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**SOMMAIRE**

M. Denechaud, M. Laval, J. Robert, D. Ducassou
Efficacité thérapeutique de l'adriamycine chez des souris porteuses de tumeurs solides autochtones ou de tumeurs greffées

M. Beer-Gabel, A. Yerushalmi
Altered glucose metabolism in intact and tumor bearing rats subjected to local hyperthermia

J. Gueret, L. Montagner, J. P. Burisson, R. Royer
Action cytostatique de nitro-2 naphtofurannes sur des cellules de la leucémie murine L 1210 en milieu semi-solide

J. E. Burhers, R. L. Faman, G. J. Juillard
Induction of canine in vitro reactivity to alloantigen following intralymphatic immunization

D. Maraninch, J. A. Gastaut, N. Tubiana, Y. Carcassonne
Étude de la Vindesine en perfusion de 5 jours dans le traitement des leucémies et lymphomes

C. Aubert, J. P. Cano, J. P. Riaut, J. F. Seitz, Y. Carcassonne
Pharmacokinetics of 5-fluorouracil: Impact of the measurement of the 5,6-dihydrofluorouracil (Note)

F. Saiz-Garcia, V. Rodriguez-Valverde
Serum factors inhibiting E-rosette formation in patients with lung malignancies (Note)

J. P. Dero, P. Chambord, Ph. Rouger, C. Faevert
Échec de la chimiothérapie des cancers de la thyroïde (Note)

J. Hamez, P. Blondiaux, M. Mirgoux
Cutaneous angiosarcoma arising in a mastectomy scar after therapeutic irradiation (Cas clinique)

Association Française pour l'Étude du Cancer : Forum de Cancérologie 1981 (Résumés)

J. Y. Cahn, P. Hurteleur, J. F. Rosset, S. Schraub
La morphine par voie orale dans les douleurs des cancéreux : A propos des complications digestives majeures. (Lettre à la Rédaction)

J. A. de Brux
Lésions frontières de l'ovaire (étude histo- et cytologique). (Nouvelles et Travaux)

Informations

Contents in English language

See overleaf
Induction of Canine in vitro Reactivity to Alloantigen Following Intralymphatic Immunization

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SUMMARY:

An experimental model has been developed using the dog to study the induction of systemic cell-mediated immunity following intralymphatic immunization (ILI) with allogenic cells. As detected in one-way mixed lymphocyte cultures, blastogenically-reactive immune peripheral blood lymphocytes were observed after the third ILI with 10^6 cells. The in vitro reactivity was augmented by a fourth ILI to a node not previously injected indicating that a response in one node was followed by the trafficking of memory cells to other nodes. No immune PBL were detected after four ILI with lower doses of 10^5 or 10^4 cells. However, these dogs subsequently responded to a single injection of 10^7 cells with high levels of immune lymphocytes which were detectable for up to 30 days. Apparently, ILI with 10^5 or 10^6 cells, while insufficient to produce detectable levels of alloreactive lymphocytes were sufficient for lymphocyte priming. Results obtained with this model may also suggest the potential for the delivery of immune blood has been described as a convenient source of lymphocytes for perfusion MLC (14). Our results indicate that ILI is an effective mode of inducing high levels of prolonged systemic anti-alloantigen CMI in the dog. Furthermore, immunological priming was detectable with antigen doses as low as 10^3 cells. The development of a canine model to study ILI-induced immunity will expedite understanding the role of ILI in immunotherapy of malignant disease.


INTRODUCTION

Intralymphatic immunization (ILI) using tumor cell vaccines has been used in the past as sole therapy for the treatment of both canine and human malignant disease (4, 5). This route of administration has been demonstrated to deliver a high concentration of cellular material to the lymphoid tissue where it is efficiently trapped for prolonged periods (3), thus maximizing the potential for the generation of an antitumor immune response. Clinically, tumors in patients receiving ILI have been observed to both stabilize and, in some cases, regress in size during treatment (5, 6). In those cases of tumor regression subsequent to ILI with autologous vaccines, it has seemed likely that this effect was brought about by a systemic antitumor immune response although direct evidence for this has been lacking. Other investigators have used ILI. Two dogs, 66 and 60 were treated with 10^5 x 10^6 cells alone, responder PBL alone or with 10^5/ml phytohemagglutinin, or PBL with irradiated autologous PBL instead of allogenic PBL. On the last day of culture, 1 µCi of ^3H-thymidine (specific activity 40 Ci/m mole) was added to each well for the final eight hours of culture, and the cultures were then harvested onto glass fiber filters with multiple automatic sample harvester (Brandel) for scintillation counting. The blastogenic response is presented as stimulation index (S.I.) which was calculated as the ratio of counts per minute (CPM)^3H incorporated by PBL in the presence of allogenic stimulator cells to the CPM^3H incorporated by PBL in the presence of irradiated autologous PBL.

RESULTS

Initially, it was necessary to establish whether the PBL to be used as immunogen were histoincompatible with the recipient dogs and able to stimulate recipient dogs' PBL to undergo blastogenesis in vitro. Therefore, primary MLC were established using PBL taken prior to therapy (day 0). Two dogs, 66 and 60 were found to be stimulatory towards dogs 28, 33 and 60 and dogs 22, 66 and 99 respectively (table I). Although the amount of stimulation varied, the day 6 response was always or greater...
Table I. — Primary in vitro PBL lymphoblastic responses prior to ILI

<table>
<thead>
<tr>
<th>Responder No.</th>
<th>Stimulator No.</th>
<th>Stimulation Index</th>
<th>Ratio d5</th>
<th>d14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>2.4</td>
<td>2.7</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
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<td>4</td>
<td>66</td>
<td>1.0</td>
<td>1.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table II. — In vitro PBL lymphoblastic responses following intralymphatic immunization

<table>
<thead>
<tr>
<th>Immunizing Dose</th>
<th>Responder No.</th>
<th>Stimulator No.</th>
<th>Stimulation Index</th>
<th>Ratio d5</th>
<th>d14</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁴</td>
<td>22</td>
<td>60</td>
<td>1.0</td>
<td>1.7</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>60</td>
<td>1.7</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>60</td>
<td>1.5</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>60</td>
<td>2.1</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>10⁵</td>
<td>66</td>
<td>66</td>
<td>0.7</td>
<td>1.2</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>66</td>
<td>0.7</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>66</td>
<td>1.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>66</td>
<td>2.1</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>10⁶</td>
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<td>99</td>
<td>1.5</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>99</td>
<td>1.5</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>99</td>
<td>3.1</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>99</td>
<td>6.0</td>
<td>0.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

A. Triplicate mixed-lymphocyte cultures of 2 x 10⁵ responder PBL from the indicated dogs were stimulated by varying numbers of irradiated PBL either from dog 60 or 66. Duplicate plates were 3H labelled and harvested on day 4 or 6.

B. The lymphoblastic response is expressed as stimulation index and calculated as:

S.I. = 100 x CPM of Responder lymph / CPM of Autologous lymph

C. Allogeneic stimulus lymph.

The capacity of PBL from dogs 60 and 66 to stimulate an immune response had been established, intralymphatic immunizations were begun. Dogs 28, 33 and 66 were immunized with 10⁶, 10⁷ and 10⁸ irradiated PBL, respectively, from dog 66. Dogs 28, 33 and 66 were reciprocally immunized against each other raising the possibility of a graft versus host reaction following ILI. This was not a problem, however, since all cells used as immunogen were irradiated with 2,000 rads prior to injection. After each ILI, blood was drawn on day 5 and 14 for culture in a 4-day MLC, and the ILI were repeated every 21 days. The first three immunizations were given in the front right leg while the fourth ILI was administered to the right rear leg. As table II indicates, the dogs receiving 10⁵ and 10⁶ cells per ILI did not generate detectable levels of immune PBL while the dogs receiving 10⁷ cells per ILI (dogs 99 and 60) had reactive PBL five days after the third immunization. The fourth ILI was into lymphatics and nodes not previously injected, PBL drawn on day 5 after the fourth ILI from dogs 99 and 60 showed strong in vitro reactivity and the day 14 response was greater than the day 14 response following the third ILI. PHA reactivity on the other hand, remained high throughout the protocol and was not seen to vary as a function of ILI (unpublished data).

In order to study the kinetics of the appearance of immune PBL, two dogs (22 and 66) which had not responded to 4 ILI with low cell doses were given a fifth ILI of 10⁵ PBL from the same dog against which they had previously been immunized. PBL drawn from these two dogs one day prior to immunization and on days 1, 3, 5, 6, 10, 17, 24, 28 and 36 thereafter were used in four-day MLC to detect PBL reactive with the immunizing cells. As can be seen in figure 1, immune PBL were not detected until days 3 and 6 after ILI at which time the S.I. rose to 10 to 20 fold. With some fluctuation, the activity remained high for several weeks and significant responses were detected as long as 24 days after immunization.

DISCUSSION

The intralymphatic route of immunization is presently being used in experimental immunotherapy of spontaneous malignant disease (2, 5, 6). The principal advantage of this approach is that large amounts of cellular vaccine material may be efficiently and completely trapped in lymph nodes for prolonged periods of time, thus maximizing the opportunity for an effective immune response (3). Tumors of ILI patients, both human and canine, have been observed to regress even when the ILI has been to nodes.
which do not drain the tumor site, implying the possibility that a systemic immune response is central in tumor immunity.

In order to study the capacity of ILI to induce systemic cell-mediated immunity, the dog was chosen as an experimental model because of technical limitations on lymphatic cannulation in smaller species. The capacity of canine PBL to detect cell-associated antigens in MLC (table 1) has previously been described (14), and the cell MLC has been used notably to detect histocompatibility antigens but autochthonous lymphoma-associated antigens as well (15). In the present study was conducted to establish an experimental model to monitor the development of cell-mediated immunity following ILI. PBL were selected as the source of responder cells both for the sake of convenience and because the detection of immune PBL following ILI would support the notion that cellular immunogen which had previously been immunized with low cell doses and had not generated detectable levels of immune PBL were each given a single ILI of 10^7 cells and their PBL tested at intervals thereafter in MLC. The first, and highest, responses were observed on day 3 and day 6, with the in vitro reactivity falling off after that. The earlier day 3 response seen in one dog may relate to the fact that this dog had previously received 4 ILI of 10^6 cells each while the other dog which did not respond until day 6 had 4 previous ILI of only 10^6 cells each. Both dogs did not receive the final ILI of 10^6 cells, however, in contrast to dogs 99 and 60 which also received 10^6 cells per ILI but did not respond until after the third injection. Apparently, immune PBL were detectable but insufficient to produce detectable levels of immune PBL. The rapidity with which immune PBL were detected indicates that the trafficking of immune lymphocytes, in contrast to cellular immunogen, into the peripheral blood via the thoracic duct is not temporally impeded by the lymph node. Significant levels of immune PBL were detected as long as 24 days after immunization, with levels falling to background after that. Lymphocyte responsiveness to mitogen PHA stimulation was not observed to vary as a function of ILI (unpublished data), indicating that the increased in vitro reactivity to alloantigen following ILI was not due to non-specific hyper-reactivity.

Intralymphatic immunization, then, is an effective method of inducing high levels of systemic cell-mediated immunity against cell-surface associated antigens. Furthermore, the dog provides a convenient experimental model with which to study the intralymphatic route of immunization. High levels of systemic cell-mediated alloimmunity were attained after 3 ILI of 10^6 cells without adjuvant and were maintained for several weeks. Even extremely low antigen doses (10^4 cells) administered intralymphatically were able to generate local immune responses as indicated by the kinetics of systemic immunity following subsequent ILI with 10^7 cells. The capacity of the dog to serve as an experimental model with which to maximize and utilize the effects of ILI will be of particular importance as an adjunct to the ongoing human ILI trials.

Acknowledgments

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