

# The Comparison of the Expression of Activation Antigens on Peripheral Blood Mononuclear Cells in Chronic Lymphocytic Leukemia and in Hairy Cell Leukemia

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## Introduction

Based on the results of the immunophenotypical analysis both B-cell chronic lymphocytic leukemia (B-CLL) and B-cell hairy cell leukemia (B-HCL) appear to be derived from activated lymphocytes [1–4]. However, CLL-B cells represent an earlier stage of differentiation than the cells of HCL. The data of the gene rearrangement studies also suggest that HCL derives from the clonal expansion of a cell at a later developmental stage than the CLL [5].

In this study the peripheral blood mononuclear (PBM) cells of patients with CLL and HCL were characterized by the presence of a variety of cell surface differentiation and activation antigens.

## Patients and Methods

Twenty CLL patients were studied. Their age ranged from 42 to 79 years (mean: 63.0). The male to female ratio was 0.67:1. Five HCL patients in the leukemic phase of the disease were selected from the 24 investigated HCL patients (age 22–79 years, mean: 54.3). All patients were untreated for at least 3 months at the time of the study. Eight healthy age- and sex-matched persons served as controls. Indirect immuno-

fluorescence was used to detect the differentiation and activation antigens on PBM cells.

T cell-associated antibodies were CD 3 (T3), CD 2 (T11), CD 8 (T8), CD 4 (T4) from Ortho Diagnostic System and (Raritan, New Jersey, USA), CD 5 (Leu-1) from Becton-Dickinson (Mountain View, California, USA). B cell-associated antibodies were CD 19 (B4) and CD 20 (B1) from Coulter Corporation (Hialeah, Florida, USA). Class II antigen was detected with HLA-DR from Becton-Dickinson. Anti-immunoglobulin M (IgM) antibody (Heintel Vienna, Austria) was used as directly conjugated with fluorescein isothiocyanate (FITC). RAB-1 monoclonal antibodies (moAbs) were used to detect the hairy cells [6]. The Immune Monitoring Kit of Becton-Dickinson was used to detect activated T cells. The monoclonal antibodies (moAbs) from the panel of the IVth International Workshop on Human Differentiation Antigens (CD 25, CD 30, CD 40, CD 69, CD 70, CD 39, CD 71) were used to detect the activation antigens. Analysis of all samples was done using a FACSTAR flow cytometer (Becton-Dickinson).

## Results

The cells of 20 CLL patients were found to be B-cell phenotype when studied with Abs directed against CD 19, CD 20, HLA-DR and surface immunoglobulin (sIg) antigens. Furthermore, significant percentage of the cells gave a positive reactions with moAbs to CD 5 [7]. The

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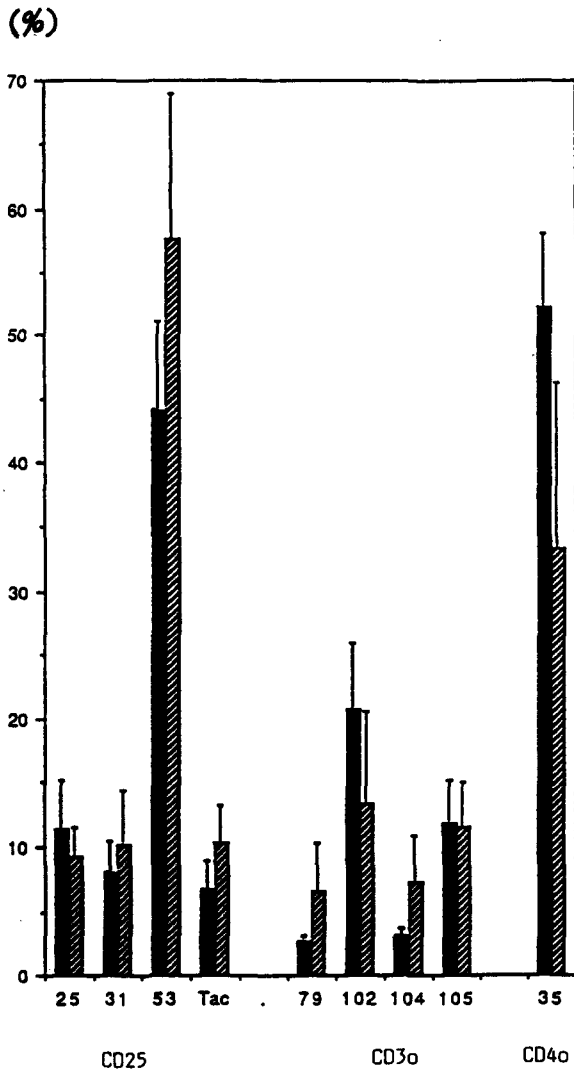


Fig. 1. The comparison of activation antigen expression on CLL (black bars;  $n = 20$ ) and HCL (hatched bars;  $n = 5$ ) cells I

PBM cells from 24 HCL patients showed similar antigen profile: CD19, CD20, HLA-DR and sIg positivity, but CD5 negativity. The moAbs to hairy cells RAB-1 showed a positive reaction both on CLL and HCL cells, but the expression of RAB-1 was found to be significantly higher in HCL than in CLL. Neither CLL nor the HCL cells expressed the CD21 antigen (C3d receptor, EBV receptor).

Investigating the presence of various activation antigens using 18 moAbs grouped into seven clusters, both CLL and HCL PBM cells were found to carry a significant proportion of activated cells when compared to the healthy controls. Comparing the CLL and HCL cells with

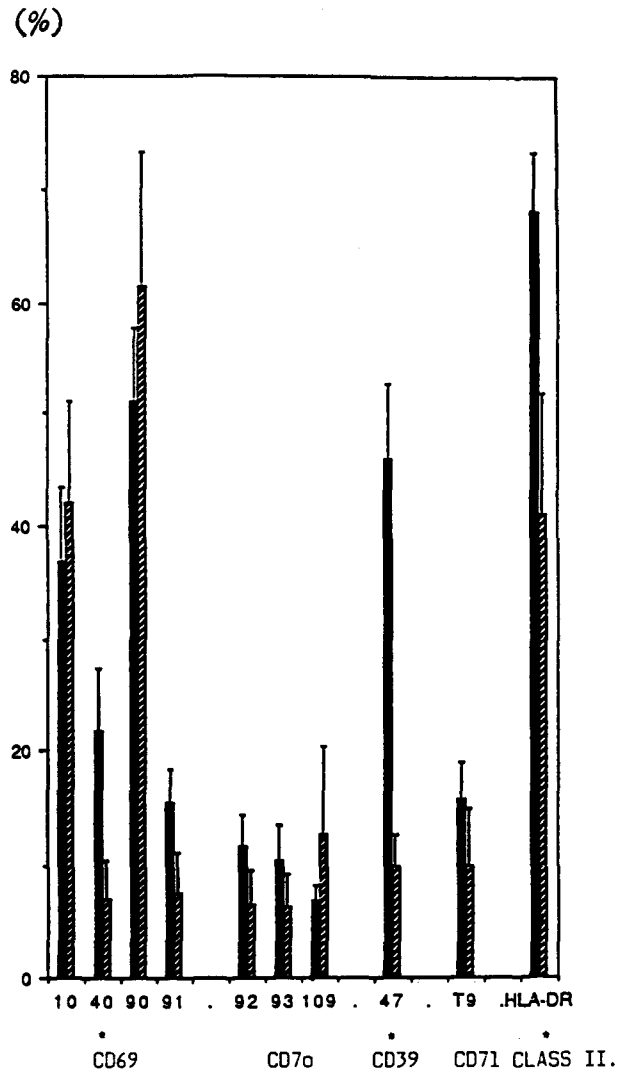


Fig. 2. The comparison of activation antigen expression on CLL (black bars;  $n = 20$ ) and HCL (hatched bars;  $n = 5$ ) cells II

each other, the activation stage of PBM cells in CLL and HCL differed only in two out of the 18 activation antigens (Figs. 1, 2). The expression of the CD39 cluster as well as the expression of a single antigen of the CD69 clusters were significantly higher in CLL than in HCL.

## Discussion

Our data confirm and extend earlier suggestions that both CLL and HCL might be derived from subpopulations of activated lymphocytes. The CLL cells were characterized by the expression of CD25, CD30, CD39, CD40, CD69 and CD70 activation antigens, while the HCL cells

were bearing CD25, CD40 and CD69 activation antigens. The expression of RAB-1 antigen on CLL lymphocytes suggests that RAB-1 might be also an activation marker [8]. The interleukin (IL-2) receptor (CD25) and two early activation antigens, CD40 and CD69 [9], were found to be expressed in cells of both CLL and HCL while the CD25 antigen positivity was significantly higher in HCL than in CLL.

The antigens of the CD30 and CD70 clusters which are present on the cell lines that related to activated T and B lymphoma cells were found to be expressed only in CLL cells, suggesting that HCL cells represent a later stage of differentiation.

## References

1. Freedman AS, Boyd AW, Bieber FR, Daley J, Rosen K, Horowitz JC, Levy DN, Nadler LM (1987) Normal cellular counterparts of B cell chronic lymphocytic leukemia. *Blood* 70:418–427
2. Dörken B, Moldenhauer G, Pezzutto A, Schwartz R, Feller A, Kiesel S, Nadler LM (1986) HD39 (B3), a B lineage-restricted antigen whose cell surface expression is limited to resting and activated human B lymphocytes. *J Immunol* 136:4470–4479
3. Posnett DN, Wang CY, Chiorazzi N, Crow MK, Kunkel HG (1984) An antigen characteristic of hairy cell leukemia cells is expressed on certain activated B cells. *J Immunol* 133:1635–1640
4. Posnett DN, Duggan A, McGrath H (1990) Hairy cell leukemia-associated antigen (HC2) is an activation antigen of several hemopoietic cell lineages, inducible on monocytes by IFN- $\gamma$ . *J Immunol* 144:929–933
5. Foroni L, Catovsky D, Luzzatto L (1987) Immunoglobulin gene rearrangements in hairy cell leukemia and other chronic B cell lymphoproliferative disorders. *Leukemia* 4:389–392
6. Berrebi A, Ashhar Z, Linder A, Guedj L, Avaraham H (1986) RAB-1: a new monoclonal antibody to leukemic hairy cells. *Leuk Res* 10:1071–1078
7. Pálózi K, Pócsik É, Mihalik R, Benczur M, Demeter J, Solti V, Petrányi Gy, Hollan RS (1990) Detection of activation antigens on chronic lymphocytic leukaemia cells. *Leuk Lymphoma* 3:31–36
8. Demeter J, Pálóczi K, Földi J, Hokland M, Hokland P, Benczur M, Lehoczky D (1989) Immunological and molecular biological identification of a true case of T-hairy cell leukaemia. *Eur J Haematol* 43:339–345
9. Stein H, Schwarting R (1989) Activation antigens. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGK (eds) *White cell differentiation antigens*. Oxford University Press Oxford, pp 388–513