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京都大学
A New Technique of Microsurgical Adult Thymectomy in Mice

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Abstract

A new technique of microsurgical adult thymectomy in mice was described. An operative mortality less than 5% was obtained ensuring a complete removal of thymic lobes by this microsurgery. The main factors contributing to this increased survival are the application of microscopical magnification and the dissection with well-controlled aspirators.

Accordingly, immunological responses in relation to T cell subsets to anti-tumor immunity can be investigated satisfactorily.

Introduction

The thymus is known to be essential for the differentiation of T lymphocytes that are closely related to the cellular immunity, and adult thymectomy may be helpful for the analysis of the role of T cell subsets in the anti-tumor cellular immunity.

The major procedures for adult thymectomy have been described in the literatures1,2,3,4,5. In order to remove thymic lobes more completely without cutting the sternum and to avoid the operative damages, especially pneumothorax, as much as possible, a modified microsurgical adult thymectomy was designed.

Materials and Methods

Instruments:

iris forceps and scissors, microdissection scissors and forceps, Michel's surgical clips and forceps, vacuum pumping-set (Model MA-2, Nishizawa LTD) and two aspirators (devised Pasteur pipettes), devised operating board and operating binocular dissecting microscope (Fig. 1).

Key words: Microsurgical adult thymectomy, Anti-tumor cellular immunity, Life-span of T cell subsets, Kinetics of T cell subsets.

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Ether is preferable for general anesthesia since the recovery period is short. Although there is some difficulty in mucous secretion, this trouble can be avoided by premedication with atropine sulphate (0.01 mg/mouse) injected subcutaneously. This medication, however, is not always needed.
Animals:

6- to 8-week-old C57BL/6 mice from Animal Center of Kyoto University were used throughout the experiments.

Adult thymectomy:

1. The anesthetized mouse is put on the supine position with the head turned to the operator (Fig. 1).
2. After cleaning the skin of the neck region with 70% ethanol, a small midline longitudinal skin incision of about 7 mm is made over the sternum, which is not splitted. It is possible to open into the thoracic cavity by mild retraction ventrally. If pneumothorax has taken place, air should be evacuated from the thoracic cavity postoperatively.
3. The submandibular glands are then freed and retracted anteriorly. The sternohyoid muscles are separated, and the trachea and manubrium sternum are exposed. It is

Fig. 2. Photographic view of thymic lobes and surrounding structures after lifting the sternum ventrally.
important that the sternohyoid muscle should be carefully and gently separated and retracted, since the bilateral carotid arteries and jugular veins are present in the close vicinity.

4. The sternum is then lifted ventrally with the aid of retractors and thereby the thoracic cavity is opened. The anterior ends of bilateral thymic lobes are exposed overlying the mediastinum (Fig. 2).

5. The larger suction cannula is then used with gentle manipulation; the lobes can be sufficiently aspirated together with applying the microdissection scissors and forceps. Cares should be taken not to injure the adjacent vagus and recurrent laryngeal nerves and vessels (especially the superior vena cava) as well as the heart and lungs.

6. Thereafter, with the smaller suction cannula, the total removal of thymic lobes should be confirmed as carefully as possible. Speedy and correct microsurgical procedures are mandatory after opening the thoracic cavity, to keep the operative mortality minimum.

7. The wound is closed with Michel’s surgical clips and forceps. The neck clips are removed on the 7th postoperative day.

** The mice with thymic lobes and remnants should be discarded from the study. However, we had no instances of incomplete adult thymectomy confirming by autopsy at the end of study.

** Sham-thymectomy: age- and sex-matched mice were used for sham-thymectomized mice, opening the thoracic cavity.

** A binocular dissecting microscope was enabled the operator to observe clearly all anatomical structures. Magnification of approximately 10–20 was sufficient.

** Results**

Mortality due to surgery was approximately 3.7% (Table 1). This modified microsurgical technique has been used in 846 mice. The cause of death were mainly due to uncontrollable

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<th>Table 1. Operative results and causes of death</th>
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<tr>
<td>success rate</td>
</tr>
<tr>
<td>adult thymectomy (ATx)</td>
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<tr>
<td>sham thymectomy (STx)</td>
</tr>
<tr>
<td>total</td>
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<tr>
<td>2. Causes of death:</td>
</tr>
<tr>
<td>venous bleeding</td>
</tr>
<tr>
<td>pneumothorax</td>
</tr>
<tr>
<td>excessive anesthesia</td>
</tr>
<tr>
<td>postoperative epilepsy</td>
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bleeding and excessive anesthesia. The average operating time was about 15 minutes per mouse.

Comment

We have described a technique of modified microsurgical adult thymectomy in mice which carried a high survival rate and always ensured a complete removal of thymic lobes.

The main factors contributing to this increased success rate are the application of microscopic magnification and the dissection with well-controlled aspirators.

By this method, the effects of adult thymectomy on the growth of tumor and the kinetics of T cell subsets in tumor-bearing mice have been successfully investigated.

In order to gain more informations on the surface phenotypes in relation to the life-span of T cell subsets and to resolve the kinetics of killer T cell induction in brain tumor, the effects of adult thymectomy on the growth of tumor have been investigated.

Acknowledgements

We are grateful to Professor M. HANAOKA and Dr. Y. NAMBA for valuable advices on the analysis of immunological responses to the experimental tumor.

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References

手術顕微鏡的成熟マウス胸腺摘出術

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胸腺は、細胞性免疫の中枢的役割を担い、胸腺由来のT細胞は、腫瘍免疫における複雑なネットワークの主要な免疫担当細胞である。従って、胸腺摘出は、T細胞に関連した抗腫瘍細胞性免疫機構の検索に際し、ひとつの優れた手段であると考えられる。

従来の胸腺摘出術に手術顕微鏡的技術（microsurgery）を導入し、手術成功率及びマウス死亡率が各々100％、3.7％と好成績を得た。