages I and II, localized disease, regardless of histopathologic subtype, have an excellent prognosis (90% curability) when treated with combined modalities. Consequently, with the objective of reducing acute toxicity and adverse long-term consequences of treatment, current trials aimed at lessening the intensity of therapy for children with Stage I or II disease are underway in many centers and cooperative groups. Optimal management of advanced stages, III and IV, requires categorical separation of lymphoblastic (T) from non-lymphoblastic (B) cases. Lymphoblastic lymphomas, primarily mediastinal, should be treated like high-risk acute lymphoblastic leukemia, with multiple drugs and CNS prophylaxis, without mediastinal radiation. Using such a strategy, the 3-year disease-free survival rate of advanced stages of lymphoblastic lymphomas in our experience and others is approximately 75%. Advanced stages III and IV of Burkitt-type NHL have been treated successfully with intensive combination chemotherapy protocols of short duration (6 months) incorporating high doses of cyclophosphamide, high dose methotrexate, cytosine arabinoside, and other agents in combination with intrathecal prophylaxis. Using such a strategy, several groups (SJCRH Total Therapy 'B', SFOP LMB-01-02, and BFM 81-83) have reported major improvements (to 65-75%) in the curability of Stage III-IV Burkitt-type NHL, though Burkitt's lymphoma in a leukemic phase (B-ALL) remains grave. 5-10% of all cases of childhood NHL are either nodular, mixed, pleomorphic peripheral T-cell type, mediastinal non-lymphoblastic, or essentially unclassifiable and require an individualized approach to management. In summary, using a modern strategy for treatment, the majority of children with NHL are curable.

30 RESULTS OF THE BFM THERAPY FOR CHILDHOOD NON-HODGKIN'S LYMPHOMA. IMPROVEMENT OF PROGNOSIS BY ADAPTION OF CHEMOTHERAPY TO STAGE AND TYPE.

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Treatment of childhood NHL has been increasingly aggressive, but mostly did not take into account the heterogeneity of these diseases. In the BFM study 1975/81, childhood NHL was treated uniformly with a chemotherapeutic regimen, which has been proven to be very effective in childhood ALL. Results depended primarily on clinical stage and histologic/immunologic type. In disseminated disease (stage III and IV according to Murphy), the probability of continuous complete remission (CCR) after 8 years is excellent for Non-B-NHL (78%, n=42), but poor for B-NHL (34%, n=29). B-NHLs relapsed exclusively within 7 months after diagnosis.

Therefore, in 1981 a new therapeutic regimen was developed for B-NHL and B-ALL, while the therapy of Non-B-NHL underwent no maior changes. Two slightly different, alternating chemotherapy blocs were given in B-Neoplasias: Bloc 1: Cyclophosphamide 200 mg/m2 daily day 1-5; Methotrexate 500 mg/m2 with leucovorin rescue day 1; i.th. Methotrexate day 1; VM 26 165 mg/m2 and ARA-C 300 mg/m2 day 5. Bloc 2: As bloc 1, but ADR 50 mg/m2 instead of VM 26/ARA-C day 5. Patients with stage I and resectable stage II B-NHL (stage "II-R", mostly intraabdominal) received 4 of these blocs within 8 weeks, disseminated and not resectable intraabdominal B-NHL (stage "II-NR") 8 blocs within approximately 20 weeks. No continuation therapy was given. Second look laparatomy was performed in most patients with initially non resectable intraabdominal B-NHL. Prophylactic cranial irradiation was given to patients with disseminated B- or Non-B-NHL only.

3 years after initiation of this stage- and type-adapted regimen, the probabilities of CCR are: All NHL-patients (n=99): 80%; Non-B-NHL, stage I/II (n=9): 89%; Non-B-NHL, stage III/IV (n=33): 79%; B-NHL, stage I/II-R (n=19):100%; B-NHL, stage II-NR/IV (n=37): 67%. B-ALL (n=22): 49%. In conclusion, our results indicate, that treatment of B-NHL and B-ALL has to be different from treatment of Non-B-Neoplasias. The prognosis of B-NHL and even B-ALL has been greatly improved by the BFM therapy designed for more specific treatment of these diseases.

31 IMPROVEMENT OF SURVIVAL OF STAGE IV B-CELL NON HODGKIN LYMPHOMA (NHL) AND B ACUTE LEUKEMIA (B-ALL). A STUDY OF THE FRENCH PEDIATRIC ONCOLOGY SOCIETY (SFOP)

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Advanced stage (st.) diffuse B-cell NHL and moreover B-ALL are known to be of very bad prognosis. For these (extended head and neck st. II, all st. III, IV and B-ALL), a new protocol, called LMB, was designed in February 1981. The protocol was as follows : A. Induction with : 1) "COP-COPAD-M" course : CPM : 0,5 g/m2 Day (D) 0 and 1 g/m2 D6, 7, 8 ; VCR : 2 mg/m2 D0, 5, 10 ; PRED : 2 mg/kg/d from D0 to D10 ; ADR : 60 mg/m2 D6 ; HD-MTX : 3 g/m2 D5 ; IT-MTX : 15 mg/m2 D0, 5, 10. 2) "COPAD-M" course as before from D5 to D10. 3) "CAMAD" course : ARA-C : 100 mg/m2/d in continuous infusion D1 to D5 ; L-ASP : 1000 U/kg D2 to D6 ; HD-MTX : 3 g/m2 D0 ; ADR : 45 mg/m2 D5 and 6 ; IT-MTX : 15 mg/m2 D1 ; IT-ARA-C : 30 mg/m2 D6. 4) "MINI-BACT" course : BCNU : 60 mg/m2 D0 ; ARA-C : 100 mg/m2 D0 to 4 ; CPM : 0,5 g/m2 D1, 2, 3 and 6-TG : 150 mg/m2 D0 to 4. B. No radiotherapy at all. C. Maintenance made of two monthly alternative courses : 1) HD-MTX : 3 g/m2 D0 ; CPM : 0,5 g/m2 D0, 1 ; ADR : 60 mg/m2 D1 ; VCR : 2 mg/m2 D2 ; PRED : 2 mg/kg D0 to 4 and IT-MTX : 15 mg/m2 D1. 2) BCNU : 60 mg/m2 D0 ; ARA-C : 100 mg/m2 D0 to 4 ; IT-ARA-C : 30 mg/m2 ; L-ASP : 1000 U/kg D1 to 4 ; 6-TG : 150 mg/m2 D0 to 3. The treatment was to be completed within one year.

Due to toxicity, this protocol was modified after the first 32 patients in November 1981 : CPM was diminished and delayed in COP-COPAD-M : 0,3 g/m2 D0 and 0,5 g/m2 D8, 9, 10. ADR was removed from CAMAD.

From February 1981 to October 1983, 123 patients from 17 centers in France have been included in a non randomised study. According to Murphy's staging (st. IV being defined by less than 25 % blast cells in bone marrow), there were : 10 st. II, 79 st. III, 13 st. IV (6 CNS involvement) and 20 B-ALL (8 had CNS involvement and 10 blast cells in blood). Actuarial survival for all patients is 74 %, 100 % for st. II, 78 % for st. III, 54 % for st. IV, 53 % for B-ALL.

Thus, according to these criteria, there is no difference in survival between st. IV and B-ALL. In fact, with this treatment, the critical prognostic factor is the initial CNS involvement in both st. IV and B-ALL. The actuarial survival is 80 % for the 17 st. IV and B-ALL patients without CNS involvement and 30 % for the 16 with CNS involvement.

In conclusion, this protocol has considerably improved survival for st. IV NHL and B-ALL. For a next study, we consider to diminish lenght of protocol for all patients without CNS involvement and to intensify it for patients with CNS involvement.

32 THERAPY OF UNDIFFERENTIATED (INCLUDING BURKITT'S) AND LYMPHOBLASTIC LYMPHOMAS IN CHILDREN AND YOUNG ADULTS.

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Seventy five patients with diffuse non-Hodgkin's lymphomas, aged 2-35 years have been treated according to an intensive protocol in which a 42 hour infusion of methotrexate (total 2.7 gm/m^2) with leukovorin rescue is administered 10 days (first 6 cycles) or 14 days (last 9 cycles) after a combination of cyclophosphamide (1.2 g/m^2, given alone in the first cycle), vincristine (1.4 mg/m^2), adriamycin (40 mg/m^2) and prednisone (40 mg/m^2 daily x 5). Intensive intrathecal therapy is given as CNS prophylaxis. Radiation is used only in exceptional circumstances (e.g., paraplegia). Approximately 70% of patients had advanced stage disease. All patients have been followed for at least one year. Overall complete response rate was approximately 90% and continuous disease-free survival (DFS) for all patients was approximately 60% at 3 years. Patients with extensive bone marrow involvement, regardless of histology, had the worst prognosis (less than 20% DFS at 3 years), and patients with lymphoblastic lymphoma (without marrow involvement) or completely resected abdominal undifferentiated lymphoma had the best prognosis (over 85% DFS at 3 years). The latter patients are treated with only 6 cycles of therapy. Patients without bone marrow involvement had a DFS of approximately 70% at 3 years. Patients classified as Murphy stage III had a DFS of over 60% at 3 years. No differences in prognosis were observed in patients less than 16 yrs or greater than 17 yrs, regardless of histology, but all partial responders were 16 or more years old. The best predictor of response was tumor burden, as measured by clinical stage, or biochemical parameters such as serum LDH and uric acid levels. Relapse in the CNS was the commonest site of recurrence in children, but 4 such relapses occurred prior to the introduction of intrathecal prophylaxis, which appears to be effective. In adults, the commonest site of relapse was the bone marrow. These results are of interest since a) this protocol is effective in both lymphoblastic and undifferentiated lymphomas in the absence of bone marrow involvement; b) radiation is not used; and c) patients with completely resected disease do well with only 6 cycles of therapy.

33 EVOLUTION OF THE THERAPEUTIC APPROACHES OF NON-HODGKIN'S LYMPHOMA OF CHILDHOOD - Cl. JACQUILLAT, D. KHAYAT, M. WEIL

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During the past decades, a huge improvement has been made concerning the understanding and the prognosis of pediatric (ped.) non-hodgkin's lymphoma (NHL). Until the late sixties, the NHL of childhood were considered as non different from the adults NHL and were treated very slightly, mainly with radiotherapy and/or surgery. The results obtained with those historical controls were of about 10 % (extended forms) to 40 % (localized forms) long term survival. The bone marrow involvement led to classify some NHL as leukemias and therefore were not included in thoses series. During the seventies, a better understanding of the disease led to separate the ped. NHL from the adult NHL. Some features appeared very characteristic of the ped. NHL : 1°) A very rapid and agressive clinical course which has to be opposed by a vigourous induction treatment ; 2°) A high incidence of central nervous system (CNS) involvement justifying a systematic CNS prophylaxis ;- 3°) The better accuracy of the Murphy's staging (st) than the Ann Arbor st system. During this period, all of us pointed out the great value of the histological features of the ped. NHL : 1°) being almost always of the diffuse histologic pattern ;2°) with about 40 % of them originated from B-lymphocytes (most of them classified as Burkitt type) with proeminant abdominal symptomatology ;3°) and most of the rest with T-lymphocytes lineage features (lymphoblastic) explaining the occurence of mediastinal extension in about one third of the children. The therapeutic approach became therefore more specific with immediate and heavy treatment, systematic CNS prophylaxis, giving 85 % overall long-term survival in localized form and about 50 % in non-localized NHL, using chemotherapeutic regimens such as "LSA_2-L_2" and "COMP". This study of Wollner and others studies pointed out the different therapeutic approaches that should be done according to the B or T-lymphocyte origine of the proliferation, on which are based now most of the up to date treatments : non localized lymphoblastic type NHL should be treated agressively, almost like acute leukemias with regimens which include Cytosine Arabinoside and Asparaginase, such as the "VIRCALL" or the "LSA_2-L_2",. The results of such treatments in this indication reach probably now a better than 75 % cure rate. The treatment of non-localized B-lymphocyte type NHL can be slightly less agressive with regimens such as "COMP" which should include Methotrexate and Cyclophosphamide, those two drugs highly improving the results in non-differentiated or Burkitt NHL with about 60 % long-term survival. Except perhaps for some very localized non-lymphoblastic forms, CNS prophylaxis is always imperative but skull irradiation may not be mandatory.

34

CHILDHOOD HODGKIN'S DISEASE: TREATMENT WITH ABVD CHEMOTHERAPY AND LIMITED FIELD RADIOTHERAPY. F.Fossati-Bellani, R.Kenda, F.Lombardi, C.Gianni, M.Gasparini, P.Pizzetti, R.Musumeci, and G.Bonadonna. Istituto Nazionale Tumori, Milan 20133, Italy.

With the objective of reducing acute and late complications from surgical diagnostic procedures and from extensive radiation therapy (RT), since 1979 a new therapeutic approach has been devised and applied to children and adolescents with nodal extent of Hodgkin's disease admitted and treated at our Institute. Laparotomy with splenectomy was omitted, and staging procedures utilized lymphangiography, CA scan, bone marrow biopsy and laparoscopy to evaluate the extent of disease. The initial treatment for all patients, regardless of stage and histologic subtype, consisted of three monthly cycles of ABVD (ADM 25 mg/m^2, BLM 10 mg/m^2, VLB 6 mg/m^2, DTIC 375 mg/m^2). Subsequently, RT was delivered according to the type of clinical response induced by chemotherapy: 30-35 Gy to involved area(s) in complete and partial responders, respectively; 25 Gy to the adjacent area(s). Three additional courses of ABVD were then given only to children with stage IIB and IIIA and B. Thirty-four consecutive children (age 3.4 to 15.4 yrs, median 10 yrs) were staged as follows: IA 12, IIA 7, IIB 6, IIIB 2, IIIS A+B 7. According to histology patients were classified into the following subtypes: LP 2, MC 12, NS 20. Following initial ABVD, CR was achieved in 16 of 19 patients (84%) with stage I and IIA, and PR >50% in 3 patients. Stage IIB and IIIA and B attained CR in 6 of 15 (40%) and PR >50% in 9 cases. After a median follow-up of 30 months (range 15-51) 33 of 34 children remain alive and progression-free. Only one child with stage IIIB relapsed with disseminated disease two months after completion of therapy. Nausea and vomiting represented the only immediate toxic effect of this treatment program and were almost always severe. No patient showed either cardiac or respiratory abnormalities. In children followed for more than 2 years growth as well as endocrine and gonadal function were uneffected. Although this combined approach appears very effective in inducing high remission rates and durable CR, a longer follow-up is needed to establish the actual cure rate and treatment-related late sequelae.

35

COMBINED TREATMENT MODALITY WITH REDUCED CHEMO- AND RADIOTHERAPY IN HODGKIN'S DISEASE: RESULTS FROM 300 CHILDREN IN 2 CONSECUTIVE STUDIES

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Since 1978 two consecutive therapy studies for Hodgkin's disease in children are in progress in Germany and Austria. Between June 1978 and Nov 1981 170 protocol pts from 47 hospitals were enrolled in the 1st study HD-DAL 78 (age 1 - 16 yrs, median 12;2 yrs, male/female ratio 1.79). Laparotomy was done in 164 pts and splenectomy in 159 pts. Chemotherapy consisting of 2 OPPA cycles (Oncovine, Procarbazine, Prednisone, Adriamycine) were administered to all pts prior to irradiation. Additional 4 COPP cycles (C = Cyclophosphamide) were given after irradiation to stage IIB to IV pts. All involved fields (IF) were irradiated with 36 - 40 Gy. To the extended fields (EF) 36 - 40 Gy (group I) or 18 - 20 Gy (group II) were given in a randomised manner. Projected disease free survival rates after 5 yrs are 91 % in the total group and 89 % (I) and 94 % (II) in the 2 randomisation groups. Thus, radiation dose to EF can be reduced, if a combined treatment modality is used.

In Dec 1981 the non-randomised 2nd study HD-DAL 82 was started. Pts are stratified into 3 groups with different chemotherapy duration: stage I/IIA 2 x OPPA, stage IIB/IIIA 2 x OPPA plus 2 x COPP, stage IIIB/IV 2 x OPPA plus 4 x COPP. Irradiation is limited to IF, the dose depending on extent of chemotherapy (35, 30 or 25 Gy). Laparotomy is done in all children, but splenectomy is performed only in selected cases (approx. 36 %) following a new intraoperative strategy, which was developed on the basis of an analysis of 154 pts of the study HD-DAL 78 [Klin. Pädiat. 194 : 242 - 250 (1982)]. Until Dec 1983 130 protocol pts from 46 hospitals entered the study (age 3 - 6 yrs, median 12 yrs, male/female ratio 1.82). All pts achieved complete remission. Until now 1 child (stage $IV_{L,B}$) had a relapse in its lungs. 2 pts (stage IIIB) died of infections. Projected disease free survival rates after 2 yrs are 100 % for stage I/IIA (n=64) as well as for stage IIB/IIIA (n=36) and 86 % for stage IIIB/IV (n=30).

36 HODGKIN'S DISEASE (HD) IN CHILDHOOD : TREATMENT WITH CHEMOTHERAPY AND LOW-DOSE RADIATION. RATIONALE AND FEASIBILITY

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Dramatic improvement in survival and relapse free survival (RFS) has been obtained in childhood HD employing extended fields radiotherapy (RT) alone or in conjunction with multiagent chemotherapy (CT). Nevertheless it is everyone concern to minimize the side effects of such treatment while maintaining good RFS.

With this aim, 4000 rads radiation was first limited to involved fields (IF) and chemotherapy lowered to 3 cycles of MOPP as the first step. In 65 clinically staged patients (CS IA-CS IVB) the overall 5 years survival was 93 % and RFS was 83 %.

Based upon these data and those of other published series on low-dose radiation, the French Society of Pediatric Oncology associated with Hôpital Saint-Louis (Paris) started in 1982 a new study as a second step of the therapeutic decrease. It aims at answering to 2 questions : 1) effectiveness of ABVD alone compared to alternating MOPP-ABVD in remission induction ; 2) effectiveness of 2000 rads in IF when associated to CT. No staging laparotomy is performed. Treatment is based on CT followed by RT. Thus, according to the disease extent and systemic symptoms, 2 different schemes of CT and RT are used : 1) CSI-IIA : randomized CT : 4 ABVD vs alternating 2 MOPP-2 ABVD then RT 2000 rads to IF (stages I upper neck disease are excluded from randomization but given 4 ABVD before RT). 2) CSIB-IIB-III-IV : alternating 3 MOPP-3 ABVD then RT 2000 rads to IF and lomboaortic and splenic fields in all the cases.

The RT dose is not randomized but remission is evaluated at the end of CT. Patients (pts) who do not attain "good remission" (defined as complete remission or at least reduction of tumor $\geq$ 70 % at the end of CT) are given the previous dose of 4000 rads.

In December 1983, 50 pts have been included in the study : 28 CSI-IIA, 7 CSI-IIB, 8 CSIII, 7 CSIV. 37 pts completed CT : 29 pts achieved CR and 5 pts good remission, these pts were given 2000 rads. 1 patient had partial remission ($<$ 70%) after 4 ABVD but attained CR with MOPP. Only 2 pts (IIIB-IVA) were considered as CT failures for they presented early relapse before RT and had 4000 rads RT. All the pts in the study are still now in first CR with follow-up from 1 month to 20 month (median 11 months).

37 HODGKIN'S DISEASE (HD) IN CHILDHOOD AND ADOLESCENCE.RESULTS OF CHEMOTHERAPY-RADIOTHERAPY (CT-RT) IN CLINICAL STAGES (CS) IA-IIB.

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From 4/1972 to 5/1980, 72 children and adolescents (age, range 5-19 years, median 16) with HD, CS IA-IIB (IA:18, II2 A, 2 areas involved on the same side of the diaphragm : 23, II3 + A, 3 areas or more : 16, IIB : 15) were prospectively treated by 2 successive clinical trials (H72 and H77). CS IA and II2A received 3 MOPP and supradiaphragmatic RT (40 gy) ; no laparotomy was performed. CS II3 + A and IIB received 6 MOPP (H72), 3 MOPP or 3 CVPP (CCNU, Vinblastine, Procarbazine, Prednisone) (H77) and had a subsequent laparotomy followed by supradiaphragmatic RT with a lumboaortic field if positive laparotomy. Patients (pts) without evidence of mediastinal involvement did not have mediastinal RT. At completion of therapy, 70/72 pts were in complete remission (CR) (1 failure, 1 death under treatment). Eight pts relapsed (in situ : 1, marginal : 1, non irradiated subdiaphragmatic area : 6) after 3 to 57 months of CR (median 20 months) ; 1 pt died after relapse. There were 3 deaths in CR (infection : 2 ; AML : 1, actuarial risk : 1.8 %). In 11/1983 median follow-up was 75 months (range 27-132 months) ; actuarial probabilities for survival and freedom from relapse for all pts were respectively 91.6 % and 87.3 %. There was no statistical difference according to CS, age ($>$ 15 or $\leq$ 15 years), sex, 6 or 3 cycles of CT. Bone growth defects related to RT could be reduced particularly in the 29 pts who did not receive mediastinal RT (none of them had a mediastinal relapse). Azoospermia was the rule for studied male pts, but CT allowed small girls and young women to retain reproductive integrity. The 38 non splenectomized pts were subtracted to the infection risk of splenectomy

38 TREATMENT OF CHILDHOOD HODGKIN'S DISEASE STAGE I AND II WITHOUT RADIOTHERAPY

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Radiotherapy is the major modality in the treatment of Hodgkin's Disease stage I and II in adults, while chemotherapy is mainly used as an adjuvant before or after radiotherapy. However, combination chemotherapy has become the main modality for patients with advanced stages of Hodgkin's Disease, and at least half of the patients with stage III and IV can be cured with chemotherapy alone. Because good results are obtained with this strategy, children with Hodgkin's Disease have been treated in most centres as if they were small adults. However, during the past decade it has become more apparent that the late sequelae from radiotherapy are more serious in children. With decreasing age the late effects from radiotherapy, such as growth disturbances in irradiated areas, hypoplasia of breasts in pubertal girls, hypothyroidism, pericardial fibrosis, radiation pneumonitis and aspermia are increasingly prominent; in addition radiation-associated tumours can be expected. Consequently radiotherapeutic management of children with early stage Hodgkin's Disease has been adapted in several treatment centres. Extended fields have been reduced to involved fields, and it has been shown that the 4000 Rad dose can be decreased to 2500 Rad if the radiotherapy was sandwiched between periods of chemotherapeutic treatment.
In 1975 we decided to select from our patients with Hodgkin's Disease CS I and II a group of children who presented with only small lymphnode swellings, i.e. lymphnode tumours with a diameter less than 4 cm. These patients were treated with six MOPP-cycles at monthly intervals, without radiotherapy. The other children with CS I and II, having tumour masses with a diameter of more than 4 cm, were also treated with six cycles of MOPP, to which involved field radiotherapy was added after the third MOPP cycle. The radiation dose was 2500 Rad, given over a period of 3 weeks. From 1975 to 1982 18 consecutive children aged 5-14 years with CS I and II were treated according to the above mentioned programme. No child underwent staging laparotomy with splenectomy, but the other usual stage-screening methods were completely performed, including lymphangiography. Nine patients had small tumours with a diameter less than 4 cm, and they were treated with six cycles of MOPP without radiotherapy. Complete remission was easily obtained in all patients and up until now no relapse have occurred. These patients are followed for 11-103 months (median 59.6 months). Nine patients, having tumour masses in excess of 4 cm, received bimodal treatment with MOPP and involved field radiotherapy. From this group one child developed a relapse outside the irradiated area after 26 months, and he died of progressive disease in spite of agressive treatment with full dose radiotherapy and heavy chemotherapy. No relapses were seen in the other eight patients of this group, and they are followed for 22-72 months (median 49.9 months).
The data derived from this study, although preliminary, indicate that stage I and II of childhood Hodgkin's Disease can be successfully managed with chemotherapy alone. This was also the conclusion of dr. Olweny et al. based on their experience with the treatment of 48 children with Hodgkin's Disease in Uganda, where no radiotherapeutic facilities were available. However, much more consideration must be given to the late complications of this type of treatment. The risk of infertility in boys, who are treated with alkylating agents during or after puberty is subtantial. Second malignancies are also to be expected in the group of patients who are treated with MOPP, especially in those cases where chemotherapy was combined with radiotherapy.
It is not only our task to define the minimum effective therapy for children stage I and II Hodgkin's Disease, but also to treat them without damaging late effects. Although by far the majority of these patients can be cured by the MOPP-combination without radiotherapy, we must look for other chemotherapeutic combinations which do not contain alkylating agents, but have the same curative properties without its potentially injuring sequelae.

39 SELECTIVE SPLENECTOMY IN CHILDREN WITH HODGKIN'S DISEASE: PROSPECTIVE USE OF A NEW INTRAOPERATIVE STRATEGY IN 109 CHILDREN.

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By means of an analysis of 154 splenectomised children with Hodgkin's disease (study HD-DAL 78) we tried to find out a method to predict splenic involvement (IS) on the basis of clinical and/or intraoperative findings. 6 out of 17 pre- and intraoperative findings showed significant correlation to IS: B-symptomatology, palpable spleen, mediastinal enlargement, nodular changes of the splenic surface (SS+), enlarged lymphnodes of the hilus of spleen and/or the tail of pancreas (SH/TP+), enlargement of other upper-abdominal lymphnodes. Multivariat analysis showed, that the two most evident findings SS+ and SH/TP+ gave almost the entire information which can be obtained about IS. All other parameters were no longer significant when combined with these two.
Based on these results an intraoperative strategy has been developed. Splenectomy is restricted to those pts, which present the criteria SS+ and/or SH/TP+ (expected incidence 36 %). If abdominal lymphnode biopsies are positive in non-splenectomised children, irradiation will include the spleen, because the probability for IS in these cases is 70 %. In an additional 9 % of the non-splenectomised pts an IS remains undetected, i. e. our strategy of selective splenectomy has to be used in combination with chemotherapy.
Until Nov 1983 the new method was applied prospectively in 109 children of the therapy study HD-DAL 82. Pts receive 2, 4 or 6 cycles of chemotherapy (depending on the stage of HD) followed by involved field irradiation. 39/109 pts (36 %) were splenectomised, 26 pts by the criteria SS+ and 13 pts by SH/TP+. The spleen was involved in 28 of the 39 pts (72 %). 4 non-splenectomised pts received irradiation of their spleen due to positive abdominal lymphnode biopsies. These figures correspond very well with the expectations from the retrospective analysis. After a median observation time of 12 mths (range 1 - 24 mths) only 1 pt has relapsed (lungs). These results confirm the usefulness of our new strategy making it possible to omit splenectomy in about two thirds of pts and still to obtain detailed information about infradiaphragmatic spread.

40 ENZYMES INVOLVED IN ADENOSINE METABOLISM, IN NORMAL OR LEUKEMIC LYMPHOCYTES

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Enzymes involved in adenosine metabolism and adenine nucleotide catabolism are particularly important in lymphocytes, as compared to other cell types, mainly because lymphocytes are almost devoided of *de novo* purine synthesis. Moreover adenosine appears to be involved in lymphocyte differentiation and function. Deficiencies in some of these enzymes are correlated with abnormal maturation levels, abnormal lymphocyte function, and it was unambiguously shown that inherited deficiencies of some adenosine metabolizing enzymes led to immunodeficiencies.

We studied several of these enzymes both in human and mouse, in normal lymphocyte sub-populations, in leukemic cells and in lymphoblastoid cell lines.

For several enzymes, no significant differences were found among various populations: adenosine kinase, deoxyadenosine kinase, AMP kinase, S-adenosyl-homocysteine hydrolase, S-adenosyl-homocysteine synthesis, cytosolic 5'nucleotidase.

On the opposite, ecto-5'nucleotidase, ecto-ADPase, ecto-ATPase, AMP-deaminase were found higher in mature than in immature cells. Adenosine deaminase was found lower in mature than in immature cells.

In human lymphoblastoid cell lines also, low 5'N/ADA ratios and low AMP-deaminase activities seemed to be correlated with high TdT (Terminal deoxynucleotidyl Tranferase) levels and immature character, while high 5'N/ADA and high AMP-deaminase activities were found in mature cells with low TdT levels.

In lymphocytes from patients with some B-cell type leukemias we found low activities for both adenosine deaminase and ecto-5'nucleotidase, while in T-ALL patients low ecto-5'nucleotidase and normal adenosine deaminase activities were observed.

41 PURINE NUCLEOTIDE METABOLISM IN NORMAL AND PATHOLOGICAL LYMPHOID CELL DIFFERENTIATION.

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A normal purine metabolism is necessary for proper functioning of lymphoid cells. For *normal lymphoid cell differentiation*, lymphocytes in various maturation stages differ considerably in make-up of enzymes of purine metabolism. E.g., within the T-cell lineage, the activity ratio adenosine deaminase (ADA) / purine nucleoside phosphorylase (PNP) is twofold higher in small immature lymphocytes in the thymus cortex than in medium-sized cells in the thymus medulla, and blood T-cells reveal a value 20-fold lower than thymocytes. The variation in enzyme make-up is related with cell function. E.g., for thymocyte subpopulations there is a significant correlation between the activity ratio ecto-5'-nucleotidase (ecto-5'NT) / deoxycytidine kinase (which ratio determines the net capacity of the cell to convert (deoxy)nucleosides to toxic (deoxy)ribonucleotides) and the capacity of (deoxy)nucleosides to inhibit proliferative responses of the cell.
For *pathological lymphoid cell differentiation*, especially lymphoreticular malignancies (leukemia and lymphoma), immunological phenotyping has proved to be of value in addition to histopathology in assessment diagnosis and prognosis. The evaluation of purine enzymes has revealed considerable differences between various forms of leukemia. E.g., childhood T-cell acute lymphoblastic leukemia (T-ALL) distinguishes from other forms of ALL by a relatively high ADA and low ecto-5'NT enzyme activity. Within one type of leukemia subgroups can be discerned, e.g. in B-cell chronic lymphocytic leukemia, cells from patients with paraproteinemia have higher ecto-5'NT and lower ADA activities than cells from patients without paraproteinemia.
Apart of being markers in diagnosis, the assessment of purine enzyme make-up shares with immunological phenotyping the possibility to relate pathological lymphoid cells with normal lymphoid cell differentiation. In this, the enzyme make-up of T-ALL resembles that of immature T-cells found in the thymus. From its relation with cell function, the purine enzyme make-up (by giving insight in privileged pathways in purine nucleotide metabolism) may open possible ways of treatment, which include either inhibition of enzyme activities (e.g., ADA by deoxycoformycin) or therapy with purine analogues which are easily converted in pathological cells to toxic compounds. The variable succes of deoxycoformycin treatment of ALL may be based on variation in enzyme make-up, especially in ecto-5'NT activity (which dephosphorylates toxic ribonucleotides accumulating due to blocked ADA activity).

Most studies on purine nucleotide metabolism have been performed on leukemia and need extension to lymphoma. The detailed analysis of purine enzyme make-up in pathological cells in lymphoma may add to a better classification and prognosis of the disorder, and may open putative approaches of treatment by enzyme-directed chemotherapy.

42 EXPRESSION OF AN ECTOENZYME-CASCADE ON HUMAN LEUKEMIC AND LYMPHOBLASTOID CELLS. BIOCHEMICAL AND IMMUNOLOGICAL STUDIES AND CLINICAL SIGNIFICANCE.

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Earlier observations of Silber et al. on differential expression of the ectoenzyme 5′-nucleotidase (5′-N) on peripheral blood lymphocytes of normal subjects and patients with CLL were extended into the field of acute leukemias. Here a remarkable correlation of high activities of this enzyme with the expression of the common ALL antigen was found. The two surface antigens are not identical and plasmamembrane subfractionation studies with the cell line Nalm 1 show, that they are not closely associated on the membrane level. Further ectoenzymes like ATPase, ADPase and a nucleoside-diphosphate-kinase were characterized in their membrane orientation and enzymatic properties in lymphoblastoid B-cell-lines. ATPase, ADPase and 5′-N seem to be organized in form of an enzyme-cascade, since all three members are enriched in specific plasmamembrane subfractions. A coordinate expression of ATPase and 5′-N is found in a series of different B-cell-lines, but this does not apply to T-cell-lines or blast cells in different forms of acute leukemias. A number of findings in this investigation could be substantiated or extended using inhibiting polyclonal or monoclonal antibodies against 5′-N. Ecto-5′-N as a biochemical diagnostic marker is especially useful for the distinction of lymphoid and myeloid blast crisis in CML. Its clinical significance was further evaluated within a prospective study on acute leukemias. Our own data on ectoenzyme-expression in malignant lymphomas do not yet allow any general conclusions.
By virtue of their distinct differences in the patterns of surface expression lymphocytes and lymphoblastoid cells are regarded as good models for the study of the normal physiological function of ectoenzyme-cascades.

43 ENZYMATIC AND ULTRASTRUCTURAL PROPERTIES OF THE PLASMA MEMBRANE IN HUMAN LEUKEMIAS, NON-HODGKIN'S LYMPHOMAS AND IN HUMAN LYMPHOBLASTOID CELLS.

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The cellular plasmalemma is an effective organelle which enables many physiological events to be carry out through the involvement of associated enzymes, antigens and other macromolecular constituents.Their characteristic levels may undergo changes during pathological onset, following stimulation, perturbation of homeostasis or by adaptation to the various stages of cell differentiation and maturation.

The amplitude of variations of enzymatic and ultrastructural markers was investigated in the plasma membrane of cells with acute lymphoblastic leukemia or isolated from NH lymphomas and in human leukemic cell lines assigned to a definite stage of the B cell lineage.The enzymatic analysis of membranes obtained by discontinuous isopycnic centrifugation revealed not only characteristic enzymatic make-up in the various leukemic cell lines but also different activity profiles of each enzyme within a given cell population. At the opposite, a freeze-fracture analysis of intact cells revealed normal particle density distributions on the plasma membrane with a minor scattering of the particle density between the various cell lines.

These findings may reflect a continuous rearrangement of those constituents located on the outer leaflet of the plasma membrane while integral entities ensuring the intimate architecture of the cell envelope distributed more uniformly in proliferating cells. Membrane dynamics partially explains activity variations and should be ascribed to cell proliferation and maturation rather than to pathogenic events. It could also elucidate the shedding off of membrane fragments enriched with single membrane enzyme and antigen, which have been identified in cell culture supernatants and in sera of leukemic patients.

Resting human blood lymphocytes and more mature lymphoid cells express a more uniform distribution of their membrane constituents.

44 ENZYME PATHWAYS IN MALIGNANT LYMPHOMAS. A.V. Hoffbrand, Department of Haematology, Royal Free Hospital and School of Medicine, London, U.K.

Recent biochemical studies have shown close similarities between normal lymphoid cells and the leukaemias and lymphomas which are thought to arise by clonal expansion from them. The enzymes found to be of particular value diagnostically are adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and 5'-nucleotidase (5'NT) which are concerned in purine degradation, terminal transferase (TdT), ecto-ATPase, thymidine kinase (TK) and lactate dehydrogenase (LDH). Normal thymic cortical "blasts" and most Thy-ALL cases and some T lymphomas show high ADA, low PNP and 5'NT and raised TdT. In comparison to normal B cells, poorly or well differentiated B lymphomas and myeloid cells, early T cells show a highly efficient multienzyme complex for DNA synthesis but the ability of early T cells to degrade DNA precursors is low, endogenous production of deoxyadenosine is low and early T cells are susceptible to toxicity by deoxyribonucleosides, or to the ADA inhibitor deoxycoformycin (dCF). Thus, dCF and deoxyadenosine or deoxyguanosine plus PNP inhibitors could be used to selectively kill T tumour cells in bone marrow prior to autologous transplantation. More mature normal T cells and mature T cell tumours (e.g. Sezary, some T lymphomas, T-CLL) show higher PNP and 5'NT levels with low ADA and absent TdT. Although normal OKT_8 cells show higher 5'NT levels than OKT_4 cells, this pattern is not clearly reproduced in chronic T cell disorders. The LDH isoenzyme pattern changes as T cells mature and this pattern is also reproduced in the corresponding "early" and "late" T cell tumours.

The earliest recognisable B cell tumours e.g. c-ALL and pre B-ALL and the normal equivalent cells show the presence of TdT. It is possible that TdT has a role in the generation of diversity during immunoglobulin gene rearrangements in these early B cells. These cells have intermediate levels of ADA, PNP and 5'NT, the ADA:PNP ratio being higher than in more mature B cells or B cell tumours. TdT is absent from SIg secreting B cells and the B cell lymphomas derived from them. CLL shows low levels of all three purine degradative enzymes, absent TdT, but higher ecto ATPase levels than in mature B or T cells. TK is of fetal (TK_1) type in less well differentiated non-Hodgkin's lymphomas but of normal adult (TK_2) type in diffuse well-differentiated lymphomas. In CLL, TK_2 occurs except in clinically aggressive cases but in hairy cell leukaemia, TK_1 surprisingly dominates.

45 LYMPHOCYTE UROPORPHYRINOGEN SYNTHASE ACTIVITY IN LYMPHOPROLIFERATIVE DISORDERS - A VALUABLE DIAGNOSTIC TEST. +*M. Lahav, +O. Epstein, +N. Schoenfeld, "M. Shaklai and +*A. Atsmon. +The Laboratory of Biochemical Pharmacology, *Department of Internal Medicine B and "The Hematology Unit, The Beilinson Medical Center, Petah Tiqva, Israel.

Patients with lymphoproliferative diseases (LPD) were shown to have a significantly elevated activity of lymphocyte uroporphyrinogen synthase (1-URO-S). The mean values of 1-URO-S activity of a control group (n=70) and of LPD patients (n=70) were 24.7 (SD=5.2) and 87.2 (SD=44.0) pmol porphyrins/mg protein/hr, respectively. There was almost no overlap in the 1-URO-S activity of patients with LPD and of the control group. L-URO-S activities of patients with other malignant diseases and with viral and bacterial infections were within the normal range. The specifity of the determination of 1-URO-S activity in the diagnosis of LPD was 98% and the sensitivity was 97%. The positive predictive value of the test was over 90%. L-URO-S activity was determined in 49 patients clinically suspected of harboring LPD. In 45 of them a final diagnosis was established. In 15 of the latter the test was positive and the diagnosis was subsequently confirmed by other means such as a lymph node biopsy. In 27 patients the test was negative and other causes for the symptoms were established. In three patients, suffering from diseases other than LPD, the values obtained were slightly above the highest value of the controls. These data indicate that the determination of lymphocyte uroporphyrinogen synthase activity may be of considerable assistance in the diagnosis of lymphoproliferative diseases.

46 BIOCHEMICAL MARKERS IN NON-HODGKIN'S LYMPHOMA STAGES III AND IV AND PROGNOSIS - A MULTIVARIATE ANALYSIS.

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The prognostic value of different pretreatment laboratory and clinical findings at diagnosis was analyzed in a series of 141 patients with generalized non-Hodgkin's lymphoma. Univariate and multivariate survival analysis (Cox's regression model) was performed using serum analysis of deoxythymidine kinase (S-TK), β_2-microglobulin (S-β_2m), lactic dehydrogenase (S-LDH), α-1-acid glycoprotein = orosomucoid (S-α_1 AGP), haptoglobin and ferritin. In addition hemoglobin and erythrocyte sedimentation rate were measured. The clinical variables were age, presence or absence of B-symptoms, histopathology ("low-grade"; "intermediate grade" and "high-grade" malignancy, respectively), and bone-marrow involvement. Among the eight biochemical markers all were found to relate significantly to survival except hemoglobin and sedimentation rate. Among the clinical variables, B-symptoms and histopathology were found to relate significantly to survival. Using a multivariate analysis to all variables, S-TK was found to be the best factor predicting duration of survival. The only significant additional information was given by S-α_1 AGP. When only the clinical variables were taken into account it was found that histopathology contributed significant information to B-symptoms in the prediction of the survival time. If the biochemical variables were added to this model only S-TK gave significant additional prognostic information.

TK is an enzyme involved in the DNA synthesis. It converts deoxythymidine to deoxythymidinemonophosphate (dTMP). The activity of TK is high in dividing cells and very low in resting cells. When measuring S-TK we used a very sensitive method recently described (Gronowitz et al. Int. J. Cancer, January 1984).

We conclude that S-TK seems to be the most important prognostic biochemical marker in NHL.

47 PURINE DEGRADATIVE ENZYMES IN THE MALIGNANT CELLS OF PATIENTS WITH B-CELL LEUKEMIA.

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Previous studies have shown that investigations of the purine degradative enzymes adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and 5'-nucleotidase (5'-NT) are of value in defining subsets of lymphoid malignancies of T-cell origin. The significance of these enzymes in B-cell derived malignancies is still unknown. We have studied the activities of these enzymes in the circulating malignant cells of 13 patients with B-chronic lymphatic leukaemia (B-CLL), 15 patients with leukaemic immunocytoma (IC), 4 patients with centrocytic lymphomas (CC), 3 patients with B-prolymphocytic leukaemia (B-PLL). Diagnosis was established by morphology (cytology or histology) according to Kiel classification, immunologic marker analysis with monoclonal antibodies against B-cell differentiation antigens (HD-6, HD-21, HD-28, HD-39), and studies of surface and intracytoplasmic immunoglobulins.

Malignant cells of B-CLL were characterised by low activities of ADA (mean ± SD = 1.62 ± 1.04 U/10^8 cells), PNP (mean = 65.8 ± 31.9 U/10^6 cells), and 5'NT (mean = 1.97 ± 1.67 U/10^6 cells). In malignant cells of IC, low activity of ADA (mean = 1.64 ± 1.40 U/10^8 cells) was also observed, but the activities of PNP (mean = 99.9 ± 30.5) and 5'NT (mean = 22.6 ± 13.4) were relative high. The differences in PNP ($p < 0.05$) and in 5'NT ($p < 0.001$) between B-CLL and immunocytoma were significant. In CC, ADA activity was again low (mean 0.95 ± 0.85 U/10^8 cells), but PNP (mean = 86.9 ± 21.3 U/10^6 cells) and 5'NT (mean = 13.6 ± 9.7 U/10^6 cells) activities were moderately high. Circulating cells of PLL were shown to have low levels of ADA (mean = 1.59 ± 1.09 U/10^8 cells), PNP (55.9 ± 36.0 U/10^6 cells) and 5'NT (mean 1.43 ± 1.36 U/10^6 cells). These findings suggest that quantitation of purine degradative enzymes can be useful in classifying subsets of B-cell malignancy. In IC, for example, the enzyme activities were comparable to those measured in normal peripheral B-cells. These results support the conception that immunocytoma cells are well-differentiated whereas the B-CLL cells are immature with respect to the B-cell axis. Studies of these enzymes may be also of importance in defining maturation stages of B-cell malignancies.

48 TRANSFERRIN RECEPTOR EXPRESSION IN NON HODGKIN LYMPHOMAS (NHL). AN IMMUNOHISTOCHEMICAL STUDY.

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The monoclonal antibody OKT9 is directed at a transferrin receptor, which is present on the cytoplasmic membrane of proliferating cells, while it is not expressed by resting elements. Recently Habeshow et al. found that the percentage of OKT9 positive cells in lymphomatous nodes was related to disease activity and survival, showing that the low grade lymphomas of the Kiel classification had significant fewer positive elements than the high grade ones. These Authors, however, studied cell suspensions, which do not permit any correlation between the immunological analysis and structural characteristics of the examined tissues. Therefore, in order to get more reliable, information, we tested by an immunoperoxidase - ABC - method the expression of OKT9 on lyophilized frozen section of 24 lymph nodes from patients with different types of NHL diagnosed according to Kiel . Our results confirm a well defined OKT9 reactivity pattern, which corresponds to the grade of malignancy. Moreover, within the low grade lymphomas, a group of cases dysplaied higher number of positive cells; this support the view that, between low and high grade lymphomas, a third group of tumors with an intermediate behaviour could be defined. Finally according to the results obtained in the cases of centroblastic centrocytic lymphoma in centroblastic trasformation, it must be outlined that the recognition of areas with a higher content of OKT9 positive cells might have prognostic relevance, expecially in low grade cases with an initial evolution into a more aggressive form.

49 BANDED CHROMOSOME ABNORMALITIES IN NON-BURKITT'S, NON-HODGIN'S LYMPHOMA. CORRELATIONS WITH MORPHOLOGY AND IMMUNOLOGIC PHENOTYPE.

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The malignant lymphomas were among the first human neoplasms to be studied systematically when the new banding techniques became available. When appropriate techniques are used, clonal chromosome abnormalities can be found in the neoplastic tissue of almost all cases. We initially studied involved lymph nodes or other tumor masses in 94 patients with malignant lymphoma (Cancer Research 43:2975, 1983). Clonal chromosome abnormalities were identified in 91, including all 81 B-lymphomas, but only 6 of 9 T-lymphomas. Many recurring chromosome abnormalities were found. Most common numerical alterations involved gains of chromosome 12 (19% of patients), chromosome 18 (13% of patients), chromosome 7 (12% of patients), and chromosome 21 (10% of patients). Structural abnormalities were more frequent than numerical alterations. Most commonly involved chromosome regions were 14q (71% of patients), 18q (36% of patients), 6q (31% of patients), 1p (24% of patients), and 8q (19% of patients). Seven recurring translocations were identified and all except one involved 14q32. The most frequent were t(14;18)(q32;q21), t(8;14)(q24;q32) and t(1;14)(q42;q32). Deletions most frequently involved the long arm of chromosome 6 at band q21 or q23.

The common recurring chromosome abnormalities were correlated with histology, using the International Working Formulation for Clinical Usage, and with immunologic phenotype. Four abnormalities were significantly associated with specific histologies. Eighty-two percent of patients with t(14;18)(q32;q21) were follicular. Similarly, 82% of patients with del(6)(q21) had large cell lymphoma. Lymphomas with trisomy 7 were either diffuse, large cell or follicular. Patients with t(8;14)(q24;q32) were primarily diffuse, large cell; one patient had malignant lymphoma small lymphocytic type. A significant association with immunologic phenotype was seen for t(14;18) only. All patients with this translocation had either B or C' lymphomas, and the heavy chain was more commonly γ and less frequently δμ than among the total B-lymphoma population. Interestingly, both cases with t(3;14)(p21;q32) expressed μ heavy chain and both cases with t(14;19)(q32;q13) expressed δμ. Finally, preliminary analysis in our lymphomas indicates that recurring chromosome abnormalities are frequently in areas to which oncogenes have been localized.