Variants of an Interspecies Hybridoma with Altered Tumorigenicity and Protective Ability against Mouse Myeloma Tumors

R. Baumal, E. Musclow, H. Farkas-Himsley, and A. Marks

Department of Pathology, The Hospital for Sick Children [R. B.], Department of Laboratories, Mount Sinai Hospital [E. M.], and Department of Microbiology and Parasitology [H. F-H.] and Banting and Best Department of Medical Research [A. M.], University of Toronto, Toronto, Ontario M5G 1X8, Canada

ABSTRACT

Recent isolates of RX54-3 hybridoma cells (new cells) protect BALB/c mice against subsequent challenge with the tumorigenic myeloma parent cells used to construct this hybridoma. In contrast, hybridoma cells which have been maintained in tissue culture for long periods of time (old cells) are not protective. In the present study, we compared a number of properties of the new and old hybridoma cells and determined which line was more similar to the parent myeloma. We found that new hybridoma cells resembled myeloma cells in: (a) possessing A- and C-type viral particles on transmission electron microscopy and a relatively smooth surface on scanning electron microscopy; (b) being sensitive to a hypotonic solution containing the dye propidium iodide; (c) having similar DNA histograms on flow cytometric analysis; (d) being sensitive to the bacteriocin colicin HSC 10; and (e) being tumorigenic in nude mice. In contrast, old hybridoma cells differed in all of these characteristics from new hybridoma and myeloma cells. Therefore, in order to protect against challenge with the tumorigenic myeloma parent, hybridoma cells must retain properties of that parent.

INTRODUCTION

Production of hybridomas by fusion of a tumorigenic and nontumorigenic cell line is now a well-established laboratory technique. We have been utilizing an interspecies hybridoma, produced by fusion of the P3X63/Ag8 mouse myeloma cell line and rabbit lymphocytes, referred to as RX54-3 cells, to study whether it will protect a host against the tumorigenic parent used to construct the hybridoma (1). We showed that, although P3X63/Ag8 myeloma cells were tumorigenic in BALB/c mice, the hybridoma cells were not, probably due to the presence of rabbit antigenic determinants on them, leading to their rejection by the host. Furthermore, when hybridoma cells were administered to these mice prior to the parent P3X63/Ag8 mouse myeloma line, protection against tumorigenicity was seen. New clones of hybridoma cells, when maintained in tissue culture for prolonged periods of time, became altered and formed old RX54-3 hybridoma cells. Only the new clones were effective in protecting mice against myeloma tumors induced by administration of P3X63/Ag 8 cells.

In a previous study, the new and old RX54-3 hybridoma cells were partially characterized (1). New hybridoma cells possessed 63 chromosomes, produced mouse and rabbit immunoglobulins, and were killed by complement and an anti-myeloma cell antiserum which had been raised by immunizing rabbits with a nonimmunoglobulin-producing variant of the MPC-11 myeloma cell line. Old hybridoma cells possessed 40 chromosomes, did not produce mouse or rabbit immunoglobulins, and were not killed by anti-myeloma cell antiserum and complement. These results infer that old hybridoma cells had lost some myeloma antigenic determinants and, because of this, could no longer induce an immune response against the myeloma component of the hybridoma. Consequently, old hybridoma cells were no longer effective in protecting against subsequent administration of myeloma cells. In the present study, we have further characterized new and old RX54-3 hybridoma cells utilizing (a) TEM and SEM, (b) sensitivity to hypotonicity, (c) flow cytometry, (d) ability of the cells to react with bacteriocin, and (e) tumorigenicity in nude mice to further elucidate the differences in these 2 cell lines, which are accompanied by alterations in their tumorigenicity and protective ability. We have shown that new hybridoma cells resemble myeloma cells more closely than do old hybridoma cells. Since new but not old hybridoma cells protected BALB/c mice against the development of myeloma tumors, protection appeared to be dependent on the retention of myeloma-like properties by the new cells.

MATERIALS AND METHODS

The RX54-3 hybridoma cell line was constructed by fusion of P3X63/Ag 8 mouse myeloma cells and rabbit lymphocytes, as described previously (1). New hybridoma cells consisted of a clone which had been propagated in tissue culture for only 1 to 2 months, while old hybridoma cells had been in tissue culture for periods of 8 to 12 months or longer. The IgG-producing P3X63/Ag 8 mouse myeloma tissue culture line was obtained from Dr. J. Harris of the Department of Medical Research, Banting and Best Institute, University of Toronto, Toronto, Canada. The hybridoma and myeloma cell lines were grown in Petri dishes at 37° in a humidified atmosphere of 5% CO2, using a growth medium (Flow Laboratories, Inc., MacLean, Va.) supplemented with 15% fetal calf serum and 2 mM glutamine.

For TEM and SEM, 5 × 106 hybridoma and myeloma cells were centrifuged at 1000 rpm for 5 min, and the cell pellets were resuspended in the appropriate fixatives for TEM and SEM. After 1 hr in fixative, the cells were spun, processed for electron microscopy, and viewed with a Philips 201 (TEM) or a Jeol JSM-35 (SEM) microscope.

DNA histograms of hybridoma and myeloma cells were constructed by staining 1 × 106 cells with propidium iodide (Sigma Chemical Co., St. Louis, Mo.) by the method of Kriahan (8) and analyzing them on a Coulter TPS-1 flow cytometer (Coulter Electronics, Miami, Fla.) as described previously (4). To test the ability of hybridoma and myeloma cells to react with the bacteriocin colicin HSC 10, cells were incubated for 43 hr in medium without fetal calf serum, supplemented with either bacteriocin at 1 growth-inhibitory unit or with 0.03 mM Tris buffer, pH 7.8, the bacteriocin diluent, as a control (2).

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2 To whom requests for reprints should be addressed.

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The ability of hybridoma and myeloma cells to induce tumors in nude and BALB/c mice was assessed by i.p. administration of 25 x 10^6 cells suspended in 0.2 ml 140 mM NaCl:3 mM KCl:0.15 mM KH2PO4:8 mM Na2HPO4:7H2O, pH 7.4. Mice were primed by injection of 0.5 ml Pristane (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Company, Milwaukee, Wis.) 1 week before. The mice were then observed for the development of ascites, and autopsies were performed to assess the presence of i.p. tumor implants.

RESULTS

TEM and SEM of Hybridoma and Myeloma Cells. RX54-3 new hybridoma and P3X63.Ag8 myeloma cells resembled each other in possessing large numbers of intracisternal A-type and smaller numbers of extracellular C-type viral particles (Fig. 1A). Similar viral particles have been described previously in P3 myeloma cells (6). Old hybridoma cells completely lacked these viral particles (Fig. 2A). Moreover, new hybridoma and myeloma cells had surfaces which were relatively smooth and characterized by the presence of short, stubby microvilli (Fig. 1B). In contrast, old hybridoma cells possessed more complex surfaces with numerous prominent ruffles and folds (Fig. 2B). Therefore, these morphological studies indicated that new hybridoma cells possessed the same viral particles and appeared similar in their cell surface configuration to myeloma cells, whereas old hybridoma cells lacked viral particles and had a more complex cell surface.

Sensitivity of Hybridoma and Myeloma Cells to Hypotonicity. Hybridoma and myeloma cells were incubated for 10 min in hypotonic solution (B), and DNA was then stained using the dye propidium iodide. Ninety-five % of the myeloma cells and 89% of the new hybridoma cells reacted with the propidium iodide, whereas only 50% of the old hybridoma cells took up this dye (mean of 3 experiments). When the plasma membrane of the new and old hybridoma and myeloma cells was damaged by fixation with ethyl alcohol, all 3 cell lines reacted equally well with the propidium iodide. Therefore, the diminished sensitivity of the old hybridoma cells to the hypotonic solution emphasizes a difference in the plasma membrane of these cells.

Flow Cytometric Analysis of Hybridoma and Myeloma Cells. Hybridoma and myeloma cells were analyzed for their content of DNA, using flow cytometry on a fluorescence-activated cell sorter (Chart 1). A single peak, with a maximum reading at Channels 37 to 39, was observed for the DNA histogram of P3X63.Ag8 myeloma and new hybridoma cells (Chart 1, A and B). In contrast, old hybridoma cells demonstrated a different DNA histogram in that they showed 2 peaks of DNA, one similar to that described for the new hybridoma and myeloma cells and a second peak (Peak X) at Channel 20 containing one half the DNA content (Chart 1C). Therefore, new hybridoma cells resembled myeloma cells with this method of analysis, but old hybridoma cells differed.

Ability of Hybridoma and Myeloma Cells to Interact with Bacteriocin. Bacteriocin, an antibiotic which reacts preferentially with malignant as opposed to benign cells (3), was utilized to determine whether new and old hybridoma cells reacted differently with this agent. Cells were incubated in a-medium without fetal calf serum, containing 1 growth-inhibitory unit of bacteriocin. DNA content of the hybridoma cells was assessed by flow cytometry on a fluorescence-activated cell sorter (4). New hybridoma cells were sensitive to bacteriocin (Chart 2A).

The DNA histogram indicates that the cell number in G0-G1, and G2-M dropped by about 30%, whereas the cell number of the "pre-G1" phase increased more than 2 times as compared to the controls (no bacteriocin). In contrast, the DNA histogram of the old hybridoma cells was not affected appreciably by the bacteriocin (Chart 2B). Similar results were obtained in 2 additional experiments, and the overall mean for all 3 experiments is shown in Chart 3. In the presence of bacteriocin, pre-G1 cells accumulated in the case of P3X63.Ag8 myeloma and new hybridoma cells to the extent of 29 and 23%, respectively, over untreated controls. On the other hand, myeloma and new hybridoma cells in the G0-G1 phase of growth were reduced by 18 and 12%, respectively, over control cells not treated with bacteriocin. In contrast, old hybridoma cells were barely affected by bacteriocin at either stage. This difference in sensitivity was statistically highly significant with p less than 0.001. Therefore, these studies showed that new hybridoma cells reacted with bacteriocin in a manner similar to mouse myeloma and other malignant cells, whereas the old hybridoma cells did not.
Chart 2. Effect of bacteriocin on RX54-3 new (A) and old (B) hybridoma cells as assessed by flow cytometry. Coefficient of variation in A without bacteriocin is 7.5% and with bacteriocin is 10%.

Table 1

Tumorigenicity of RX54-3 hybridoma and P3X63Ag8 myeloma cells in nude and BALB/c mice

<table>
<thead>
<tr>
<th>Cells (2.5 x 10^5)</th>
<th>Nude</th>
<th>BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3X63Ag8 myeloma</td>
<td>4/5 (80)</td>
<td>9/10 (90)</td>
</tr>
<tr>
<td>New hybridoma</td>
<td>5/5 (100)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Old hybridoma</td>
<td>0/5 (0)</td>
<td>0/10 (0)</td>
</tr>
</tbody>
</table>

DISCUSSION

The foregoing studies have shown that new RX54-3 hybridoma cells were more similar to P3X63Ag8 myeloma cells than were old hybridoma cells, as assessed by (a) the presence of A- and C-type viral particles on TEM and relatively smooth cell surfaces, characterized by short microvilli rather than ruffles and folds, on SEM; (b) sensitivity to a hypotonic solution containing propidium iodide; (c) similar DNA histograms on flow cytometric analysis; (d) sensitivity to the bacteriocin colicin HSC 10; and (e) tumorigenicity in nude mice. The fact that new hybridoma cells were not tumorigenic in BALB/c mice, in contrast to P3X63Ag8 myeloma cells, was probably a consequence of their being rejected, due to the presence of rabbit antigenic determinants. Old hybridoma and myeloma cells differed from new hybridoma cells in that they (a) lacked viral particles on TEM and possessed complex surfaces on SEM, (b) were relatively insensitive to a hypotonic solution containing propidium iodide, (c) showed a different DNA histogram on flow cytometric analysis, (d) were not sensitive to bacteriocin action, and (e) were not tumorigenic in mice. These altered
properties of old hybridoma cells were accompanied by a loss of their tumorigenicity; the latter was undoubtedly a consequence of chromosomal loss by these cells. Previously, we showed that the chromosome number, immunoglobulin-producing capacity, and cytotoxicity by anti-myeloma cell antiserum and complement of new and old hybridoma cells differed. The present studies indicate that new hybridoma cells are more similar to myeloma cells than they are to old hybridoma cells. The results are summarized in Table 2.

A number of investigators have shown that immunization of experimental animals with hybridomas, formed by fusion of tumor cells with either allogeneic or xenogenic cells, protects them against subsequent challenge with the syngeneic tumor cell parent (5, 7, 9, 10). This raises the possibility that such hybridomas could theoretically be used as vaccines to complement other therapeutic approaches in the treatment of cancer in humans, provided it can be shown in experimental systems that hybridomas are also effective in protecting the host when administered after the malignant cells. Further experiments are required to clarify this issue. The present and previous studies have shown, in an experimental model system, that the ability of hybridoma cells to protect BALB/c mice against myeloma tumors is dependent on the retention of myeloma-like properties by these cells. Therefore, if immunotherapy with hybridomas should prove to be effective in treating cancer in experimental and possibly clinical situations, consideration must be given to the fact that the hybridoma should retain characteristics of the malignant parent.

REFERENCES

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