Brief Definitive Report

EXPRESSION OF THE 6C3 ANTIGEN ON MURINE HEMATOPOIETIC NEOPLASMS

Association with Expression of *abl*, *ras*, *fes*, *src*, *erb*B, and Cas NS-1 Oncogenes but Not with *myc*

BY HERBERT C. MORSE III,* GEORGE F. TIDMARSH,§ KEVIN L. HOLMES,* TORGNY F. FREDERICKSON,* JANET N. HARTLEY,* JACALYN H. PIERCE,‡ WALLACE Y. LANGDON,* MORRIS O. DAILEY,‖ AND IRVING L. WEISSMAN§

*From the *Laboratory of Immunopathology, National Institutes of Allergy and Infectious Diseases; and the ‡Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; the §Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305; and the ‖Department of Pathology, University of Iowa School of Medicine, Iowa City, Iowa 52242*

Clinical (1) and experimental (2) analyses of transformation indicate that two or more alterations of normal cell function are required to induce autonomous growth of malignant cells. In many instances, one of these events appears to be associated with deregulated expression of a protooncogene. Studies of murine B lineage neoplasms have shown that a variety of oncogenes, including *abl* (3), *fes*, Ha-*ras*, Ki-*ras*, *bas*, *src* (4), *myc* (5), *erb*B (7) and the transforming gene carried by Cas NS-1 (8), can contribute to induction of tumors of this lineage. In contrast, little is known about the postulated second events that participate in the development of these neoplasms. Recently, it was found that pre-B cell tumors, but not fibroblasts transformed by the *abl*-containing Abelson murine leukemia virus (A-MuLV) regularly express high levels of a cell surface glycoprotein of 160 kD ($gp160^{6C3}$) that is not a product of genes in the A-MuLV complex (9, 10); $gp160^{6C3}$ contains an epitope detected by the monoclonal antibody 6C3. Although this antigen is expressed at low levels on some normal thymocytes and bone marrow cells (10), these cells appear to acquire the molecule by transfer from stromal elements, whereas the molecule is actively synthesized by transformed hematopoietic cell lines (10). The observation that expression of the 6C3 antigen appears after A-MuLV-infected cells become independent of feeder layers for growth in vitro suggests that activation of the gene encoding this molecule is associated with acquisition of the fully transformed phenotype. If this suggestion is correct, this gene could be considered an oncogene with the

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potential for complementing *abl* and possibly other oncogenes to induce transformation of a variety of cell types.

In this study we undertook an examination of a large series of hematopoietic tumors for the presence of the 6C3 antigen, gp160^{6C3} was found on most B-lineage spontaneous tumors and each B-lineage tumors induced by replication-defective MuLV containing the oncogenes *fes*, *abl*, H-*ras*, *bas*, *src*, *erb*B, and Cas NS-1, but not on T cell lymphomas, or myelomonocytic leukemias or erytholeukemias. By comparison, none of the early B-lineage lymphomas induced by v-*myc* or involving translocated c-*myc* sequences bear the antigen.

Materials and Methods

Cells. Cell lines containing *abl*, *fes*, *ras* (including Ha-*ras*, Ki-*ras*, and *bas*), *src* and *erb*B were produced by infection of fetal liver or bone marrow cells in vitro with Moloney or 4070A pseudotypes, or with replication-defective viruses, as described (4). Cell lines induced by Friend spleen focus-forming virus (F-SFFV) and Cas NS-1 were adapted to growth in vitro from primary tumors or passaged in vivo. Five in vitro–adapted cell lines from A-MuLV plasmacytoid lymphosarcoma (ABPL) tumors with 5′-truncated *myb* genes have been shown to be of myelomonocytic origin (G. L. C. Shen-Ong, K. L. Holmes, H. C. Morse, III, manuscript submitted for publication). One IL-3-dependent cell line with a 3′-truncated *myb* gene and myeloid characteristics has been described (15).

Viruses. Virologic characteristics of CWD/Ag1 (12), Bio.NZN (13), and NFS.*Akv-1* (14) mice are published. Data concerning the structure and transforming activities of J-3, J-5, J-2, and J-1 recombinant viruses are presented elsewhere (6). Northern blot hybridization studies of mRNA produced by Cas NS-1–transformed pre-B cells indicate that the Cas NS-1 transforming gene is unrelated to *abl*, *myc*, *myb*, Ki-*ras*, *mos*, *fos*, *raf*, *erb*B, *fms*, *sis*, *Pim*-1, *src*, p53, *ets*, *fes*, or *bcl*-2 (11).

Characterization of Tumors. All the tumors were characterized as to cell lineage and state of differentiation by flow microfluorometry (FMF) studies using a large panel of antibodies to cell surface antigens (4, 8). Gross and histological studies were carried out on tissues obtained at autopsy from mice.

Polyacrylamide Gel Electrophoresis. Immunoprecipitation and SDS-PAGE have been described before (9).

Results

To determine if expression of gp160^{6C3} is limited predominantly to pre-B cells induced by A-MuLV, we analyzed a large series of hematopoietic tumors for expression of this antigen (Table I). All the tumors diagnosed as T cell lymphomas were Thy-1$^+$ and variably expressed Ly-1, Ly-2, L3T4, or Tla. Pre-B cell lymphomas were all surface immunoglobulin (sIg)-negative and Lyb-2$^+$, and consisted mostly of large pre-B cell tumors. The B cell lymphomas were uniformly sIg$^+$ and all plasmacytomas were PC-1$^+$. The diagnosis of erythroleukemia was made primarily on histologic grounds, as these tumors are predominantly null for the antibodies used in the FMF studies (8). Finally, all the myelomonocytic tumors were Mac-1$^+$, Ly-17$^+$ (alleles of Ly-17 define polymorphisms of the Fcγ-R) and most were Ia$^+$. Studies of these tumors for expression of the 6C3 antigen showed that, with one exception, only tumors of the B lymphocyte lineage were 6C3$^+$. Thus, 53 of 59 (90%) pre-B cell lymphomas and 15 of 31 (48%) B cell lymphomas were 6C3$^+$, whereas none of the 44 T cell lymphomas or 11 erythroleukemias expressed this antigen (Table I). The only one of 13 myelomonocytic tumors found to be 6C3$^+$ was the macrophage cell line, P388D1. This

TABLE I

Expression of 6C3 Antigen on Hematopoietic Tumor Cells

Virus	Transduced oncogene	Frequency of 6C3$^+$ tumors					
		T	Pre-B	B	PCT	Erythroid	Myelo-monocytic
A. Virus-induced primary tumors							
Cas NS-1	Cas NS-1		12/13				
Abelson	*abl*		3/3	1/1			
J-3	*myc*	0/4	0/1	0/3	0/10		0/5
J-5	*myc*	0/9	0/1	0/1			
J-2	*raf* + *myc*	0/4		0/6		0/5	
J-1	*raf*					0/5	
Moloney	—	0/9					
Cas NS-6	—	0/8		1/1			0/1
SL3-3	—	0/5					
Gross passage A	—	0/1					
C2S	—	0/2					
B. Primary spontaneous tumors of high-virus mice							
CWD	—	0/1	1/1	10/14			
B10.NZW	—		1/1				
NFS.*Akv-1*	—	0/1					
C. Cell lines induced by viruses containing oncogenes or pristane-induced PCT							
	abl		7/7	1/1			
	ras		7/9				
	fes		3/3				
	src		6/6				
	erbB		5/5				
	myc				0/3		
	Cas NS-1		1/1				
	F-*SFFV*					0/1	
	myb						0/6
D. Cell lines with no oncogene defined							
			7/8	2/4			1/1
Total:		0/44	53/59	15/31	0/13	0/11	1/13

Figures indicate the number of 6C3$^+$ tumors (T, T cell lymphoma; pre-B, pre-B cell lymphoma; B, B cell lymphoma; PCT, plasmacytoma) over the total number of tumors examined.

apparent discrepancy may be explained by the recent finding that this tumor has many characteristics of cells in the B lymphocyte lineage, including expression of Lyb-2 and Ly-5 (B220), and rearrangements of Ig heavy and light chain genes (16).

Almost all of the pre-B cell lymphomas induced by retroviruses containing the Cas NS-1 transforming gene, *abl*, *ras* (including H-*ras*, Ki-*ras*, and *bas*), *fes*, *src*, or *erb*B were 6C3$^+$. By comparison, none of 20 pre-B, B cell, or plasmacytic tumors induced by viruses expressing v-*myc* were positive for this antigen (Table I). In addition, more than 10 pre-B lymphomas of mice transgenic for c-*myc* driven by Ig enhancer sequences (17) have been examined, and none express 6C3 antigen (W. Y. Langdon, A. W. Harris, J. M. Adams, and S. Cory, personal communication). It is known that ~60% of these tumors have pre-B cell characteristics (17). This indication that deregulated expression of *myc* is incompatible with high levels of 6C3 expression is supported indirectly by two findings: First, the c-*myc* genes of all seven 6C3$^+$ pre-B and B cell lymphoma cell lines included

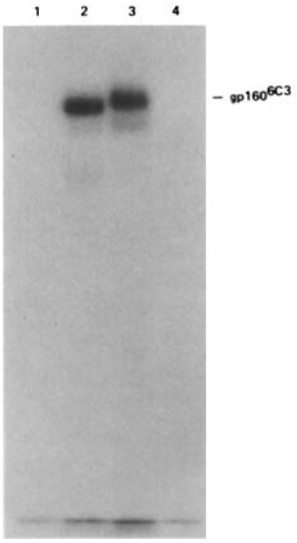

FIGURE 1. Cells from the Cas NS-1-induced pre-B cell lymphoma, NFS-467 (lanes *1* and *2*), and the Abelson virus–induced pre-B cell lymphoma L1-2/23 (lanes *3* and *4*) were surface labeled with ^{125}I using lactoperoxidase (9), lysed, and reacted with mAb 6C3 (lanes *2* and *3*) (9, 10), or isotype-matched (rat IgG2a) mAb R7D4 (10). The immune complexes were precipitated with goat anti–rat Ig and analyzed by SDS-PAGE (8% gel) under reducing conditions (10).

in Table I *D* are not rearranged and these cells contain c-*myc* transcripts at levels consistent with their stages of differentiation (11; J. F. Mushinski, W. F. Davidson, H. C. Morse III, unpublished observations). Second, three plasmacytomas with translocated c-*myc* loci and elevated *myc* RNAs were 6C3$^-$.

Comparisons of ^{125}I-labeled cell surface proteins precipitated from a pre-B cell lymphoma (NFS-467) induced by the Cas NS-1 transforming virus and a pre-B cell lymphoma (L1-2) induced by A-MuLV showed that the predominant proteins reactive with 6C3 were of 159 and 160 kD, respectively (Fig. 1). It is likely that the 6C3-reactive antigens present on cells transformed by A-MuLV and other replication-defective or -competent viruses are very similar, with molecular mass differences possibly reflecting variations in glycosylation of a common protein.

Discussion

These results demonstrate that expression of gp160^{6C3} antigen is often associated with neoplastic transformation of early B lymphocytes. These results support our earlier findings that expression of 6C3 antigen by tumor cells is confined to the B lineage (9). Additionally, we present evidence here that the 6C3 antigen is expressed in early B leukemias that have been induced with a variety of "cytoplasmic" oncogenes but never with the "nuclear" *myc* oncogene. These findings suggest that alternative 6C3$^+$ and 6C3$^-$ pathways may be involved in the transformation of B lineage cells with otherwise indistinguishable phenotypes. There is no evidence to suggest that mAb 6C3 defines leukemias of a B sublineage distinct from a sublineage transformed by *myc*.

If the assumption is made that transformation of normal B lineage cells, like other primary cells (2), requires the interaction of at least two kinds of oncogene products (nuclear and cytoplasmic), there are several possible models for coexpression of the 6C3 antigen with a cytoplasmic but not a nuclear oncogene. For example, functions provided to normal cells by the gene encoding the 6C3 antigen may be similar to those provided by *myc*. The observation that this antigen is expressed on the surface of tumor cells is not incompatible with the suggestion that its location in normal cells is nuclear, but that like the simian virus 40 large T antigen, both nuclear and surface membrane functions can contribute to the immortalization and transformation of cells (18). In a second model, aberrant expression of $gp160^{6C3}$ on the cell surface may drive the expression of a nuclear oncogene that can complement the different cytoplasmic viral oncogenes for transformation of B lineage cells. In either model, the provision of a nuclear signal via deregulated *myc* expression would preclude the requirement to engage either the nuclear or surface membrane functions postulated for the 6C3 antigen. In a third model, different cytoplasmic oncogenes may indirectly induce or augment expression of the gene encoding $gp160^{6C3}$ in a way unrelated to any contribution of the antigen to the induction or maintenance of the transformed phenotype. While several other models are also possible, each of the above leads to a specific, testable prediction.

Summary

The monoclonal antibody 6C3 was used to test a wide variety of murine hematopoietic neoplasms for cell surface expression of a 160 kD glycoprotein ($gp160^{6C3}$) previously shown to be expressed by neoplastic pre-B and some B lymphocytes transformed by Abelson murine leukemia virus (A-MuLV). This antigen was expressed on many pre-B and B cell lymphomas, but not on A-MuLV-transformed fibroblasts, T cell lymphomas, or myelomonocytic leukemias. $gp160^{6C3}$ was expressed by most early B-lineage spontaneous tumors, and early B tumors induced by replication-defective MuLV-containing oncogenes the products of which are associated with the cytoplasmic aspect of the plasma membrane, i.e., *fes*, *abl*, H-*ras*, *bas*, *src*, *erb*B, and Cas NS-1. By comparison, none of the early B lineage lymphomas induced by the "nuclear" oncogene avian v-*myc* MuLV, or arising in mice transgenic for a murine c-*myc* gene, or later B cell lineage stages bearing translocations of the c-*myc* locus expressed this antigen.

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