Tumorigenicity and Intracisternal A-Particle Expression of Hybrids between Murine Myeloma and Lymphocytes

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ABSTRACT

Hybrids of BALB/c lymphocytes and a murine myeloma, a tumor that expresses intracisternal A-particles, were obtained with polyethylene glycol as the fusogen. The karyotype, tumorigenicity, and A-particle expression of the hybrid clones were assessed. All the hybrid clones analyzed were tumorigenic and expressed intracisternal A-particles even when they were the result of a fusion event between two lymphocytes and one myeloma cell in which no loss of chromosomes was detected. The tumors that developed following inoculation of hybrid cells into BALB/c mice (1 x 10⁶ cells/mouse) were karyotypically identical to the inoculated cells. It appears that the two myeloma cell phenotypic traits analyzed (tumorigenicity and A-particle expression) are dominant.

INTRODUCTION

It is now generally accepted that tumorigenesis is due to permanent, inheritable changes in the genome of tumor cells. Some disagreement exists, however, on the role of viruses and environmental factors in causing these genetic changes. In particular, it is still unclear whether tumorigenesis is due to the addition or to the inactivation of genetic traits in normal cells, i.e., whether tumorigenicity behaves as a dominant or a recessive trait. The genetic control of a given phenotypic trait can be studied by a parasexual approach involving the use of cellular hybrids (for a discussion of these techniques and of their limitations, see Ref. 9). Using these techniques, many authors have investigated the genetic control of cancer. Croce and Koprowski (6, 7) found that fusion of SV40-transformed human fibroblasts with normal cells yielded transformed hybrids and gave rise to tumors in nude mice (5). Experiments suggesting that cancer can be suppressed were first performed in the laboratories of Harris et al. (10, 12) who reported that hybrids between Ehrlich tumor cells and A9 mouse cells expressed low tumorigenicity. These data were confirmed in other laboratories (18, 20). More recently, Jonasson et al. (11) observed that subclones of nonmalignant melanoma × lymphocyte hybrids recovered the ability to grow as tumors following the loss of specific chromosomes that were assumed to carry the “suppressor” genes. The experiments presented here were designed to assess the ability of myeloma × lymphocyte hybrids to express the malignancy of a cell line of murine myeloma, a tumor that contains virus-like intracisternal A-particles but for which the causative agent is unknown. The need for these experiments was prompted by the fact that only 2 myeloma × lymphocyte hybrid clones have been analyzed and found to be tumorigenic when injected into mice at very high doses (10⁷ cells/mouse) (14). Moreover, the cells of these 2 clones contained 10 chromosomes less than expected in an ideal hybrid, raising the possibility that repressor genes had segregated. It was felt that the malignancy of several hybrid clones that had undergone very little or no loss of chromosomes had to be investigated before a conclusive statement on the genetic regulation of myeloma tumorigenicity could be made.

Because of the presence of intracisternal A-particles in all murine myelomas analyzed (4), their expression in myeloma × lymphocyte hybrids was also investigated. Little is known on the genetic regulation of the expression of intracisternal A-particles. Biochemical data indicate that sequences complementary to A-particle-specific RNA are present in murine myeloma and in normal mouse cells in equal amounts (16). These findings suggest that these virus-like particles are transmitted vertically and are therefore suppressed in normal cells. Conversely, the presence of a few intracisternal A-particles in mammary tumor x 3T3 cell cybrids (17) suggest a horizontal mode of transmission of intracisternal A-particles. For these reasons, the expression of A-particles in myeloma × lymphocyte hybrids was investigated. The data presented here suggest that both tumorigenesis and A-particle expression in murine myeloma are dominant traits.

MATERIALS AND METHODS

Cells. The myeloma cell line P3X63Ag8 derived from a MOPC-21 cell line (22) was obtained from Dr. A. Williams of the University of Oxford. These cells lack hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) (13). They were grown in Dulbecco’s medium supplemented with 10% fetal calf serum previously heated at 56°C for 30 min. P3X63Ag8 cells contain 65 ± 5 (S.D.) chromosomes of which 2 are biarmed (marker chromosomes). When 24 cultures of these cells (5 x 10⁶ cells/culture) were grown in HAT medium¹ (15), no growth of HAT medium-resistant clones was observed, indicating a reversion rate of less than 1 in 10⁷. Inoculation (s.c.) of 1 x 10⁶ P3X63Ag8 cells into 2-month-old BALB/c mice caused development of solid tumors in more than 80% of the mice, whereas injection of 2 x 10⁶ cells/mouse failed to cause development of tumors. P3X63Ag8 cells contain intracisternal A-particles, a morphological characteristic of all murine myelomas analyzed (4). Lymphocytes were obtained from the spleens of BALB/c or C57BL mice; they contain 40 acrocentric chromosomes. In some experiments, mice were given s.c. injections of 0.2 ml of complete Freund’s adjuvant 10 days before spleen cells were collected (stimulated lymphocytes).

Production of Cellular Hybrids. Ten million P3X63Ag8 cells

¹ The abbreviation used is: HAT medium, Dulbecco’s medium containing hypoxanthine (10⁻⁴ M), aminopterin (4 x 10⁻⁵ M), and thymidine (2 x 10⁻⁵ M).
were mixed with $1 \times 10^8$ lymphocytes in a small volume of 50% (v/v) polyethylene glycol (M.W. 1000) in Dulbecco's medium according to the method of Pontecorvo (19) as modified by Davidson and Gerald (8). Following washing to eliminate polyethylene glycol, cells were suspended in Dulbecco's medium supplemented with 20% fetal calf serum, and 1-ml aliquots were distributed into plastic wells (from $5 \times 10^4$ to $3 \times 10^5$ myeloma cells per well). Twenty-four hr later, 1 ml of Dulbecco's medium and enough hypoxanthine, aminopterin, and thymidine were added to each well to yield 2 ml of HAT medium which was replaced every 3 to 4 days. Vigorous growth of HAT medium-resistant hybrids occurred after 3 weeks. Only those hybrid clones obtained in experiments where clones grew in less than 20% of the wells were collected and studied, thus minimizing the possibility of collecting 2 hybrid clones from the same well.

**Karyological Analysis of Cellular Hybrids.** Cultures were exposed to Colcemid (0.1 $\mu$g/ml, 1 hr), subjected to hypotonic shock in 0.56% KCl, fixed in methanol:acetic acid (3:1), and stained with Giemsa. For each clone, the total number of chromosomes and biarmed chromosomes (marker chromosomes of P3X63Ag8 cells) was assessed. From these data, it was possible to assess the type and number of cells that participated in the fusion event and whether loss of parental chromosomes had occurred.

**Tumorigenicity of the Cellular Hybrids.** One million hybrid cells were injected s.c. into groups of 6 to 8 BALB/c mice, and the development of the tumor was assessed by palpation. Tumor incidence was defined as the number of "takes" over a 45-day period.

**Electron Microscopy.** Expression of A-particles in the hybrid clones was assessed by electron microscopic analysis of the cells ($1 \times 10^7$) fixed in glutaraldehyde and OsO$_4$, as described by Stubblefield and Brinkley (21). Intracisternal A-particles appeared mostly in clusters of 10 or more particles. Presence of A-particles in more than 30% of the cell sections analyzed was scored as positive for A-particle expression.

**RESULTS**

Ten hybrid clones were selected at random from those obtained in a fusion experiment between $1 \times 10^7$ P3X63Ag8 myeloma cells and $1 \times 10^8$ unstimulated BALB/c lymphocytes. The karyotype, the ability to assemble intracisternal A-particles, and the tumorigenicity of these 10 clones were assessed. All the hybrid clones that were analyzed for tumorigenicity (5 of 10) caused an incidence of tumors in BALB/c mice comparable to that of the tumorigenic parental cells (Table 1). All the clones analyzed for their A-particle content (6 of 10) were positive for this morphological characteristic, thus resembling the myeloma parental cells (Table 1). The karyological analysis of these clones suggested that loss of chromosomes had occurred. In fact, instead of the 100 to 105 chromosomes expected to be present in an ideal hybrid cell, the clones that were obtained contained an average of only 69 to 83 chromosomes (Table 1). The cells of one clone (Table 1, clone 3) contained an average of 112 chromosomes, but 3 of these were biarmed; thus suggesting that this clone was derived from the fusion of 2 myeloma cells with one lymphocyte and had undergone a heavy loss of chromosomes. The intracisternal A-particle content of 2 hybrid clones obtained by fusion of cells and lymphocytes of C57BL, a strain of mice that does not develop myelomas, was assessed. As shown in Table 2, both clones contained A-particles (>70% of the cellular sections contained clusters of these virus-like particles) and had undergone loss of parental chromosomes.

To minimize the loss of chromosomes from the hybrids and because it was felt that chromosomes from the nongrowing parental cell (i.e., the lymphocyte) are more likely to segregate, the P3X63Ag8 myeloma cells were fused with BALB/c lymphocytes that were obtained from mice that were given injections previously with complete Freund's adjuvant (see "Materials and Methods"). Freund's adjuvant stimulates spleen cell proliferation and causes enlargement of the spleen. Twenty-one hybrid clones obtained from a fusion experiment between $1 \times 10^7$ P3X63Ag8 cells and $1 \times 10^6$ stimulated BALB/c lymphocytes were karyotyped. Of these, 8 clones (reported in Table 3) contained a high number of chromosomes and seemed to have undergone very limited or no loss of chromosomes. One clone (Table 3, clone 1) derived from the fusion of one lymphocyte and one myeloma cell (as indicated by the presence of only 2 biarmed chromosomes) suffered no loss of chromosomes. Similarly, no loss of chromosomes was detected in 2 clones (Table 3, clones 4 and 5) derived from the fusion of 2 lymphocytes and one myeloma cell. In the other clones reported in Table 3, fewer chromosomes were lost than in the clones obtained by fusion of unstimulated lymphocytes with myeloma cells (Tables 1 and 2). The tumorigenicity of all the clones reported in Table 3 is very high regardless of the type and number of cells participating in the fusion event. In most cases, all mice given injections of $10^6$ hybrid cells developed tumors within 45 days. Similarly, all the clones expressed intracisternal A-particles (Table 3).

The possibility existed that the tumors observed were due to the in vivo outgrowth of a subpopulation of hybrid cells that differed karyotypically from the average cell in the clone. To assess this possibility, tumors were excised, and the cells derived from the tumors were cultured for 2 days in Dulbecco's

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**Table 1**

Tumorigenicity and A-particle expression of P3X63Ag8 x BALB/c lymphocyte hybrids

<table>
<thead>
<tr>
<th>Clone</th>
<th>No. of chromosomes</th>
<th>Tumor$^a$ incidence</th>
<th>A-particles expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79 (75-82)$^b$</td>
<td>7/7</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>74 (71-78)</td>
<td>7/7</td>
<td>+</td>
</tr>
<tr>
<td>3$^c$</td>
<td>112 (101-123)</td>
<td>6/8</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>71 (67-74)</td>
<td>5/6</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>80 (70-90)</td>
<td>7/7</td>
<td>ND$^d$</td>
</tr>
<tr>
<td>6</td>
<td>69 (63-74)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>83 (79-86)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>78 (65-98)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Control$^e$</td>
<td>65 (61-70)</td>
<td>9/12</td>
<td>+</td>
</tr>
</tbody>
</table>

$^a$ Forty-five days after s.c. injection of $10^8$ cells/mouse.
$^b$ Numbers in parentheses, range.
$^c$ Clone due to fusion of 2 myeloma cells and 1 lymphocyte.
$^d$ ND, not determined.
$^e$ P3X63Ag8 cells ($10^6$/mouse).

**Table 2**

Expression of A-particles of P3X63Ag8 x C57BL lymphocyte hybrids

<table>
<thead>
<tr>
<th>Clone</th>
<th>No. of chromosomes</th>
<th>A-particles expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81 (73-84)$^*$</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>64 (62-67)</td>
<td>+</td>
</tr>
</tbody>
</table>

$^*$ Numbers in parentheses, range.
medium. The karyotype of these cells was found to be virtually identical to that of the injected hybrid cells with respect to both the total number of chromosomes and the number of biarmed, marker chromosomes (Table 3). Intracisternal A-particles were also present in the one tumor analyzed (Table 3, clone 6).

**DISCUSSION**

Fusion of P3X63Ag8 myeloma cells with BALB/c lymphocytes has consistently yielded hybrid clones that were tumorigenic in BALB/c mice at relatively low doses (10^6 cells/mouse) and expressed intracisternal A-particles (Tables 1 and 3). Similarly, Myeloma × C57BL lymphocyte hybrids expressed A-particles (Table 2). When spleen cells from mice nonstimulated with Freund’s adjuvant were used as the normal parental cell (Tables 1 and 2), loss of chromosomes could not be avoided. The data obtained with these hybrids are not readily interpretable because of the possibility that the chromosomes lost carried the genetic information for factors that suppress tumorigenicity and expression of A-particles. Fusion of myeloma cells with lymphocytes stimulated with Freund’s adjuvant yielded clones (30%) that revealed little or no loss of chromosomes. Even the hybrid clones with a full chromosomal complement (Table 3, clones 1, 4, and 5) were tumorigenic and contained intracisternal A-particles. It is possible, however, that the growth of tumors was due to cells that had segregated the chromosome(s) carrying the repressor gene(s). The karyological analysis of the tumors produced by the hybrid clones excluded this possibility. In fact, the striking similarity between the karyotypes of the tumors and of the injected hybrid cells (Table 3) indicates that no selection of cell subpopulations occurred during tumor growth.

One may conclude, therefore, that in murine myeloma cells tumorigenicity is a dominant trait. The possibility that BALB/c lymphocytes contain insufficient amounts of repressor to inhibit phenotypic expression of tumorigenicity cannot be completely excluded. Indeed, it could constitute the biological basis for BALB/c mouse susceptibility to myeloma. However, such an hypothesis does not appear very probable in view of the fact that even clones derived from 2 lymphocytes and one myeloma cell (Table 3, clones 4, and 5) resemble the tumorigenic parent. The data reported here also suggest that the expression of murine myeloma intracisternal A-particles is a dominant trait. However, since intracisternal A-particles have been found in lymphocytes of normal BALB/c mice (23) as well as in embryonic tissues of several strains of mice (1–3), the possibility must be entertained that the clones studied were the result of a fusion event involving lymphocytes phenotypically positive for A-particles. Thus, the evidence available makes it impossible to firmly conclude that the expression of murine myeloma A-particles is a dominant character.

**ACKNOWLEDGMENTS**

This research was performed during a stimulating sabbatical year spent at the Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. The author wishes to thank Dr. H. Harris for allowing him to use the excellent facilities of the Dunn School and for critical discussions and Dr. A. Williams for providing the myeloma cell line. The author also wishes to gratefully acknowledge the help received from Dr. D. Kay and M. Bergin in the performance of electron microscopy.

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