EXPRESSION OF THE 6C3 ANTIGEN ON MURINE HEMATOPOIETIC NEOPLASMS

Association with Expression of abl, ras, fes, src, erbB, and Cas NS-1

Oncogenes but Not with myc

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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), erbB (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 (gp1606C3) that is not a product of genes in the A-MuLV complex (9, 10); gp1606C3 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

This work was supported in part by contract NO1AI-22673 to Microbiological Associates, Inc., Bethesda, MD, and by grants from the American Cancer Society (CD 227) and the National Institutes of Health (CA-32031). G. Tidmarsh is a predoctoral fellow supported by grant CA-09302 from the National Cancer Institute. Address correspondence to H. C. Morse, Building 7, Room 304, National Institutes of Health, Bethesda, MD 20892.
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, gp1606C3 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, erbB, and Cas NS-1, but not on T cell lymphomas, or myelomonocytic leukemias or erythroleukemias. 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 erbB 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/Agl (12), Bio.NZN (13), and NFS.Aku-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, erbB, fms, sis, Pim-1, src, p53, ets, fes, or bel-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 gp1606C3 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 Fcy-R) and most were la+. 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
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 (13-220), 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 erbB 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

| 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            | 12/15 |
| Abelson       | abl                 | 5/5  | 1/5  |    |     |           |                |
| J-3           | myc                 | 0/4  | 0/5  | 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  |     |           |                |
| SL-3-3        | —                   | 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       | —                   | 0/1  | 1/1  |     |     |           |                |
| NFS.Akt-1     | —                   |     | 0/1  |     |     |           |                |
| C. Cell lines induced by viruses containing oncogenes or pristane-induced PCT |                     |    |       |    |     |           |                |
| abl           |                     | 7/7  | 1/1  |     |     |           |                |
| ras           |                     | 7/9  |       |     |     |           |                |
| fes           |                     | 5/5  |       |     |     |           |                |
| src           |                     | 6/6  |       |     |     |           |                |
| erbB          |                     | 5/5  |       |     |     |           |                |
| myc           |                     |     | 0/3  |     |     |           |                |
| Cas NS-1      |                     | 1/1  |     |     |     |           |                |
| F-SSPV        | myb                 |     | 0/1  |     |     |           |                |
| 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.
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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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., \textit{fes}, \textit{abl}, \textit{H-ras}, \textit{bas}, \textit{src}, \textit{erbB}, 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.

We gratefully acknowledge the expert secretarial assistance of Ms. Susan Grove and Janice Mason in the preparation of this manuscript.

Received for publication 14 October 1986.

References

1. Klein, G., and E. Klein. 1985. Evolution of tumors and the impact of molecular oncology. \textit{Nature (Lond.)}. 315:190.
2. Land, H., L. F. Parada, and R. A. Weinberg. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. \textit{Nature (Lond.)}. 304:596.
3. Rosenberg, N. E. 1981. Abelson leukemia virus. Curr. Top. Microbiol. Immunol. 95:101.
4. Holmes, K. L., J. H. Pierce, W. F. Davidson, and H. C. Morse, III. 1986. Murine hematopoietic cells with pre-B or pre-B/myeloid characteristics are generated by in vitro transformation with retroviruses containing fes, ras, abl, and src oncogenes. J. Exp. Med. 164:443.
5. Shen-Ong, G. L. C., E. J. Keath, S. P. Piccoli, and M. D. Cole. 1982. Novel myc oncogene RNA and abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell. 51:443.
6. Morse, H. C., III, J. W. Hartley, T. N. Fredrickson, R. A. Yetter, J. L. Cleveland, C. Majumdar, and U. R. Rapp. 1986. Tumors of newborn NFS/N mice infected with murine retroviruses containing v-myc. Curr. Topics Micro. Immunol. 132:17.
7. Pierce, J. H., A. Gazit, P. P. Fiore, M. Kraus, C. Y. Pennington, K. L. Holmes, W. F. Davidson, H. C. Morse, III, and S. A. Aronson. 1986. Mammalian cell transformation by a recombinant murine retrovirus containing the avian erythroblastosis virus erbB gene. Curr. Top. Micro. Immunol. 132:55.
8. Holmes, K. L., W. Y. Langdon, T. N. Fredrickson, R. L. Coffman, P. M. Hoffman, J. W. Hartley, and H. C. Morse, III. 1986. Analysis of neoplasms induced by Cas-Br-M MuLV tumor extracts. J. Immunol. 137:679.
9. Pillemer, E., C. Whitlock, and I. Weissman. 1984. Transformation-associated proteins in murine B cell lymphomas that are distinct from Abelson gene products. Proc. Natl. Acad. Sci. USA. 81:4434.
10. Tidmarsh, G. F., M. O. Dailey, C. A. Whitlock, E. Pillemer, and I. L. Weissman. 1985. Transformed lymphocytes from Abelson-derived mice express levels of a B lineage transformation-associated antigen elevated from that found on normal lymphocytes. J. Exp. Med. 162:1421.
11. Mushinski, J. F., W. F. Davidson, and H. C. Morse, III. 1987. Cancer Invest. In press.
12. Angel, J. M., and H. G. Bedigian. 1984. Expression of murine leukemia viruses in B-cell lymphomas of CWD/Agl mice. J. Virol. 52:691.
13. Morse, H. C., III, R. A. Yetter, J. H. Stimpfling, O. M. Pitts, T. N. Frederickson, and J. N. Hartley. 1985. Greying with age in mice: relation to expression of murine leukemia viruses. Cell. 41:439.
14. Frederickson, T. N., H. C. Morse, III, and W. P. Rowe. 1984. Spontaneous tumors of NFS mice congenic for ecotropic murine leukemia virus induction loci. J. Natl. Cancer Inst. 73:521.
15. Holmes, K. L., E. Palaszynski, T. N. Frederickson, H. C. Morse, III, and J. N. Ihle. 1985. Correlation of cell-surface phenotype with the establishment of interleukin 3-dependent cell lines from wild-mouse murine leukemia virus-induced neoplasms. Proc. Natl. Acad. Sci. USA. 82:6687.
16. Bauer, S. R., K. L. Holmes, H. C. Morse, III, and M. Potter. 1986. Clonal relationship of the lymphoblastic cell line P388 and the macrophage cell line P388D1 as evidence by immunoglobulin gene rearrangement and expression of cell surface antigens. J. Immunol. 136:4695.
17. Adams, J. M., A. W. Harris, W. Y. Langdon, C. A. Pinkert, R. L. Brinster, R. D. Palmiter, W. S. Alexander, M. Graham, and S. Cory. 1986. C-myc-induced lymphomagenesis in transgenic mice and the role of Pet-1 locus in lymphoid neoplasia. Curr. Top. Microbiol. Immunol. 132:1.
18. Lanford, R. E., and J. S. Butel. 1982. Intracellular transport of SV40 large tumor antigen: a mutation which abolishes migration to the nucleus does not prevent association with the cell surface. Virology. 119:169.