

SPECIFICITIES OF MONOCLONAL ANTIBODIES B-LY7 AND HML-1 ARE IDENTICAL

To the Editor:

In their report in *BLOOD* 74:320, 1989, Visser et al reported on a new monoclonal antibody designated B-ly7 that they suggested to be specific for hairy cells and some scattered normal B cells. Using their antibody (courtesy of Dr Poppema) we can essentially confirm the close association of B-ly7 antigen expression with hairy cell leukemia. However, regarding the specificity of this reagent we would like to add some new significant aspects.

Most of the normal (scattered) B-ly7 positive lymphocytes found in the white and red pulp of the spleen and in any other lymphoid organ are not of B-cell origin. They carry a CD8+ CD3+ phenotype.

In the intestine, B-ly7 MoAb consistently stains intraepithelial and interepithelial mucosal CD8+ T lymphocytes.

The low number of B-ly7 positive peripheral blood lymphocytes can be greatly enlarged to approximately 60% by lectin stimulation following a slow kinetic time course with the maximum of positive cells around day 8. The majority of these cells are CD8/CD3 positive.

We recently established a number of HTLV1+ positive T-cell lines of which a proportion was strongly positive for B-ly7.

Knowing this reactivity, which is not or incompletely described in the report by Visser et al, we were very much reminded of the reactivity pattern of the recently described MoAb HML-1 that labels mucosa-associated T cells and enteropathy associated T-cell lymphomas.¹⁻³ In search of new MoAb against lymphoid activation antigens, we obtained one MoAb designated Ber-ACT8 that exhibited a similar binding pattern. Comparison of this MoAb with B-ly7 and HML-1 MoAb provided firm evidence that all three MoAbs (B-ly7, HML1, and Ber-ACT8) recognize the same antigen based on the following: (1) The distribution pattern on various normal T- and B-cell targets is identical; (2) all three antibodies reacted with

all cases of hairy leukemia, mucosa-associated T cells, and enteropathy-associated T-cell lymphomas; and (3) all three MoAbs consistently precipitated the same molecular trimer with molecular weights (mol wts) 170,000, 140,000, and 110,000 from both hairy cell leukemias and certain permanent T-cell lines (Visser et al report only one band of 145 Kd corresponding probably to the mol wt 170,000 band, which is by far the strongest component). Identity of the HML-1, B-ly7, and Ber-ACT8 antigens was ascertained by sequential immunoprecipitation.

In conclusion, the B-ly7 MoAb is neither B-lineage specific nor specific for hairy cell leukemia. Furthermore, most of the HML-1/B-ly7 positive normal lymphocytes are not candidates for being the normal counterpart of hairy cell leukemia because they exhibit T-cell phenotype. However, it is not too surprising that the expression of this interesting new antigen on both hairy cell leukemia and mucosa-associated T-cell lymphoma has not yet been discovered since both types of lymphomas are rare.

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RESPONSE

Schwartzing et al report some new exciting information on the reactivity of antibody B-ly7.¹ In addition to its reactivity with hairy

cell leukemia (that has now been confirmed to us on more than 70 cases by several participants of the IVth Leucocyte Antigen Work-

shop in Vienna) they found that B-ly7 also reacted with a case of enteropathy-associated T-cell lymphoma. The reactivity of B-ly7 appeared to them to be similar to that of HML-1, an antibody that has been described to label mucosa associated T cells with CD3+, CD7+, CD8+; or CD3+, CD7+, CD8-; or CD3-, CD7+, CD8- immunophenotypes,² as well as cases of enteropathy-associated T-cell lymphoma. This was originally described by Spencer et al on a series of eight cases, that had CD3+, CD7+, CD8-, or CD3-, CD7+, CD8- immunophenotypes,³ and subsequently confirmed by Stein et al⁴ on one case with a CD3+, CD8+ immunophenotype. Schwarting et al have also found that HML-1 reacts with cases of hairy cell leukemia. The molecular weight of the antigen recognized by HML-1 that had not previously been reported was found by them to be similar to that of B-ly7. They describe a major band at 170 Kd with additional weaker bands at 140 and 110 Kd. The major band may indeed correspond to the 144-Kd band described in our report. The weak band that can be seen at approximately 115 Kd in our gel has been considered by us as a degradation product and not as a component of a dimer or trimer.

With regards to the normal cell population recognized by B-ly7, we had concentrated our efforts on peripheral blood cells and found that more than 98% of B-ly7+ cells in normal peripheral blood (usually less than 0.5% of the lymphocytes) are B cells. We did not obtain convincing and quantitative results on tissue sections of lymphoid organs, since the evaluation of double stains for B-ly7 and CD3 or CD8 was seriously hampered by the fact that the majority of the cells surrounding the scattered B-ly7+ cells are CD3 and CD8 positive. At the IVth Leucocyte Antigen Workshop it came to our attention that a high proportion of intraepithelial lymphocytes of the gut are B-ly7 positive (personal communications: Dr P. Möller, Heidelberg, and Dr K. Beiske, Oslo, February 1989). Subsequently we performed double staining on frozen tissue sections and found a majority of these cells to be B-ly7 and CD8 positive. We also stained a series of mucosa-associated lymphomas of B-cell origin, the so-called centrocyte-like lymphomas,⁵ monocytoid B-cell lymphomas, or parafollicular B-cell lymphomas that are considered to be related to hairy cells by some investigators⁶ and found these cases to

be B-ly7 negative. However, one case of mucosa-associated T-cell lymphoma of the ileum in a 41-year-old woman, which was morphologically classified as a plasmacytoid T-cell lymphoma and had a CD2-, CD3-, CD4-, CD5-, CD7+, CD8-, CD25+ immunophenotype and a TCR beta chain rearranged genotype, was found to be B-ly7 positive in the tumor of the ileum as well as in a lesion in the lung.

An interesting aspect is that antibodies B-ly7 and HML-1 that both react with very infrequent malignancies and small subpopulations of B cells and T cells and were established in totally different ways now appear to recognize the same antigen. This will have to be confirmed in the next Leucocyte Antigen Workshop to enable designation to a cluster (CD).

The functional significance of the B-ly7/HML-1 antigen is not known. We found that phorbol ester stimulation of B cells, purified by depletion of T cells, monocytes and most CD5 positive B cells with MT1 (CD43) coated magnetic beads (greater than 98% CD20 positive), leads to the expression of the B-ly7 antigen in up to 30% of the B cells on day 3 (L. Visser and S. Poppema, submitted for publication). In addition, we found that PHA stimulation as well as phorbol ester stimulation of T cells, purified by depletion of B cells with B-ly1 (CD20) coated magnetic beads, also results in the expression of B-ly7 antigen, with a particularly strong expression after phorbol ester stimulation, with up to 40% positive T cells at day 3. The results indicate that normal, peripheral blood B and T cells can be driven by phorbol ester to express B-ly7 antigen. This suggests that B-ly7 and HML-1 recognize an activation antigen that is expressed on a small normal B-cell subpopulation and on intestinal T cells as well as on their corresponding neoplastic counterparts, being hairy cell leukemia and enteropathy-associated T-cell lymphoma.

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