Protective Effect of an Interspecies Hybridoma on the Tumorigenicity of Mouse Myeloma Cells

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ABSTRACT

A rabbit lymphocyte-mouse myeloma hybridoma (RX54-3) was administered to BALB/c mice, in order to determine whether hybridoma cells have any protective effect on the tumorigenicity of the P3X63/Ag8 mouse myeloma cell line, one of the parents of this hybridoma. Two sublines of RX54-3 were studied. (a) "new" cells, which produce rabbit light chains and mouse immunoglobulin, are killed by anti-myeloma cell antiserum and complement and possess a high chromosome count. And (b) "old" cells, which have lost all immunoglobulin-producing capacity, are no longer killed by anti-myeloma cell antiserum and complement and possess a low chromosome count. In contrast to myeloma cells, the hybridoma cells were nontumorigenic in normal BALB/c mice, even at very high cell doses. When hybridoma cells were administered prior to challenge with P3X63/Ag8 myeloma cells, protection against development of myeloma tumors was much more effective with new cells than with old cells. Development of protection was dependent on the dose of hybridoma cells, their route of administration, their viability and ability to divide, and the time interval between administration of the hybridoma and myeloma cells. The protection produced by the new hybridoma cells was comparable to that seen after administration of a subtumorigenic dose of living myeloma cells. With both the hybridoma and myeloma cells, protection was seen only against challenge with P3X63/Ag8 myeloma cells but not against another myeloma tumor. These studies indicate that administration of somatic cell hybrids is an effective method of protecting syngeneic hosts against challenge with mouse myeloma tumor cells.

INTRODUCTION

Immunization of animals with somatic cell hybrids, formed by fusion of tumor cells with either allogeneic or xenogeneic cells, has been shown to protect the hosts against subsequent challenge with the syngeneic tumor cell parent (2-4, 9). Adoptive transfer experiments have suggested that this immunity is mediated by thymus-derived or T-lymphocytes (3). Since hybrid cells express surface antigens of both parents (10), heterologous hybrids are rejected by the host because of their foreign histocompatibility antigens, and consequently they are nontumorigenic. This raises the possibility that somatic cell hybrids may be used as vaccines to complement other therapeutic approaches to cancer.

In order to extend the generality of this approach in animal models which are used to study neoplastic disease, we have initiated experiments on the protective effect in mice of an interspecies hybridoma, formed by fusion of rabbit lymphocytes and mouse myeloma cells, against subsequent challenge with the tumorigenic mouse myeloma cell parent. We have found that administration of hybridoma cells protects the mice against a subsequent challenge with myeloma cells and is as effective as the protection produced by administration of subtumorigenic doses of myeloma cells. Nontumorigenic myeloma or hybridoma cells at equivalent doses did not confer any protection. The degree of protection varied with the route and schedule of administration of the hybridoma cells. Loss of mouse myeloma surface antigens by the hybridoma cells during prolonged culture in vitro considerably decreased the ability to confer protection against challenge with myeloma cells. These results suggest that the protection observed probably depends on a complex immunological response directed against surface antigens of the myeloma cell parent and that somatic cell hybrids offer definite advantages over live or killed tumor cells as vaccines against cancer.

MATERIALS AND METHODS

Cell Lines. The IgG,-producing P3X63/Ag8 mouse myeloma tissue culture cell line was obtained from Dr. John Harris of the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada, while the IgA-producing MOPC 315 myeloma tumor was obtained from Litton Bionetics Inc. (Kensington, Md.). The RX54-3 hybridoma cell line was provided by Dr. George Kohler of the Basel Institute for Immunology (Basel, Switzerland). It was established by fusing rabbit lymphocytes with the P3X63/Ag8 mouse myeloma cell line. The myeloma and hybridoma cell lines were grown in Petri dishes at 37° in a humidified atmosphere of 5% CO₂, using Alpha growth medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 15% fetal calf serum and 2 mM glutamine. When large numbers of cells were required, growth was carried out in spinner bottles. Viable cell counts were performed using a hemocytometer, and the cells were centrifuged, resuspended in PBS (3) and used for injection of mice. The MOPC 315 tumor was passaged serially in BALB/c mice by i.p. injection.

Immunization and Assessment of Tumorigenicity. Female BALB/c mice (6 to 8 weeks old) were primed by i.p. injection of 0.5 ml of Pristane (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Co., Inc., Milwaukee, Wis.). The mice were given injections of variable numbers of hybridoma cells in 0.5 ml, 1 week after priming, by the i.p., s.c., or i.v. routes. One month later, the mice were challenged i.p. with a dose of myeloma...
cells determined in dose-response experiments to be lethal for
100% of mice. In the case of the P3X63.Ag8 myeloma cell
line, mortality was occasionally less than this in some experi-
ments for unknown reasons, even though an appropriate dose
of cells had been injected. Animals were observed for de-
myeloma and the new hybridoma cells were killed by complement and either rabbit anti-mouse myeloma cell antiserum or goat anti-hybridoma cell antiserum (Chart 2). Normal rabbit and goat sera were not toxic for the myeloma and hybridoma cells (data not shown). Old hybridoma cells were killed by the anti-hybridoma cell antiserum but not by the anti-myceloma cell antiserum. These results indicate that determinants which are recognized by the anti-myceloma cell antiserum were not present on the old hybridoma cells.

Karyotypes of Myeloma and Hybridoma Cells. Chromosome counts were performed on P3X63/Ag8 myeloma and RX54-3 old and new hybridoma cells (Table 1). P3X63/Ag8 mouse myeloma cells contained only telocentric chromosomes (mean number, 56). Old hybridoma cells demonstrated biarmed and telocentric chromosomes (mean number, 40), the former presumably derived from the rabbit parent and the latter from the mouse or both parents. New hybridoma cells also contained biarmed and telocentric chromosomes and were made up of 2 populations having mean chromosome counts of either 42 or 63. These results suggest that old hybridoma cells were constantly being generated from new cells by loss of chromosomes.

Tumorigenicity of MOPC 315 and P3X63/Ag8 Myeloma and RX54-3 Hybridoma Cells in BALB/c Mice. Varying numbers of MOPC 315 and P3X63/Ag8 myeloma cells and RX54-3 hybridoma cells were injected i.p. into Pristane-primed BALB/c mice. The development of ascites and myeloma tumors and the length of time required for death of the mice were noted. Both MOPC 315 and P3X63/Ag8 myeloma cells were tumorigenic in BALB/c mice, and the percentage of mice developing ascites and myeloma tumors increased with the dose of myeloma cells administered (data not shown). However, there was a marked difference in the tumorigenic potential of MOPC 315 and P3X63/Ag8. Injection of 0.01 x 10^6 MOPC 315 cells, which were not adapted to tissue culture and which had been derived from solid tumor fragments, was tumorigenic in 100% of mice, whereas 25 to 30 x 10^6 P3X63/Ag8 myeloma tissue culture cells were needed from 100% tumor induction. A reduced tumorigenicity of tissue culture mouse myeloma cells, as opposed to cells derived from solid tumors not adapted to tissue culture, has been noted previously (5, 8). Histological examination of tumor masses showed that they were composed of sheets of highly anaplastic pleomorphic plasma cells, and there were many bizarre appearing multinucleated cells undergoing mitosis. In contrast to these results, injection of either RX54-3 old or new hybridoma cells failed to produce tumors in BALB/c mice, even at a dose as high as 80 x 10^6 cells.

Effectiveness of Administration of RX54-3 Hybridoma Cells in Protecting Mice against Challenge with P3X63/Ag8 Myeloma Cells. Pristane-primed BALB/c mice were injected with varying numbers of RX54-3 old or new hybridoma cells, challenged 1 month later with 25 x 10^6 P3X63/Ag8 myeloma cells, and observed for the development of ascites and myeloma tumors and the time required for death to occur (Chart 3). When 80 x 10^6 RX54-3 hybridoma cells were used, both old and new cells were effective in protecting mice against a subsequent challenge with P3X63/Ag8 myeloma cells. When
hybridoma cells were injected i.p. into Pristane-primed BALB/c mice. One month receiving preinjections of RX54-3 hybridoma cells. Varying numbers of RX54-3 used in each group. O, old; N, new.

mice developing ascites and myeloma tumors was noted. A total of 12 mice was later, 25 x 10^6 P3X63/Ag8 myeloma cells were injected i.p. The percentage of tumor was present only along the needle track where the ever, partial protection was seen in the case of the mice development of ascites, or myeloma tumors, or death. How neither subline of hybridoma cells completely prevented the dose of hybridoma cells was reduced to 0.2 x 10^6 or less, protection against a subsequent challenge with P3X63/Ag8 mye

depended on their route of administration (Chart 4). Mice were administered.

When BALB/c mice were challenged with P3X63/Ag8 myelo

the dose of hybridoma cells was reduced to 0.2 x 10^6 or less, neither subline of hybridoma cells completely prevented the development of ascites, or myeloma tumors, or death. How

ever, partial protection was seen in the case of the mice injected with the new hybridoma cells. This was manifested by the development of very little ascites and small myeloma tumors which were localized as 2 or 3 lumps in the peritoneal cavity, rather than being diffuse and distributed widely. Very often, tumor was present only along the needle track where the myeloma cells had been injected. The mice preinjected with 0.2 x 10^6 new hybridoma cells lived considerably longer (up to and beyond 60 days) than mice preinjected with the same dose of old hybridoma cells (death at 21 days). In the range of 2 to 20 x 10^6 hybridoma cells/injection, the new cells were completely protective while the old cells failed to provide protection against a subsequent challenge with P3X63/Ag8 myeloma cells. These and the previous results indicate that, concurrent with loss of immunoglobulin production and ability to be killed by anti-myeloma cell antiserum and complement, the RX54-3 old hybridoma cells demonstrated markedly decreased ability to induce protection against subsequent challenge with the P3X63/Ag8 myeloma cell parent, relative to new hybridoma cells.

Experiments were next carried out to determine whether protection against development of myeloma tumors, produced as a result of administration of RX54-3 hybridoma cells, depended on their route of administration (Chart 4). Mice were given injections of 20 x 10^6 old or new hybridoma cells by the i.p., s.c., or i.v. routes and were challenged with P3X63/Ag8 myeloma cells 1 month later. As expected, the old hybridoma cells afforded no protection when administered by any of these routes. New hybridoma cells were effective when administered i.p. and were totally devoid of protection when administered i.v. Partial protection (as defined above) was seen when new hybridoma cells were administered by the s.c. route, even though the percentage of mice developing tumors was the same as with the old cells. Therefore, the protection against development of myeloma tumors afforded by the new hybridoma cells depended on the route by which these cells were administered.

A comparison was next made of the relative effectiveness of live and dividing, live but nondividing, and dead hybridoma cells in conferring protection against the development of myeloma tumors (Chart 5). Live hybridoma cells were prevented from dividing by treatment with mitomycin C or were killed by repeat freezing and thawing. Old hybridoma cells, whether capable of cell division, nondividing, or dead, did not protect against subsequent challenge with myeloma cells. In contrast, i.p. administration of live and dividing new hybridoma cells was effective in protecting against the development of myeloma tumors when P3X63/Ag8 cells were injected 1 month after the hybridoma cells. However, new hybridoma cells which had been prevented from dividing by incubation with mitomycin C or killed by freezing and thawing were no longer protective. Therefore, the protection afforded by the new hybridoma cells depended on their viability and their ability to divide.

When BALB/c mice were challenged with P3X63/Ag8 myeloma cells 1 day or 1 week following administration of RX54-3 hybridoma cells, less protection was observed than when the myeloma cells were administered 1 month after the hybridoma cells (Chart 6). Both RX54-3 old and new hybridoma cells were ineffective in protecting mice from developing myeloma tumors when administered 1 day prior to P3X63/Ag8 myeloma cells. When hybridoma cells were injected 1 week prior to myeloma cells, the old cells failed to protect, whereas the new hybridoma cells produced partial protection, as manifested by a reduced
Relative Effectiveness of P3X63/Ag8 Myeloma Cells in Protection against Challenge with P3X63/Ag8 Myeloma Cells. Mice can be protected against developing myeloma tumors by immunizing them with subtumorigenic doses of myeloma cells. Experiments were carried out to determine whether hybridoma cells produced protection comparable to myeloma cells. Mice were injected with either new or old hybridoma cells or with living or dead (achieved by repeat freezing and thawing) P3X63/Ag8 myeloma cells (Chart 7). In the case of the hybridoma cells, the dose was either 2 x 10^6 cells, which was effective in conferring protection against challenge with myeloma cells, or 0.2 x 10^6 cells, which was not effective. In the case of the myeloma cells, the dose chosen was 2 x 10^6 living or dead cells, since larger numbers of viable cells were tumorigenic themselves, or 0.2 x 10^6 cells, a markedly subtumorigenic dose. In all cases, the mice were challenged with 25 x 10^6 P3X63/Ag8 myeloma cells 1 month later. As expected, old hybridoma cells were devoid of protection, whereas a dose of 2 x 10^6 new hybridoma cells was fully protective (Charts 3 and 7). Two x 10^6 live but not dead myeloma cells also conferred complete protection against a subsequent challenge with P3X63/Ag8 myeloma cells, whereas 0.2 x 10^6 cells were devoid of protection. Therefore, these studies indicate that new hybridoma and live myeloma cells demonstrate comparable ability to protect mice against challenge with the P3X63/Ag8 myeloma cell line.

Inability of RX54-3 Hybridoma or P3X63/Ag8 Myeloma Cells to Produce Protection against Challenge with Other Myeloma Lines. Experiments were carried out to determine whether administration of RX54-3 hybridoma or P3X63/Ag8 myeloma cells produced protection against a myeloma cell line other than the P3X63/Ag8 cell line, which was one of the parent cell lines used for fusion to obtain the RX54-3 hybridoma. Mice were given injections of either 20 x 10^6 old or new hybridoma cells or 2 x 10^6 P3X63/Ag8 myeloma cells and were challenged 1 month later with either 25 x 10^6
P3X63/Ag8 or 0.01 × 10^6 MOPC 315 myeloma cells (Chart 8). In the case of the hybridoma cells, old cells failed to protect against either of the 2 myeloma lines while new cells produced protection against challenge with P3X63/Ag8 myeloma cells but not against MOPC 315 myeloma cells. In the case of the myeloma cells, protection was also observed against P3X63/Ag8 myeloma cells but not against MOPC 315 tumor cells. Therefore, protection produced by both myeloma and hybridoma cells against development of myeloma tumors did not extend to another myeloma (MOPC 315 cells) different from that used to construct the hybridoma.

**DISCUSSION**

Our results confirm and extend previously reported experiments in animal models which show protection against tumor challenge by somatic cell hybrids (2-4, 9). In the present experiments, administration to BALB/c mice of a hybridoma cell line (RX54-3), formed by fusion of P3X63/Ag8 mouse myeloma cells with rabbit lymphocytes, was evaluated for its ability to protect BALB/c mice against challenge with the syngeneic mouse myeloma tumor cell parent. Two different populations of hybridoma cells were used, new and old, the latter having been generated from the former as a result of loss of mouse chromosomes during prolonged maintenance of the cells in tissue culture (Table 1). This chromosomal segregation further resulted in a loss of a number of additional mouse myeloma markers by the old hybridoma cells (i.e., they no longer produced mouse immunoglobulin chains and were not killed by a rabbit anti-mouse myeloma cell antiserum), suggesting a loss of myeloma surface antigenic determinants (Charts 1 and 2).

The presence of myeloma antigenic determinants, including the MOPC 21 or P3 idotype, was necessary for eliciting effective protection against P3X63/Ag8 myeloma cells, since old hybridoma cells were 50-fold less potent than new hybridoma cells (Chart 3). It is not known whether these myeloma antigenic determinants, which presumably operated by producing an immune response in the host, were specific products or myeloma cells, were proteins coded for by transforming viruses, or were budding viral particles. However, such determinants are not ubiquitously shared by all myeloma cells since the RX54-3 hybridoma cell line conferred protection only against the parental P3X63/Ag8 myeloma cells and not against another (MOPC 315) mouse myeloma cell line (Chart 8).

Although adoptive transfer experiments in other systems suggest that the protection observed is mediated by T-lymphocytes (3), several features are still obscure. For example, there is insufficient experimental data at present to relate the requirement for a specific route of administration and the prolonged time interval on the effectiveness of hybridoma cells in producing protection (Charts 4 and 6). Likewise, the basis for the advantage of dividing over nondividing or nonviable cells in conferring protection (Chart 5) is not known. It is possible that tumor antigens are removed from the surfaces of cells by proteolytic enzymes in serum and that cell division is necessary for the continued expression of these antigens. Similar results have also been reported but not explained in previous studies (3).

An important practical and fundamental question raised by these experiments is whether incorporation of tumor antigens into a foreign plasma membrane and their possible association with foreign histocompatibility antigens increases their immunogenicity. No previous study has addressed itself directly to this question. Our results show that, at equivalent doses, immunization with hybridoma cells was not more effective than immunization with a subtumorigenic dose of myeloma cells in conferring protection against the myeloma cells (Chart 7). This finding indicates that the 2 approaches do not differ substantially and suggests that the antigenicity of tumor cell antigens was not increased after incorporation into the hybridoma cell membrane.

The transplantable mouse myeloma tumor system is considered a model for the human disease multiple myeloma, since in both species there is a neoplastic proliferation of immunoglobulin-producing plasma cells which ultimately proves fatal (6). Our results are relevant to the potential use of interspecies hybrids as vaccines for multiple myeloma in particular and other cancers in general. However, it is unlikely that such an approach will be effective against tumors in the absence of additional therapeutic agents. Since immune responses against tumor antigens are very likely already stimulated maximally in patients with cancer, further immunization with hybrid cells would probably have no additional beneficial effect. However, several arguments, based on our experimental data, suggest that administration of somatic cell hybrids may have a definite role as therapeutic adjuncts, for example, for maintaining patients in drug-induced remissions following surgical removal of a tumor. (a) Hybridoma cells were nontumorigenic in mice at all doses tested, presumably due to a rejection reaction produced by rabbit histocompatibility antigens. Therefore, somatic cell hybrids formed between heterologous parents are safe to use as vaccines. (b) Live hybridoma cells were more effective in conferring immunity than dead tumor cells (Chart 7) (3). (c)
Protection observed with live tumor cells was dose dependent (Chart 8), suggesting that use of live tumor cells for therapy may be both difficult and unacceptable in clinical situations. And (d) with presently available technology, it should be readily possible to dissociate resected tumor cells in vitro, fuse them with a heterologous cell line, isolate a hybrid clone, and use the hybrid cells to immunize patients in whom remission has been achieved by surgery and/or drugs. In this respect, our results emphasize a number of technical problems which must be controlled if this approach is to succeed, such as the choice of the heterologous parent to be used for fusion and the period of maintenance of the hybrids in culture. We have shown that chromosomal segregation during in vitro culture of interspecies hybrids can lead to loss of the antigenic determinants necessary to confer protection (Chart 3).

The results obtained in these experiments are summarized in Table 2. It is clear that prior administration of hybridoma cells protects BALB/c mice against subsequent challenge with the myeloma parent. Of greater interest than prevention, however, would be a therapeutic role for hybridomas when administered following myeloma cells, analogous to the situation which exists in humans with malignant disease. Such studies have been performed by Kim (3) using a mouse myeloma-L-cell hybridoma, in which it was reported that spleen lymphocytes from hybridoma-injected mice produced transient regression of myeloma tumor growth. Experiments are presently underway to determine whether RX54-3 hybridoma cells also prevent myeloma when administered after P3X63/Ag8 myeloma cells. If these studies prove promising, then hybridomas can be considered as therapeutic, possibly immunotherapeutic, agents, and their role in the treatment of other types of tumors will require assessment.

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REFERENCES

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