Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

TLym 1, a Stage-Specific Transforming Gene Which Shares Homology to MHCI Genes and Encodes a Secreted Protein

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We previously reported the identification of five different genes activated in human and mouse leukemias and lymphomas [1]. Three of these genes were found to be activated in a stage-specific manner in B cell lineage neoplasms. The gene activated in multiple human intermediate B stage tumors, BLym 1 which shares substantial homology with chicken BLym 1 [2] was isolated from human Burkitt's lymphomas. This gene encodes a small protein of about 8 kilodaltons, the bulk of which is present in the nucleus of transformed cells (Nieman and Cooper, personal communication).

We recently isolated a second stage-specific transforming gene from mouse T lymphomas, representative of an intermediate stage of T lymphocyte differentiation. This gene differs by restriction endonuclease sensitivity from the transforming gene activated in multiple mature T neoplasms. TLym 1 has been found to be activated in four human and 12 mouse T cell neoplasms, but has not been found to be activated in cells from other lineages or cells at other stages of differentiation. TLym 1 shares no homology with Blym 1 nor does it share homology with any of the identified retrovirally transduced oncogenes. Thus, it represents a new cellular transforming gene highly specific for T lymphocytes at an intermediate stage of differentiation [3].

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TLym 1 was isolated from the S49 BALB/c T lymphoma by sib-selection and transfection of a transforming gene-enriched recombinant library prepared in the bacteriophage Charon 30. The isolated bacteriophage clone had a transforming efficiency of 10^4 foci per microgram cell DNA insert as assayed on NIH 3T3 cells. From Southern blot analysis using a flanking sequence probe, we determined that the gene did not become activated as a result of gross inversions, deletions, or rearrangements. TLym 1 is a small gene, approximately 3 kilobases in length and was originally thought to be a member of a small gene family, consisting of only two genes as defined by sequences hybridizing to the flanking region probe. Recent findings described in the following paragraphs, however, may alter this conclusion [3].

Hybridization of TLym 1 to T cell RNA indicated that three major messages could be identified in helper T cells which were 0.6, 0.7, and 1.6 kilobases in length. An additional message of 1.8 kilobases was identified in a suppressor T cell clone. As the size classes of meassages identified by TLym 1 were quite similar to those reported by Peter Rigby for his Set 1 genes, thought to encode a major histocompatibility class I gene [4], it was of interest to us to determine whether our gene shared homology to MHCI genes as well.

Southern blot hybridization was carried out using the following probes: pAG64C, a cDNA clone obtained from P. Rigby containing half of exon 4, and exons 5 and 6 of his Set 1 gene; pAG64E, which contained the Set 1 transposon-like direct repeat se-

quence; and pMHCI, and MHCI cross-reactive probe containing exons 2, 3, 4, and 5 which was obtained from John Seidman [5]. Additional hybridizations carried out by Steven Hunt in the laboratory of Lee Hood included hybridization utilizing MHC cross-reactive 5′ and 3′ probes. These studies indicated that our TLym 1 gene shared homology to genes mapped to the MHCI region and that the direct repeat sequence described by Rigby and co-workers was present in our gene. This sequence has also been detected by Hood and co-workers and was found in the third intron of their QA pseudogene 27.1 [6]. We were also able to determine from these hybridizations the orientation of our gene in the pBR322 plasmid to which it was subcloned.

As TLym 1 contained a ClaI site, some further analysis was possible based upon the report by Steinmetz et al. [7] defining 13 clusters containing 36 genes within the BALB/c MHCI region. From their reported analysis of ClaI sites within the gene clusters. Clusters 1 and 6 map to QA regions, while clusters 3 and 5 map to TL regions as determined by these authors. If the genes contained in the 13 clusters constitute all of the genes encoding MHCI sequences, then this retrospective analysis further localizes TLym 1 to the QA/TL region of the MHCI complex.

We have determined that our gene differs from that identified by Rigby and coworkers based upon hybridization of our flanking sequence probe to mouse DNA digested with the enzyme BAMH1 which identified two bands of 8.6 and 18.0 kilobases while hybridization of Rigby and coworkers' pAG64C probe to the same filter identified bands of 6.0, 3.5, 3.2, and 2.2 kilobases, as the 8.6 kilobases genomic BamH1 fragment is analogous to the cell DNA insert which contains the TLym 1 transforming gene isolated in Charon 30. We concluded that although these genes share some homology, they are in fact different genes. Genes within the MHCI region may share as much as 80% homology. Thus, analysis of these genes at the level of Southern blot hybridization is sometimes complicated, however, utilization in this case of low or single-copy probes has facilitated this analysis.

To analyze the protein encoded by TLym 1 we utilized a series of monoclonal or heteroantibodies prepared against H-2, TL, or QA region determinants. Immunoprecipitations carried out utilizing these reagents have failed to detect the TLym 1-encoded protein. Utilization of an antiserum cross-reactive with all MHCI-encoded proteins, however, detected a 44 kilodaltons protein in the supernatants of NIH 3T3 cells transformed by TLym 1 which was not present in supernatants from normal NIH 3T3 cells, spontaneously transformed 3T3 cells, NIH 3T3 cells transformed by ras^H, or NIH 3T3 cells transformed by the Blym 1 gene. These findings further substantiate the relatedness of the TLym 1 product to MHCI-encoded proteins and indicate that TLym 1 is the first transforming gene found to encode a secreted protein product. While most MHCI products have been localized to the cell surface, Jay and co-workers have reported a liver-specific MHCI protein which appears to be secreted, but which is not involved in transformation [8].

As TLym 1 appears to encode a secreted protein, we have begun preliminary experiments to determine whether this protein can behave as a growth factor. We therefore utilized supernatants from untransformed NIH 3T3 cells and supernatants from NIH 3T3 cells transformed by TLym 1 in a soft agar colony growth factor assay [9] with NIH 3T3 cells as the target cells.

After 14 days in culture, plates were scored for numbers of colonies having 20 or more cells. A total of 77 colonies were detected on seven plates treated with supernatants from NIH 3T3 cells whereas 960 colonies were detected on seven plates treated with supernatant from NIH 3T3 cells transformed by TLym 1. It will be of great interest to determine whether supernatants from TLym 1-transformed NIH 3T3 cells will have similiar effects upon T lymphocytes.

To summarize these findings, TLym 1 is an intermediate T-specific transforming gene which transforms NIH 3T3 cells with high efficiencies. This gene appears to share homology with genes encoded in the MHCI region and may be the transforming analog of a TL/QA region gene. TLym 1 encodes a

secreted protein of 44 kilodaltons which preliminary evidence indicates behaves as a transforming growth factor.

Acknowledgments. The authors wish to thank Dr. John Seidman, Dr. Peter Rigby, Dr. Leroy Hood, Dr. Stephen Hunt, F. W. Shen, E. A. Boyse and Gordon Freeman for reagents and useful discussions. This work was supported by CA 33108. M. A. Lane is a scholar of the Leukemia Society of America.

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