氏名	李 湛 卓（り たん たく）
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学位論文名	Antibody deposition in rat hearts that develop transplant vasculopathy in (donor×recipient) F1 environment （ラット心移植後動脈硬化症における病的血管内膜への抗体の沈着）
論文審査委員	（主査） 教 授 白倉 良太 （副査） 教 授 宮坂 昌之　教 授 祖父江憲治

論文内容の要旨

〔目的〕 In clinical heart transplantation, transplant vasculopathy remains the principal limiting factor for the long-term survival of heart transplantation recipients. The pathogenesis of transplant vasculopathy has not been elucidated and no optimal procedures for the prevention and treatment have been developed. The essential role of allo-T cells in the development of transplant vasculopathy has been demonstrated. However conflicting evidence has been reported on the pathogenic role of alloantibodies. We investigated the development of transplant vasculopathy in (donor × recipient) F1 environment to clarify the observed discrepancy between the requirement for antibody production in transplant vasculopathy and its development in a syngeneic environment.

〔方法〕 Minor-histoincompatible WKY (WKY/Crj : RT-1^{l}) hearts were heterotopically transplanted to LEW (LEW/Crj : RT-1^{l}) rats and retransplanted to (WKY×LEW) F1 rats 3 or 5 days after the initial grafting (3 or 5-d reTx group). Grafts were removed 1, 14, 30, or 60 days after retransplantation then subjected to RNA extraction and histological analysis.

Genes preferentially expressed in the 5-d reTx grafts late after retransplantation were identified by mRNA differential display. To verify the results of the differential mRNA display and to assess immune reactions in retransplanted cardiac allografts, the immunoglobulin kappa chain (Ig κ), B-lymphocyte chemoattractant (BLC), and CD40 ligand (CD40L) gene expressions were detected using quantitative RT-PCR. Histological changes were analyzed by Elastica van Gieson and immunohistochemical staining.

〔結果〕 Sixty days after retransplantation, severe vascular stenosis which is typical to transplant vasculopathy disease was only developed in cardiac allografts retransplanted to (WKY×LEW) F1 rats 5 days after the initial grafting (5-d reTx group), but not in those retransplanted on the third day (3-d reTx group). A significant vascular lesion first appeared 30 days after the retransplantation and gradually progressed thereafter.

We performed PCR-based mRNA differential display to find out the promoting factor(s) for transplant

vasculopathy, that is, to identify the genes induced in the 5d-reTx grafts at 14 or 30 days after retransplantation, but not that were expressed on the first day or in the 3d-reTx grafts. One candidate gene product was identified ; its sequence was 99% identical to the constant region of the rat immunoglobulin kappa chain (Ig κ) gene.

To confirm the result from differential display study we also performed real-time quantitative RT-PCR experiment. The relative level of the Ig κ gene expression was minimal in both 5-d and 3-d reTx grafts 1 day after retransplantation. Significant and strong induction in the 5d-reTx grafts wes first seen on the 14th day and the peak of expression was on the 30th day. Ig κ gene induction was also observed in the 3d-reTx grafts on the 30th day, but the level of the induction was less than 10% of the level in comparable 5d-reTx grafts. Immunohistochemistry revealed that CD45R-positive B-lymphocytes preferentially infiltrated 5d-reTx grafts and were concentrated in the pathological neointima and adventitia of the diseased vessels. We also measeured the expression levels of B-lymphocyte chemoattractant (BLC) and CD40 ligand (CD40L), as B cells could have been passively trapped in the grafts due to the change in the vasculature. The expression patterns of the genes were nearly identical to the expression pattern of the Ig κ gene, and their induction peaked on the 30th day, at the beginning of pathological neointimal progression.

Immunohistochemistry also revealed strong depositions of IgM on the neointima and IgG on both the neointima and the media of the diseased vessels of 5d-reTx grafts 30 days after retransplantation, but no antibody deposition was detected on the 1st day, indicating that the antibody depositions were formed after the retransplantation into the (donor $\times$ recipient) F1 animal.

〔総括〕 In summary, we observed B-lymphocyte infiltration and antibody deposition during the development of transplant vasculopathy in a (donor $\times$ recipient) F1 environment. Autoantibody production needs to be considered as a likely factor in the pathogenesis of transplant vasculopathy.

論文審査の結果の要旨

心臓移植の臨床において、心移植後動脈硬化症は遠隔期成績を左右する最も重大なリスクファクターである。しかし、その病態はほとんどわかっておらず、従って治療法もないのが現状である。動物実験から慢性拒絶反応であろうと言われてきたが、移植心を5日後にドナーの系（syngeneic or F1）に戻しても血管病変が発生することが明らかとなった。

本論文は、このラット心房し移植モデルにおいて発症する移植後冠状動脈硬化病変の発生機序を検討したものである。戻し移植を3日目に行うと60日後も病変は軽微であることから、3日目戻し移植（3d-reTx）群と5日目戻し移植（5d-reTx）群を比較検討した。病変発現に関与すると思われる遺伝子発現を検索した結果、5d-reTx 群に、再移植後 30 日をピークに免疫グロプリンの κ 鎖遺伝子が特異的に発現増加することを見いだした。また、病変が完成する時期に病変内に活性化されたB細胞が浸潤してくるのと軌を一にすること、免疫染色で病変部に抗体の沈着が見られることを確認しており、心移植後動脈硬化症の発症に自己免疫の可能性も含めた抗体産生系の関与を示唆したもので、本症の予防と治療に結びつく重要な発見であり、博士の学位を授与するに値すると考える。