Brief Definitive Report

EXPRESSION OF A MONOCLONAL ANTIBODY-DEFINED, B-LINEAGE TRANSFORMATION ANTIGEN SPECIFICALLY IDENTIFIES ABELSON-DISEASED ANIMALS

Genetically Determined Resistance to Abelson Murine Leukemia Virus Acts before Induction of gp160^{6C3}

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Studies examining Abelson/Moloney murine leukemia virus [A-MuLV(Mo)]-induced leukemia in mice have revealed that there is a marked variation in the susceptibility to disease among commonly used inbred strains of mice (1). While nearly all strains examined develop characteristic disease symptoms after injection of newborn mice with the virus, most mouse strains are resistant when inoculated as adults. For example, C3H/HeN and C57BL/6 mice are almost completely resistant to tumor induction as adults, BALB/c and DBA/2 mice are among the most susceptible strains, and A/J mice show an intermediate- to low-level incidence. Although two susceptibility loci have been identified using inbred and hybrid mouse strains, the mechanism of tumor resistance remain unknown.

We have previously described the appearance of a cell surface glycoprotein, gp160^{6C3}, at high levels on virtually all in vitro (2) and in vivo (3) Abelson MuLV (A-MuLV)-induced B lineage tumors. After in vivo infection with A-MuLV(Mo), susceptible hosts develop a lymphoid replacement of the bone marrow, splenomegaly, and often lymphadenopathy, all due to proliferation and spread of malignant cells (4). We have shown (3) that the antigen 6C3Ag marks these cells and that the 6C3Ag$^+$ cells score as transformed clonogenic cells in an in vitro agar colony assay. Because it was possible that one or more A-MuLV(Mo) resistance genes acts before or after expression of 6C3Ag on infectecd cells, we undertook an examination of the mouse strain distribution of expression of 6C3Ag on cells of A-MuLV(Mo)-infected hosts.

In this report we show that strains that are not susceptible to disease when injected as adults do not develop cells expressing high levels of 6C3Ag. In fact, expression of 6C3Ag by bone marrow, lymph node, or spleen cells is confined to diseased animals in susceptible strains. These data support our conclusion that high-level 6C3Ag expression is specific for the population of lymphocytes transformed by the virus in diseased animals, and supports the hypothesis that

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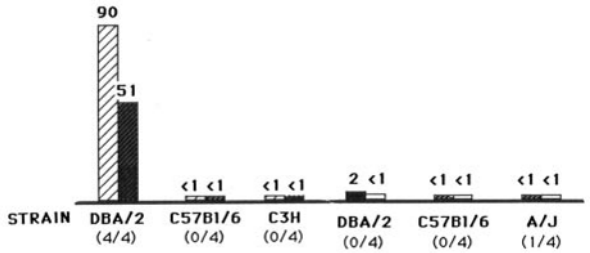

FIGURE 1. Percentage of 6C3Ag^{hi} bone marrow cells in various strains of inbred mice exposed to A-MuLV(Mo). At 3.5 wk (*left*) or 8 wk (*right*) after birth, various strains of inbred mice were injected i.v. with a lethal dose of A-MuLV(Mo). Mice from each group were killed when disease symptoms became apparent (young DBA/2 mice), or at 4 wk (striped bars), 8 wk (shaded bars), or 12 wk (open bars) (in the case of the mice injected at 8 wk of age). Numbers above each bar indicate the percentage of cells that stain with mAb 6C3 above that found in bone marrow of uninjected mice. Numbers in parenthesis below strain designation represent the number of animals that developed disease symptoms or died, and the number of animals injected. One A/J mouse died before an analysis could be done, therefore that mouse is not represented in this data.

expression of the antigen-bearing proteins at high levels may be necessary for growth of A-MuLV(Mo) tumors in vivo.

Materials and Methods

Mice. All inbred mice were bred at mouse colonies at Stanford University. The recombinant inbred mice CxB.H and CxB.G were purchased from The Jackson Laboratories, Bar Harbor, ME.

Reagents. mAb 6C3 has been described previously (2, 3). Goat anti–rat Ig was purified from serum purchased from PelFreez (Rogers, AR) by protein A chromatography, and was subsequently conjugated to FITC by the method of Goding (5).

Staining. Immunofluorescent stainings and FACS analysis were carried out as previously described (3).

Virus. Virus was harvested from confluent, clonal Moloney MuLV (M-MuLV)-producing cells originally provided by O. N. Witte (Molecular Biology Institute, University of California, Los Angeles). This virus was then used to rescue the A-MuLV genome from a confluent culture of ANN-1 cells, a procedure resulting in A-MuLV(Mo).

Results

6C3Ag^{hi} Cells Are Found Only in Bone Marrow Cell Suspensions from A-MuLV(Mo)-infected Mice Known to Be Susceptible to Disease. To test the correlation between Abelson disease and the appearance of cells expressing high levels of 6C3Ag (6C3Ag^{hi} cells), we injected a high–disease susceptibility mouse strain (DBA/2) and several low-susceptibility mouse strains (C3H/HeN, C57BL/6, A/J) with A-MuLV(Mo) (1). In addition, we injected two different ages of DBA/2 and C57BL/6 mice; 3.5 wk and 8 wk after birth. Fig. 1 (*left*) shows that only DBA/2 mice injected 3–4 wk after birth develop Abelson disease symptoms and that only 3–4-wk-old DBA/2 mice developed 6C3Ag^{hi} bone marrow cells. 8-wk-old DBA/2 mice did not succumb to the virus and did not develop a significant percentage of 6C3Ag^{hi} cells in bone marrow (Fig. 1, *right*). A low percentage (2%) of 6C3Ag^{hi} cells were found in one older DBA/2 mouse, which could reflect incipient disease. From this and other (3) experiments, there appears to be a

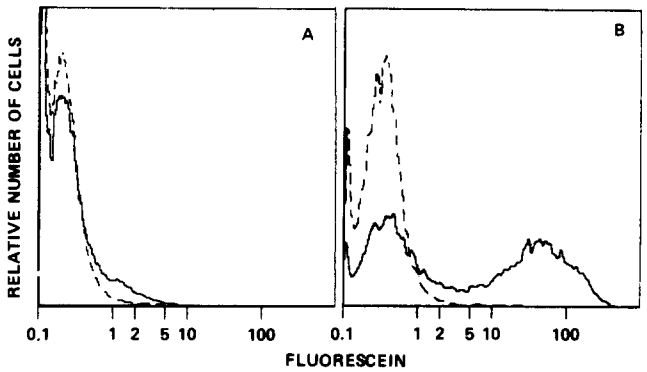

FIGURE 2. Appearance of 6C3Ag^{hi} cells corresponds to Abelson disease susceptibility in recombinant inbred strains. CxB.G (*A*) and CxB.H (*B*) mice were injected at 3.5 wk of age, and each was killed 4 wk later when the CxB.H mice showed signs of Abelson disease. The CxB.G mouse appeared normal, with none of the disease symptoms obvious in the CxB.H mouse analyzed in *B*.

complete correlation between disease and high-level expression of 6C3Ag on a high percentage of bone marrow cells.

6C3Ag^{hi} Cells Are Found Only in Recombinant Strains that Succumb to Abelson Disease. As a further test of the hypothesis that 6C3Ag^{hi} expression is completely associated with the Abelson disease state and that genetic restrictions to A-MuLV(Mo) leukemogenesis prevent appearance of 6C3Ag^{hi} cells, A-MuLV(Mo) was injected i.v. into disease-resistant and -susceptible recombinant inbred strains. These animals were derived from inbreeding pairs of (C57BL/6 × BALB/c)F_2 (CxB) mice. As published (1), CxB.G is resistant to tumor induction while CxB.H is relatively susceptible (1). 3 wk after injection, CxB.H mice begin to develop signs of tumor, whereas CxB.G appeared normal. At this time, one injected animal of each strain was killed, and a bone marrow cell suspension was made and stained with mAb6C3. Fig. 2*A* is the FACS profile derived from analysis of immunofluorescent staining of a CxB.G mouse, while Fig. 2*B* represents the profile from the diseased CxB.H strain; only the CxB.H (susceptible) mouse showed 6C3Ag^{hi} cells. Thus the appearance of 6C3Ag^{hi} cells is predictive of the diseased state and genetic susceptibility to the disease. In total, three CxB.H mice with symptoms of Abelson disease were analyzed for 6C3Ag, and all display this pattern of expression (Fig. 2*B*). With a longer latency, some of the CxB.G mice developed symptoms of Abelson disease. When analyzed for 6C3Ag, bone marrow, lymph node, and spleen cells from such mice were found to express high levels of the antigen (Fig. 3). Therefore, we have not yet found a mouse with Abelson disease that lacks a population of cells expressing high levels of 6C3Ag, regardless of genetic resistance or susceptibility.

Discussion

Genetically linked susceptibility to A-MuLV(Mo) leukemogenesis in vivo appears to involve two independent loci, either of which modify the development of leukemia rather than cause regression of established tumors (1). Thus these restrictions probably limit events before the emergence of frankly leukemic cells.

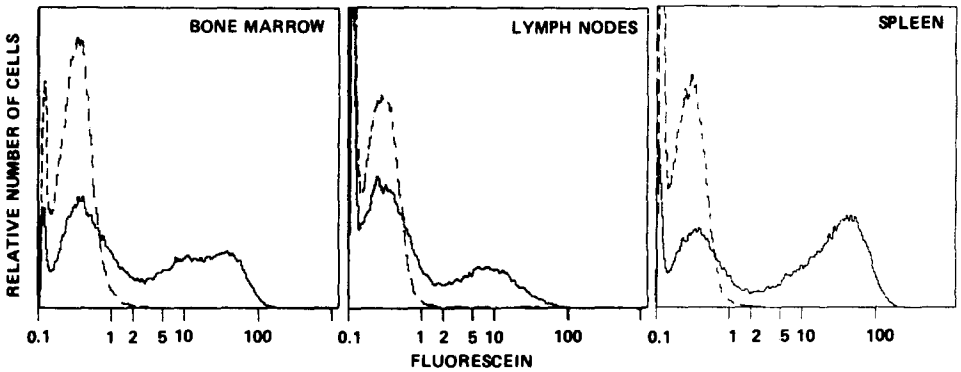

FIGURE 3. The appearance of 6C3Ag^{hi} cells in CxB.G mouse lymphoid tissues correlates with disease symptoms. 8 wk after i.v. injection of A-MuLV(Mo) into CxB.G mice some developed typical disease symptoms. This observed longer latency is consistent with that observed previously (1). A mouse with symptoms but still alive was killed and all tissues were stained with mAb 6C3. This figure represents the FACS profiles derived from bone marrow, lymph node, and spleen cell suspensions. There was no detectable staining in any secondary lymphoid organ from animals not injected with the virus (data not shown). In addition, no 6C3Ag^{hi} cells were detected in the thymus of the animal represented here.

After integration of A-MuLV into host cells, viral-encoded *gag-abl* fusion protein is expressed as a cytoplasmic, plasma membrane–associated tyrosine kinase. This expression of the v-*abl* oncogene is not sufficient for full neoplastic transformation in vitro in liquid cultures, as proliferation of cells expressing *gag-abl* is dependent on bone marrow–derived stromal feeder cells characteristic of the Whitlock-Witte in vitro pre-B culture system (6). These *gag-abl*$^+$ cells are 6C3Ag$^-$ at this stage of development (2). Fully malignant, feeder layer–independent pre-B leukemias emerge; these cells either are 6C3Ag^{hi} or give rise to progeny that become 6C3Ag^{hi} (G. F. Tidmarsh, manuscript in preparation). Incidentally, the cloned stromal cells from Whitlock-Witte pre-B cultures that support growth of premalignant *gag-abl*$^+$ *6C3Ag*$^-$ cells are themselves synthesizing and expressing high levels of cell surface 6C3Ag, providing evidence that the expression of 6C3Ag is linked to growth control of both normal and preneoplastic pre-B cells (Whitlock, Tidmarsh, Muller-Sieburg, and Weissman, manuscript submitted for publication). Herein, we sought evidence as to whether genetically limited susceptibility to A-MuLV(Mo)-induced pre-B leukemia acted before or after the stage of 6C3Ag expression. Mouse strains genetically resistant to A-MuLV(Mo) develop neither 6C3Ag$^+$ bone marrow cells nor Abelson leukemias, consistent with the model that restriction occurs before 6C3Ag expression at some earlier stage of neoplastic progression, perhaps after *gag-abl* expression in infected cells.

It has recently (7) been shown that several other viral oncogenes, when expressed as cytoplasmic, membrane-associated phosphokinases, can induce pre-B leukemias after infection of bone marrow cells in vitro with a murine retrovirus vector (7); these include *abl, src, fes, ras,* and *erb B.* These pre-B tumors also express high levels of surface 6C3Ag (H. C. Morse, K. L. Holmes, W. Y. Langdon, T. N. Fredrickson, J. W. Hartley, J. H. Pierce, G. F. Tidmarsh, M. O. Dailey, I. L. Weissman, manuscript submitted for publication; and J. Adams, W. Langdon, A. Harris, and S. Cory, personal communication). It will be interesting to test

whether any (or all) of these are also genetically restricted as leukemogens in the mouse strains resistant to A-MuLV(Mo). In contrast to these phosphokinase oncogenes, v-*myc*-containing vectors induce and c-*myc*-transgenic mice with immunoglobulin H chain enhancer sequences develop marrow-derived pre-B leukemias, and none of these are $6C3Ag^+$. It will be interesting to see whether these tumors also differ from the phosphokinase oncogene leukemogens by the above-described mouse strain genetic restrictions.

In these and previously published experiments (2, 3) there is an absolute correlation between induction of Abelson disease and expression of cell surface 6C3Ag on presumably malignant cells. This is consistent with the hypothesis that expression of high levels of 6C3Ag on pre-B/early B cells is a marker for the malignant stage of neoplastic progression after A-MuLV(Mo) infection, and that proteins bearing 6C3Ag are directly involved in expression of the malignant phenotype in these cells.

The observation that 6C3Ag is undetectable in secondary lymphoid organs in the absence of disease, yet is readily detectable in these organs when they are lymphomatous makes the antigen an excellent marker for diagnosis of disease. Current efforts are directed toward developing polyclonal antisera and mAbs that recognize an analogous molecule on human tumors. Identification of such a determinant may be of potential benefit for diagnosis and/or therapy of pre-B cell or non-B/non-T cell leukemias and lymphomas in man.

Summary

Mice genetically susceptible or genetically resistant to the leukemogenic effects of A-MuLV(Mo) were tested for their expression of the B-lineage neoplastic transformation–associated antigen, 6C3Ag. Genetically resistant inbred strains and recombinant inbred lines developed neither cells expressing high levels of 6C3Ag ($6C3Ag^{hi}$) in their hematolymphoid tissues nor Abelson leukemias. Genetically susceptible inbred strains and recombinant inbred lines developed high percentages of $6C3Ag^{hi}$ hematolymphoid cells concomitant with development of Abelson leukemias and lymphomas. Thus the genetically-determined resistance to A-MuLV(Mo) leukemogenesis appears to act at some step(s) after virus infection but before the stage of malignant progression, which is marked by 6C3Ag expression.

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