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Immunity to varicella-zoster virus in immunocompromised patients

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Immune response to varicella-zoster virus before and after renal transplantation

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ABSTRACT

Background

Herpes zoster (HZ) risk is high in renal transplant recipients, but live-attenuated varicella-zoster virus (VZV) vaccination is contra-indicated after transplantation. Vaccination prior to transplantation may be considered. It is however unclear how VZV immunity evolves following transplantation. Aim of the current study was to investigate VZV immunity before and after renal transplantation, in order to increase the understanding of VZV-specific immune responses in this patient group.

Methods

Immunity to VZV was determined prior to and 2-3 years after renal transplantation in the same 60 adult patients, and 62 matched healthy controls. VZV-specific cellular immunity was measured by interferon gamma (IFNγ) enzyme-linked immunospot (ELISpot) assay and by analyzing T-cell functionality using flowcytometry. VZV-specific IgG antibodies were measured using an in-house glycoprotein enzyme-linked immunosorbent assay (gpELISA). Medical history, including infection history, was assessed by reviewing medical records and for VZV infections using a questionnaire.

Results

Numbers of IFNγ-producing cells did not change after transplantation using a paired analysis, but were significantly lower in transplant recipients than in controls (p = 0.028). Patients in whom the post-transplant period was complicated by rejection or any acute infection (excluding herpes zoster) had a lower number of IFNγ-producing cells than patients who did not. VZV-IgG levels did not significantly differ from controls, but a significant decrease was observed after transplantation (p < 0.0001).

Conclusion

Cellular immunity, but not humoral immunity, against VZV is decreased in renal transplant recipients compared to healthy controls and is lower in patients who experienced rejection and acute infections other than herpes zoster in the post-transplant period. Cellular immunity to VZV did not significantly change following renal transplantation.

INTRODUCTION

Herpes zoster (shingles) is characterized by neuralgia and a vesicular rash. Neuralgia can last for months or even years, known as postherpetic neuralgia [1,2]. The pain can have a major effect on a patients' quality of life, but is often difficult to treat [3,4]. Cause of herpes zoster is the reactivation of a latent varicella-zoster virus (VZV) infection. After VZV vaccination or a primary VZV infection, known as varicella or chickenpox, the virus remains latently present for life in in sensory neurons of the dorsal root ganglia [1,5].

Being intensively treated with immunosuppressive medication, renal transplant recipients are known to be at increased risk of herpes zoster. Incidence in this group is estimated to be 28 to 37 per 1000 person years [6,7], which is 6 to 11 times higher than in the general population [8]. Also a high prevalence of postherpetic neuralgia of up to 48.7% has been reported [9,10]. Disseminated disease and visceral involvement are rare complications that occur mainly in immunocompromised patients and may have a lethal outcome [11].

Vaccination can contribute to prevention and decreased severity of varicella and herpes zoster [12,13]. In contrast to the United States, routine vaccination to prevent varicella in children is not recommended in The Netherlands [14,15]. In The Netherlands almost the whole population has experienced varicella in childhood, thereby gaining VZV immunity through natural infection [16].

The currently only licensed vaccine to prevent herpes zoster in adults contains the same virus strain as the childhood varicella vaccine, but is at least 14 times more potent [17]. The zoster vaccine is thought to prevent reactivation of the latent varicella-zoster virus by boosting pre-existing virus specific cellular immunity, and in particular CD4+ T cell responses, in persons latently infected with VZV [18,19]. It was shown to reduce the incidence of herpes zoster by 51% and of postherpetic neuralgia by 67% in healthy people above 60 years of age [12].

As the zoster vaccine contains live attenuated virus and theoretically could induce disseminated disease in immunosuppressed persons, even when VZV seropositive, the American Society of Transplantation (AST) in 2013 and the United States Advisory Committee on Immunization Practices (ACIP) in 2008 did not advise zoster vaccination after transplantation regardless of VZV serostatus [20,21]. The AST furthermore stated that there was insufficient data to suggest that zoster vaccination prior to transplantation will reduce the risk of VZV reactivation posttransplant [20].

Since that time zoster vaccination has been shown to be efficacious in patients with chronic kidney diseases above 60 years of age, with a 50% lower herpes zoster risk in vaccinated compared to unvaccinated patients [22]. It however remains unclear in what way VZV-specific immunity evolves surrounding renal transplantation and consequently, whether zoster vaccination prior to transplantation could be efficacious to prevent herpes zoster in transplant recipients. Aim of the current study was to investigate VZV immunity before and after renal transplantation, in order to increase the understanding of VZV-specific immune responses in this patient group.

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METHODS

Study population

Patients who received a renal transplant in the University Medical Center Groningen (UMCG) 2-3 years before inclusion and were still under supervision in this center, were eligible for participation. Blood was drawn at out-patient clinic visit in order to obtain serum and peripheral blood mononuclear cells (PBMC). Serum and PBMC of the same patients collected before administering induction therapy, immediately prior to the most recent transplantation, were retrieved from diagnostic archives from the department of Transplantation Immunology. Healthy control subjects were age and sex-matched to patients.

Immediately before and 4 days after renal transplantation standard induction therapy using basiliximab (2 doses of 20 mg) was administered. Immunosuppression after transplantation consisted of a combination of tacrolimus or cyclosporine, in combination with mycophenolate mofetil and prednisolone. Prednisolone dose was gradually tapered and usually discontinued after three months. Choice of anti-rejection therapy depended on type of rejection, but generally consisted of intravenous prednisolone (3 x 1000 mg intravenously, possibly repeated) which was followed by antithymocyte globulin or alemtuzumab in case of an unsatisfactory result. Amidst the study period, the protocol was adopted to prophylactically treat CMV-positive recipients or CMV-seronegative recepients receiving a graft from a CMV-positive donor with valganciclovir for the first 6 months after transplantation.

Health insurance is mandatory in The Netherlands, covering also most specialized treatment. For those with low-incomes, insurance costs are compensated by the government.

The study was approved by the institutional review board of the University Medical Centre Groningen (METc 2014/305 and 2012/375). All patients and controls gave written informed consent.

Clinical data, including the occurrence of varicella, herpes zoster and other infections

Patient characteristics and medical history (age at time of transplantation, cause of renal failure, multiple transplantations, renal function replacement therapy, herpes zoster, medication use including use of prophylactic valganciclovir after transplantation, episodes of rejection) were retrieved from medical records. Furthermore, medical records were reviewed for the occurrence of acute infections in the period between transplantation and study inclusion (2-3 years after transplantation). Positivity for herpes viruses, hepatitis viruses or BK virus were not regarded as acute infections. In The Netherlands it is custom to first consult a general practitioner in case of health problems, who will only refer in case of more serious problems. Therefore the problems that come to attention of a hospital specialist and, consequently, that are documented in hospital medical records, are generally more serious.

In addition to review of medical records, patients and controls were asked about their history and timing of varicella and herpes zoster using a questionnaire. Vaccination to prevent varicella in children is not part of routine immunizations, and zoster vaccination is not recommended for adults in The Netherlands. Therefore the whole study population can be regarded as non-vaccinated.

Isolation, storage and thawing of PBMC and serum

Immediately after collection of venous blood in lithium heparin containing tubes, PBMC were isolated according to standard protocols and stored in liquid nitrogen until use. Upon thawing, cell viability was evaluated by trypan blue staining. Serum was stored at -20 °C until use.

Interferon-γ (IFNγ) ELISpot assay

Interferon-γ (IFNγ) ELISpot assay was performed as previously described [23]. In short, per well 2 x 10^5 PBMC were added. Cells were stimulated using 10 µl UV-inactivated varicella vaccine (Provarivax; MSD, 1350 PFU/0.5 ml) or 5 µg/ml of concanavalin A (positive control), while a negative control consisted of PBMC in culture medium alone. Experiments were done in duplicate, except for the positive control. After staining and drying of the plates, spots were counted using an automated reader (AID EliSpot Reader; Autoimmun Diagnostika GmbH). Results were only accepted in case the concanavalin A filled well (positive control) yielded a convincingly positive result. The mean number of spots in the VZV-stimulated wells was corrected for the mean number of spots in the negative control wells. Results are referred to as the number of IFNγ spot-forming cells per 2 x 10^5 PBMC.

Flowcytometric analysis

PBMC (1.2 x 10^6/tube) were stimulated using 20 µl UV-inactivated varicella vaccine, 5 µg/ml staphylococcal enterotoxin B (SEB; Sigma-Aldrich, positive control) or left unstimulated (PBMC in medium alone, negative control), except for the positive control in the presence of 10µg/ml anti-CD28/CD49d (Beckton Dickinson (BD)). PBMC were stimulated for 18 hours, of which the last 16 hours in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich). Fluorescent T cell barcoding staining and immunostaining with anti-CD3, anti-CD8, anti-CD69, anti-IFNγ, anti-tumour necrosis factor alpha (TNFα) and anti-interleukin 2 (IL-2) was performed as described previously [24], with the addition of anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (anti-CTLA-4 from BioLegend, all other antibodies from BD). Analyses were done using Kaluza software (Beckman Coulter). CD4+ and CD8+ T cell populations were gated as CD3+CD8- and CD3+CD8+, respectively. Results were expressed as the percentage of CD69+ cytokine/CTLA-4/PD-1 expressing CD4+ or CD8+ T cells within the total CD4+ or CD8+ T cell population. Furthermore, relative percentages of the 7 possible cytokine expression profiles within the cytokine-producing CD4+ T cell

population are shown. In this analysis a minimum number of 30 cytokine-producing cells per subject were required, in order to obtain reliable results.

Antibody levels to VZV

VZV-specific IgG antibodies were quantified using an in-house glycoprotein (gp) enzyme-linked immunosorbent assay (ELISA), which was previously developed and validated using a quantitative Serion classic VZV IgG ELISA (Institut Virion/Serion) and the institution's standard diagnostic test for VZV serology [23]. As antigen, VZV purified glycoproteins (EastCoastBio) were used. Pooled human serum with known levels of anti–glycoprotein VZV was used as standard. According to recommendations of Institut Virion/Serion, VZV-IgG levels from 50 to 100 mIU/ml were considered as borderline, while values above 100 mIU/ml were considered positive.

Statistical analysis

The Wilcoxon signed rank test, intended for paired analyses, was used to compare continuous variables before and after transplantation. Results and characteristics of subgroups of patients were compared using a Mann-Whitney test or Fisher's exact test. Comparisons between transplant recipient (post-transplantation) and healthy control groups were done using a Mann-Whitney U test or Fisher's exact test when appropriate. For correlations, Spearman's rho was used.

P-values ≤0.05 (2-sided) were considered significant. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, USA).

RESULTS

Study population

Sixty renal transplant recipients and 62 healthy control subjects were included. Their characteristics are summarized in Table 1. At the time of inclusion, only 1 (2%) transplant recipient was in need of renal replacement therapy, following rejection of the transplant kidney. There were no significant differences in gender and age between patient and control group. Moreover, no significant age/gender differences were found between patients who experienced rejection or infection after transplantation and those who did not. Patients with a history of more than 1 transplantation tended to be younger (median 45.7 versus 55.7 years, p=0.088).

Occurrence of herpes zoster and other acute infections

Questionnaire results, asking about history of varicella and herpes zoster, were available for 53 (88%) of transplant recipients. Combined with results from medical record review, it was determined that 13 patients (22%) had a history of herpes zoster. In four of these patients, timing was not known. In six, herpes zoster occurred within 2-3 years after most recent transplantation. One patient experienced herpes zoster only 3 weeks

Table 1. Characteristics of healthy controls and transplant recipients

	HC n=62	Tx n=60
Female gender, no. (%)	33 (53)	30 (50)
Age, median (range) years	58.4 (25.3-72.6)	55.6 (25.7-72.5)
Time since transplantation, median (range) months	NA	33.7 (25.6-38.4)
Cause of renal failure, no. (%)	NA	
Glomerulonephritis		25 (42)
IgA nephropathy		14 (23)
MPGN		2 (3)
FSGS		3 (5)
Anti-GBM		1 (2)
SLE		2 (3)
AAV		2 (3)
Cause unknown		1 (2)
Genetic		7 (12)
Vascular		4 (7)
Diabetic nephropathy		5 (8)
Congenital		2 (3)
Urologic		3 (5)
Chronic TIN		3 (5)
Unknown		9 (15)
Other		2 (3)[a]
History of > 1 transplant, no. (%)	NA	15 (25)[b]
RRT pre-Tx, no.	NA	
None/HD/PD		17/29/14
VZV serostatus		
Negative/positive	0/62	0/60
History of herpes zoster, no. (%)	4 of 32 (13)	13 (22)[c]
Prophylactic valganciclovir, no. (%)	NA	5 (8)
No. (%) of patients with post-Tx complications	NA	
Rejection	NA	11 (19)[e]
Infection, any/bacterial/viral/fungal[f]	NA	40/25/27/4[e]

No.: number, Tx: transplantation, MPGN: membranoproliferative glomerulonephritis, FSGS: focal segmental glomerulosclerosis, GBM: glomerular basement membrane, SLE: systemic lupus erythematosus, AAV: ANCA-associated vasculitis, TIN: tubulointerstitial nephritis, RRT: renal replacement therapy, HD: haemodialysis, PD: peritoneal dialysis, VZV: varicella-zoster virus.
[a] One patient developed renal failure because of acute tubulus necrosis (after acute aorta rupture surgery) and one patient underwent nephrectomy of his one functional kidney because of transitional cell carcinoma.
[b] Two patients received a transplant organ other than kidney before their most recent renal transplantation; one received a heart transplant and one received multiple liver transplants.
[c] In six patients, timing of herpes zoster was known to have occurred after most recent transplantation (within 2-3 years before study inclusion).
[d] Seven patients experienced a primary CMV infection in the post-transplantation period (within 2-3 years before study inclusion).
[e] Missing information on rejection in 2 patients and on infection (other than herpes zoster) in 1 patient.
[f] More detailed information on the occurrence of infections in the 2-3 years after transplantation is provided in Supplementary Table S1.

after transplantation, and another patient immediately after rejection therapy (with methylprednisolone and leflunomide). No herpes zoster episodes occurred during prophylactic use of valganciclovir.

Of the healthy control group, questionnaire results were available for 32 (52%) persons. Four (13%) stated to have experienced herpes zoster. In one subject this occurred only a few months prior to study participation. In the other three persons, herpes zoster occurred at least 15 years prior to study inclusion.

The occurrence of acute infections in the 2-3 year period between transplantation and study inclusion is presented in Table 1. A more detailed summary of infections occurring in this period is provided in Supplementary Table S1.

Numbers of IFNγ spot-forming cells in response to VZV stimulation

Number of IFNγ spot-forming cells in response to VZV stimulation did not significantly change following renal transplantation. A lower number of IFNγ spot-forming cells was found in transplant recipients than in healthy controls (p = 0.028) (Figure 1A).

A history of more than 1 transplantation was not found to be of influence on numbers of IFNγ spot-forming cells to VZV, also when assessing results prior to most recent transplantation. The same held true for patients who were transplanted pre-emptively versus patients treated with renal replacement therapy prior to transplantation (data not shown).

Interestingly, transplant recipients in whom the post-transplantation period was complicated by a rejection episode, were found to have a lower number of IFNγ spot-forming cells in response to VZV stimulation. This difference was already present prior to transplantation. Patients with a herpes zoster history were excluded from analysis (Figure 2A).

Transplant recipients who experienced any acute infection other than herpes zoster in the 2-3 years post-transplantation (excluding those with a herpes zoster history) also had lower number of IFNγ spot-forming cells in response to VZV stimulation than those who did not. This difference was not yet present in the same patients prior to transplantation (Figure 3A). Analyzing data separately for viral or a bacterial infections yielded similar results (Figure S1).

The occurrence of herpes zoster within 2-3 years before blood drawing was not of significant influence on number of IFNγ spot-forming cells in response to VZV stimulation, but number of subjects was low (n=6; Figure S2).

Humoral immunity to VZV

All patients and controls were VZV seropositive. After transplantation VZV-IgG level was significantly lower than before transplantation (p<0.0001). There was no significant difference between healthy controls and transplant recipients (p=0.149) (Figure 1B).

In contrast to cellular immunity results, humoral immunity to VZV was not found to be different in transplant recipients that experienced rejection of their transplant kidney
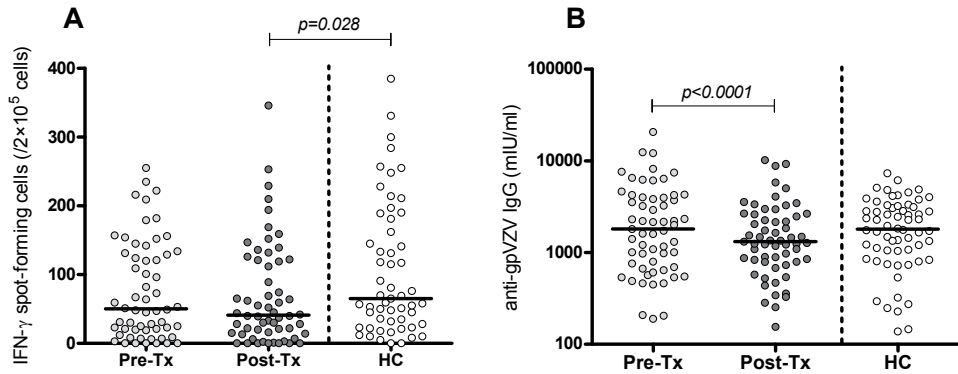
Figure 1. Numbers of interferon-γ (IFNγ) spot-forming cells in response to VZV stimulation (A) and levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale (B) in 55/62 healthy control (HC) subjects and 58/59 patients before (pre) and 2-3 years after transplantation (Tx). Lines show the median.
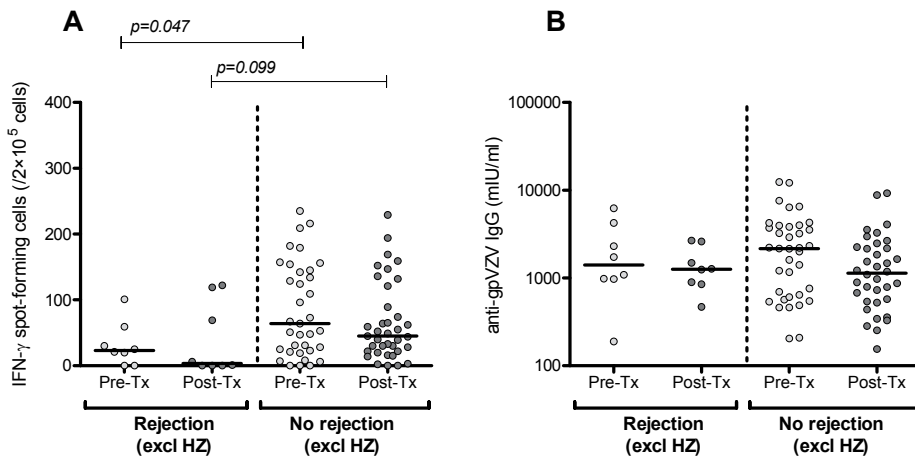
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Figure 2. Numbers of interferon-γ (IFNγ) spot-forming cells in response to VZV stimulation (A) and levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale (B) in 8 patients that experienced a rejection episode and 35-37 patients who did not, before (pre) and 2-3 years after transplantation (Tx). Patients with a history of herpes zoster were excluded from analysis. Lines show the median.

(Figure 2B). Although not statistically significant, patients that experienced an acute infection other than herpes zoster tended to have lower antibody levels to VZV than patients without any documentation of acute infections in their records (Figure 3B).

Increased percentages of cytokine-expressing T cells prior to renal transplantation

Upon stimulation with VZV, high percentages of CD4+ T cells producing TNFα and IL-2 were found in patients prior to transplantation. This phenomenon was also seen upon polyclonal stimulation using SEB (Figure 4A and C).

Within CD8+ T cells, percentage of cells producing cytokines in response to VZV stimulation was generally lower than in the CD4+ cells, except for IL-2. For this cytokine, the same phenomenon was seen as in the CD4+ cells: a significantly increased cytokine production prior to transplantation, but similar levels in transplant recipients and control subjects (Figure 4B).

In response to polyclonal stimulation, again the CD8+ T cells were shown to be less responsive than their CD4+ counterparts. For TNFα and IL-2, expression was shown to decrease after transplantation, for IL-2 to levels that were significantly lower than in healthy controls (Figure 4D).

Similar percentages of IFNγ-producing CD4+ and CD8+ T cells were found before and after transplantation, both upon stimulation with VZV and with SEB. Also, no difference in the percentage of T cells producing this cytokine were found between transplant recipients and control subjects (Figure 4A-D).
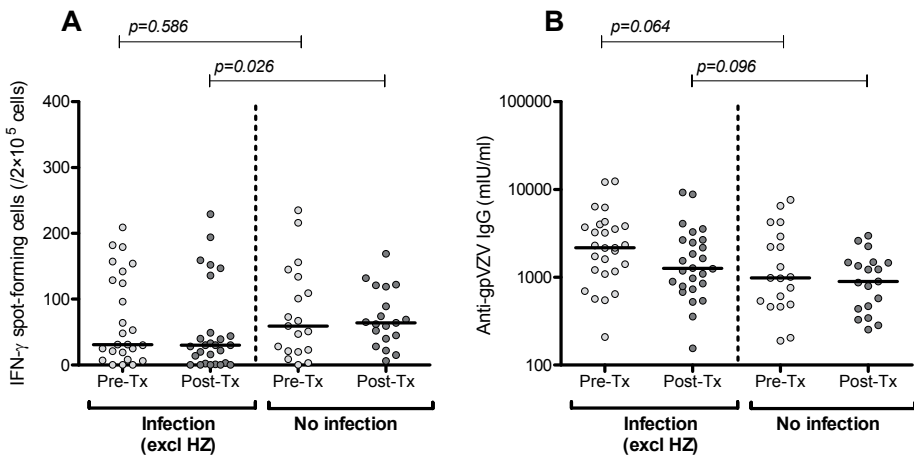
Figure 3. Numbers of interferon-γ (IFNγ) spot-forming cells in response to VZV stimulation (A) and levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale (B) in 25-27 patients that experienced an acute infection (excluding herpes zoster) in the post-transplantation period and 19 patients who did not, before (pre) and 2-3 years after transplantation (Tx). Patients with a history of herpes zoster were excluded from analysis. Lines show the median.
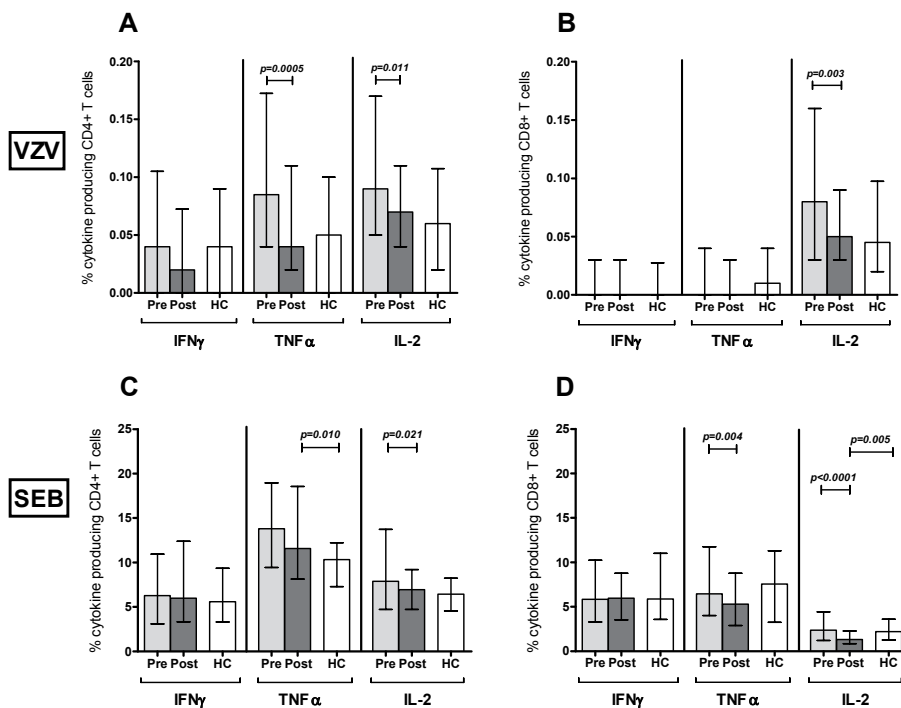
Figure 4. Frequencies of cytokine-producing CD4+ (**A**) and CD8+ (**B**) T cells upon stimulation with varicella-zoster virus (VZV), and upon stimulation with staphylococcal enterotoxin B (SEB, positive control) (**C** for CD4+ and **D** for CD8+ T cells) in 56 healthy control subjects (HC) and 59 patients before (pre) and 2-3 years after (post) receiving a renal transplant. Bars show the median and interquartile range. IFNγ = interferon gamma; TNFα = tumor necrosis factor alpha; IL-2 = interleukin-2.

No marked functional changes in VZV-specific T cells after kidney transplantation

The total VZV-specific CD4+ T cell population in transplant recipients consisted mainly of IFNγ single-positive cells. Except for a significantly lower percentage CD4+ T cells expressing both IFNγ and IL2 (p=0.0002) prior to transplantation, no changes before versus after transplantation were observed. Results were similar in transplant recipients and control subjects (Figure 5A).

Functionality of T cells was more diverse upon polyclonal stimulation, but again no marked changes following transplantation, nor differences between transplant recipients and controls were observed (Figure 5B).

Mean fluorescence intensity (MFI) of programmed cell death protein 1 (PD-1) was higher upon stimulation with VZV than upon polyclonal stimulation, both for CD4+ and CD8+ T cells. No differences between transplant recipients and control subjects were observed (Figure 6A and B). While cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)

MFI on CD4+ T cells was significantly lower in patients prior to transplantation, it was significantly increased in these patients on CD8+ T cells. No marked differences between transplant recipients and controls were seen (Figure 6C and D).

Figures representing percentages of T cells expressing PD-1 and CTLA-4 are available online (Figure S3).

Correlations

A weak correlation between age and cellular immunity to VZV (number of IFNγ spot-forming cells) (ρ = -0.389, p = 0.002) was present in transplant recipients. No significant correlations were found between age and humoral immunity to VZV, or between humoral and cellular immunity to VZV (data not shown).
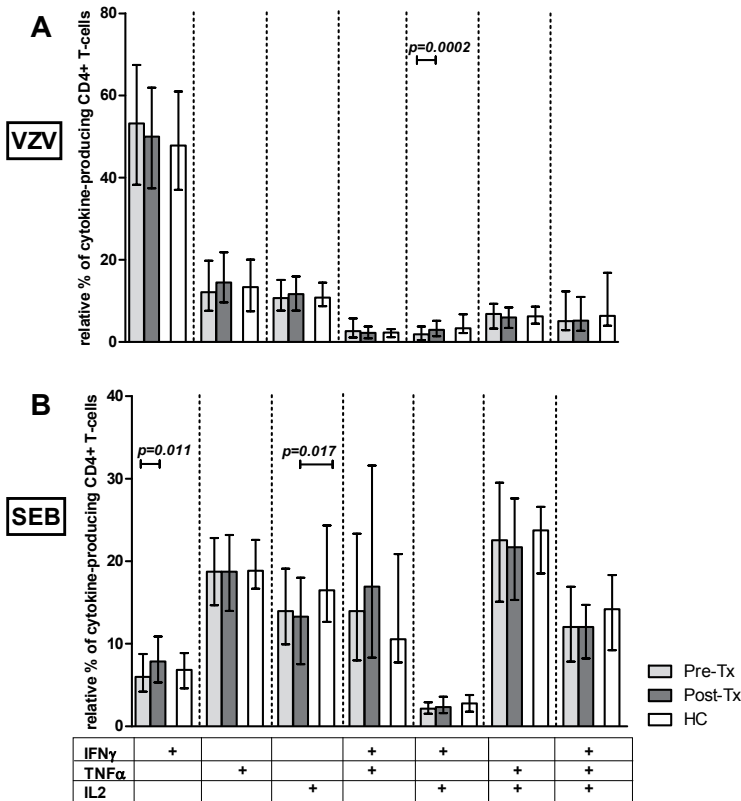
Figure 5. Cytokine-producing CD4+ T cells in response to stimulation with varicella-zoster virus (VZV) (A), and to polyclonal stimulation using staphylococcal enterotoxin B (SEB) (B) divided according to expression of interferon gamma (IFNγ), tumour necrosis factor alpha (TNFα) and interleukin-2 (IL2), and co-expression of combinations of these cytokines in 56 healthy control (HC) subjects and 59 patients before (pre) and 2-3 years after (post) receiving a renal transplant (Tx). Presented percentages are relative percentages of the total population of cytokine-producing CD4+ T cells in response to stimulation with VZV or SEB. Bars show the median and interquartile range.
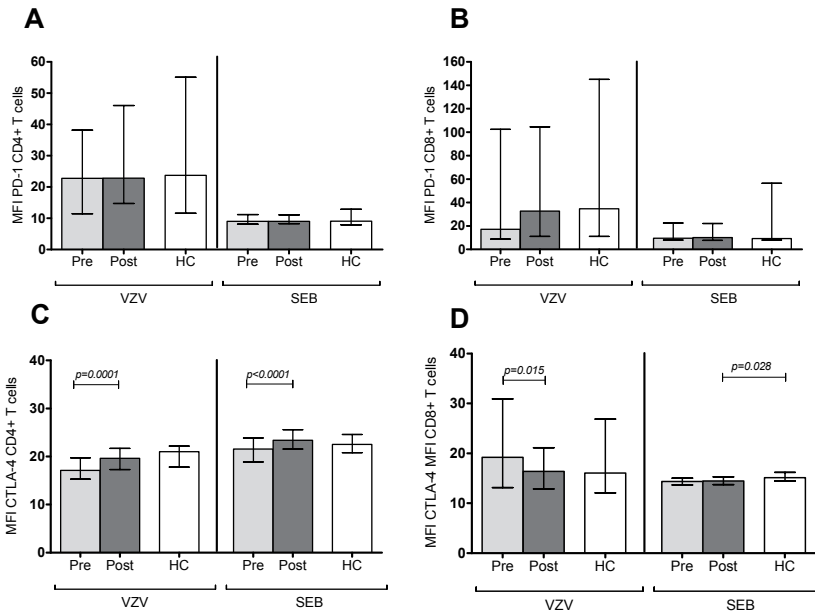
Figure 6. Mean fluorescence intensity (MFI) of programmed cell death protein 1 (PD-1) on CD4+ (**A**) and CD8+ (**B**) T cells, and of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on CD4+ (**C**) and CD8+ (**D**) T cells upon stimulation with varicella-zoster virus (VZV) and staphylococcal enterotoxin B (SEB, positive control) in 28 healthy control subjects (HC) and 59 patients before (pre) and 2-3 years after (post) receiving a renal transplant. Bars show the median and interquartile range.

DISCUSSION

In this study, VZV-specific immunity was evaluated in the same 60 patients before and after renal transplantation. Cellular immunity to VZV, as assessed by an IFNγ ELISpot assay, did not significantly change after transplantation. The number of IFNγ spot-forming cells in response to VZV stimulation was however shown to be significantly lower in transplant recipients than in control subjects. VZV-specific humoral immunity in transplant recipients did not significantly differ from controls, but a significant decrease was observed after transplantation.

Cellular immunity to VZV is considered to be essential in the immune response to VZV and prevention of herpes zoster. Number and functionality of VZV-specific CD4+ T cells have been shown to be impaired in immunocompromised patient groups at increased risk of herpes zoster [25,26]. Responding cells in the IFNγ ELISpot assay consist mostly out of CD4+ T cells. This assay, which tests functionality of VZV-specific cells, is generally considered to be a reliable method for the assessment of VZV-specific cellular immunity [27]. Previously, decreased numbers of IFNγ spot-forming cells upon VZV stimulation have been demonstrated in the elderly and different immunocompromised patient groups at increased risk of herpes zoster [23,28-31]. Our finding of a lower number of IFNγ spot-forming cells upon VZV stimulation in transplant recipients compared to controls is in line

with the reported increased herpes zoster incidence in this group [6,7,32]. Although they reported a lower percentage of VZV-specific CD8+ effector memory cells in patients, Van Besouw et al. did not find a difference in percentage of subsets of VZV-reactive CD4+ T cells between 11 renal transplant recipients and 21 controls, using intracellular IFNγ flowcytometry [33]. Flowcytometry has been shown to be less likely than ELISPOT to detect low-level responses [27].

Interestingly, in response to VZV stimulation a lower number of IFNγ spot-forming cells was present in transplant recipients in whom post-transplant period was complicated by rejection or acute infections other than herpes zoster. Anti-rejection therapy was previously identified to be a significant risk factor for the development of herpes zoster in renal transplant recipients [32], but as the difference was also present before transplantation this could indicate that a suboptimal immune system is already present in these patients prior to transplantation. Age is a well-known risk factor for herpes zoster, and has been shown to be associated with impaired cellular immunity to VZV [34]. This is in line with our finding of an inverse correlation between number of IFNγ spot-forming cells and age of transplant recipients.

Next to evaluating cellular immunity to VZV using an IFNγ ELISpot assay, we evaluated cytokine production of CD4+ and CD8+ T cells in response to stimulation with VZV. Cytokine production was shown to be high prior to transplantation, but did not differ between transplant recipients and control subjects. As this phenomenon was also seen upon polyclonal stimulation, it was not VZV-specific. Hypercytokinaemia could be an explanation of our findings, which is known to occur in chronic kidney disease patients, and is thought to be associated with uraemia [35,36].

Schub et al. reported a significantly lower percentage of CD4+ T cells concomitantly producing 3 cytokines in response to VZV stimulation in transplant recipients compared to controls, while percentage of cells only producing IFNγ was increased. They suggested that multifunctionality of specific T cells is correlated with sufficient pathogen control [25]. In the current study we did not find marked differences in VZV-specific CD4+ T cell functionality between transplant recipients and controls. In both groups, median share of cells producing only IFNγ comprised approximately half of the total VZV-specific CD4+ T cell population. The reason for the different findings is not clear, but may in part be caused by differences in patient population between countries with (Germany) and without (The Netherlands) routine childhood varicella vaccination. Furthermore, there were differences between the studies in stimulation time of T cells and using cryopreserved PBMC versus whole blood [25].

Upregulation of inhibitory receptors such as PD-1 and CTLA-4 has been identified as an important feature of exhausted T cells. Exhausted T cells, resulting from a persistent (viral) infection, are less able to exert their effector functions [37,38]. As we found the expression of inhibitory receptors PD-1 and CTLA-4 to be similar in transplant recipients and controls, we could also not confirm the finding of an increased PD-1 expression on CD4+ T cells in transplant recipients upon polyclonal stimulation, as reported by Schub et al.[25]. Mean

fluorescence intensity of PD-1 was higher upon VZV stimulation than upon polyclonal stimulation. This may be explained by the physiologic role of PD-1 in the regulation of immune responses towards a specific antigen [37,39].

Clinical VZV vaccination studies in solid organ transplant recipients or patients awaiting solid organ transplantation to date have mainly focused on prevention of varicella in VZV seronegative patients, mostly children. Vaccination generally appears to be safe and immunogenic in these patients [40-43], although immunogenicity may be reduced compared to healthy control subjects [44]. In a recent study by Kho et al., adult renal transplant candidates with undetectable VZV-IgG levels, were vaccinated using a live attenuated varicella vaccine. Cellular immunity to VZV was evaluated in 11 patients before vaccination and after transplantation (median 7.2 months after transplantation). While total number of leukocytes decreased, percentage of VZV-specific CD4+ memory T cells was shown to significantly increase, indicating that pre-transplant VZV vaccination may be useful. As cellular immunity between vaccination and transplantation was not evaluated, effect of the transplant procedure itself on VZV immunity however remained unclear [45]. The effect on post-transplant herpes zoster incidence of vaccinating seropositive persons before solid organ transplantation to date has not been sufficiently studied.

This study has several limitations. Firstly, information regarding herpes zoster and other infections could have been missing from medical records as these do not necessarily come to attention of a hospital specialist. Moreover, questionnaire results on herpes zoster occurrence did not seem completely reliable, with at least two patients not mentioning a herpes zoster episode that was noted in medical records. As many patients and controls did not reliable recall occurrence and timing of varicella, it could not be determined whether age at first encounter with VZV was of influence on VZV immunity. Furthermore, differences in VZV-immunity between diagnostic subgroups could not be reliably evaluated because of the limited number of patients in the different subgroups. Lastly, T cell tests are not standardized and results differ greatly between publications. Therefore comparing different studies is difficult.

In conclusion, no marked changes in cellular immunity to VZV following renal transplantation were observed. However, transplant recipients were shown to have a lower cellular immunity to VZV than matched control subjects, corresponding with a high herpes zoster risk in these patients. Transplant recipients that experienced rejection or infection in the post-transplant period seemed to be at even higher risk. In contrast to cellular immunity, humoral immunity to VZV was shown to decrease significantly following transplantation, but resulting levels were not different from levels in healthy control subjects. Future research should focus on the post-transplant effect on herpes zoster incidence of vaccinating VZV seropositive patients before solid organ transplantation.

ACKNOWLEDGEMENTS

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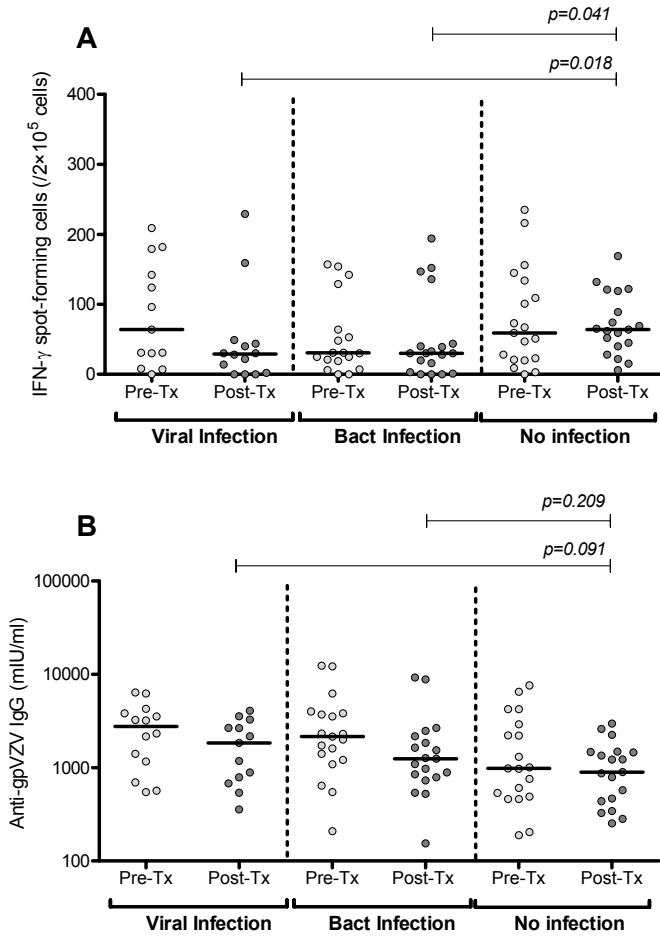
SUPPLEMENTARY DATA

**Supplementary Table 1.** Infections in the 2-3 years post-transplant period

	No. (%)
Viral	27 (46)
Herpes zoster	6 (10)[a]
Gastroenteritis (mostly norovirus)	10 (17)
Primary CMV infection	5 (8)
Primary EBV infection/EBV reactivation	4 (7)
Rhinovirus	2 (3)
Influenza	2 (3)
Herpes simplex	1 (2)
Bacterial	25 (42)
Urinary tract infection	12 (20)
Gastroenteritis	7 (12)
Skin infection requiring antibiotic treatment	4 (7)
Pneumonia	3 (5)
Ear/nose/throat infection requiring antibiotic treatment	3 (5)
Spondylodiscitis	1 (2)
Fungal	4 (7)

No.: number, CMV: cytomegalovirus, EBV: Epstein-Barr virus
[a] Total of self-reported herpes zoster infections (using questionnaire) and herpes zoster infections documented in medical records was 13. Three cases occurred before the most recent transplantation. In four cases, timing was unknown.
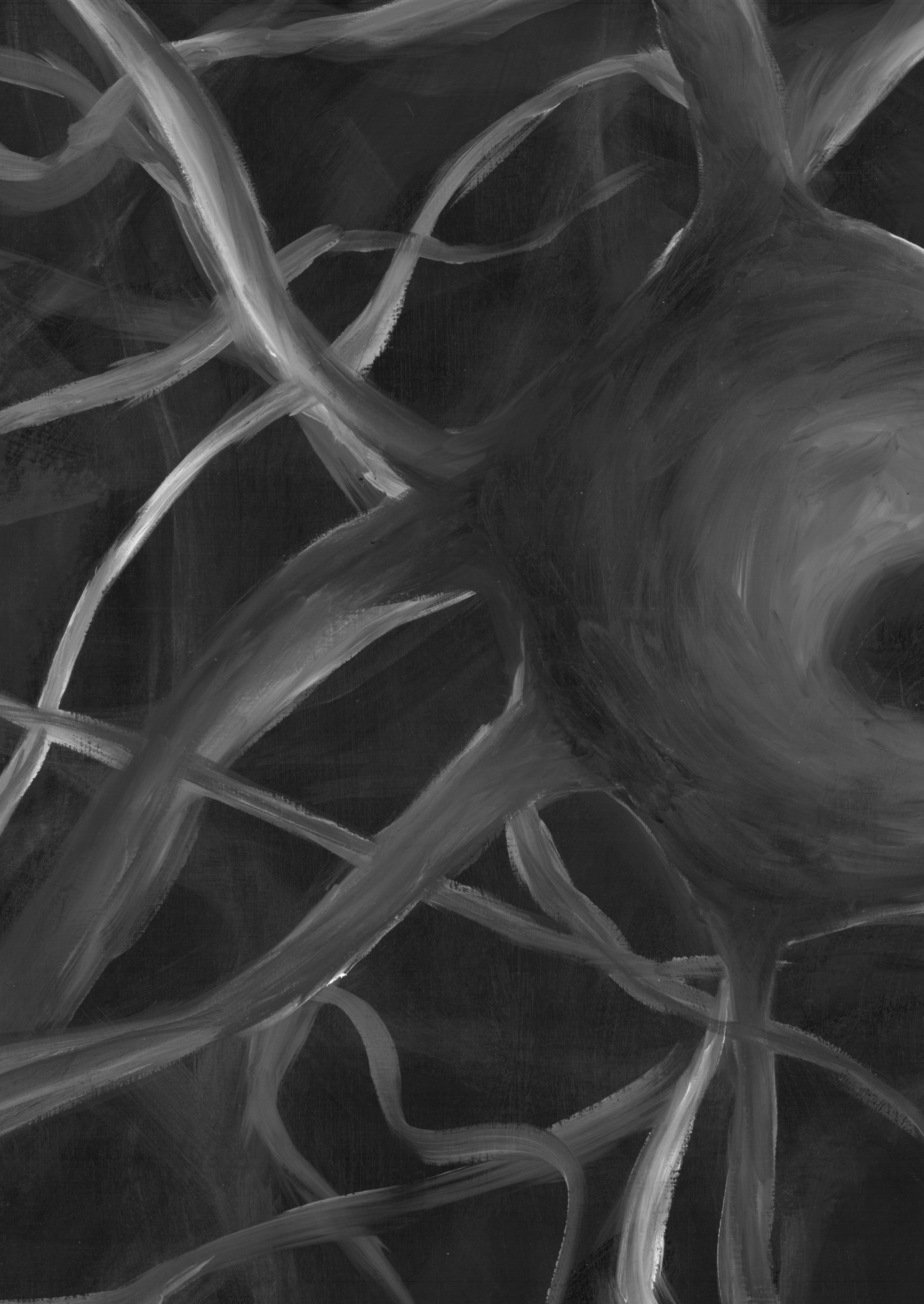
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Supplementary figure 1. Numbers of interferon gamma (IFNγ) spot-forming cells in response to VZV stimulation (A) and levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale (B) in 13/14 patients who experienced an acute viral infection, 18/19 patients who experienced an acute bacterial infection and 19 patients without documented infections in the 2-3 years after transplantation. Results before (pre) and after (post) transplantation (Tx) are shown. Patients with a history of herpes zoster were excluded from analysis. Lines show the median.
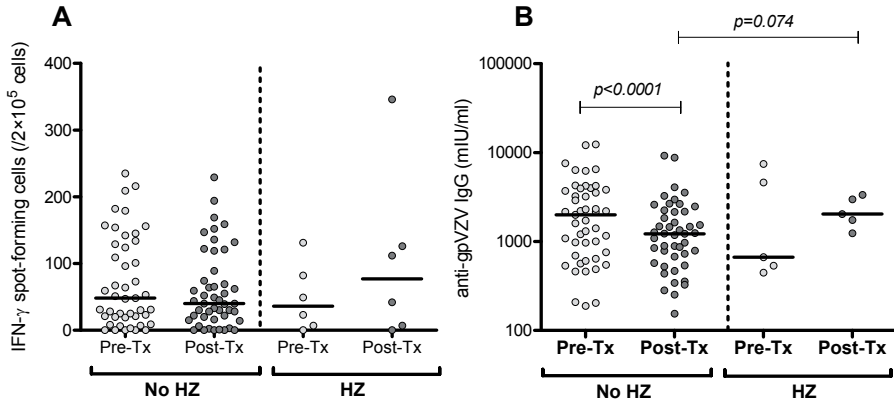
Supplementary figure 2. Numbers of interferon gamma (IFNγ) spot-forming cells in response to VZV stimulation (A) and levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale (B) in 6 patients who experienced herpes zoster (HZ) in the 2-3 years after transplantation compared to 45/47 patients without any mentioning of herpes zoster (no HZ) in records/questionnaire. Lines show the median.

6
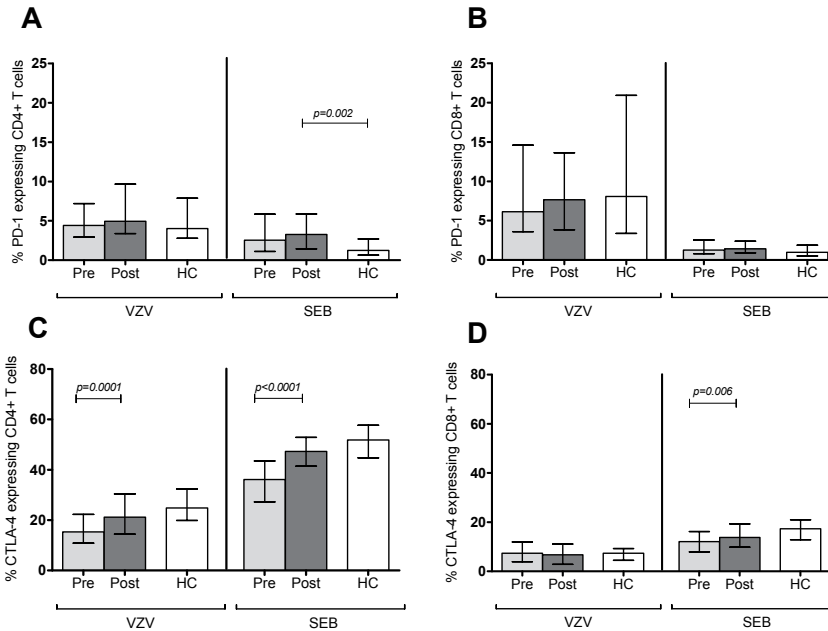
Supplementary figure 3. Percentage of cells expressing programmed cell death protein 1 (PD-1) of CD4+ (**A**) and CD8+ (**B**) T cells, and percentage of cells expressing cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) of CD4+ (**C**) and CD8+ (**D**) T cells upon stimulation with varicella-zoster virus (VZV) and staphylococcal enterotoxin B (SEB, positive control) in 28 healthy control subjects (HC) and 59 patients before (pre) and 2-3 years after (post) receiving a renal transplant. Bars show the median and interquartile range.

Part II

VACCINATION OF PATIENTS WITH
AUTOIMMUNE INFLAMMATORY
RHEUMATIC DISEASES