

Association between increased levels of regulatory T cells and soluble human leukocyte antigen G with the prevalence of cancer in kidney transplant recipients

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Introduction Kidney transplantation is the most efficient method of treatment for patients with end-stage renal disease. Over the last few years, a gradual improvement in the survival of kidney transplant recipients (KTRs) with functioning grafts has been observed. Data from registries have shown cardiovascular diseases and malignancies to be the main causes of mortality among patients with a functioning graft.¹ There is evidence that cancers are more common in KTRs than in the general population and that mortality from malignancy is higher than that observed only a few years ago.^{2,3} During the last decade, it has become evident that regulatory T cells (T_{regs}) and human leukocyte antigen G (HLA-G) are key players in inducing and maintaining immune tolerance. The available data indicate that T_{regs} and HLA-G can prevent graft rejection but could be also responsible for higher incidence and progression of cancer.^{4,5} T_{regs} play an important role in maintaining self-tolerance and immune hemostasis by suppressing a variety of physiological and pathological immune responses against self, nonself, and tumor antigens.⁴ T_{regs} have also been shown to be involved in the development and maintenance of transplantation tolerance. Circulating T_{regs} can inhibit antidonor immune responses in clinically stable transplant recipients.⁶ Increased frequencies of T_{regs} have been associated with development, progression, and poor prognosis in several solid tumors and hematological malignancies.^{7,8}

Physiologically, HLA-G has been first described as the key player in maternal–fetal tolerance.⁹ HLA-G can inhibit a broad spectrum of immune cells, and its significance has been shown in the

pathogenesis of inflammatory diseases as well as in the fields of transplantology and oncology. The increased expression of HLA-G could be beneficial in the context of transplantology because it may protect the allograft from an immune reaction, possibly through the induction of T_{regs} and tolerogenic dendritic cells. Notably, the enhanced expression of HLA-G molecules might also favor tumor development and progression.¹⁰

During the last few years, a significant progress has been made in the understanding of interactions between HLA-G and T_{regs}. HLA-G may promote T_{reg} survival and proliferation, which may prevent graft rejection but may also lead to a higher incidence of cancer. Because these 2 important immune components have never been analyzed in KTRs, we decided to assess the relationships between the frequencies of T_{regs} and concentrations of soluble HLA-G (sHLA-G) in the blood of KTRs with and without cancer.

Patients and methods The study included 51 patients (18 women and 33 men) and 20 healthy volunteers (median age, 59.3 years; range, 20–85 years; 12 women and 8 men). Cohort A included 39 patients after kidney transplantation without cancer (median age, 56 years; range, 22–76 years; 11 women and 28 men). Cohort B included 12 patients after kidney transplantation with cancer (median age, 63.5 years; range, 32–72 years; 7 women and 5 men). Among patients with cancer, hepatocellular cancer, skin cancer, and kidney cancer in the original kidney were reported. In addition, 1 case of lung, breast, ovarian, and

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vaginal cancer, 1 case of melanoma, and 1 case of anaplastic lymphoma were found.

The most common cause of nephropathy before kidney transplantation was primary glomerulonephritis (observed in 18 cases). Other underlying diseases included polycystic kidney disease (12 cases), diabetic kidney disease (8 cases), hypertensive renal disease (3 cases), and congenital disease (10 cases).

Plasma was obtained from blood samples by centrifugation. The plasma levels of sHLA-G were assessed using an enzyme-linked immunosorbent assay (BioVendor, Prague Czech Republic), according to the manufacturer's protocol. sHLA-G concentrations were assessed using a microplate reader, VICTOR3 (PerkinElmer, Waltham, Massachusetts, United States). Peripheral blood mononuclear cells were isolated by Ficoll density gradient centrifugation. The surface antigens CD4 and CD25 were stained with antibodies (BD Biosciences, San Jose, California, United States). Cells were then permeabilized and stained with an anti-FOXP3 (eBiosciences, San Diego, California, United States). After incubation, the cells were washed with phosphate-buffered saline and analyzed by flow cytometry for the expression of FOXP3 on CD4+CD25^{high} T_{regs}. Flow cytometry was performed using a FACS CantoII (BD Biosciences). Each time, 100 000 cells were acquired.

The study was approved by the Local Ethics Committee (No. KE-0254/92/2011), and the patients were informed about the use of their blood for scientific purposes.

Statistical analyses were performed using GraphPad Prism 5 (La Jolla, California, United States). The Mann-Whitney test and 1-way analysis of variance were used to evaluate the differences between the study subgroups. The correlations of variables were calculated with the Spearman rank correlation coefficient. All results were presented as median values and ranges. Statistical significance was defined as a *P* value of less than 0.05.

Results Increased percentage of regulatory T cells in kidney transplant recipients with cancer

The frequencies of T_{regs} in peripheral blood were assessed in healthy volunteers (*n* = 20) and 2 subgroups of KTRs. We observed that the median frequency of T_{regs} was higher in cohort A than in controls, but without statistical significance (1.36% [range, 0.0%–16.78%] vs 0.8% [range, 0.22%–3.2%]; *P* = 0.08). The median frequency of T_{regs} in cohort B was significantly higher than that in cohort A (3.8% [range, 0.1%–21.0%] vs 1.36% [range, 0.0%–16.7%]; *P* = 0.008) and controls (3.8% [range, 0.1%–21.0%] vs 0.8% [range, 0.22%–3.2%]; *P* = 0.003. Data are presented in **FIGURE 1A**. We found no difference in the median frequency of T_{regs} between older (>60 years old) and younger patients (<60 years old): 1.77% vs 1.36%; *P* = 0.4. The frequency of T_{regs} did not correlate with the percentage of white blood cells (*r* = 0.07; *P* = 0.63). We did not find differences

in the percentage of T_{regs} between patients who were less than 5 years and those who were more than 5 years after transplantation (*P* = 0.06), but there was a trend for a higher percentage of T_{regs} in the latter group (**FIGURE 1B**).

Levels of soluble human leukocyte antigen G in kidney transplant recipients

We found significantly higher concentrations of sHLA-G in the plasma of cohort A patients when compared with controls (147.5 U/ml [range, 4.05–183.8 U/ml] vs 5.34 U/ml [range, 0.0–40.0 U/ml]; *P* < 0.0001). In addition, we observed higher concentrations of sHLA-G in cohort B than in controls (177.3 U/ml [range, 54.44–193.6 U/ml] vs 5.34 U/ml [range, 0.0–40.0 U/ml]; *P* < 0.0001). We noted that the concentrations of sHLA-G tended to be higher in cohort B than in cohort A, but without statistical significance (*P* = 0.13). Data are shown in **FIGURE 1C**. There was no correlation between the concentrations of sHLA-G and frequency of T_{regs} either in cohort A or in cohort B (*P* = 0.27 and *P* = 0.73, respectively). In addition, we observed no difference in the concentrations of sHLA-G in older (>60 years) and younger patients (<60 years): 142.6 U/ml [range, 4.053–193.6 U/ml] vs 167.5 U/ml [range, 18.96–190.1 U/ml]; *P* = 0.56). We did not find differences in sHLA-G levels between patients who were less than 5 years and those who were more than 5 years after transplantation (149.2 U/ml [range, 4.053–186.3 U/ml] vs 151.7 U/ml [range, 18.96–193.6 U/ml]; *P* = 0.6).

Incidence of cancer after transplantation

In the current study, we assessed an association between the time after transplantation and incidence of cancer. Patients who developed a malignancy after kidney transplantation underwent transplantation earlier and received immunosuppressive therapy longer than patients without cancer (9.5 vs 5.0; *P* = 0.016). In addition, patients with cancer were older than those without cancer (63.5 years vs 56.0 years, *P* = 0.046).

Discussion This is the first study to have evaluated the individual relationships between tolerogenic factors, such as T_{regs} and sHLA-G, and cancer in KTRs. It is widely known that the prevalence of cancer is increased in KTRs owing to continuous immunosuppression, which may limit the survival benefit of posttransplant patients.¹ Immunosuppressants, which are used to prevent graft rejection, are linked to the development of certain types of cancers in transplant patients, such as skin cancer or lymphoma.¹¹

We observed a significantly higher frequency of T_{regs} in KTRs with cancer. T_{regs} are key players in inducing and maintaining immune tolerance, and, together with immunosuppressants, they could facilitate tumor development. Our results are in line with those reported by Hope et al,³ who observed an increased frequency of T_{regs} in KTRs with solid organ cancer and proved that high levels of T_{regs} in peripheral blood are among

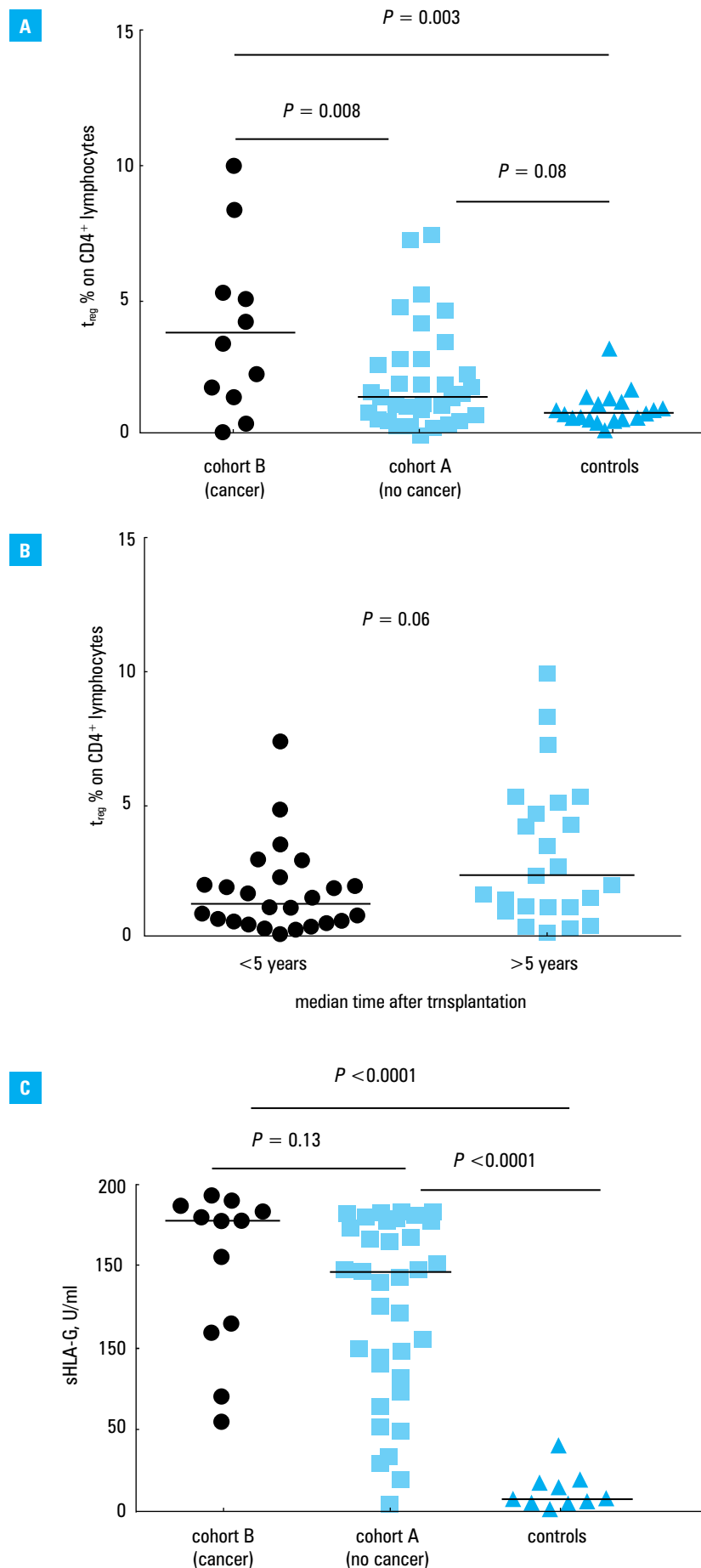


FIGURE 1 **A** – frequency of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells (T_{regs}) in kidney transplant recipients ($n = 39$), kidney transplant recipients with cancer ($n = 12$), and in healthy volunteers ($n = 20$); **B** – median frequency of T_{regs} in patients within a short (<5 years, $n = 26$) and long (>5 years, $n = 25$) period after transplantation; **C** – soluble human leukocyte antigen G (sHLA-G) levels in kidney transplant recipients ($n = 39$), kidney transplant recipients with cancer ($n = 12$), and healthy volunteers ($n = 20$)

the risk factors for recurrent cutaneous squamous-cell carcinoma. However, this finding may not be generalizable to all cancers. Hope et al³ observed that the percentage of T_{regs} decreased after tumor resection. It might suggest that the cancer itself may induce T_{reg} proliferation. Our results might support this hypothesis because the frequency of T_{regs} in KTRs with cancer was insignificantly higher than that in KTRs without cancer.

A number of studies suggested that HLA-G might also be involved in the induction of T_{regs}. In the studies of Liang et al¹² and Ristich et al,¹³ myeloid-derived antigen-presenting cells stimulated with HLA-G were able to induce T_{regs}. In our study, we found significantly higher levels of sHLA-G in KTRs with and without cancer than in controls. Interestingly, there were no significant differences in the concentrations of sHLA-G between KTRs with and without cancer; only a trend towards increased concentrations of sHLA-G in KTRs with cancer was observed. Xiao et al¹⁴ reported increased concentrations of sHLA-G in KTRs with a functioning transplant compared with controls. Our results indicate another possible role of sHLA-G, which together with T_{regs} might facilitate cancer development in KTRs. It might be due to the activation of these 2 important immune tolerance mechanisms or even direct induction of T_{regs} by sHLA-G. Because we did not find any correlation between the frequency of T_{regs} and the concentration of sHLA-G, and we observed a tendency for elevated concentrations of sHLA-G in patients with cancer, our results should be interpreted with caution. However, in a recent experimental study, Carosella et al¹⁰ confirmed the induction of T_{regs} by HLA-G molecules.

In conclusion, our study highlights the relationship between immunosuppressive components of the immune system (ie, T_{regs} and sHLA-G) and the incidence of cancer in KTRs. T_{regs} and sHLA-G may act as a double-edged sword. On the one hand, they can play an important role in the induction and maintenance of tolerance in donor alloantigens in vivo, but on the other, they may facilitate tumor development and progression.

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