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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 cells 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 literatures[1,2,3,4,5].

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.
索引語 手術顕微鏡的胸腺摘出術，抗腫瘍細胞性免疫，T細胞サブセットの寿命，T細胞サブセットの動態.
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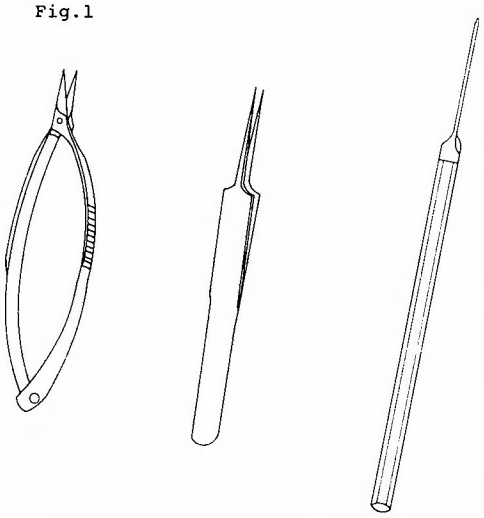
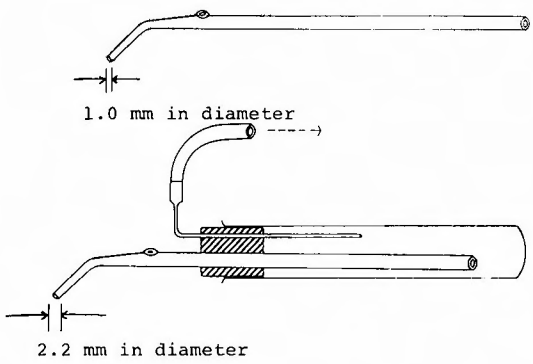
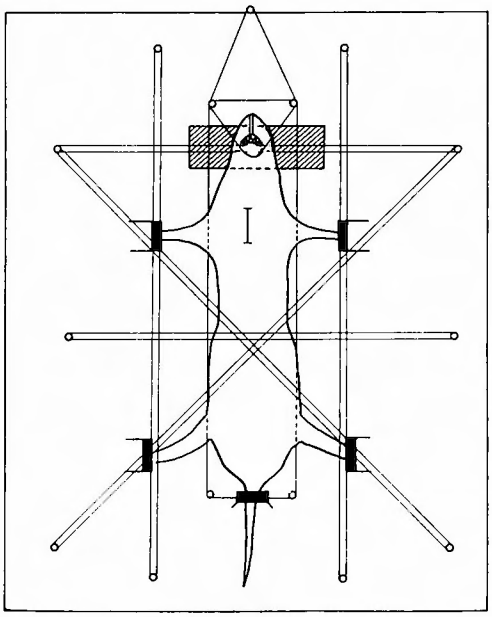
Fig.1



microdissection scissors and forceps



devised aspirators



Operating board

Fig. 1. Diagrams showing operating board, microdissection scissors and forceps and two devised aspirators.
The operating board is designed as illustrated in the figure, which enables satisfactory fixations of an anesthetized adult mouse and a comfortable support for the operator.
A suction flask set-up is needed to collect thymic lobes totally removed through a glass cannula. The specimen is able to be examined macroscopically and histologically.
The two cannulas are made as illustrated in the figure. Their diameters depend on the size of thymic lobe to be removed; larger one should be slightly smaller than the normal diameter of thymic lobe. For example, it should be approximately 2.2mm for adult mice. The other should be much smaller, approximately 1.0 mm, to be used to confirmed total surgical removal.

Anesthesia:

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

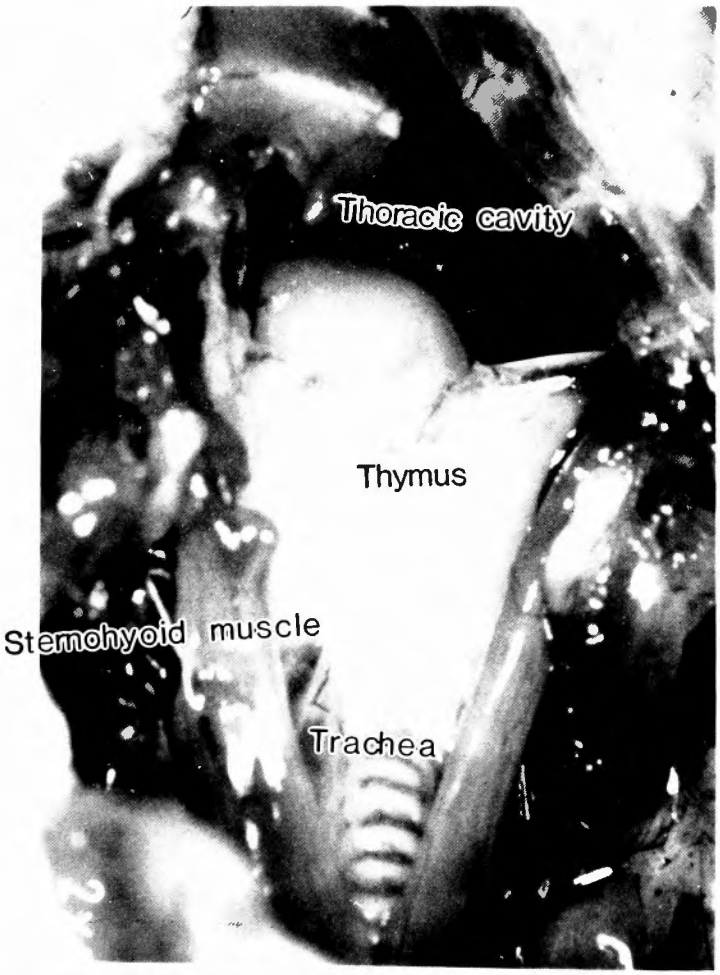


Fig. 2. Photographic view of thymic lobes and surrounding structures after lifting the sternum ventrally.

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 reccurent 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 enable 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

Table 1. Operative results and causes of death

1. Results:		
	success rate	death
adult thymectomy (ATx)	420/444 (94.6%)	24
sham thymectomy (STx)	395/402 (98.3%)	7
total	815/846 (96.3%)	31
2. Causes of death:		
venous bleeding	11 (ATx; 9 STx; 2)	
pneumothorax	13 (ATx; 10 STx; 3)	
excessive anesthesia	8	
postoperative epilepsy	2	
unknown	5	

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 microscopical 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[6].

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

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和文抄録

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．