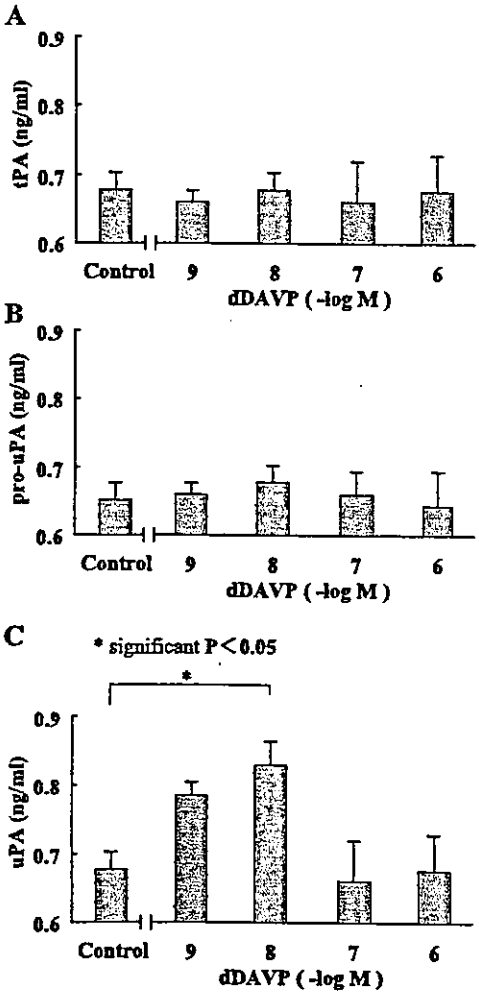

Fig. 4. Dose effect of dDAVP on tissue-type plasminogen activator (tPA, *A*), pro-urokinase-type plasminogen activator (pro-uPA, *B*), and uPA (*C*) concentrations in the medium after lymphocyte incubation. Values represent the average ± SD of 3 independent experiments.

after the incubation (Fig. 5). We also made sure that the level of fibrinolytic activity in the medium after dDAVP treatment was decreased to the basal level by the addition of PAI-1 (data not shown).

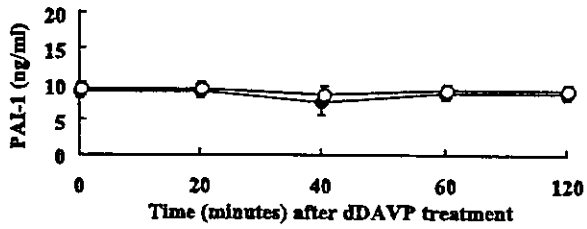

Fig. 5. Plasminogen activator inhibitor (PAI)-1 concentration in the medium after incubation of lymphocytes with (●) and without (○) 10^{-8} M dDAVP. Values represent the average ± SD of 3 independent experiments.

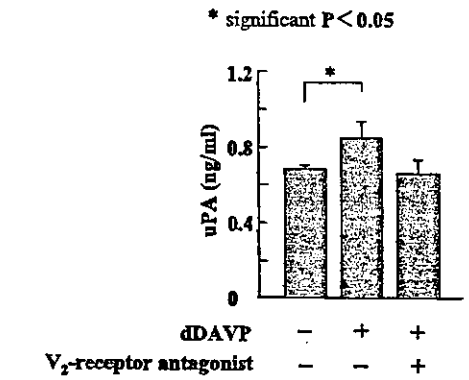

Fig. 6. Effect of the V_2 receptor antagonist on dDAVP-induced uPA increase in the medium after lymphocyte incubation. Values represent the average ± SD of 3 independent experiments.

Effect of receptor antagonists on dDAVP-induced uPA increase. We then examined whether the uPA increase in the medium was due to receptor-mediated response in lymphocytes. When lymphocytes were preincubated with the 10^{-8} M V_2 receptor antagonist [Adamantaneacetyl^1,O-Et-$_D$-Tyr^2,Val^4, Aminobutyryl^6,Arg^{8,9}]-vasopressin, uPA increase after incubating lymphocytes with dDAVP (10^{-8} M) was not detected as it had been without the antagonist (Fig. 6). We made sure that there was no change in uPA concentration after incubation only with the V_2 receptor antagonist. Preincubation with a V_{1a} receptor antagonist, [β-Mercapto-β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin, resulted in more increased levels of uPA concentration than those by dDAVP treatment alone, although the antagonist by itself did not affect the increase (Fig. 7). However, the uPA increase by the combination of a V_{1a} receptor antagonist and dDAVP was undetectable by the 10^{-7} M V_2 receptor antagonist (Fig. 8).

DISCUSSION

In the present study, we observed increased levels of fibrinolytic activity in the medium after incubating human peripheral

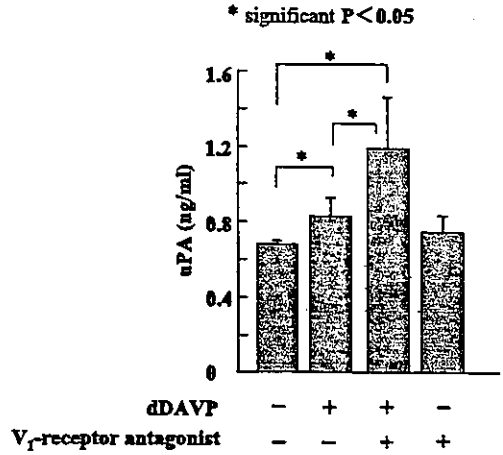

Fig. 7. Effect of the V_1 receptor antagonist on dDAVP-induced uPA increase in the medium after lymphocyte incubation. Values represent the average ± SD of 3 independent experiments.

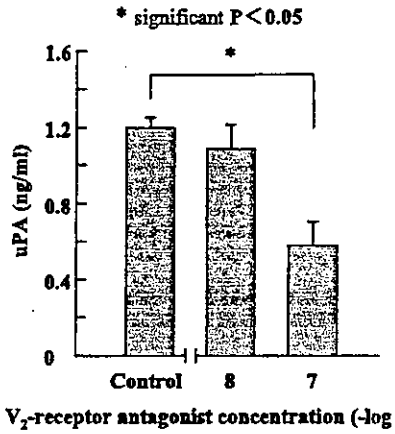

Fig. 8. Effect of the V_2 receptor antagonist on the V_1 antagonist and dDAVP combination-induced increase of uPA concentration in the medium after lymphocyte incubation. Lymphocyte samples were preincubated with the V_2 receptor antagonist (10^{-8} M and 10^{-7} M) for 20 min at 37°C. After preincubation with the V_2 receptor antagonist, samples were incubated with the V_1 receptor antagonist (10^{-8} M) for 20 min and then with dDAVP (10^{-8} M) for another 20 min at 37°C. Values represent the average ± SD of 3 independent experiments.

blood lymphocytes obtained from dDAVP-infused volunteers, possibly due to uPA released from lymphocytes. This uPA induction appeared to be involved in an AVP V_2 receptor-mediated reaction that is expressed in human lymphocytes (Fig. 1).

The levels of fibrinolytic protease activity in the medium were the highest when lymphocytes were incubated with 10^{-8} M dDAVP in vitro. The dDAVP-induced protease activity and increase in the uPA concentration showed similar dose-response curves, bell-shaped (Fig. 3*A* and Fig. 4*C*). No increase was observed in the levels of PAI-1 up to 120 min after the incubation (Fig. 5), and the level of fibrinolytic activity in the medium after dDAVP treatment was decreased to the basal level by the addition of PAI-1 (data not shown). In the absence of lymphocytes, the treatment of culture medium with dDAVP did not result in an increase of uPA concentration (data not shown). These findings suggest that the increased levels of the fibrinolytic protease activity correspond at least in part to an increase in uPA release in the medium after incubation of human peripheral blood lymphocytes with dDAVP.

The mechanism by which lymphocytes release uPA after dDAVP treatment remains unclear. We determined the population of receptors in our preparation by PCR and showed that lymphocytes express AVP V_{1a}, V_2, and OT receptors (Fig. 1). Human vascular endothelial cells have been shown to express OT receptors (37), and, in LLC-PK_1 renal epithelial cells, the uPA release by OT via V_2 receptor reaction has been reported (8). It could be explained by the sequential activation of various AVP/OT receptors. However, we propose that the V_2 receptor system makes a large contribution to this observed uPA release, because the preincubation of lymphocytes with the V_2 receptor antagonist completely inhibited the dDAVP-induced uPA increase in the medium (Figs. 6 and 8).

By contrast, the V_1 receptor antagonist enhanced the dDAVP-induced increase in uPA (Fig. 7). Some V_1 receptor antagonists demonstrate an agonist effect (21). The agonist property of the V_1-receptor antagonist used in this study, [β-Mercapto-β,β-cyclopentamethylenepropionyl,O-Me-Tyr^2, Arg^8]-vasopressin, was reported to be involved in the activation of the phosphoinositide-signaling pathway (30). In our experiment, this V_1 receptor antagonist alone showed little effect on the uPA induction, although preincubation of lymphocytes with the antagonist enhanced uPA increase by dDAVP (Fig. 7). Interestingly, in Chinese hamster ovary cells transfected with the V_{1a} and the V_2 receptor cDNAs, the V_2 receptor-induced cAMP accumulation was potentiated by stimulation of the PLC pathway via the V_{1a} receptor (10). If this antagonist acts as an agonist to the V_{1a} receptor, the signal transduction system may lead to the stimulation of the V_2 receptor, resulting in the enhancement of the uPA induction. Another possibility is that the V_1 receptor in human peripheral blood lymphocytes has an inhibitory effect on the V_2 receptor function. In the presence of the V_1 receptor antagonist, this inhibition may have been cleared, so that the V_2 receptor fully functioned to induce the uPA increase by dDAVP.

The uPA increase under the combination of a V_1 receptor antagonist and dDAVP was undetectable after V_2 receptor antagonist preincubation at the highest dose (10^{-7} M) (Fig. 8). This result may suggest that the enhanced uPA induction is also a V_2 receptor-mediated reaction. Although the exact mechanisms of the enhancement and its inhibition remain unclear, the V_1 receptor may be involved in the V_2 receptor-mediated uPA induction.

In our study, AVP alone did not increase the levels of fibrinolytic activity at any dose examined (Fig. 3*B*). We also examined the effect of the V_1 receptor antagonist on uPA releasing activity by AVP, because we apprehended the possibility that the combination of the V_1 receptor antagonist and AVP might be able to increase the fibrinolytic activity. However, there was no difference in uPA concentration among lymphocytes treated only with AVP, those pretreated with the V_1 receptor antagonist, and control lymphocytes (data not shown). From this result, we might speculate that dDAVP has its own V_2-like receptor that is inhibited by the V_2 receptor antagonist and that pretreatment of the V_1 receptor antagonist helps dDAVP to bind its receptor more efficiently by occupying the neighboring V_1 receptors on lymphocytes. We showed two amplified DNA bands in analyzing the expression of V_2 receptor mRNA (Fig. 1). The expression of an alternative form of V_2 receptor mRNA in human peripheral lymphocytes might be the explanation for this receptor mechanism. RT-PCR analysis also showed that OT receptors are expressed in human lymphocytes (Fig. 1). Most of the V_{1a} receptor antagonists, including the one that we used in this study, have high affinity with OT receptors as well as with V_{1a} receptors. Although the affinity is higher for V_{1a} receptors, we could not completely exclude the possibility of cross-talk between V_2 and OT receptors. Very recently, a highly specific OT receptor antagonist, FE 200 440 (Ferring), was developed with an affinity for human cloned OT receptors that was ~300-fold that for V_{1a} receptors, whereas other OT receptor antagonists bind well to both receptors (22). When this newly developed antagonist is made available, we will be able to perform further experiments that should help to more fully explain the mechanism underlying uPA release through the AVP/OT receptor function in human lymphocytes.

To our knowledge, this is the first study to report that the levels of uPA increase in the medium after incubation of lymphocytes with dDAVP. uPA is an extracellular serine endoprotease with a multimodular structure; it has been critically involved in various biological activities, such as tissue remodeling and cell migration (14). The activities trigger a protease cascade, including digestion of the extracellular matrix and activation of latent growth factors, such as transforming growth factor-β and pro-hepatocyte growth factor (20, 27). Although the protease cascade is intimately associated with inflammation and tissue repair, little is known regarding the impact of lymphocytes on these processes. In this regard, it is interesting to note that human peripheral blood lymphocytes can produce uPA. Under normal physiological conditions, the level of plasma AVP concentration is much lower than that of the dDAVP we treated in our study. So, in normal conditions, the release of uPA from lymphocytes may not occur in humans. However, we emphasize the potential significance of lymphocytes releasing uPA under special conditions, such as inflammation, perhaps leading to the increasing sensitivities of the receptors on the activated T cells or the elevated concentration of vasopressin in tissues. Increased plasma concentrations and hypothalamic content and release of AVP were reported in inflammatory disease-prone Lewis rats (24, 25).

uPA released from human peripheral blood lymphocytes might be ubiquitous at the sites of inflammation or tissue repair; therefore, lymphocytes may promote tissue remodeling and angiogenesis. uPA, as an element of the fibrinolytic cascade, also takes part in regulating cell-mediated immunity in cardiac allograft acceptor mice. Histological analysis revealed that accepted cardiac allografts express uPA in mononuclear cells (1). In human renal allograft transplantation, there has been no study reporting the role of uPA on graft acceptance. If a difference in the activity of uPA release from lymphocytes exists between the renal transplant patients and normal subjects, it would be useful to know the pathophysiology of graft acceptance. Such kinds of studies are currently in progress, and the results will be reported elsewhere.

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REFERENCES

1. **Bickerstaff AA, Xia D, Pelletier RP, and Orosz CG.** Mechanisms of graft acceptance: evidence that plasminogen activator controls donor-reactive delayed-type hypersensitivity responses in cardiac allograft acceptor mice. *J Immunol* 164: 5132–5139, 2000.
2. **Bossmar T, Åkerlund M, Szamatowicz J, Laudanski T, Fantoni G, and Maggi M.** Receptor-mediated uterine effects of vasopressin and oxytocin in nonpregnant women. *Br J Obstet Gynaecol* 102: 907–912, 1995.
3. **Conrad KP, Gellai M, North WG, and Valtin H.** Influence of oxytocin on renal hemodynamics and electrolyte and water excretion. *Am J Physiol Renal Fluid Electrolyte Physiol* 251: F290–F296, 1986.
4. **Culpepper RM, Hebert SC, and Andreoli TE.** The posterior pituitary and water metabolism. In: *Textbook of Endocrinology*, edited by Wilson JD and Foster DW. Philadelphia, PA: Saunders, 1985, p. 614–652.
5. **Forsling ML and Brimble MJ.** The role of oxytocin in salt and water balance. *In: Oxytocin: Clinical and Laboratory Studies*, edited by Amico JA and Robinson AG. Amsterdam: Elsevier, 1985, p. 167–175.
6. **Hasunuma K, Yamada K, Tamura T, and Yoshida S.** Cardiovascular and rennin responses to desmopressin in humans: role of prostacyclin and adrenergic systems. *Am J Physiol Heart Circ Physiol* 260: H1031–H1036, 1991.
7. **Hunter WM and Greenwood FC.** Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 194: 495–496, 1962.
8. **Jans DA, Pavo I, and Fahrenholz F.** Oxytocin induced cAMP-dependent protein kinase activation and urokinase-type plasminogen activator production in LLC-PK$_1$ renal epithelial cells is mediated by the vasopressin V$_2$-receptor. *FEBS Lett* 315: 134–138, 1993.
9. **Katusic ZS, Shepherd JT, and Vanhoutte PM.** Vasopressin causes endothelium-dependent relaxation of the canine basilar artery. *Circ Res* 55: 575–579, 1984.
10. **Klingler C, Ancellin N, Barrault MB, Morel A, and Corman B.** Potentiation of receptor-mediated cAMP production: role in cross-talk between vasopressin V$_{1a}$ and V$_2$ receptor transduction pathways. *Biochem J* 330: 1023–1028, 1998.
11. **Kobrinsky NL, Doyle JJ, Israels ED, Winter JSD, Cheang MS, Walker RD, and Bishop AJ.** Absent factor VIII response to synthetic vasopressin analogue (DDAVP) in nephrogenic diabetes insipidus. *Lancet* 1: 1293–1294, 1985.
12. **Lethagen S.** Desmopressin (DDAVP) and haemostasis. *Ann Hematol* 69: 173–180, 1994.
13. **Liard JF.** Characterization of acute hemodynamic effects of antidiuretic agonists in conscious dogs. *J Cardiovasc Pharmacol* 11: 174–180, 1988.
14. **Loscalzo J and Braunwald E.** Tissue plasminogen activator. *N Engl J Med* 319: 925–931, 1988.
15. **Lyness J, Robinson AG, Sheridan MN, and Gash DM.** Antidiuretic effects of oxytocin in Brattleboro rat. *Experientia* 41: 1444–1446, 1985.
16. **Mannucci PM, Canciani MT, Rota L, and Donovan BS.** Response of factor VIII/von Willebrand factor to DDAVP in healthy subjects and patients with haemophilia A and von Willebrand's disease. *Br J Haematol* 47: 283–293, 1981.
17. **Mannucci PM and Rota L.** Plasminogen activator response after DDAVP. A clinico-pharmacological study. *Thromb Res* 20: 69–76, 1980.
18. **Michell RJ, Kirk CJ, and Billah MM.** Hormonal stimulation of phosphatidylinositol breakdown, with particular reference to the hepatic effects of vasopressin. *Biochem Soc Trans* 7: 861–865, 1979.
19. **Nakamura S, Yamamura Y, Itoh S, Hirano T, Tsujimae K, Aoyama M, Kondo K, Ogawa H, Shinohara T, Kan K, Tanada Y, Teramoto S, Sumida T, Nakayama S, Sekiguchi K, Kambe T, Tsujimoto G, Mori T, and Tominaga M.** Characterization of a novel nonpeptide vasopressin V$_2$-agonist, OPC-51803, in cells transfected human vasopressin receptor subtypes. *Br J Pharmacol* 129: 1700–1706, 2000.
20. **Naldini L, Vigna E, Bardelli A, Follenzi A, Galimi F, and Comoglio PM.** Biological activation of pro-HGF (Hepatocyte Growth Factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 270: 603–611, 1995.
21. **Neuenschwander S and Pliska V.** Effects of neurohypophyseal hormone analogues on blood clotting factor VIII and fibrinolytic activity in sheep. *Eur J Pharmacol* 323: 205–213, 1997.
22. **Nilsson L, Reinheimer T, Steinwall M, and Åkerlund M.** FE 200 440: a selective oxytocin antagonist on the term-pregnant human uterus. *Br J Obstet Gynaecol* 110: 1025–1028, 2003.
23. **North WG, Fay MJ, and Du Jinlin.** MCF-7 breast cancer cells express normal forms of all vasopressin receptors plus an abnormal V2R. *Peptides* 20: 837–842, 1999.
24. **Patchev VK, Kalogeras KT, Zelazowski P, Wilder RL, and Chrousos GP.** Increased plasma vasopressin concentrations, hypothalamic content and release in inflammatory disease-prone, hypothalamic CRH-deficient Lewis rat. *Endocrinology* 131: 1453–1457, 1992.

25. **Patchev VK, Mastorakos G, Brady LS, Redwine J, Wilder RL, and Chrousos GP.** Increased arginine vasopressin secretion may participate in the enhanced susceptibility of Lewis rats to inflammatory disease. *Neuroendoendocrinology* 58: 106–110, 1993.
26. **Richardson DW and Robinson AG.** Desmopressin. *Ann Intern Med* 103: 228–239, 1985.
27. **Sato YR, Tubori R, Lyons R, Moses H, and Rifkin DB.** Characterization of the activation of latent TGF-β by co-cultures of endothelial cells and pericytes or smooth muscle cells: a self-regulating system. *J Cell Biol* 111: 757–763, 1990.
28. **Schwartz J, Liard JF, Ott C, and Cowley Jr AW.** Hemodynamic effects of neurohypophysial peptides with antidiuretic activity in dogs. *Am J Physiol Heart Circ Physiol* 249: H1001–H1008, 1985.
29. **Sekiya S, Oosaki T, Suzuki N, and Takamizawa H.** Invasion potential of human choriocarcinoma cell lines and the role of lytic enzymes. *Gynecol Oncol* 22: 324–333, 1985.
30. **Son MC and Brinton RD.** Vasopressin-induced calcium signaling in cultured cortical neurons. *Brain Res* 793: 244–254, 1998.
31. **Sugita K, Suzuki N, Isogai E, and Niimi H.** Insusceptibility of Cockayne syndrome-derived lymphocytes to plasminogen activator-like protease protease induction by ultraviolet rays and its abolition by interferon. *Mutat Res* 262: 139–144, 1991.
32. **Sugita K, Suzuki N, Isogai E, and Niimi H.** Serum factors responsible for unusual induction of plasminogen activator activity in tuberous sclerosis. *J Neurol Sci* 108: 202–206, 1992.
33. **Sugita K, Suzuki N, Shimizu N, Takanashi J, Ishii M, and Niimi N.** Involvement of cytokines in N-methyl-N′-nitro-N-nitrosoguanidine-induced plasminogen activator activity in acute disseminated encephalomyelitis and multiple sclerosis lymphocytes. *Eur Neurol* 33: 358–362, 1993.
34. **Takahashi S, Chi XJ, Nomura J, Sugaya S, Kita K, and Suzuki N.** A novel method of analyzing environmental chemical mutagens in human cell systems. *Water Sci and Technol* 42: 133–138, 2000.
35. **Tepperman J.** *Metabolic and Endocrine Physiology*. Chicago, IL: Year Book Medical Publishers, 1980, vol. 78.
36. **Thibonnier M, Conarty DM, Preston JA, Wilkins PL, Berti-Mattera LN, and Mattera R.** Molecular pharmacology of human vasopressin receptors. In: *Vasopressin and Oxytocin*, edited by Zingg HH, Bourque CW, and Bichet DG. New York: Plenum, 1998.
37. **Thibonnier M, Conarty DM, Preston JA, Plesnicher CL, Dweik RA, and Erzurum SC.** Human vascular endotherial cells express oxytocin receptors. *Endocrinology* 140: 1301–1309, 1999.
38. **Wickramasinghe SN, Valdimarsson H, and Williams B.** Interaction between a synthetic analogue of vasopressin and human peripheral lymphocytes. *J Clin Lab Immunol* 7: 67–70, 1982.
39. **Yamada K, Hatakeyama E, Sakamaki T, Nishimura M, Arita S, Sakamoto K, Hamaguchi K, Nakajima K, Otuka M, and Tanabe K.** Involvement of platelet-derived growth factor and histocompatibility of DRB 1 in chronic renal allograft nephropathy. *Transplantation* 71: 936–941, 2001.
40. **Yamada K, Nakayama M, Nakano H, Mimura N, and Yoshida S.** Endothelium-dependent vasorelaxation evoked by desmopressin and involvement of nitric oxide in rat aorta. *Am J Physiol Endocrinol Metab* 264: E203–E207, 1993.

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CLINICAL NEPHROLOGY – EPIDEMIOLOGY – CLINICAL TRIALS

Oxidative stress is enhanced in correlation with renal dysfunction: Examination with the redox state of albumin

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Oxidative stress is enhanced in correlation with renal dysfunction: Examination with the redox state of albumin.

Background. Cardiovascular disease is known to be the most important complication among patients with renal failure, and oxidative stress has been proposed to play a major role as the source of such complications. Human serum albumin (HSA) is composed of human mercaptoalbumin (HMA) with cysteine residues having reducing powers, of reversibly oxidized human non-mercaptoalbumin-1 (HNA-1), and strongly oxidized human non-mercaptoalbumin-2 (HNA-2).

Methods. We used the "redox state of HSA" as a marker to investigate the current status of oxidative stress in predialysis patients with renal failure. The subjects were 55 nondialysis patients (31 males and 24 females) with chronic renal diseases, and having various degrees of renal function. The subjects' redox state of HSA was determined by a high-performance liquid chromatographic (HPLC) procedure, and the results presented in terms of the ratios between HNA-total(HNA-1 + HNA-2) and HNA-2.

Results. The values for each fraction of HNA-total (f(HNA-total)) and f(HNA-2) were increased with a decrease of renal functions, and a significant positive correlation with serum creatinine ($R = 0.529$, $P < 0.0001$ and $R = 0.618$, $P < 0.0001$) was detected. Multiple (forward stepwise) regression analysis using f(HNA-total) and f(HNA-2) as the criterion variables was performed, and creatinine was adopted as significant explanatory variable in both equations.

Conclusion. We found that even before dialysis, oxidative stress was enhanced in correlation with the level of renal dysfunction among patients with chronic renal failure. In the future, antioxidant strategies should become part of treatment for predialysis renal failure.

Key words: oxidative stress, predialysis, chronic renal failure, redox state of albumin, creatinine clearance, mercapto group.

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Cardiovascular disease (CVD) is the most important complication for patients with end-stage renal disease (ESRD). The mortality rate associated with CVD is extremely high, accounting for approximately 9% per year (approximately 45% of total death) among ESRD patients that undergo dialysis, and it is the primary cause of mortality. What characterizes the death of ESRD patients is the fact that mortality rate hardly seems to be reflected by age [1], unlike patients without renal failure.

While risks associated with CVD among ESRD patients who reach dialysis are widely acknowledged, those involving patients with renal failure before dialysis are in the process of being properly identified, although not well established yet. When compared with subjects with normal renal function, the frequency of CVD has been reported to be higher not only among dialysis patients but also among predialysis patients with renal failure [2], suggesting that factors promoting complications of the cardiovascular system may already exist in predialysis patients with renal failure.

Oxidative stress has recently been proposed to play a major role in the development of CVD among renal failure patients [3]. In the past, backgrounds similar to those of patients without renal failure, such as high blood pressure and atherosclerosis, have been postulated, but several reports have made it difficult to conceive these as major causes [2]. To date, increases in blood concentrations of reactants resulting from oxidative stress have mainly been reported among dialysis patients [4–7]. Further, other studies have reported that while administration of antioxidant substances, such as vitamin E and acetylcysteine, reduce cardiovascular system events among dialysis patients [8, 9], vitamin E did not exhibit apparent effects among patients without renal failure [10]. From these findings, that oxidative stress is, at least in part, involved in CVD among chronic renal failure patients, seems virtually unquestionable.

The aim of this study was to clarify whether oxidative stress is already increased in predialysis chronic renal failure patients. We adopted "the redox state of human serum albumin (HSA)" as the marker of oxidative stress. HSA is a protein composed of 585 amino acids. The amino residue at position 34 from the N-terminus is a cysteine having a mercapto group (SH group). The mercapto group deoxidizes other substances according to the degree of surrounding oxidative stress, and is, itself, oxidized. From the view point of cysteine residue, HSA is a mixture of human mercaptoalbumin (HMA) in which the mercapto group is not oxidized, human non-mercaptoalbumin-1, which is the disulfide bond formation reversibly oxidized by either cysteine or glutathione (HNA-1) [11], and human non-mercaptoalbumin-2, which is strongly oxidized and becomes sulfenic (–SOH), sulfinic ($-SO_2H$), or sulfonic ($-SO_3H$) [12].

We, thus, performed measurements of the redox state of HSA concurrently with various blood and urine tests in 64 nondialysis patients with chronic renal disease having different levels of renal function. As a result, our study revealed a tight correlation between the oxidation-reduction state of HSA and renal function, and confirmed that oxidative stress was already increased in predialysis chronic renal failure patients even before reaching dialysis.

Table 1. Patient characteristics

Age *years*	58 ± 14
Male: Female	31: 24
Primary disease	
CGN	
IgA nephropathy	16
FSGS	4
MN	1
HSPN	1
FGO	1
MPGN	1
Crescentic GN	1
Not biopsy-proven	14
Nephrosclerosis	9
Diabetes nephropathy	3
Hereditary nephritis	1
Lupus nephritis	1
Polycystic kidney	1
Donor of RTx	1
Prescription	
Calcium antagonist	26
ACEI	24
ARB	27
Loop diuretics	12
Thiazide diuretics	6
Allopurinol	8
Eicosapentaenoic acid	16
HMG-CoA RI	8
Probucol	2
Alpha-tocopherol	1
Predonisolone	6

Abbreviations are: CGN, chronic glomerulonephritis; FSGS, focal segmental glomerulosclerosis; MN, membranous nephropathy; HSPN, Henoch-Schoenlein purpura nephritis; FGO; focal glomerular obliterans; MPGN, membranoproliferative glomerulonephritis; crescentic GN, crescentic glomerulonephritis; RTx, renal transplantation, ACEI; angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; HMG-CoA RI, HMG-CoA reductase inhibitor.

METHODS

The study involved 55 patients; 31 males and 24 females aged between 40 and 70 (58 ± 14) years with chronic renal disease (whose estimated creatinine clearance was lower than 80 mL/min) who attended the Sakura National Hospital for at least 6 months. Patients' characteristics are shown in Table 1. Regardless of the primary renal disease, a total of 15 patients presented with diabetes mellitus.

Physical measurements including height, weight, and blood pressure were taken, and then blood samples were collected to measure the redox state of HSA; 2 mL was drawn from the serum obtained by centrifugation, and stored at –80°C for 2 to 4 weeks until analysis. In addition, the following hematologic and biochemical tests were performed: total protein, albumin, ALT, urea nitrogen, creatinine, uric acid, sodium, chlorine, potassium, total calcium, inorganic phosphorus, total iron, total cholesterol, HDL cholesterol, C-reactive protein, and hemoglobin. Estimated endogenous creatinine clearance (CCr) was calculated by the Cockcroft-Gault formula [13].

We also instructed each patient to institute a 24-hour urine collection, and used the concentration of sodium and urea nitrogen from this to calculate the amount of sodium excretion and the protein intake.

Measurement of the redox status of HSA was performed by the method previously reported by Hayashi et al [14], a high-performance liquid chromatographic (HPLC) method. The chromatograph was equipped with a fluorescence detection system that consisted of a model AS-8010 autosampler, model CCPM double-plunger pump, and model FS-8000 fluorescence detector in conjunction with a model SC-8020 system controller (all from Tosoh Co., Tokyo, Japan). A Shodex-Asahipak ES-502 column (10- × 0.76-cm inner diameter, DEAE form for ion-exchange HPLC; Showa Denko Co., Tokyo Japan; column temperature, 35° ± 0.5°) was used in this study. Elution was performed by linear gradient elusion with increasing ethanol concentrations from 0% to 5% for serum in 0.05 mol/L sodium acetate and 0.40 mol/L sodium sulfate mixture (pH 4.85) at a flow rate of 1.0 mL/min. Deaeration of the buffer solution was performed by bubbling helium.

From HPLC profiles of HSA obtained from these procedures, the value for each fraction of HMA, HNA-1, and HNA-2 to total HSA (f(HMA), f(HNA-1), f(HNA-2)) were calculated. Hematologic and biochemical tests were measured with standard laboratory techniques.

Table 2. The results of clinical data

	Mean ± SD	Range
Height *cm*	160.8 ± 7.6	145.0–175.0
Weight *kg*	58.7 ± 9.1	41.0–80.0
Body mass index	22.6 ± 2.5	18.3–29.4
Systolic blood pressure *mm Hg*	129.9 ± 17.0	90–175
Diastolic blood pressure *mm Hg*	78.7 ± 10.9	55–109
f (HMA) %	69.44 ± 7.26	47.64–83.02
f (HNA-1) %	28.59 ± 6.93	15.34–48.69
f (HNA-2) %	1.98 ± 0.69	1.13–4.11
f (HNA-total) %	30.57 ± 7.27	16.98–52.36
Total protein *g/dL*	7.0 ± 0.6	5.6–8.1
Albumin *g/dL*	4.1 ± 0.4	2.6–4.6
Alanine aminotransferase *IU*	16 ± 8	5–52
Urea nitrogen *mg/dL*	31 ± 17	12–84
Creatinine *mg/dL*	2.33 ± 2.35	0.75–12.62
Uric acid *mg/dL*	7.7 ± 2.0	3.9–11.5
Sodium *mmol/L*	139 ± 2	132–144
Chloride *mmol/L*	104 ± 3	98–110
Potassium *mmol/L*	4.6 ± 0.5	3.8–5.8
Total calcium *mg/dL*	9.4 ± 0.6	6.7–10.4
Inorganic phosphate *mg/dL*	3.8 ± 0.8	2.5–7.0
Total iron *μg/dL*	89 ± 26	26–168
Total cholesterol *mg/dL*	209 ± 36	155–304
HDL cholesterol *mg/dL*	57.5 ± 15.7	30.1–104.5
C-reactive protein *mg/dL*	0.1 ± 0.3	0.0–1.8
Hemoglobin *g/dL*	12.2 ± 1.8	8.8–15.9
Creatinine clearance *mL/min*	39.9 ± 18.5	4.1–73.9
Salt excretion *g/day*	8.61 ± 2.93	3.08–18.69
Estimated protein intake *g/kgBW/day*	0.68 ± 0.26	0.20–1.35

Statistical analysis

Values were expressed as mean ± standard deviation unless otherwise stated.

We used the statistical software Stat View 5.0 (SAS Institute, Inc., Cary, NC, USA). For the magnitude of the correlation, we used Pearson's correlation coefficient (R). Item-category data (gender, primary disease, prescription) were introduced into the analysis as dummy variables. The correlation was determined to be significant when P value was less than 0.05 (5%) with Fisher's Z transformation.

RESULTS

Table 2 shows the results of clinical data. Serum creatinine in patients was a minimum of 0.75 mg/dL and a maximum of 12.62 mg/dL. CCr was between 4.1 and 73.9 mL/min. The redox states of HSA, f(HMA), f(HNA-1), and f(HNA-2) were between 47.64% and 83.02%, 15.34% and 48.69%, and 1.13% and 4.11%, respectively. The fraction of total HNA (f(HNA-total), the sum of f(HNA-1) of f(HNA-2)) was between 16.98% and 52.36%.

Relationships between the oxidized HSA (HNA-total and HNA-2) and serum creatinine are shown in Figure 1. A significantly positive correlation between creatinine and both f(HNA-total) and f(HNA-2) was found ($R = 0.529$, $P < 0.0001$ and $R = 0.618$, $P < 0.0001$).

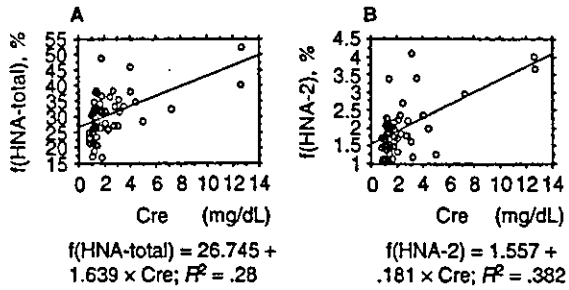

Fig. 1. The relationship between serum creatinine and f(HNA-total) (*A*) and f(HNA-2) (*B*). Each value, f(HNA-total) and f(HNA-2), shows a statistically significant positive correlation with serum creatinine.

CCr was also showed significant relationship with the redox state of HSA (Fig. 2). Namely, CCr was correlated negatively with f(HNA-total) and f(HNA-2) ($R = -0.525$, $P < 0.0001$ and $R = -0.539$, $P < 0.0001$). These findings indicate that decreased renal function contributes to the oxidation of HSA.

Table 3 shows the results of correlation analyses between the redox state of HSA and characteristics, whereas Table 4 shows the results of correlation analyses between the redox state of HSA and clinical data. The items which showed statistically positive correlation with either f(HNA-total) or f(HNA-2) were adopted as the explanatory variables of multiple regression analysis.

Multiple (forward stepwise) regression analysis was performed, in which the above adopted items were determined to be the explanatory variables, whereas f(HNA-total) and f(HNA-2) were the criterion variables. Results are shown in Table 5. Multiple regression equations using f(HNA-total) and f(HNA-2) as the criterion variables were all formulated as significant equations. In the former equation, creatinine and uric acid were adopted as significant explanatory variables, whereas creatinine, magnesium, and age were adopted in the latter. Namely, only creatinine was adopted as significant explanatory variables in both equations.

DISCUSSION

Several pieces of collateral evidence have pointed to the possibility of oxidative stress being promoted among patients with chronic renal failure. However, if we look at the status of oxidative stress in terms of an entire organism, the situation is much like the traditional Indian parable of "The blind men and the elephant" because no appropriate markers exist that could globally evaluate the entire organism [3].

In this article, we evaluated the current status of oxidative stress in predialysis renal failure patients, with the "redox state of serum albumin" as our marker. In healthy adult males, HMA commonly makes up 75% of

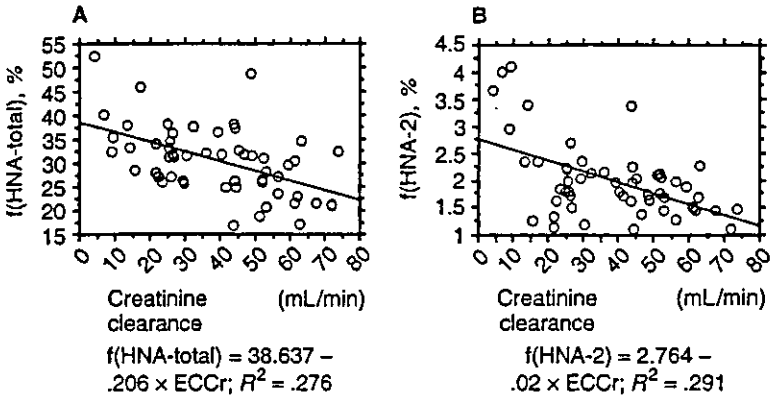

Fig. 2. The relationship between creatinine clearance and f(HNA-total) (*A*) and f(HNA-2) (*B*). Each value, f(HNA-total) and f(HNA-2), shows a statistically significant negative correlation with creatinine clearance.

Table 3. The correlation between f(HNA-total), f(HNA-2), and patient characteristics

	f (HNA-total)		f (HNA-2)	
	R	*P* value	*R*	*P* value
Age	−.001	.9920	.084	.5439
Gender	−.054	.6934	−.077	.5743
DM or NDM	.219	.1080	.091	.5107
Prescription				
Calcium antagonist	.051	.7136	.246	.0706
ACEI	−.073	.5976	.023	.8700
ARB	−.103	.4532	−.030	.8256
Loop diuretics	.400[a]	.0025	.241	.0766
Thiazide diuretics	.056	.6828	−.075	.5850
Allopurinol	.033	.8106	−.091	.5100
Eicosapentaeoic acid	.172	.2085	.059	.6703
HMG-CoA RI	−.034	.8035	−.183	.1811
Probucol	.124	.3688	−.152	.2667
Alpha-tocopherol	−.016	.9062	−.018	.8965
Predonisolone	.146	.2888	−.120	.3839

[a] $P \leq 0.05$.

Table 4. The correlation between f(HNA-(1+2)), f(HNA-2), and clinical data

	f(HNA-total)		f(HNA-2)	
	R	*P* value	*R*	*P* value
Systolic blood pressure	.265	.0510	.133	.3317
Diastolic blood pressure	.133	.3322	.051	.7138
Body mass index	.004	.9762	−.040	.7575
Total protein	−.087	.5293	−.027	.8426
Albumin	−.241	.0763	−.080	.5628
Alanine aminotransferase	−.148	.2807	.027	.8429
Urea nitrogen	.454[a]	.0005	.508[a]	<.0001
Creatinine	.529[a]	<.0001	.618[a]	<.0001
Uric acid	.466[a]	.0003	.244	.0730
Sodium	−.066	.6304	−.202	.1392
Chloride	.009	.9492	.006	.9658
Potassium	.037	.7880	.135	.3269
Calcium	−.136	.3228	−.216	.1131
Inorganic phosphate	.011	.9352	.157	.2533
Magnesium	.328[a]	.0145	.443[a]	.0007
Total cholesterol	−.173	.2068	−.218	.1103
HDL cholesterol	−.125	.3646	−.215	.1141
Total iron	−.213	.1181	−.240	.0776
C-reactive protein	.118	.3913	.144	.2931
Hemoglobin	−.439[a]	.0008	−.284[a]	.0355
Creatinine clearance	−.525[a]	<.0001	−.539[a]	<.0001
Salt excretion	−.103	4557	−.218	.1091
Estimated protein intake	−.334[a]	.0126	−.193	.1586

f(HNA-total), fractions of human non-mercaptalbumin-1 and 2; f(HNA-2), fractions of human non-mercaptalbumin-2; *R*, regression coefficient.
[a] $P \leq 0.05$.

the entire serum albumin, and HNA-1 constitutes most of the remainder [15]. Forty percent of HSA is found within blood vessels, and the remaining 60% outside blood vessels in extracellular fluid [16]. Intravascular and extravascular HSA moves beyond vessel walls and works as a carrier of many substances. It has shown that approximately 10 g per hour of HSA travels in and out of blood vessels [17]; this means that within an hour, approximately half of intravascular HSA is replaced with extravascular HSA. These findings suggest that the redox state of HSA may represent the global redox state of the entire organism, in other words, that it directly reflects the degree of oxidative stress.

Besides the redox state of HSA, many kinds of oxidative stress markers have been widely used, including 8 hydroxy 2′ deoxyguanine oxidized low-density lipoprotein and F2 isoprotanes. These markers are in vivo substances, such as DNA and lipids, which have been denatured due to oxidation. All these are useful and suitable for evaluating the degree of influence oxidative stress on various in vivo substances, but the redox state of HSA might be more appropriate when evaluating oxidative stress of an entire organism.

There have been reports in the past on the proportion of oxidized albumin being significantly increased among hemodialysis [15, 18–20] and peritoneal dialysis [20] patients; but similar examinations among predialysis patients with renal failure have not been reported. To our knowledge, our paper is the first to clarify that the proportion of oxidized albumin is already increased before dialysis, and that it exhibits a negative correlation with remaining renal functions.

Table 5. The result of multiple regression analysis

	f(HNA-total) ($P < .0001$)		f(HNA-2) ($P < .0001$)	
	R	*P* value	*R*	*P* value
Creatinine	.395	.0036	.597	<.0001
Uric acid	.271	.0415	—	—
Age	—	—	.240	.0203
Magnesium	—	—	.293	.0054

Abbreviations are: f(HNA-total), fractions of human non-mercaptalbumin-1 and 2; f(HNA-2), fractions of human non-mercaptalbumin-2; *R*, standard regression coefficient; SBP, systolic blood pressure.

To date, it is still unclear why a decrease of renal function promotes oxidative stress. Although there are many reports on the relationship of in vivo antioxidant enzymes and renal function, opinions seem to diverge when it comes to analyze the relationship between renal function, antioxidant enzyme activity of renal cortex, and that of serum [4–7, 21, 22].

Meanwhile, accumulated uremic toxins (which include reactive carbonyl compounds), per se, may be inducible oxidative stress. It is reported that by performing dialysis on patients with renal failure, decreased activity of serum glucose-6-phosphate dehydrogenase, superoxide dismutase, and calatase all began to show normal values [4], and the proportion of oxidized albumin began to decrease [15, 18, 19]. Furthermore, Wratten et al incubated various uremic substances (carboxymethyllysine, methyl glyoxal, p-cresol, hippuric acid) together with bovine serum albumin at 37°C and observed a decrease of the sulfhydril group, formation of dityrosine, and advanced oxidation protein products [22]. These reports seem to indicate the strong possibility that the primary culprit of oxidative stress in chronic renal failure could possibly be the accumulation of "oxidative" uremic toxins.

Our examination demonstrated that oxidative stress is increased before dialysis among patients with chronic renal failure. The following facts support the validity and significance of antioxidant treatment for predialysis renal failure patients in terms of prevention of cardiovascular disease: (1) a large number of renal failure patients already present with cardiovascular disease at the time they reach dialysis [1]; (2) incidences of cardiovascular disease are higher in renal failure patients than among subjects with normal renal function, even before dialysis [2]; and (3) dialysis patients who received antioxidant treatment exhibited some positive results [8, 9].

Furthermore, results obtained from examinations using animal models of renal failure also suggest the possibility that antioxidant substance administration inhibits histologic renal damage [23–25]. In other words, antioxidant treatment may not only be useful for the prevention of cardiovascular disease, but also for the amelioration of renal damage progression.

Two types of approaches are conceivable in terms of antioxidant treatment for predialysis renal failure: the administration of antioxidant substances, and the removal of "oxidative" uremic toxins. With regard to the former, the clinical effects of vitamin E [8] and acetylcysteine [9] have already been reported, and their administration might be a valid course, although more studies need to be performed in order to conform these results. Otherwise, some animal experiments further suggest the usefulness of buckwheat extract [23], green tea polyphenol [24], and lazaroid [26]. With regard to the latter, these animal experiments suggest the usefulness of the oral administration of activated charcoal [27] and the early initiation of peritoneal dialysis [27]. Further examinations are, however, required to assess the usefulness and merits of these therapies among predialysis patients with renal failure.

Besides creatinine, uric acid, magnesium, and age were adopted as significant explanatory variables with multiple (forward stepwise) regression analysis. Although this result is interesting, the meaning of it is uncertain at the present.

CONCLUSION

By measuring the redox state of HSA, we demonstrated that oxidative stress correlates with the degree of renal dysfunction and is promoted even before patients with chronic renal failure reach dialysis. Therefore, in the future, antioxidant strategies should be encouraged to become part of the treatment of patients with predialysis renal failure.

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REFERENCES

1. Foley RN, Parfrey PA, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 32:S112–119, 1998
2. Wannamethee SG, Shaper AG, Perry IJ: Serum creatinine concentration and risk of cardiovascular disease. *Stroke* 28:557–563, 1997
3. Himmelfarb J, Stenvinkel P, Ikizler TA, *et al*: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 62:1524–1538, 2002
4. Chauhan DP, Gupta PH, Nampoothiri MR, *et al*: Determination of erythrocyte superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, reduced glutathione and malonyldialdehyde in uremia. *Clin Chim Acta* 123:153–159, 1982
5. Richard MJ, Arnaud J, Jurkovitz C, *et al*: Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. *Nephron* 57:10–15, 1991

6. Mimic-Oka J, Simic T, Djukanovic L, *et al*: Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. *Clin Nephrol* 51:233–241, 1999
7. Martin-Mateo MC, Sanchez-Portugal M, Iglesias S, *et al*: Oxidative stress in chronic renal failure. *Ren Fail* 21: 155–167, 1999
8. Boaz M, Smetana S, Weinstein T, *et al*: Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): Randomized placebo-controlled trial. *Lancet* 356:1213–1218, 2000
9. Tepel M, van der Giet M, Statz M, *et al*: The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: A randomized, controlled trial. *Circulation* 107: 992–995, 2003
10. Lonn EM, Yusuf S, Dzavik V, *et al*: Effects of ramipril and vitamin E on atherosclerosis: The study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation* 103:919–925, 2001
11. Era S, Kuwata K, Imai H, *et al*: Age-related change in redox state of human serum albumin. *Biochim Biophys Acta* 1247:12–16, 1995
12. Era S, Hamaguchi T, Sogami M, *et al*: Further studies on the resolution of human mercapt- and nonmercaptalbumin in the elderly by high-performance liquid chromatography. *Int J Peptide Protein Res* 31:435–442, 1988
13. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
14. Hayashi T, Era S, Kawai K, *et al*: Observation for redox state of human serum and aqueous humor albumin from patients with senile cataract. *Pathophysiology* 6:237–243, 2000
15. Sogami M, Era S, Nagaoka S, *et al*: HPLC-studies on nonmercaptmercapt conversion of human serum albumin. *Int J Peptide Protein Res* 25:398–402, 1985
16. Peters T, Jr.: *All About Albumin: Biochemistry, Genetics, and Medical Applications*, San Diego, Academic Press, 1995
17. Joffe P, Henriksen JH: Bidirectional peritoneal transport of albumin in continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant* 10:1725–1732, 1995
18. Sogami M, Era S, Nagaoka S, *et al*: High-performance liquid chromatographic studies on non-mercapt mercapt conversion of human serum albumin. II. *J Chromatogr* 332:19–27, 1985
19. Soejima A, Kaneda F, Manno S, *et al*: Useful markers for detecting decreased serum antioxidant activity in hemodialysis patients. *Am J Kidney Dis* 39:1040–1046, 2002
20. Kumano K, Yokota S, Go M, *et al*: Quantitative and qualitative changes of serum albumin in CAPD patients. *Adv Perit Dial* 8:127–130, 1992
21. Shurtz-Swirski R, Mashiach E, Kristal B, *et al*: Antioxidant enzymes activity in polymorphonuclear leukocytes in chronic renal failure. *Nephron* 71:176–179, 1995
22. Wratten ML, Sereni L, Tetta C: Oxidation of albumin is enhanced in the presence of uremic toxins. *Ren Fail* 23:563–571, 2001
23. Yokozawa T, Kim HY, Nonaka G, *et al*: Buckwheat extract inhibits progression of renal failure. *J Agric Food Chem* 50:3341–3345, 2002
24. Yokozawa T, Cho EJ, Nakagawa T: Influence of green tea polyphenol in rats with arginine-induced renal failure. *J Agric Food Chem* 51:2421–2425, 2003
25. Van den Branden C, Deman A, Ceyssens B, *et al*: Vitamin E protects renal antioxidant enzymes and attenuates glomerulosclerosis in adriamycin-treated rats. *Nephron* 91:129–133, 2002
26. Vaziri ND, Oveisi F, Ding Y: Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int* 53:1748–1754, 1998
27. Motojima M, Nishijima F, Ikoma M, *et al*: Role for "uremic toxin" in the progressive loss of intact nephrons in chronic renal failure. *Kidney Int* 40:461–469, 1991

●特集—パンフの分類

臓器移植と分子病理学

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●特集—バンフの分類

臓器移植と分子病理学

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I. はじめに

ヒトの臓器移植においては，いろいろな機序で移植された臓器は障害を受ける。免疫学的には拒絶反応であり，血管，胆管，尿管などの管腔の狭窄，血栓，縫合不全，阻血・灌流障害による細胞変性，さらに細菌，真菌，ウイルスによる感染症，薬剤中毒，原病の再発などと病態は多方面にわたる。その中で，分子病理学には，主として拒絶反応の診断と予後予測の分野で大きな期待が寄せられている。拒絶反応は基本的には血管-間質系の反応であり，その機序は液性免疫と細胞性免疫に大別される。前者は超急性，急性促進性，急性液性拒絶に，そして後者は急性細胞性拒絶と慢性拒絶に関係する。特に慢性拒絶反応には免疫学的機序の他に，動脈の狭窄による虚血性障害や薬剤毒性の機序も関係するが，急性拒絶が繰り返し発症し，慢性拒絶に至る機序が重要な予後不良因子であることには変わりない。急性拒絶の診断の遅れが拒絶反応の進展を受け入れ，非可逆的な慢性拒絶を誘導してしまう[1]。

拒絶の診断は生検を gold standard としている。生検には，術中生検，移植臓器の機能状態に関係なく計画的に実施される定期生検 (protocol biopsy)，移植臓器の機能低下あるいは何らかの異常が現れてから実施される偶発生検 (episode biopsy)，そして退院時生検がある。また，subclinical rejection といわれ，臨床的に機能障害がなくとも定期生検により拒絶反応が見出されることがあり，その際には免疫抑制剤の増量が考慮される。そのため，生検の時期を逃すと治療の機会を逸することになる。これらのことから，患者の負担や危険を伴わず，より簡便かつ頻回にでき，診断の感度が高く，拒絶反応に特異性をもった分子病理学的検査法が望まれている。

この総説では，拒絶反応の診断，短期的予知，そして長期的な予後の予測に分子病理学がどの程度まで実用化されているかを腎移植を中心にまとめてみた。

II. 臓器生検の限界と分子病理学の必要性

臓器生検が，移植拒絶反応の診断，治療，予後予測，疾患の理解の面で主役を演じてきたが，生検には以下の限界が指摘されている。第1に，形態はいくつかの病態に対応しているため，組織診断にあたって形態学的状況証拠を提示し，鑑別診断をあげ，臨床データとつきあわせて疾患や病態を診断してきた。特に，移植病理はその特徴として，機序的にも時間的にも異なったいろいろな病態が生検の形態に折り畳まれているため，生検の時期や治療歴，その他の臨床データから，織り込まれた形態像を解きほぐすことが必要となる。その点で，拒絶に特異性の高い補助的診断が求められている。第2に，生検は拒絶の結果を診断するだけで拒絶反応の予測ができない。急性拒絶の発症を未然に予測しその発症を抑えることが，長期的観点での慢性拒絶への進展を予防し，また遅らせることができる。第3に，頻回の生検は患者に負担がかかるばかりでなく，ごく限られた領域から採取されるためサンプル・バイアスがかかる。陽性所見が発見できれば拒絶の診断が可能となるが，陰性の場合には false negative を絶えず考慮しなければならない。

以上の生検の限界を踏まえて，分子病理学に期待されていることは，拒絶反応の診断と予測において，患者に負担を与えず (non-invasive)，特異性 (specificity)

と感度（sensitivity）の高い補助的役割を果たすことである。移植臓器の機能障害が生検における炎症反応に対応するが，炎症反応，主としてリンパ球浸潤が組織内に出現する前に，臓器には免疫学的な準備状態が起こる。すなわち，サイトカイン，接着因子，T細胞の共刺激因子（costimulatory factor），アポトーシス関連蛋白などの組織内発現が炎症細胞浸潤に先行するといわれる。それらを分子病理学的に組織内（intragraft）で証明することができれば，急性拒絶発症の予知につながる。一方，患者に侵襲的な生検を頻回に行うことなしに，拒絶反応を末梢血液中のリンパ球や尿から感知できる手法があれば，拒絶をモニターし，その進展を把握することもできる。

III. これまでの臓器移植に関する分子病理学の歩み

分子病理学的手法は多方面にわたる。組織切片上の *in situ* hybridization (ISH)，*in situ* PCR や mRNA ISH 法による分子形態学的診断，FISH 法による染色体異常の解析，PCR による感染症の診断などが挙げられる。さらに，臓器移植後の拒絶に関する免疫学的なモニタリングは，移植患者の血液細胞，移植臓器からの逸脱酵素，血清や尿中の免疫関連蛋白を標的として，種々の抗体を用いて，ELISA，flow cytometry あるいは免疫組織化学的手法により行ってきた。しかし最近は，より再現性が高く安定な RT-PCR（reverse transcription-polymerase chain reaction）を用いて，移植臓器，血液，尿中の mRNA の発現を半定量的に調べる手法が主流をなしている。

RT-PCR を用いた戦略としては，第1に，免疫学的知識からあらかじめ候補となる遺伝子を想定して，その数種の候補遺伝子に関して，それぞれの mRNA から RT-PCR により cDNA を増幅して，半定量的に発現量を測定し，拒絶反応のマーカーとして最も信頼性のあるコンビネーションを探り当てる方法である。第2の方法としては，検体内の mRNA から RT-PCR により cDNA を増幅産生し，発現している遺伝子群を microarray により網羅的に調べ，対照群の非拒絶臓器での発現と比較することにより，拒絶症例に特異的な遺伝子発現をパターン認識するものである。検索対象は移植臓器片で行うのが信頼性が高いが，末梢血中の単核球や尿，胆汁，肺内洗浄液で行うこともある。

腎移植の分野では，Strom TB（Harvard Medical School）と Suthanthiran M（Cornell Medical Center）の功績が分子病理学と臓器移植の実践を大幅に結びつけた[2,3]。その歴史をたどることが，この分野の理解に最も適していると思う。彼らは pyogenic pro-inflammatory cytokine の解析から始めた。移植片における IL-1 (interleukin-1)，TNFα (tumor necrosis factorα)，そして，IL-6 について semiquantitative RT-PCR（QRT-PCR）を用いて，移植腎組織における mRNA の発現量を調べたが，再現性のある結果が得られなかった。さらに，IL-2 や IFNγ などのサイトカインについても同様に調べたが成果が得られなかった。特に，Th1 型リンパ球関連のサイトカインについては，あらかじめ治療で投与される calcineurin inhibitor をはじめとした免疫抑制剤の影響が強く，その結果として，false negative の症例数が true positive の症例を上回ったため実用に供しないことがわかった[3-5]。

その後，cytotoxic T lymphocyte (CTL) effector molecule に着目した[6]。CTL に内在する serine proteinase である granzyme B と perforin は，従来，CTL による細胞融解性機構に関与するといわれ，免疫組織化学的には granzyme A，B が急性拒絶反応症例の尿細管上皮に浸潤するリンパ球に多く発現するという報告があった[7]。また，この分子は急性拒絶反応に特異性が高く，尿細管間質の炎症の強さとは相関しないものの，移植拒絶腎以外の間質炎の症例では発現がなかったという[6]。Fas ligand (FasL) は活性化 T 細胞の表面に表出し，Fas を表出している細胞にアポトーシスを誘導するため，granzyme B や perforin とともに CTL effector molecule のひとつとされている[8]。これらの分子の mRNA 発現は，CTL の活性相（activating phase）に著明に発現し，休止期（resting phase）には発現が起こらないことから，2値（black and white）的な捉え方が可能で，その点で検出の感度（sensitivity）を上げることができたこと，そして，CTL 以外の腎実質組織によって発現されないことの理由から急性拒絶に対する特異性（specificity）も高い結果となった[9,10]。これらの成果は腎生検材料を用いることによって得られたが，さらに非侵襲性と簡便性の観点から，末梢リンパ球や尿沈渣の転写について同様に解析する方向に向かっている。

mRNA から cDNA を RT-PCR で増幅する段階で定量性を持たせることでも工夫が見られる。すなわち，glyceraldehyde-3-phosphate dehydrogenase (GAPDH) などのもともと腎組織に内在して発現する遺伝子（consitutively expressed gene）に着目し，

その単位あたりの granzyme B の cDNA 量を調べることで，個体間でのばらつきを標準化することができた。通常，mRNA の増幅には 10 μg の total RNA か 500 ng の mRNA が必要とされるが，RNA の抽出法の最近の進歩も手伝って針生検材料でも実用が可能となった[11]。上記の CTL の serine proteinase の再現性の他に，IL-10，IL-7，IL-15 などのサイトカイン，T cell receptor の constant region も再現性が高く実用性のある指標であることがわかった[3-5]。

以上の経緯を踏まえて，以下，分子病理学がどの程度生検の役割を補足しているかを中心に概説する(図 1)。

IV. 組織生検を用いた拒絶反応における mRNA の発現

急性拒絶腎において，活性化 $CD3^+$ cytotoxic T cell や $CD56^+$ natural killer cell (NK cell) の顆粒に含まれる cytotoxic effector molecule である perforin，granzyme B，FasL は，腎から組織を採取し RT-PCR にて mRMA を定量化した結果，軽度の炎症細胞浸潤を認める症例においても specificity，sensitivity とも 100%の信頼性があった[5]。しかし，上記の cytotoxic marker の発現の上昇は，血清クレアチニンの最大値やバンフ分類における rejection grade と相関はなかった[12]。一方，治療抵抗性の急性拒絶症例 (7 例) において，治療に反応した症例 (8 例) に比して FasL だけが有意に発現が上昇していたため，治療抵抗性の指標になる可能性がある[12]。granzyme B，perforin，IL-2，IL-4，IL-10，IFNγ のうち granzyme B，IL-10，そして IL-2 が急性拒絶反応と相関したが[13]，IL-2，IL-4，IFNγ は急性拒絶症例においても検出率が悪く，最近の免疫抑制治療の IL-2 と IFNγ に対する有効な治療のためだとしている[5]。その他，IL-7 や IL-15 も急性拒絶に発現していたが，RANTES と IL-8 は sensitive であるものの，急性拒絶に specificity の低いマーカーといわれる[5]。患者の負担の軽い吸引細胞 (fine needle aspirates) を用いた研究では，移植後 10 日間に連続して IL-2，IL-4，IL-6，IL-10，IFNγ についての mRNA の発現を追跡した結果，最初の 4 日間では IL-10 のみが亢進し，その後，腎機能の低下症例，非低下症例にかかわらず，IL-2 と IFNγ が炎症細胞浸潤に先行して亢進していた[14]。一方，光顕的に拒絶反応のない症例においては，IL-2 か IFNγ のどちらかしか検出されなかった[14]。心臓移植においても，拒絶の程度や機能の低下に相関する mRNA として，granzyme A が挙げられている[15]。また，IL-2 receptor，IL-1β，TNFα の mRNA の発現がステロイド反応性の症例で，ステロイド抵抗性の症例より有意に低値であったため，ステロイド反応の指標になるという報告もある[16]。

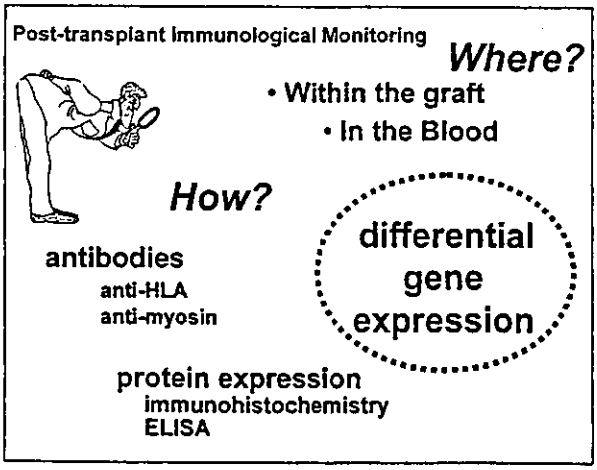

図 1 第 7 回 Banff 会議において，Dr. M. Gerbase de Lima の講演に使用されたスライドの 1 枚[31]。テーマは "心臓移植における分子病理" であったが，ストーリー性のある内容であった。本稿もその講演を参考にしている。

一方，慢性拒絶に TGFβ1 が相関するという点では諸家の文献は一致している[5,12,17]。non-heart beated donner (心停止ドナー) は heart-beated donner (脳死ドナー) に比して，虚血障害からくる腎機能回復の遅延が高頻度であるにもかかわらず，心停止ドナーと脳死ドナーの間で，少なくとも移植後 1 週間目には糸球体内の線維化関連遺伝子に関する mRNA の発現には差がなかった[18]。また，急性拒絶腎の糸球体にも同様な線維化関連遺伝子 (collagen III，collagen IV，MMP-2，TIMP-1，TIMP-2，tenascin) の発現はなかった[19]。その他のサイトカインに関しては心移植での成果がある。bFGF (basic fibroblast growth factor) の mRNA が移植後第 1 週以内に発現した場合に，移植後 1 年以内に拒絶反応が起きる可能性が高い[20]。また，AIF-1 (allograft inflammatory factor 1) の mRNA が発現すると，allograft vasculopathy になる確率が高いといわれる[21]。

腎機能が正常で，腎生検でバンフ分類の急性拒絶か，あるいは境界領域変化 (Borderline changes) を認める症例は，subclinical rejection といわれるが，この拒絶の適切な診断も分子病理的手法によりなされて

いる。Lipmanらは症例を，正常，borderline subclinical rejection, acute subclinical rejectionに分けて，TNFα，IL-1β，TGFβ，IFNγ，IL-2，IL-4，IL-10，IL-15のサイトカインに対するmRNAの発現を定量的に調べたところ，acute subclinical rejectionと正常症例では明らかな差があり，borderline subclinical rejectionはその中間であったことを報告している[22]。そして，subclinical rejectionの症例は長期間における腎機能に障害性に関連するため，治療を考慮すべき対象としている[22]。

急性拒絶反応の発症の予知に関して，granzyme B，perforin，FasLは組織にリンパ球が浸潤する前の時期，すなわち，腎機能と形態像が正常な時期から，mRNAの転写が始まるといわれる(pro-inflammatory gene transcript)。心生検標本を，急性拒絶のない時期，急性拒絶の始まる7～15日前の時期，拒絶の始まりから7～15日の時期に分け，そのCD40L，IFNγ，FasLのmRNAを比較すると，拒絶の始まる7～15日前の時期からこれらのcytotoxic effector moleculesのmRNAが増強していることがわかり，急性拒絶発症の予知が可能であった[23]。また，Tリンパ球の共刺激因子(costimulatory factor)のひとつであるT-cell immune response cDNA 7 (TIRC7)が心移植組織において上昇し，末梢血中の単核球において減少することも急性拒絶発症の予知につながるという[24]。

急性拒絶反応における接着因子の研究では，ICAM1とVCAM-1のmRNAの発現が拒絶反応と関係している。ICAM1とVCAM-1は正常と移植腎においてほぼ相補的な分布を示すが，急性拒絶の筋性小動脈の内皮にもmRNAの発現を認め，また，細動脈の血管拒絶を認める症例では血管壁の平滑筋細胞にもICAM1とVCAM-1のmRNAが発現することを*in situ* hybridizationにより証明している[25]。

V. 末梢リンパ球，尿を用いた拒絶反応におけるmRNAの発現

末梢血の単核球を用いて急性拒絶に関するmRNAの発現を調べることは，生検に比して非侵襲性で，頻回に施行できることから，より理想的な分子病理学的手法といえる。しかし現在は，より多くの証拠を集積している段階である。腎移植においては，急性拒絶症例の末梢リンパ球にgranzyme B，perforin，FasLのmRNAが増強していた[26]。また，末梢のCD 4$^+$ T細胞におけるCD40 ligand (CD40L)遺伝子のmRNA発現を，非移植対照腎，急性・慢性拒絶腎，移植腎で腎機能の正常な症例の3群において比較したところ，急性拒絶のBanff 97 scoreと傍尿細管毛細血管病変の程度に相関していたという[27]。さらに，CD40L遺伝子のmRNA発現は末梢CD4$^+$ T細胞に対するCsAやFK506の*in vitro*での反応性のよい指標になったという[27]。末梢リンパ球でのFasL mRNAの発現が慢性拒絶腎に亢進し，それによって誘導されたアポトーシスが慢性拒絶腎の組織障害に関与するとの報告もある[8]。尿中に排出された細胞を用いた研究では，perforinとgranzyme BのmRNAの発現を定量的に測定してcut off値を決め，急性拒絶腎の診断が可能であったという報告がある[28]。また，尿中のBKウイルス感染の診断にBKVP1に対するmRNAの検出が有効であった[29]。急性拒絶と急性尿路系感染との区別を尿中に排出された細胞から調べると，granzyme B mRNAの発現によって鑑別診断が可能であったという報告がある[30]。心移植の急性拒絶に関しても，末梢リンパ球のperforin，granzyme B，IFNγのmRNA発現の亢進とIL-8，TNFα mRNAの発現が低下する結果が出ている[31]。mRNAの増幅による手法ではないが，mass spectrometryを用いて，急性拒絶腎に特異的な尿蛋白出現パターンを同定して診断に役立てている論文も見られる[32]。

VI. cDNA microarray analysis

移植臓器組織に発現しているすべてのmRNAに着目し，RT-PCRにより対応するcDNAを増幅し，それをすでにわかっている既存の多種のoligonucreotideによる遺伝子チップに反応させ，疾患固有のパターン(finger print)を見ようとするDNA microarray法も有効な手段である[33,34]。cDNA microarray analysis (techonology)はbioinformaticsの発達とともに実用面に大きく近づいた[35]。すなわち，cluster analysis (hierarchical cluster analysis)やself organizing mapsの作製，そしてpriciple component analysisなどが可能となり，これまでの個々の遺伝子mRNA発現の解析では不可能であった急性拒絶や慢性拒絶における亜型の診断を可能としている。

Sarwalらは，67症例の腎移植症例の腎生検材料を用いて，cluster解析を併用してcDNA microarray analysisを行い，腎生検，臨床経過，治療反応と対応させたところ，急性拒絶型，薬物中毒型，慢性拒絶型，正常型の4つ群に分けることができた。さらに，急性

拒絶群は免疫活性化と細胞増殖に関する遺伝子群により3つの亜型に分けられた。そして，その中の1つの亜型がCD20$^+$ B細胞の浸潤がある症例群で，臨床的にステロイド抵抗性で移植腎機能廃絶に相関したという興味ある結果が得られている[36]。high-density oligoarray (GeneChip, Affymetrix, Santa Clara, CA) を用いた研究では，6800のヒト遺伝子中32から219の間の遺伝子が拒絶のない対照群に対して4倍以上の高値を示した。すなわち，7例中7例全例にmRNAの発現の亢進した遺伝子として，INFγに誘導されたmonokine, T-cell receptor active β chain protein, interleukin-2 stimulated phosphoprotein, そして，RING4 (a transporter involved in antigen presentation) の4つが挙げられ，7例中6例にmRNAの亢進していた遺伝子としては，interferon-stimulated growth factor-3, complement factor 3, nicotine amide *N*-methyltransferase, macrophage inflammatory protein-3β, myeloid differentiation protein, そして，CD18が挙げられた。この遺伝子チップを使用する限り，以前から報告のあるcytotoxic T-cell effector分子の亢進はなかったという[37]。

慢性拒絶症例に関しては，移植後6カ月と12カ月とを比較し，移植後12カ月目に慢性拒絶に進展した症例に10個の遺伝子セットが関係し，それにより6カ月後の予後が予測できたという報告がある[38]。また，慢性拒絶，嚢胞腎末期，正常腎の症例において，7KヒトcDNA microarrayを用いて解析した結果，細胞代謝，輸送，シグナル，翻訳活性，接着，免疫反応に関する571遺伝子のヒエラルキー・クラスター解析で上記の3つの疾患を鑑別することができた[39]。そして，慢性拒絶群においては2つの異なった亜型 (subset) のheterogeneityが見いだされた[39]。末梢血の単核球を用いたmicroarrayの仕事はわずかであるが，長期生着症例の末梢リンパ球を解析した研究[40]も出始めている。

以上，cDNA microarrayを用いた臓器移植の研究は，光顕診断では区別がつかない急性拒絶や慢性拒絶の亜型を鑑別し，予後の予測や発症の予知に貢献する可能性がある。

VII. microdisection法の応用

腎臓は形態的にも機能的にも高度に分化した臓器で，糸球体と間質尿細管で，構成細胞も機能も大きく分かれている。そのため，遺伝子発現の解析のために採取された腎組織における細胞のheterogeneityが研究成果の再現性に障害となる可能性がある。その観点から，microdisection法を組み合わせて，特定の組織の場所を切り出し，そのmRNAの発現をみる手法は有効である。臓器移植に関するこの方面の研究はまだ黎明期にあるといえる。急性拒絶の大部分は尿細管間質に病変が起こり糸球体には変化が起こらないため，microdisectionを併用することなしに成果が出ているのかもしれない。最近のKretzlerらによる総説は今後の指針を立てる意味で参考になる[41,42]。

VIII. おわりに

臓器移植の分野における分子病理学はこれまで述べてきたような方向に進んできたと思う。移植後の経過中に，感染症や合併症，さらに薬剤中毒を避けることはいうまでもないが，現在の臓器移植の治療学において，有効かつ実用的な手段は免疫抑制剤の適正な使用であり，また，現在，持ち合わせている治療法を効果的に使用できる余地のある分野でもある。それにより，急性拒絶の頻度と強度を最小限に抑え，慢性拒絶への進展を防ぐか，少くともその時期を延ばすことが，古くて新しい移植治療のひとつの課題であるかもしれない。

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文 献

1) Almond PS, Matas A, Gillingham K, Dunn DL, Payne WD, Gores P, Gruessner R, Najarian JS. Risk factors for chronic rejection in renal allograft recipients. Transplantation 1993; 55: 752-756.
2) Strom TB, Suthanthiran M. Prospects and applicability of molecular diagnosis of allograft rejection. Semin Nephrol 2000; 20: 103-107.
3) Suthanthiran M. Acute rejection of renal allografts: mechanistic insights and therapeutic options. Kidney Int 1997; 51: 1289-1304.
4) Suthanthiran M. Molecular analyses of human renal allografts: differential intragraft gene expression during rejection. Kidney Int Suppl 1997; 58: S15-21.
5) Strehlau J, Pavlakis M, Lipman M, Shapiro M, Vasconcellos L, Harmon W, Strom TB. Quantitative detection of immune activation tran-

scripts as a diagnostic tool in kidney transplantation. Proc Natl Acad Sci USA 1997; 94: 695-700.
6) Kummer JA, Wever PC, Kamp AM, ten Berge IJ, Hack CE, Weening JJ. Expression of granzyme A and B proteins by cytotoxic lymphocytes involved in acute renal allograft rejection. Kidney Int 1995; 47: 70-77.
7) Lipman ML, Stevens AC, Strom TB. Heightened intragraft CTL gene expression in acutely rejecting renal allografts. J Immunol 1994; 152: 5120-5127.
8) Kasprzycka M, Klodos K, Nowaczyk M, Wyzgal J, Podobinska I, Durlik M, Gorski A. Expression of FasL gene in T cells of renal allograft recipients. Immunol Lett 2002; 80: 9-13.
9) Strehlau J, Pavlakis M, Lipman M, Maslinski W, Shapiro M, Strom TB. The intragraft gene activation of markers reflecting T-cell-activation and -cytotoxicity analyzed by quantitative RT-PCR in renal transplantation. Clin Nephrol 1996; 46: 30-33.
10) Pavlakis M, Lipman M, Strom TB. Intragraft expression of T-cell activation genes in human renal allograft rejection. Kidney Int Suppl 1996; 53: S7-12.
11) Suthanthiran M. Human renal allograft rejection: molecular characterization. Nephrol Dial Transplant 1998; 13 Suppl 1: 21-24.
12) Nickel P, Lacha J, Ode-Hakim S, Sawitzki B, Babel N, Frei U, Volk HD, Reinke P. Cytotoxic effector molecule gene expression in acute renal allograft rejection: correlation with clinical outcome; histopathology and function of the allograft. Transplantation 2001; 72: 1158-1160.
13) Suthanthiran M. Clinical application of molecular biology: a study of allograft rejection with polymerase chain reaction. Am J Med Sci 1997; 313: 264-267.
14) McLean AG, Hughes D, Welsh KI, Gray DW, Roake J, Fuggle SV, Morris PJ, Dallman MJ. Patterns of graft infiltration and cytokine gene expression during the first 10 days of kidney transplantation. Transplantation 1997; 63: 374-380.
15) Alpert S, Lewis NP, Ross H, Fowler M, Valantine HA. The relationship of granzyme A and perforin expression to cardiac allograft rejection and dysfunction. Transplantation 1995; 60: 1478-1485.
16) Baan CC, Niesters HG, Balk AH, Mochtar B, Zondervan PE, Weimar W. The intragraft cytokine mRNA pattern reflects the efficacy of steroid antirejection therapy. J Heart Lung Transplant 1996; 15: 1184-1193.
17) August P, Suthanthiran M. Transforming growth factor beta and progression of renal disease. Kidney Int Suppl 2003; 87: S99-104.
18) Jain S, Bicknell GR, White SA, Williams ST, Furness PN, Nicholson ML. Comparison of the expression of fibrosis-associated genes in glomeruli after renal transplantation between conventional cadaveric and non-heart-beating donors. Br J Surg 1999; 86: 1264-1268.
19) Brook NR, White SA, Waller JR, Bicknell GR, Nicholson ML. Fibrosis-associated gene expression in renal transplant glomeruli after acute renal allograft rejection. Br J Surg 2003 (8); 90: 1009-1014.
20) de Groot-Kruseman HA, Baan CC, Loonen EH, Mol WM, Niesters HG, Maat AP, Balk AH, Weimar W. Failure to down-regulate intragraft cytokine mRNA expression shortly after clinical heart transplantation is associated with high incidence of acute rejection. J Heart Lung Transplant 2001; 20: 503-510.
21) Autieri MV, Kelemen S, Thomas BA, Feller ED, Goldman BI, Eisen HJ. Allograft inflammatory factor-1 expression correlates with cardiac rejection and development of cardiac allograft vasculopathy. Circulation 2002; 106: 2218-2223.
22) Lipman ML, Shen Y, Jeffery JR, Gough J, McKenna RM, Grimm PC, Rush DN. Immune-activation gene expression in clinically stable renal allograft biopsies: molecular evidence for subclinical rejection. Transplantation 1998; 66: 1673-1681.
23) Shulzhenko N, Morgun A, Franco M, Souza MM, Almeida DR, Diniz RV, Carvalho AC, Pacheco-Silva A, Gerbase-Delima M. Expression of CD40 ligand, interferon-gamma and Fas ligand genes in endomyocardial biopsies of human cardiac allografts: correlation with acute rejection. Braz J Med Biol Res 2001; 34: 779-784.
24) Shulzhenko N, Morgun A, Rampim GF, Franco

M, Almeida DR, Diniz RV, Carvalho AC, Gerbase-DeLima M. Monitoring of intragraft and peripheral blood TIRC7 expression as a diagnostic tool for acute cardiac rejection in humans. Hum Immunol 2001; 62: 342-347.
25) Park SY, Kim HW, Moon KC, Hong HK, Lee HS. mRNA expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in acute renal allograft rejection. Transplantation 2000; 69: 2554-2560.
26) Vasconcellos LM, Schachter AD, Zheng XX, Vasconcellos LH, Shapiro M, Harmon WE, Strom TB, Schachter D. Cytotoxic lymphocyte gene expression in peripheral blood leukocytes correlates with rejecting renal allografts. Transplantation 1998; 66: 562-566.
27) Shoker A, George D, Yang H, Baltzan M. Heightened CD40 ligand gene expression in peripheral $CD4^+$ T cells from patients with kidney allograft rejection. Transplantation 2000; 70: 497-505.
28) Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001; 344: 947-954.
29) Ding R, Suthanthiran M. Noninvasive diagnosis of BK virus nephritis by measurement of messenger RNA for BK VP1 virus in urine. Transplantation 2003; 75: 1752-1754.
30) Dadhania D, Muthukumar T, Ding R, Li B, Hartono C, Serur D, Seshan SV, Sharma VK, Kapur S, Suthanthiran M. Molecular signatures of urinary cells distinguish acute rejection of renal allografts from urinary tract infection. Transplantation 2003; 75: 1752-1754.
31) Gerbase de Lima. Immune response gene expresssion in cardiac transplantation. Abstract Book, 7th Banff Conference on Allograft Pathology, 2003.
32) Schaub S, Rush D, Wilkins J, Gibson IW, Weiler T, Sangster K, Nicolle L, Karpinski M, Jeffery J, Nickerson P. Proteomic-based detection of urine proteins associated with acute renal allograft rejection. J Am Soc Nephrol 2004; 15: 219-227.
33) Chua MS, Mansfield E, Sarwal M. Applications of microarrays to renal transplantation: progress and possibilities. Front Biosci 2003; 8: S913-923.
34) Chua MS, Sarwal MM. Microarrays: new tools for transplantation research. Pediatr Nephrol 2003; 18: 319-327.
35) Kurella M, Hsiao LL, Yoshida T, Randall JD, Chow G, Sarang SS, Jensen RV, Gullans SR. DNA microarray analysis of complex biologic processes. J Am Soc Nephrol 2001; 12: 1072-1078.
36) Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O Jr. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling N Engl J Med 2003; 349: 125-138.
37) Akalin E, Hendrix RC, Polavarapu RG, Pearson TC, Neylan JF, Larsen CP, Lakkis FG. Gene expression analysis in human renal allograft biopsy samples using high-density oligoarray technology. Transplantation 2001; 72: 948-953.
38) Scherer A, Krause A, Walker JR, Korn A, Niese D, Raulf F. Early prognosis of the development of renal chronic allograft rejection by gene expression profiling of human protocol biopsies. Transplantation 2003; 75: 1323-1330.
39) Donauer J, Rumberger B, Klein M, Faller D, Wilpert J, Sparna T, Schieren G, Rohrbach R, Dern P, Timmer J, Pisarski P, Kirste G, Walz G. Expression profiling on chronically rejected transplant kidneys. Transplantation 2003; 76: 539-547.
40) Zhang HQ, Lu H, Enosawa S, Takahara S, Sakamoto S, Suzuki S. Identification of novel genes associated with immunosuppression in renal transplant patients. Transplant Proc 2002; 34: 2733-2735.
41) Henger A, Kretzler M, Doran P, Bonrouhi M, Schmid H, Kiss E, Cohen CD, Madden S, Porubsky S, Grone EF, Schlondorff D, Nelson PJ, Grone HJ. Gene expression fingerprints in human tubulointerstitial inflammation and fibrosis as prognostic markers of disease progression. Kidney Int 2004; 65: 904-917.
42) Kretzler M, Cohen CD, Doran P, Henger A, Madden S, Grone EF, Nelson PJ, Schlondorff D, Grone HJ. Repuncturing the renal biopsy: strategies for molecular diagnosis in nephrology. J Am Soc Nephrol 2002; 13: 1961-1972.

進行性腎障害：診断と治療の進歩

I. 腎障害の評価と診断法
5. 腎疾患ネットワークによるデータベース構築

山田　研一　　柏原　英彦

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トピックス

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山田　研一　　柏原　英彦

要　旨

わが国は未曽有の高齢化社会を迎え，特に糖尿病，腎硬化症といった高齢者の腎不全予備軍が増大しつつある．透析患者は20万人を越え，10年以上透析者が25%を占めるにいたり，長期透析合併症の増加も顕著であるが，腎移植は低迷を続け，わが国の腎不全医療は医学的，社会的，経済的にも深刻な問題を抱えている．これに対して，国立病院・療養所による全国規模での腎疾患ネットワークが組織され，腎疾患発症予防と腎不全への進展阻止および腎不全患者QOL (quality of life) 向上にむけての，腎疾患（糖尿病性腎症，慢性腎炎（特にIgA腎症），腎透析，腎移植）患者のデータベース（基本情報，経過情報，固有情報）および腎病理支援システムの構築が開始された．〔日内会誌　93：886～895，2004〕

Key words：腎疾患ネットワーク，糖尿病性腎症，IgA腎症，腎透析，腎移植，データベース構築

はじめに

わが国は急速な生活習慣の欧米化に伴い，疾病構造の変化を認め，成人病型疾患の急増を認めている．特に糖尿病性腎症や腎硬化症進展による腎不全・透析は急増し，新規透析導入患者数は，1998年以後糖尿病性腎症が第1位のまま，増加の一途である．

わが国の慢性維持透析人口は約21万人に達し，その医療費は日本の総医療費30兆円の約3%強に達している．腎疾患医療の量的・質的変化に加わえ，医療経済的にも大きな問題となってきている．

このような状況の中で，腎疾患の疾病構造，発症，進展に対する病態特性や医療内容に関して，更に末期腎不全医療内容にまで及ぶ，一貫した全国規模でのデータベース構築は日本ではまだなされていない．

最近，全国規模で存在し，地域の中核病院でもある国立病院・療養所での腎疾患ネットワークを活用したデータベース構築が，一つのモデルとして開始された．その概説とともにデータベースより得られた成果の一部を報告し，批判を受けたい．

1. わが国の腎疾患医療の現状と特徴

2002年末での慢性維持透析患者総数は229,538人になり，新規透析導入患者は10年間で約2倍の33,710人に達し，導入者年齢も平均で64.2歳（1983年は51.9歳）と高齢化している．導入の原疾患は，1998年より糖尿病性腎症が第1位で，12,630人；37.5%を占め，第2位は慢性腎炎の10,309人；30.6%，第3位は腎硬化症の2,536人；7.5%になっている（図1，2）．一方導入後の1年，5年，10年生存率は各々，87.7%，60.9%，

やまだ　けんいち：国立病院機構千葉東病院
かしわばら　ひでひこ：聖隷佐倉市民病院