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<td>KODAMA, Hiroshi; MATSUMOTO, Kozo; ONUMA, Misao; MIKAMI, Takeshi; IZAWA, Hisao</td>
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<tr>
<td>Citation</td>
<td>Japanese Journal of Veterinary Research, 28(1-2): 31-35</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1980-05-31</td>
</tr>
<tr>
<td>DOI</td>
<td>10.14943/jjvr.28.1-2.31</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/2184">http://hdl.handle.net/2115/2184</a></td>
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<tr>
<td>Type</td>
<td>bulletin</td>
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<tr>
<td>File Information</td>
<td>KJ00003407907.pdf</td>
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**Instructions for use**

The objective of this study was to investigate the potential for heterotransplantation of Marek's disease lymphoma-derived cell lines and Marek's disease lymphoma cells to nude mice. The results indicated that these cell lines and cells were not able to successfully establish xenografts in nude mice, suggesting that nude mice may not be suitable for the study of Marek's disease lymphoma.
LACK OF HETEROTRANSPLANTATION OF MAREK'S DISEASE LYMPHOMA-DERIVED CELL LINES AND MD LYMPHOMA CELLS TO NUDE MICE

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(Received for publication, February 27, 1980)

Nude mice of BALB/c background were used for the heterotransplantation of Marek's Disease (MD) lymphoma-derived cell lines (MDCC-MSB 1, MDCC-RP 1 and MDCC-JP 2) or MD lymphoma developed in a Marek's disease virus-inoculated chicken. None out of the 57 nude mice developed tumors at the site of inoculation. These nude mice formed cytotoxic antibody against MD lymphoma-derived line cells 6-14 weeks after inoculation. The lack of heterotransplantation of cells from avian origin into nude mice is discussed in relation to the existence of natural cell-mediated immunity and of cytotoxic activity of antibody in nude mice.

INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a herpesvirus (Churchill & Biggs, '70). The host defence mechanisms against Marek's disease virus (MDV) infection and against tumor formation have been investigated (Haffer et al., '79; Kodama et al., '79, '80; Powell, '76; Powell & Rowell, '77; Ross, '77; Schierman et al., '76; Sharma & Coulson, '77; Sharma et al., '75; Sugimoto et al., '78; Witter et al., '75). Several cell lines (e.g., MDCC-MSB 1, MDCC-RP 1, MDCC-JP 2 and others) have been established from the MD lymphomas (Akiyama & Kato, '74; Nazarian et al., '77; Yamaguchi et al., '79). These cell lines as well as MD tumor cells express the MD tumor-associated surface antigen (MATSA) (Nazarian et al., '77; Witter et al., '75; Yamaguchi et al., '79) which seem to have an important role in the antitumor response of the host (Powell & Rowell, '77; Sharma & Coulson, '77; Witter et al., '75).

Inoculation of MDCC-MSB 1 cells to chickens, however, does not result in the development of a tumor at the site of inoculation (Nazarian & Witter, '75). Presumably because the MDCC-MSB 1 line cells do not proliferate in chickens, transplantation is not established in the recipient (Dot et al., '76). This may be due to the rejection of

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injected cells by host caused by differences in histocompatibility antigens. To overcome this problem and to investigate antitumor immunity against MATSA, heterotransplantation of MDCC-MSB 1 line cells to nude mice was attempted (Powell, '76). In the present study we examine the possibility of heterotransplantation of MD lymphoma derived line cells (MDCC-MSB 1, MDCC-RP 1, MDCC-JP 2) and naturally occurring MD tumors into nude mice.

MATERIALS AND METHODS

Nude mice

BALB/c-nu/nu mice were supplied from the Institute of Medical Science, Tokyo University, Tokyo, Japan. They were maintained in flexible vinyl film isolators under specific-pathogen-free conditions. Some nude mice were irradiated by sublethal doses of 200 rads X-rays prior to implantation of MD cells.

Cell lines

The MDCC-MSB 1 cell line was kindly provided by Dr. S. Kato (Research Institute for Microbial Diseases, Osaka University, Osaka, Japan). The MDCC-RP 1 cell line was obtained by the courtesy of Dr. S. Horiuchi (Poultry Disease Laboratory, National Institute of Animal Health, Seki, Japan), who had originally received it from Dr. K. Nazarian (Regional Poultry Research Laboratory, East Lansing, Michigan, USA). MDCC-JP 2 was kindly supplied by Dr. H. Kawamura (Poultry Disease Laboratory, National Institute of Animal Health, Seki, Japan). These cell lines were cultured in RPMI-1640 supplemented with 10-20% fetal calf serum, penicillin (200 U/ml), streptomycin (200 μg/ml), and fungizone (2.5 μg/ml), at 41°C in an atmosphere containing 5% CO₂. Nomenclature for cell lines used herein is as recently proposed by Witter et al. ('79).

Tumor specimens

Ovary MD lymphoma of a 42-day-old specific-pathogen-free White Leghorn line PDL-1 chicken which had been inoculated with JM strain of MDV at one day of age was used (Kodama et al., '79). Enlarged nerve tissues of the bird were also used. Tumor or nerve tissues were teased out with scissors and forceps in RPMI-1640 supplemented with 20% fetal calf serum and antibiotics. The cells were washed twice with medium by centrifugation.

Injection of line cells or tumor tissues

About 0.5 ml cell suspension (1.0 to 3.9 × 10⁶ viable cells per 0.5 ml) from line cells or tumor tissues were injected into the subcutaneous layer of the back of nude mice using 18 gauge of needle. Viability of the line cells was more than 75% as determined by the trypan blue dye-exclusion test.
Complement-dependent antibody cytotoxicity (CDAC) test

The CDAC test was carried out by the trypan blue dye-exclusion test. Twenty-five μl of line cell suspension (1 × 10^7 cells/ml), 25 μl of mouse serum and 25 μl of guinea pig complement (1:4) were mixed in the well of a microplate and incubated at 41°C for 45 min. After incubation 50 μl of 0.16% trypan blue solution was added to each well, and the viability of cells was immediately established by counting a minimum of 100 cells. Antibody titer was expressed at the highest dilutions of serum showing 50% cytotoxicity. Cytotoxicity was calculated by the following formula.

\[ \text{Cytotoxicity} (\%) = \frac{\text{viable cell \% in test sample} - \text{viable cell \% in control sample}}{\text{viable cell \% in control sample}} \times 100 \]

RESULTS AND DISCUSSION

The results of heterotransplantation of line cells and cells from MD tumor into nude mice are shown in table 1. No progressively growing tumor was observed in any of 57 nude mice inoculated during 3 months of observation. Line cells or MD tumor cells injected subcutaneously into the back of adult or newborn nude mice disap-

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<th>TRANSPLANTS</th>
<th>NUDE MICE (NU/NU)</th>
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<td>line cell or MD tumor cell number (×10^6)</td>
<td>age</td>
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**Line cell**

| MDCC-MBS 1 | newborn | - | 0/5 | ND*3 |
| MDCC-RP 1 | 3-7 wks | + | 0/3 | ND |
| MDCC-JP 2 | 5 wks | + | 0/10 | 10/10 |
| MD tumor (ovary) | newborn | - | 0/4 | 4/4 |
| (ovary) | 2 wks | - | 0/2 |
| (nerve) | 4 mos | - | 0/4 |

*1 Mice were irradiated with 200 rads of X-rays.

*2 Cytotoxic antibody was determined 6-14 weeks after inoculation against homologous line cells. Cytotoxic antibody was not determined in serum from non-inoculated nu/nu and nu/+ control mice.

*3 Not done
peared within 1 week and never regrew. The lack of heterotransplantation of these cells to nude mice indicates that some mechanisms of rejecting or inhibiting tumor growth other than T cell-mediated cellular immunity such as allogenic inhibition may exist. The immune responses may be against chicken antigen and MATSA of the transplanted cells. The recent recognition of natural cell-mediated immunity (HERBERMAN, '78) has substantially altered the concepts concerning the potential mechanisms for resistance against tumor growth. Cell-mediated cytotoxicity against tumor cells has generally been thought to be mediated by (a) T cells, (b) antibody-dependent cytotoxic cells, and (c) activated macrophages or macrophages armed with specific antibodies (CEROTTINI & BRUNNER, '74). As shown in table 1, the nude mice inoculated with line cells developed cytotoxic antibodies against line cells 6–14 weeks after inoculation. No cytotoxic antibody was detected in serum from non-inoculated nu/nu and nu/+ control mice. Antibody-dependent cytotoxic cells and antibody-coated macrophages may act as cytotoxic effector cells in rejection of transplanted cells. In addition, macrophages may themselves react with the transplant before antibodies to these cells develop. Firm identification of the cytotoxic effector cells remains to be established. POWELL ('76) reported that only 1 of 9 nude mice inoculated with MSB-1 developed generalized lymphadenopathy and had two abdominal tumors of avian origin. The reason for the discrepancy of our results with POWELL’s is not known. As discussed above, non-T cell-mediated immunological activity of nude mice against chicken antigen and MATSA might be an important factor. It will be necessary to investigate using immunosuppressants such as antimacrophage substances or anti-B cell serum to see what role these cells may play. As shown by the results of the present study, nude mice are not suitable hosts for MD tumor-derived line cells or MD tumor cell growth. Possibly because birds are phylogenetically distant vertebrates from nude mice.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan. We thank Miss Y. MUNAKATA for technical assistance and for the preparation of the manuscript.

References

Lack of heterotransplantation of MD cells to nude mice

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