the neuronal soma, without labeling of the 2nd and 3rd neuron. Our suggestion that there is a transneuronal transfer of HRP does not invalidate this neuroanatomical method since the amount of HRP transferred is very small, but it should be a factor to consider.

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## T-cell leukemia induced by 1-propyl-1-nitrosourea in Fischer rats[1]

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*Summary.* T-cell leukemia was induced at a high rate in female Fischer rats by continuous oral administration of 1-propyl-1-nitrosourea. No association of oncorna viruses were found in the leukemogenesis.

We have reported on the high incidence of thymic lymphoma induction (41/48, 85%) in female Fischer rats following the continuous oral administration of 1-propyl-1-nitrosourea (PNU)[2]. Among those thymic lymphomas induced, 25 cases were successfully transplanted into syngeneic Fischer rats i.p. Here, we have reported on the preliminary characterization of 6 cases of thymic lymphoma transplanted in Fischer rats.

Female Fischer 344 rats purchased from Charles River Japan Co. (Atsugi, Kanazawa) were used through the experiment. Hematologically, all of the transplanted tumors were considered to be lymphoblastic leukemia. It was noted that the presence of leukemic cells with convoluted nuclei, which is usually found in human adult T-cell leukemia[3,4], was always observed in all of the transplanted cases. Cytochemical staining of leukemic cells revealed the focal distribution of acid phosphatase and $\beta$-glucuronidase activity which is typical for T-cells[5]. Peroxidase and alkaline phosphatase reactions were negative. Periodic acid-Schiff staining was also negative.

The results of the cell-surface and intracellular marker test are shown in the table. Antisera used in this experiment were mouse anti-Thy 1.1 alloantiserum, Searle Lab., London, fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat IgM serum, FITC-conjugated rabbit anti-rat IgG serum, FITC-conjugated rabbit anti-mouse IgG serum, Medical & Biological Lab. Ltd, Nagoya, and rabbit anti-calf terminal deoxynucleotidyl transferase (TdT) serum, monoclonal mouse anti-calf TdT serum, Bethesda Research Lab., Bethesda. The specificities of FITC-conjugated antisera were tested by the double immunodiffusion test described previously[6]. E-rosette formation with sheep erythrocytes, EA and EAC rosettes with bovine erythrocytes sensitized with IgG antibody (EA) or IgM antibody and mouse or human complement (EAC) were tested using an assay kit purchased from the Japan Immuno-Research Lab., Takasaki. E-rosette formation with guinea-pig erythrocytes (Hartley strain) was carried out as described previously[7].

Most of the leukemic cells from the 6 cases tested possessed Thy 1.1 antigen were detected as a result of the indirect

Cell-surface and intracellular marker studies on thymic lymphoma induced by 1-propyl-1-nitrosourea in Fischer rats*

| Case No. | Cell-surface markers | | | | | | | | Intracellular markers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SmIg | | Receptors | | | | | CIg | | |
| | Thy 1.1 | M | G | E:sheep | Guinea-pig | EA(G) | EAC:human | Mouse | M | G | TdT |
| 1 | 87 | 1 | 4 | 22.5 | 26 | 43.5 | 4.0 | 2.5 | 0 | 0 | 10 |
| 2 | 85 | 1 | 0 | 26.0 | 8 | 15.0 | 2.0 | 1.5 | 0 | 0 | 10 |
| 3 | 89 | 1 | 0 | 39.5 | 18 | 73.5 | 3.0 | 11.0 | 0 | 0 | 9 |
| 4 | 92 | 1 | 0 | 41.5 | 39 | 62.5 | 7.0 | 9.5 | 0 | 0 | 11 |
| 5 | 95 | 0 | 0 | 9.5 | 15 | 2.5 | 1.5 | 2.0 | 0 | 0 | 32 |
| 6 | 92 | 0 | 0 | 8.0 | 18 | 5.5 | 1.5 | 2.5 | 0 | 0 | 11 |

* Percent of positive cells.

membrane immunofluorescence test. In contrast, only a few or no leukemic cells were found to be positive for surface membrane immunoglobulin (SmIg). The number of leukemic cells having E-receptors varied from 8 to 41.5%. The number of leukemic cells having EA(Fcγ) receptors also varied from 2.5 to 73.5%, whereas a few EAC receptor-positive cells were found in all cases. It is speculated that the finding of various percentage of Fcγ-positive cells in the various cases suggests, in turn, the presence of Fcμ-positive cells, and that PNU-induced leukemia will be the consequence of malignant transformation of a heterogeneous T-cell subset. As intracellular markers, cytoplasmic immunoglobulin (CIg) M and G, which are found in pre-B cells and plasma cells, could not be found in the cytoplasm of leukemic cells by the direct immunofluorescence test. TdT-positive cells, which are usually seen in thymus cortex[8], were found to be 9–32% of the leukemic cells by cytoplasmic immunofluorescence.

Taking these facts into consideration, it was concluded that all of the PNU-induced leukemias tested were T-cell leukemia, which is considered to be similar to T-cell malignancies found in human disorders.

Furthermore, the presence of oncorna virus-related antigens in these T-cell leukemia was examined by the cytotoxicity test using anti-Gross tumor serum[9] and anti-rat erythroblastosis virus serum[10]. None of the leukemic cases gave positive results whereas, in sharp contrast, 50% of the leukemic cells induced by the viruses were killed by the anti-Gross tumor serum with a dilution of 1:32 and by the anti-erythroblastosis serum with a dilution of 1:1024, respectively. It is again stressed that even in rat T-cell leukemia, where there is a possibility of viral implication, there is no association of oncorna virus with leukemogenesis in rats[9].

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## Effect of lithium on normal and chronic granulocytic leukemia colony forming cells (CFU-GM)

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*Summary.* The effect of lithium on normal and chronic granulocytic leukemia (CGL) granulo-monocytic precursors (CFU-GM) has been studied. Lithium slightly increases normal CFU-GM growth whereas it is withouth effect or moderately inhibitory for CGL CFU-GM. It is suggested that it is unlikely that lithium therapy enhances the proliferation of a silent leukemic clone.

Lithium, due to its stimulatory effect on granulopoiesis[2,3], has been used to treat granulocytopenias both idiopathic and secondary to cytostatic treatment[4,5]. Extensive in vitro investigation has thrown some light on the mode of action of lithium on normal hemopoietic cells, showing that its stimulatory effect depends on an enhancement of the production of colony stimulating activity as well as on a direct stimulation of hemopoietic stem cells[6–8]. On the other hand nothing is known of the effect of lithium on leukemic cells. Such information appears to be of some relevance because of recent reports of cases of leukemia during or subsequent to treatment with lithium; it has been suggested that these resulted from a transformation of a normal cell, or a stimulation of a silent malignant clone, induced by lithium[9–11]. While there is evidence against a mutagenic effect of lithium[12] it is not known whether lithium can accelerate leukemic cell proliferation[13]. For this reason we undertook studies on the in vitro effect of lithium on normal and CGL CFU-GM.

Increasing concentrations of lithium chloride (dissolved in distilled water) were added to $1 \times 10^5$ cells from 10 normal bone marrow samples and from peripheral blood of 8 CGL patients. All samples were prepared as previously described[14] in McCoys 5A supplemented medium with 15% foetal calf serum (FCS, Seromed, Munich, FRG) in agar 0.3% and plated over feeder layers prepared, according to Pike and Robinson[15], in McCoys 5A supplemented medium with 15% FCS in 0.5% agar. After 8 days' incubation in a fully humidified atmosphere with 5% $CO_2$ the dishes were scored and all aggregates of more than 40 cells were counted as colonies.

The table shows that lithium slightly increased normal CFU-GM growth, confirming the results of Tisman et al.[6] while no effect or a slight inhibition was observed in CGL cultures. At lithium concentrations between 1 and 5 mM the difference in response between normal and CGL CFU-GM was statistically significant ($p < 0.05$).

Effect of increasing concentrations of lithium on normal and CGL CFU-GM. In each experiment mean CFU-GM colony number of 3 dishes per point was determined and expressed as a percentage of control colony number. Results are means ± SD of these percentages. Colony number range in control dishes/$10^5$ cells plated: normal = 68–172; CGL = 42–184.

| | Lithium ($10^{-3}$ M) 0 | 0.5 | 1.0 | 2.5 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|
| Normal | 100 | 116 ± 13 | 118 ± 11 | 119 ± 15 | 128 ± 18 | 98 ± 26 |
| CGL | 100 | 94 ± 16 | 82 ± 18 | 79 ± 19 | 75 ± 23 | 71 ± 28 |