Endogenous RNA tumor viruses are activated during chemical induction of murine plasmacytomas

(pristane/murine leukemia virus/myeloma/chemical carcinogenesis)

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ABSTRACT Plasmacytomas are induced in BALB/c mice by the intraperitoneal injection of pristane (2,6,10,14-tetra-methylpentadecane) after a latent period of six months and more [Anderson, P. N. & Potter, M. (1969) Nature 222, 994-995]. Spleen cells, mesenteric lymph node cells, thoracic lymph node cells, and peritoneal exudate cells were prepared from pristane-treated and control uninjected BALB/c mice during the course of a 10-month period, and these cell suspensions were tested for the release of infectious murine leukemia viruses. Endogenous ecotropic and xenotropic murine leukemia viruses were expressed in pristane-treated mice during the latter part of the tumor induction period. In those cell populations in which transformed plasma cells appear, namely, peritoneal exudate cells and thoracic lymph node cells. The significance of preferential expression of both ecotropic and xenotropic murine leukemia virus in target cell populations following the administration of a carcinogen is discussed in terms of the possible formation of an oncogenic variant virus.

Plasmacytomas are readily induced in the inbred BALB/c mouse by intraperitoneal implantation of a variety of physical and chemical agents (1). Such agents cause granulomata to form on the peritoneal surface (1), and it is in these granulomata that tumors develop (2). Because the tumors characteristically produce monoclonal immunoglobulins, this experimental mouse model has been extensively studied (3) since it was described in 1959 (4).

Considerable evidence has accumulated which points to an association between induced murine plasmacytomas and RNA tumor viruses. Intracutaneous type A particles have been repeatedly observed by electron microscopy in primary and transplanted plasmacytomas (1). Although such particles possess many of the biochemical characteristics of RNA tumor viruses (5, 6), their biological activity remains unknown (1, 5, 6). Budding and extracellular type C particles, which are the etiological agent in a number of vertebrate lymphoid tumors (7), have also been described in primary, transplanted, and tissue-culture-adapted plasmacytomas (1). Three serologically distinct populations of type C particles have been demonstrated in primary and transplanted plasmacytomas by immunoelectron microscopy (8), one population bearing Gross-type murine leukemia viral envelope antigens (VEAs), another population bearing a "unique" type-specific viral envelope antigen (xVEA), the third population bearing neither of these two types of VEAs. xVEA* type C particles have been termed murine myeloma-associated viruses (9) and have also been described in spontaneously transformed clones derived from BALB/c 3T3 mouse embryo cells (9). The host range of a number of xVEA+ viruses isolated from different sources has been determined and various tropisms have been found. Thus, N-tropic [growing preferentially in mouse cells of the Fo-1⁰ genotype (10)], B-tropic [growing preferentially in mouse cells of the Fo-1⁰ genotype (10)], and xenotropic [growing in heterologous cells, but not in mouse cells (11)] xVEA+ isolates have all been described (9, 12). In addition, type C viruses produced by two different tissue-culture-adapted plasmacytoma cell lines have been reported to have NB-tropism (13, 14), i.e., similar growth in Fo-1⁰ and Fo-1⁰ mouse cells (10).

What is not known is the significance of this mixed population of murine leukemia viruses (MuLVs). Are they merely innocuous passengers, activated in established plasmacytomas, or do they include endogenous oncogenic MuLV variants generated prior to tumor development and etiologically involved in tumor induction? As an initial approach to these questions, we undertook a study of endogenous MuLV expression in BALB/c mice injected with pristane, a potent plasmacytoma-inducing oil. We here report that both ecotropic and xenotropic endogenous MuLV are expressed during tumor induction in those sites where foci of transformed plasma cells subsequently appear.

MATERIALS AND METHODS

Mice. Five-week-old BALB/cAn female mice were obtained from Charles River Breeding Laboratory, North Wilmington, MA, placed six to a cage under a filtertop, and housed in our animal care facility.

Pristane. This chemically pure oil (2,6,10,14-tetramethylpentadecane) was obtained from Aldrich Chemical Company, Metuchen, NJ. Mice were given an intraperitoneal injection of 0.5 ml of pristane at 6 weeks, at 14 weeks, and at 23 weeks of age.

Preparation of Cell Samples. Cell suspensions were prepared from spleen, mesenteric lymph node, and thoracic lymph nodes in Eagle's minimum essential medium, containing 100 units of penicillin and 100 µg of streptomycin per ml. The tissue was finely minced with scissors, the fragments were gently pressed through tantalum gauze, and final cell dispersion was achieved by repeated aspiration with a Pasteur pipette. Peritoneal exudate cells were obtained by collecting ascitic fluid when present and by flushing out the peritoneal cavity with medium. In a few instances, peritoneal exudate cells were collected from live mice by tapping the peritoneal cavity after 10 ml of medium had been injected intraperitoneally. Spleen, mesenteric lymph node, and thoracic lymph node cells were washed once; peritoneal exudate cells were washed twice to remove most of the pristane. All cell suspensions were adjusted to a final concentration of 20 × 10⁶ cells per ml.

MuLV Assays. Cells in 0.1-ml aliquots were plated as in-

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fectious centers on both BALB/cN and NIH Swiss mouse embryo cells and assayed for B-tropic and N-tropic MuLV, respectively, by using the UV-TC test (15), modified as previously described (16). Both types of MuLV were assumed to be present in an individual cell suspension if the number of infectious centers on BALB/cN mouse embryo cells and that on NIH Swiss embryo cells showed less than a 50-fold difference.

Cells were tested for xenotropic MuLV by using Peebles’ focus-induction assay (17). The indicator cell is a morphologically flat revertant of mink lung cells nonproductively infected with Moloney sarcoma virus (MSV). Superinfection of these MSV-infected cells (term S+L- mink cells) by replicating, but usually nontransforming, mammalian viruses induces foci of transformed cells. S+L- mink cells were obtained from Janet W. Hartley. S+L- cells (2 × 10⁵) in 4 ml of Dulbecco’s high-glucose modified Eagle medium (supplemented with 10% heat-inactivated fetal calf serum and 100 units of penicillin and 100 µg of streptomycin per ml) were seeded into 60 × 15 mm plastic petri dishes. The following day (day 1), the S+L- cells were pretreated with 25 µg of DEAE-dextran per ml, then incubated with 2 × 10⁵ test cells. Because foci of transformed cells were usually not induced by either the experimental or control cell samples until the indicator cells had been blind-passaged at least once, the S+L- cells were routinely passaged on day 8, again on day 15, and examined for presence of foci at low magnification (×7) on day 22. The cultures were fed twice weekly.

RESULTS

Experimental Model. Anderson and Potter reported the induction of plasmacytomas in BALB/cAn mice injected intraperitoneally with a chemically pure oil, pristane (2,6,10,14-tetramethylpentadecane) (18). Pristane causes oil granuloma on the peritoneal surface and in the draining thoracic lymph nodes and it is in these granuloma that foci of transformed plasma cells appear (2, 18). We followed Anderson and Potter’s protocol (18) and gave BALB/cAn female mice three intraperitoneal injections of 0.5 ml of pristane, at 2-month intervals, starting at 6 weeks of age. Tissue samples were obtained from groups of pristane-treated and un.injected BALB/cAn female mice at 9 weeks, 17 weeks, 22 weeks, 28 weeks, 32 weeks, 39 weeks, and 50 weeks of age. Cell suspensions were prepared and tested for infectious MuLV. Because 59 of the 72 experimental mice and 40 of the 46 control mice were killed at the time of sampling, it was not possible to compute the tumor incidence. However, the sequence of events in our pristane-treated mice mirrored that in Anderson and Potter’s series, in which the first documented plasmacytoma was noted 26 weeks after the initial pristane injection and tumors developed with increasing frequency thereafter (18).

Ecotropic MuLV Expression. Infectious ecotropic MuLV was not detected in any of the 19 cell samples from pristane-primed mice or in 18 cell samples from control mice examined at 9 weeks of age (Figs. 1 and 2). At 17 weeks and at 22 weeks, infrequent ecotropic MuLV isolations of low titer were made (from 2 of 18 pristane-primed mice and 3 of 18 control mice, representing 3 of 60 and 3 of 48 virus-positive cell samples, respectively). From 28 to 50 weeks, however, striking differences developed between the experimental and control mice. The percentage of ecotropic virus-positive mice rose sharply in the experimental group, as did the number of virus-positive cell samples. Thus, 24 of 36 (67%) pristane-primed mice and 54 of 105 (51%) cell samples were positive for ecotropic MuLV, as compared with 4 of 22 (18%) control mice and 11 of 58 (19%) control cell samples. In addition, the mean titer of positive
samples was substantially higher in the experimental group than in the control group, being more pronounced for B-tropic MuLV than N-tropic MuLV (Fig. 1). The difference in the two groups was due principally to a marked increase in virus isolation and in virus titer in both thoracic lymph node cells and peritoneal exudate cells at 32 weeks and in the peritoneal cells at 50 weeks (Fig. 2). Thus, ecotropic MuLV is expressed in those cell populations in which transformed cells occur. Of the 36 peritoneal cell samples obtained from pristane-primed mice from 28 to 50 weeks, 22 were positive for ecotropic MuLV (15 were positive for both N- and B-tropic MuLV; 7 were positive for B-tropic MuLV alone), while only 3 of 22 control peritoneal cell samples were virus positive (all 3 were N-tropic isolates, with a mean viral titer under 10 plaque-forming units). The dip in titer in both N-tropic and B-tropic MuLV, seen at 39 weeks (Figs. 1 and 2), does not appear to be an artifact because it represents data from 25 experimental cell samples and 24 control cell samples.

**Xenotropic MuLV Expression.** Infectious xenotropic MuLV was detected in both experimental and control mice. However, the percentage of virus-positive samples was consistently higher in pristane-treated mice, and xenotropic MuLV was isolated from these mice throughout the observation period (Fig. 1). In control mice, xenotropic MuLV could not be detected in any of the 40 samples obtained at 28 weeks, 32 weeks, and 39 weeks (Fig. 1). The majority of viral isolations were made from peritoneal exudate cells in both groups of mice. Because the total number of such cells was characteristically greater in pristane-primed mice than in control mice (mean counts 29 x 10^6 and 5 x 10^6, respectively), it follows that more xenotropic MuLV is released into the peritoneal cavity of pristane-primed mice than into that of control mice.

**DISCUSSION**

An important development of recent years has been the discovery that the DNA of normal mouse cells contains sequences coding for the components of a family of MuLVs (19, 20). These include ecotropic viruses, which grow only in mouse and rat cells, and xenotropic viruses, which replicate in cells from a variety of heterologous species, but which do not normally infect mouse cells (11, 21). The expression of such endogenous MuLVs varies from mouse strain to mouse strain. That there may be a causal relationship between endogenous viral expression and the occurrence of lymphoreticular tumors is evident from studies in AKR mice (22, 23). In this strain, high titers of infectious ecotropic MuLV are present from infancy and there is a high spontaneous incidence of thymic lymphomas (25). Endogenous MuLVs may also exert their oncogenic potential under certain circumstances in strains of mice that have a low spontaneous incidence of lymphoreticular tumors and that normally express little or no infectious MuLV. For example, we have studied such mice, in which reticulum cell neoplasms develop following the experimental induction of a graft-versus-host reaction (24). We have shown that the immunological disorder is associated with an enhanced expression of infectious ecotropic MuLV (25) and that the viral population includes an oncogenic member (26), which appears to be etiologically involved in the subsequent tumor development (16).

In BALB/c mice, which are of the **Fcr-1** genotype, infectious ecotropic MuLV is rarely detectable before 20 weeks of age; thereafter, first N-tropic, then B-tropic MuLV become expressed in a gradually increasing proportion of mice, but in low titer, at least during the first year (25, 27). Isolation of xenotropic MuLV from the spleen of 10-month-old BALB/c mice has also been reported (28). In our study, the mean titers of both N-tropic and B-tropic MuLV isolates rose substantially in pristane-treated mice during the latter part of the tumor induction period. The overall rise in titer was principally due to increased viral titers in those cell populations in which transformed cells eventually appear, namely, peritoneal exudate cells and thoracic lymph node cells. We do not know the significance of the dip in both N-tropic and B-tropic MuLV titers at 39 weeks and of their subsequent sharp rise. One intriguing possibility is that this sequence of events reflects a changing viral population, with the emergence of MuLV variants having an ecotropic host range. Such endogenous variants have been described in strains of mice with a high incidence of lymphoreticular tumors (29) and appear to be envelope (env) gene recombinants of ecotropic and xenotropic MuLV (30). In mice injected with pristane, circumstances may favor the formation of such recombinants. Our data show that peritoneal exudate cells produce xenotropic MuLV. The increased numbers of these cells in pristane-treated mice may result in the release of substantial amounts of such virus. Because ecotropic MuLV replication is enhanced in this same cell population, phenotypic mixing may well occur (31). Xenotropic pseudotypes can infect mouse cells (31). Once in the cell, replication proceeds normally (31), and the possibility of genetic recombinants between ecotropic and xenotropic MuLV is facilitated. The expression of both ecotropic and xenotropic MuLV in peritoneal "target tissue" cells is of particular interest because the AKR recombinant virus appears in the target organ during the prethymoma phase (29, 32). It may be that when tumors develop in pristane-treated BALB/c mice, the transformed cells provide the necessary milieu for the enhanced replication of a previously undetectable subpopulation of MuLV variant. The AKR recombinant virus itself appears to have specific tropism for the target tissue (29).

Whether or not the endogenous MuLVs expressed following pristane administration play an etiological role in plasmacytoma induction is something that remains to be determined. It is possible that the enhanced expression of both ecotropic and xenotropic MuLV during the tumor induction period merely reflects the availability of a permissive cell population permitting increased replication of background levels of endogenous virus. Pristane itself might initiate synthesis of endogenous MuLV in previously virus-negative cells or augment existing virus production, through a direct effect on cellular regulatory processes such as has been described for a variety of chemical agents (33).

It is desirable to further characterize the endogenous MuLVs expressed in pristane-treated mice and to test their ability to induce plasmacytomas in their own right. It is known that MuLV can transform plasma cells following pristane administration. Thus, when BALB/c mice are primed with a single intraperitoneal injection of pristane and subsequently infected with Abelson MuLV (34), the incidence of plasmacytomas is increased and the latent period is shortened (35). The Abelson virus is a "laboratory" strain of MuLV and appears to consist of a mixture of Moloney MuLV and of a lymphosarcoma virus in a Moloney MuLV envelope (35). In the absence of pristane priming, the Abelson virus induces nontrophic lymphosarcomas (34).

If endogenous MuLVs are etiologically involved in tumor induction in the murine plasmacytoma model, such a finding could be highly relevant for human tumorogenesis. Endogenous RNA tumor viruses probably also exist in man, because viral proteins are found in both normal and neoplastic human tissues (36). Environmental factors, many of them chemical, are now thought to be involved in a substantial proportion of human cancers (37) and could well trigger the oncogenic expression of these virogenes.
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