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科学研究費補助金研究成果報告書

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研究種目：基盤研究(C)
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研究課題名（和文）　腸－脳相関からみた移植腸管機能維持および呼吸器感染予防に関する分子生物学的研究

研究課題名（英文）　Investigation for maintaining transplanted intestinal allograft function and for preventing respiratory tract infection by the application of a concept of gut-brain axis

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研究成果の概要：
小腸移植後の免疫抑制剤大量投与下では、移植腸管の粘膜は萎縮し、また、呼吸器感染に対する抵抗力も低下している。安全に小腸移植を行うためには、移植後の小腸の機能を正常に維持すること、また、呼吸器感染を予防することが重要である。今回の研究では腸―脳相関の概念を応用し、神経ペプチドである bombesin を移植後に投与し、移植腸管の機能維持あるいは呼吸器感染予防が可能かを検討したところ、移植腸管の粘膜構造だけではなく、消化管運動に重要な役割を果たす神経節細胞やペースメーカー細胞である Cajal 細胞も bombesin が正常と同様に維持することが判明した。呼吸器感染予防に関しては現在のところ十分なデータ解析に至っていない。

交付額

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２００８年度	1,300,000	390,000	1,690,000
総　計	3,500,000	1,050,000	4,550,000

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キーワード：小腸移植、神経ペプチド、腸―脳相関、bombesin, 感染予防、粘膜免疫

１．研究開始当初の背景

これまでの我々の異所性小腸移植モデル研究で神経ペプチドである bombesin が移植腸管の粘膜萎縮を予防し、正常腸管と同様の形態を維持する機能を持つことを発見し、報告してきた。

２．研究の目的

本研究の目的は、同所性移植モデルを作成し、移植後に bombesin が粘膜の形態だけ無く、様々な腸管機能を維持することが出来るかあるいは同じ粘膜免疫系である呼吸器粘膜においても正常と同様の免疫応答性が維持出来るかを検討することである。

３．研究の方法

ラットを用いた同所性小腸移植モデルを作成し、術後に免疫抑制剤単独投与と bombesin を併用投与した群と比較することによって、小腸の粘膜構造のみならず神経節細胞、消化管運動ペースメーカー細胞、小腸および肺の粘膜免疫細胞などを形態学的あるいは免疫学的な手法を用いて解析した。

４．研究成果

従来の異所性モデルと同様、粘膜構造の維持に関してはbombesin非投与群に比較して bombesin投与群で有意に小腸粘膜のvilliの構造が維持され、また、enteric nervous

systemに関してもbombesinを投与することにより神経節細胞およびCajal細胞の萎縮が予防できることが判明した。すなわち、同所性小腸移植モデルにおいても神経ペプチドであるbombesinが急性拒絶反応を悪化させることなく、移植腸管の構造維持にきわめて有効であることが明らかとなった。

Materials and methods

1.1. Animals

Twelve male Brown-Norway rats (250-300 g) (Shimizu Laboratory Supplies Co., Ltd. Kyoto, Japan) were used as donors, and 12 male Lewis rats (300-350 g) (Shimizu Laboratory Supplies Co., Ltd. Kyoto, Japan) were used as recipients of SBTx. This study was approved by the Committee for Animal Research at Kyoto Prefectural University of Medicine (Kyoto, Japan).

1.2.Experimental design

The donor operation was performed according to a modified procedure reported by Monchik and Russell. A 20-cm segment of the distal ileum 10 to 30 cm proximal to the ileocecal junction was removed for the graft. After dissection of the right renal artery, the aorta was ligated above the origin of the superior mesenteric artery and dissected 0.5 cm below it. The portal vein was cut at the hepatic hilus, and then the graft was removed. The grafts were immediately immersed in a cold bath. A 16-gauge polyethylene tube was attached to the portal vein as a cuff with a 6-0 silk ligature. Recipient operations were carried out according to the procedure reported by Wallander et al and Toyama et al . The left renal vessels were separated and clamped with microclips as close as possible to the root, and a left nephrectomy was performed. The cuff was attached to the renal artery using a 20-gauge polyethylene tube in the same manner as for the graft portal vein. Venous reconstruction was performed between the cuffed donor PV and the left renal vein of the recipient. Arterial reconstruction was performed between the cuffed left renal artery of the recipient and the donor aorta containing superior mesenteric artery. Both ends of the intestinal graft were exteriorized as stomas (Thiry-Vella fistula). From the first postoperative day, all rats were administered FK506 intramuscularly at a dose of 0.32 mg/kg per day. On postoperative day 14, the animals were divided into 2 groups of 6 rats each to be administered BBS (10 μg/kg per day; Sigma Chemical Co, St Louis, Mo) or saline as follows: group A, allogeneic SBTx with FK506 and saline and group B, allogeneic SBTx with FK506 and BBS. Bombesin or saline was administered using an osmotic minipump (Alzet 2002, Palo Alto, Calif), which was implanted subcutaneously. This pump delivers solutions continuously for 14 days. After 2 weeks' treatment with BBS or saline, the rats were weighed, and the transplanted grafts were removed.

1.3.Histologic examination

After full-thickness segments from the grafts of each group were removed, they were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Sections were cut 6-μm thick and stained for analysis.

1.4.c-kit

Interstitial cells of Cajal were analyzed by immunohistochemistry with an anti-c-kit antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), which reacts exclusively with the ICC membrane. Immunohistochemical staining was performed using a streptavidin-peroxidase technique (Histofine SAB-PO(R) kit; Nichirei, Tokyo, Japan), with diaminobenzidine as the chromogen. The c-kit antibody was used at a dilution of 1:100 with phosphate-buffered saline. The number of clusters of c-kit-positive cells was counted in the myenteric plexus around the circumference of 5 different axial sections of each graft. The number of clusters was expressed as clusters/cross section (C/CS). The evaluation was performed in a blinded fashion.

1.5. General neuronal marker PGP 9.5

To evaluate the expression site of c-kit-positive cells, the graft enteric ganglia were stained immunohistochemically with a general neuronal marker protein gene product (PGP) 9.5 (RA95101, UltraClone Limited, Isle of Wight, United Kingdom). Analysis was performed by immunohistochemistry with a SAB-PO(R) kit with diaminobenzidine as the chromogen. The antibody PGP9.5 was used at a dilution of 1:400 with Tris-buffered saline. The ganglionic count of the myenteric plexus was obtained by counting the number of PGP9.5-positive ganglia around the circumference of 5 different axial sections of each graft and. The ganglionic count was expressed as ganglia/cross section (G/CS). This evaluation was also performed in a blinded fashion.

2.Results

2.1.Bombesin maintained allograft enteric ganglia

Acute rejection was suppressed by FK506 in both groups, even if it was administered at a low

dose. Immunohistochemical findings of graft enteric ganglia stained with PGP9.5 are shown in Fig. 1. PGP9.5-immunoreactive ganglia darkly stained with PGP9.5 were localized to the submucosal layer and intramuscular layer. The myenteric ganglia were counted around the circumference of axial sections of grafts for evaluation. The numbers of PGP9.5-positive myenteric ganglia of the allograft in group A and group B were 36.3 ± 9.2 G/CS and 53.4 ± 7.0 G/CS, respectively. There was a significant difference between the 2 groups (Table 1). Myenteric ganglia treated with BBS were well preserved compared with those in group A.

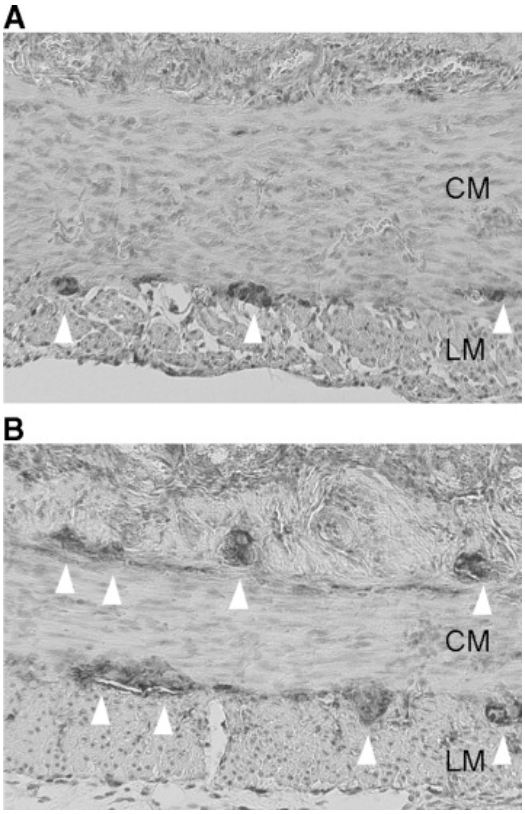

Fig. 1. Graft enteric ganglia stained by PGP9.5. Ganglia in submucosal layer of group A (A, normal saline control) were diminished because of FK506 neurotoxicity. Myenteric ganglia of group A were also decreased compared with those of group B (B, BBS treated). The BBS-treated enteric ganglia were well preserved. Arrowheads indicate PGP9.5-immunoreactive ganglia. CM indicates circular muscle; LM, longitudinal muscle.

2.2. Expression of c-kit on graft intestine with immunohistochemistry

The c-kit immunoreactivity was also localized within the intramuscular layer. The immunoreactivity of c-kit accumulates around 60% of PGP9.5-positive enteric ganglia (Fig. 2). Myenteric ganglia were demarcated by abundant ICC, c-kit–positive cells, with brown staining in both groups (Fig. 3). The overt c-kit immunoreactive cells situated at the surface of myenteric ganglia were counted as "c-kit–positive cell cluster" for this evaluation. The number of c-kit–positive cells clusters in group A was 22.3 ± 5.5 C/CS and that in group B was 36.3 ± 5.1 C/CS, respectively. Interstitial cells of Cajal were well preserved in group B. There was a significant difference between groups A and B (Table 1).

Table.1

	Ganlion cells	Cells of Cajal	
Group A	36.3±9.2	22.3±5.5	
Group B	53.4±7.0	36.3±5.1	C/CS

A: Bombesin非投与群　B: Bomesin投与群

Counts of PGP9.5-positive ganglia (Ganglion cells) and c-kit positive clusters(Cells of Cajal) Data are presented as means ± SD. $P < .001$ vs group A.

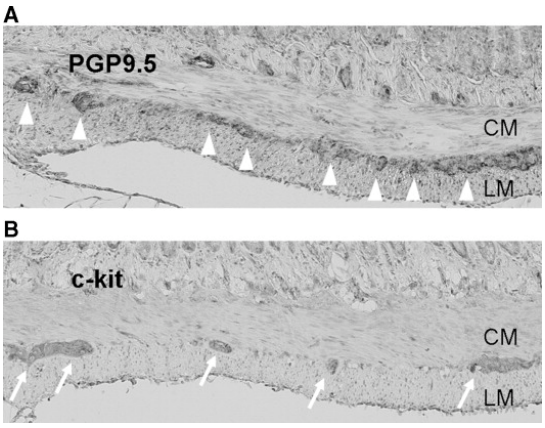

Fig. 2. Expression of immunoreactivity of PGP9.5 and c-kit in small bowel allograft. Tissue sections (A and B) were taken from the same graft treated with BBS (group B). The c-kit immunoreactive cells accumulated around 60% of PGP9.5-positive enteric ganglia (A). Arrowheads indicate PGP9.5-immunoreactive ganglia (A), and arrows indicate the clusters of c-kit immunoreactive cells (B). CM indicates circular muscle; LM, longitudinal muscle.

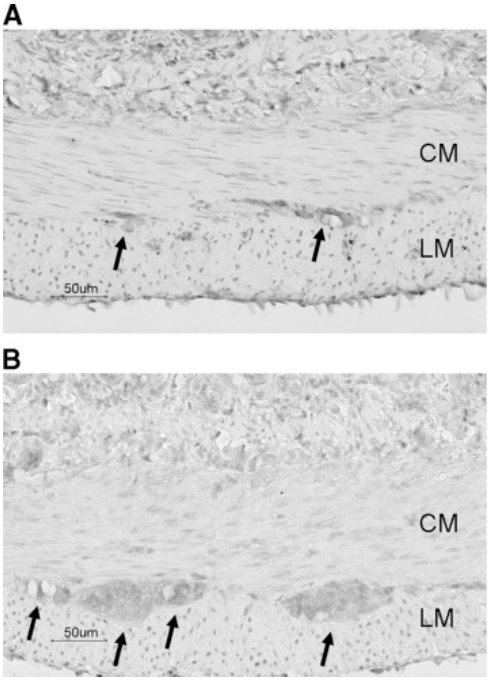

Fig.3 Findings of immunohistochemical study with anti-c-kit antibody in the intestinal allograft on postoperative day 28. Bombesin significantly preserves ICC (c-kit-positive cells) against the FK506 effect in the intestinal allograft.

次に、これらの構造維持が移植後のグラフト腸管の栄養吸収能維持、および粘膜免疫応答性の維持に有効であるかについて解析を試みたが、現在のところ、安定したデータを得ることが難しく、これらの機能維持に関してbombesin投与が有効であるかどうかの結論は得られていない。これらの点についてさらに解析を進めていくことが今後の課題である。

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計２件）

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