最終試験の結果の要旨

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主査および副査の５名は、令和元年 11 月 5 日、学位申請者 Sahar Ghavidel Darestani 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

Q1. Why did you focus on PAFR in monocytes and not other type of blood cells like neutrophils?

A1. The advantage of PBMCs is that it is an easy accessible source of human immune cells, as the cells are isolated form full blood or buffy coats, and monocytes are precursor of macrophages that play a major role for developing atherosclerosis and proteinuria.

Q2. What is the role of monocytes to develop nephropathy?

A2. Several investigations had shown macrophage accumulation in diabetic kidneys, which suggested critical roles for inflammatory processes in development of diabetic nephropathy and a pathogenic role for these cells. It is also associated with the development of albuminuria and renal fibrosis in several models, suggesting that macrophages may have a role in promoting these pathologic responses.

Q3. How did messenger levels of PAFR change during transformation from monocyte to macrophage?

A3. There is no report on the expression level of PAFR mRNA in monocytes versus macrophages. But two studies reported on the levels of PAF. Although both macrophages and monocytes can synthesize PAF following their activation, monocytes accumulate considerably more. One study showed that the accumulation of PAF in stimulated human monocytes decreased by 90% as they differentiated into macrophages.

Q4. Why did you checked CD36, IP 10, and TIMP2?

A4. Inflammation may be a key factor in the development of atherosclerosis and diabetic nephropathy and these are known as proinflammaroty genes.

Q5. PAF-PAFR interaction increases permeability of albumin in glomeruli due to neutralize anionic barrier by polycations. What are polycations?

A5. They include many kind of proteins with cationic charge, calcium ions and polyethyleneimine etc..

Q6. Are there any differences in the composition of PBMCs isolated from patients with or without diabetes?

A6 The PBMC count in our study was as follows: lymph: 16.1%; mono: 3.1% of WBC. One study reported that patients with type 2 diabetes mellitus had PBMC composition ratios comparable to healthy controls.

Q7. Is there any pathological study showing an increased invasion of macrophases in glomerular leision of diabetic nephropathy.

A7. Recently, accumulated data have emphasized the critical roles of inflammatory process in development of diabetic nephropathy. Evidence from animal models has shown that macrophages are the major immune cells infiltrating the kidney in type 1 and type 2 diabetes and their accumulation is linked to the development of hyperglycemia. The development of albuminuria in diabetic mouse kidneys is associated with glomerular macrophage accumulation and podocyte loss. Increasing numbers of kidney macrophages also are associated with the development of albuminuria and renal injury and sclerosis in several models, suggesting that macrophages may have a role in promoting these pathologic responses and contribute to the development of diabetic nephropathy.

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Q8. In addition to the levels of mRNA expression, did you check the surface protein expression on the cells using FACS study?

A8. As mentioned, the expression of PAFR on macrophages go through a complicated regulatory pathway. Based on findings of our previous study, protein expression of PAFR was similar to the mRNA expression (evaluated by western blotting); however, after PAFR-PAF interaction this pattern might change. Our study design did not allow these to be investigated.

Q9. In addition to leukocytes, structure cells like endothelial cells may have PAFR. How do you speculate the role of PAFR in your study?

A9. Although numerous cell types are able to generate PAF, the kidney is an important, if not main, source of PAF production in the body because PAF is virtually undetectable in the blood of anephric patients and experimental animals that have undergone bilateral nephrectomy. As for the PAFR, in the kidney, PAFR mRNA is ubiquitously expressed. There is a gradient of its expression levels being the richest in the renal cortex, with a lesser amount in the outer medulla, followed by the inner medulla. Within the nephron, the glomerulus demonstrates the highest PAFR expression, followed by the proximal tubule, with the other tubular segments displaying lower levels. Numerous studies have implicated PAF in the pathogenesis of renal diseases because of its detrimental effects on kidney functions.

Q10. When you think about PAF/PAFR in other situations like allergy or under treatment with anticoagulant, did you check for anticoagulants or state of allergy?

A10. Patients with the following conditions were excluded from the study: those with allergic diseases, connective tissue diseases, hepatitis C or hepatitis B infections, other glomerulonephritis, hematuria, malignant diseases in the past 3 years, ongoing steroid or immunosuppressive therapy, kidney transplantation, or nephrectomy. And the number of patients with anticoagulant therapy was not different among normo-, micro- and macro-albuminuria groups.

Q11. You concluded a common factor causes inflammation. Why didn't you include CRP in the paper?

A11. We have reported the results for CRP in our previous paper: "mRNA expression of platelet activating factor receptor (PAFR) in peripheral blood mononuclear cells is associated with albuminuria and vascular dysfunction in patients with type 2 diabetes". In the paper, we mentioned that there were no significant differences between nephropathic groups regarding the baseline characteristics (age, sex, duration of diabetes, BMI, fasting plasma glucose (FPG), HbA1c, TG, oxLDL, or hs-CRP) of the 95 participants.

Q12. You present some pathology for podocytes. So the podocyte, do you have any information about PAFR and podocyte? Is PAFR expressed in podocytes?

A12. There is a consensus that podocytes are a target of PAF because they express PAFR. PAF effects on podocytes include loss of nephrin, cytoskeletal rearrangements and decreased proteoglycan production, which leads to a decreased anionic charge of the glomerular basement membrane and, consequently, loss of its charge selectivity. On the other hand, preincubation of podocytes with a PAFR antagonist prevented the loss and redistribution of nephrin. In control podocytes overexpressing acetylhydrolase, nephrin loss was abrogated. A cross-talk between mesangial cells and podocytes is postulated as a mechanism responsible for the development of proteinuria. PAF might be one of the cytokines released by mesangial cells that mediate their communication with podocytes

Q13. How is PAFR expression in PBMC involved in the progression of diabetic nephropathy?

A13. One of the main actions of PAFR on the renal tissue is the change in charge barrier selectivity.

Q14. Are there any reports of PAFR-KO animal models? What will happen in animal lacking PAFR expression?

A14. In a study on Wild-type (WT) and PAFR knockout (KO) mice, PAFR KO animals showed less renal dysfunction, evaluated by urine protein/creatinine ratio. They also had less fibrosis evaluated by collagen deposition, type I collagen, Lysyl Oxidase-1 (LOX-1) and TGF- β gene expression, and higher expression of bone morphogenetic protein 7 (BMP-7). PAFR KO animals also showed downregulation of extracellular matrix (ECM) and adhesion molecule-related machinery genes; and lower levels of pro-inflammatory cytokines.

以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。