The Granzyme B Inhibitor SERPINB9 (Protease Inhibitor 9) Circulates in Blood and Increases on Primary Cytomegalovirus Infection after Renal Transplantation

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SERPINB9 is the only known human intracellular inhibitor of granzyme B (GrB), the effector molecule in immunity against cytomegalovirus (CMV) and in renal allograft rejection. Therefore, using specific enzyme-linked immunosorbent assays, we addressed the presence of circulating SERPINB9 during primary CMV infection, subclinical rejection, acute rejection, and uncomplicated posttransplantation course. Soluble (s) SERPINB9 circulates in blood and increases on primary CMV infection. This increase was significantly higher in symptomatic than in asymptomatic patients. In contrast, sSERPINB9 levels did not change in response to subclinical or acute rejection. We demonstrated the presence of circulating sSERPINB9/sGrB complexes, which suggests that SERPINB9 has extracellular functions as well.

After renal transplantation, recipients are threatened by cytomegalovirus (CMV) infection and clinical or subclinical rejection and, thus, are exposed to a higher risk of chronic rejection and long-term graft failure [1]. Granzyme B (GrB)–mediated cytotoxicity plays a key role in immunity against CMV and also in the effector phase of rejection [2, 3]. Previously, we have shown that expansion of a specific subset of CD8+ GrB+ T cells coincides with a temporary increase in systemic soluble (s) GrB levels during primary CMV infection. In contrast, no such increase was observed during acute allograft rejection [4]. Rapid trapping of allospecific GrB+CD8+ T cells into the graft may account for the lack of increase in circulating sGrB levels during rejection.

The intracellular serpin SERPINB9 (proteinase inhibitor 9 [PI-9]) is the only known human protein that specifically inhibits GrB [5, 6]. The in vivo distribution of SERPINB9 suggests that this enzyme protects against unwanted GrB-mediated cytotoxicity. SERPINB9 is expressed at immune-privileged sites and by endothelial, dendritic, and mesothelial cells and malignant lymphomas [7, 8]. Ex vivo experiments using NK cell lines and peripheral blood cells from healthy persons have provided us with evidence for subcellular localization and up-regulation of SERPINB9 during maturation of accessory cells and degranulation of effector cells [6]. In such systems, SERPINB9 could associate with GrB-containing granules and form intracellular complexes, leading to inactivation of cytoplasmic GrB by SERPINB9. However, whether SERPINB9 associates with sGrB released in the circulation is not yet known.

Recently, we have shown that SERPINB9 expression by tubular cells is significantly higher during subclinical rejection than during acute rejection. In contrast to acute rejection, subclinical allograft rejection is defined as the presence of a GrB+ lymphoid cell infiltrate in a renal allograft, which causes clear-cut tubulitis at the histological level but does not, however, lead to an instant deterioration of allograft function. High SERPINB9 expression in these tubules, involved in subclinical rejection, correlates with the presence of GrB+ T cells in the graft. These data point to a protective role of SERPINB9 against cytotoxic T cells [9]. We wondered whether serum levels of SERPINB9 can be used to discriminate subclinical from acute rejection and can possibly be used as a noninvasive diagnostic tool for intragraft events.

In the present study, we addressed the presence of this serpin in peripheral blood from healthy persons and renal transplant recipients during primary CMV infection, subclinical rejection, acute rejection, or no rejection.

Subjects, materials, and methods. This longitudinal study was performed retrospectively on serum samples from 46 transplant recipients (30 men and 16 women; median age, 46 years [range, 21–73 years]). Samples were obtained before transplantation, 3 times/week during hospitalization, and 1 time/week after discharge for 3 months and regularly thereafter for up to 6 months. Patients were treated with prednisolone (10 mg/day),
were 2-sided; was considered significant between groups were analyzed by Mann-Whitney Spearman rank correlation analysis, whereas differences otherwise. The correlation between parameters was assessed by the control.

YT-indy, also containing SERPINB9/sGrB complexes, served as body GB11 (microtiter plates, and biotinylated anti-GrB monoclonal anti-clonal anti–PI-9 antibodies (1 μg/mL) were used for coating the microtiter plates, and biotinylated anti-GrB monoclonal antibody GB-11 as detecting antibody. With this assay, sSERPINB9/sGrB complexes could be detected in some patients. Also, sSERPINB9/sGrB complexes were demonstrated in the above-mentioned high-molecular-weight peak of sSERPINB9. These data indicate that sSERPINB9 circulates in serum at least partially complexed to GrB.

sSERPINB9 levels increased significantly, from a median of 275 pg/mL (range, 83–688 pg/mL) at 1 week after transplantation to a median of 2324 pg/mL (range, 532–10,046 pg/mL) at the time of the peak CMV DNA load, as determined by polymerase chain reaction (PCR). This increase paralleled the increases in CMV DNA load and sGrB level. As shown in figure 1, the peak levels of sSERPINB9 in patients with symptomatic CMV infection (n = 16; median, 2780 pg/mL [range, 532–10,046 pg/mL]) were significantly higher than those in asymptomatic patients (n = 7; median, 1112 pg/mL [range, 620–2367 pg/mL]) (P < .01).

Figure 2 shows that sSERPINB9 levels rose in parallel with increases in sGrB level and CMV DNA load, as measured by PCR. In the majority of patients, the peak levels of sSERPINB9, sGrB, and CMV DNA coincided. However, the extent of the increase in sSERPINB9 did not correlate with that of sGrB (n =...
Kinetics of soluble (s) SERPINB9 and granzyme B (sGrB) levels in serum from patients with symptomatic and asymptomatic primary posttransplantation cytomegalovirus (CMV) infection. CMV DNA load, as determined by polymerase chain reaction (PCR), is expressed as copies per milliliter of whole blood. A parallel increase and nearly coinciding peak levels of sSERPINB9, sGrB, and CMV DNA were observed in symptomatic and asymptomatic patients.

sSERPINB9 and sGrB levels did not differ significantly between patients with subclinical rejection, acute rejection, and no rejection. On the basis of the median of repeated measurements of SERPINB9 levels in a given patient, we determined a median sSERPINB9 level for each group of patients. In patients experiencing a subclinical rejection, sSERPINB9 and sGrB levels were, respectively, 936 pg/mL (range, 130–8565 pg/mL) and 40 pg/mL (range, 2–4453 pg/mL). In patients experiencing an acute rejection, sSERPINB9 and sGrB levels were 555 pg/mL (range, 35–10,046 pg/mL) and 38 pg/mL (range, 3–1256 pg/mL). In patients with no rejection, sSERPINB9 and sGrB levels were 852 pg/mL (range, 162–27,300 pg/mL) and 19 pg/mL (range, 5–1244 pg/mL). No significant differences were found.

Discussion. Here, we show that the intracellular serpin SERPINB9 can be detected in the circulation. Serum levels of SERPINB9 increase during primary CMV infection after transplantation, showing a higher increase in symptomatic than in asymptomatic patients.

SERPINB9 belongs to the subfamily of intracellular serpins, which are unique in that they lack a cleavable N-terminal signal sequence and, therefore, reside and function mainly intracellularly [12]. Although intracellular serpins exert their functions primarily intracellularly, at least 2 other intracellular serpins, SERPINB2 (PAI-2) and SERPINB3 (SCCA1), are also found extracellularly. SERPINB2 is partially secreted through a yet-undefined secretory pathway [13], whereas SERPINB3 is thought to be passively released into the circulation [14]. In this study, we demonstrate the existence of sSERPINB9 in the circulation. Whether sSERPINB9 is actively secreted or passively released remains to be elucidated.

In addition, we show that sSERPINB9 in serum partially occurs as complexes with GrB. Because inhibition of target proteinases by serpins yields covalently linked complexes between these compounds, these sSERPINB9/sGrB complexes provide us with extra evidence that sSERPINB9 indeed inhibits GrB in vivo. Until now, such complex formation between serpins and their cognate proteinases, such as thrombin-ATIII, plasmin-antiplasmin, and complement factor 1 esterase–complement factor 1 esterase inhibitor, has been thought to be required as the first step in the performance of serpin’s inhibitory function.

Previously, we showed a marked and temporal increase in sGrA and sGrB levels during CMV infection after renal transplantation [4]. sSERPINB9 levels increased in parallel with the increase in CMV DNA load, as determined by PCR, and fol-
lowed or coincided in time with that of sGrB. The peak levels of CMV DNA, sGrB, and sSERPINB9 occurred on approximately the same day. In patients with symptomatic infection, sSERPINB9 levels rose to a significantly higher degree than in asymptomatic patients. A more protracted exposure to the virus could offer an explanation for this difference. Higher numbers of virally infected endothelial cells in symptomatic patients may also underlie this difference, because these cells are principal CMV reservoirs and contain high amounts of SERPINB9 [2, 7]. To escape unwanted cytotoxicity, these cells probably express SERPINB9 to a high degree. Other cell types, such as cytotoxic T lymphocytes (CTLs) and dendritic cells, may also be the source of sSERPINB9 [5, 6]. CTLs produce high levels of SERPINB9 during subclinical rejection [15], we speculate that measurement of SERPINB9 mRNA and protein in urine of patients with subclinical rejection, or uncomplicated posttransplant course. Also, levels measured on the day before transplantation did not discriminate between these clinical conditions. Apparently, hyperexpression of SERPINB9 by tubular cells during subclinical rejection, as has been shown recently by immunohistochemical studies of graft biopsy samples [9], is not reflecte by increased systemic sSERPINB9 levels. Similar data were shown previously for systemic sGrB levels during acute rejection. During acute rejection, increased numbers of GrB+ CTLs in allograft biopsy samples have been found [3]; however, sGrB levels in the circulation have not been found to be elevated [4]. Accordingly, in the present study, low GrB levels were measured during acute rejection (data not shown). Because the levels of mRNA encoding either SERPINB9 or GrB have been shown to be increased in urinary cells in recipients with acute rejection versus no rejection [15], we speculate that measurement of SERPINB9 mRNA and protein in urinary cells from patients with subclinical, acute, or no rejection may constitute a noninvasive diagnostic tool. To address this question, further studies are in progress.

In conclusion, the GrB inhibitor SERPINB9 can be detected in blood, and levels of SERPINB9 increase temporarily on primary posttransplantation CMV infection. However, because levels of sSERPINB9 are not increased during subclinical rejection, levels in the circulation do not reflect intragraft events.

References