Short-term kinetics of torque teno virus viraemia after induction immunosuppression confirm T lymphocytes as the main replication-competent cells

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Torque teno virus (TTV) is increasingly considered a universal marker of global immune function. The virus is supposed to replicate in lymphocytes, but poor information is available about fluctuations of viraemia after administration of anti-lymphocyte agents. We studied TTV kinetics in a cohort of 70 kidney ± pancreas recipients receiving one of two different anti-T-cell induction immunosuppressants. During the first 30 days after anti-T-cell antibody administration, we report kinetics of TTV viraemia compatible with replication in T lymphocytes, and highly dependent on the potency of the anti-T-cell drug administered.

INTRODUCTION

Torque teno virus (TTV) is a human anellovirus first discovered in 1997 (Maggi & Bendinelli, 2009). It has a circular, 3.8 kb, negative-sense, single-stranded DNA genome including at least four open-reading frames. Prevalence in the plasma is almost universal as primary infection is acquired early in childhood through the respiratory route and is followed by chronic infection leading to stable viraemia. Activated peripheral blood mononuclear cells were soon identified as the main replication-competent cells (Maggi et al., 2001; Mariscal et al., 2002; Okamura et al., 1999). Unfortunately, TTV cannot be cultured yet, although recently the related TTMV has been cultured on respiratory epithelium A549 cells (Galmes et al., 2013).

Our group and others have reported an association between TTV viraemia and the extent of immune deficiency, whether the latter is congenital (Maggi et al., 2011) or acquired (iatrogenic) (Focosi et al., 2010; Maggi et al., 2008), due to cancer (Zhong et al., 2001, 2002) or to chronic infections (Fogli et al., 2012). In 2008, we described, using a quantitative real-time PCR assay targeting conserved TTV genome regions in myeloma and lymphoma patients receiving high-dose chemotherapy, a clear correlation between peaks of total TTV viraemia and expansion of senescent CD8+ CD57+ T lymphocytes, which are known to decrease immune response and are correlated with reactivated CMV infections (Maggi et al., 2008). We also previously demonstrated that the time needed for TTV viraemia to return to baseline levels after the peak that inexorably follows conditioning predicts the time for recovery of immune system function. Hence, we proposed TTV viraemia as a surrogate marker of functional immune competence (Focosi et al., 2010). In another study, we confirmed the pre-eminent role of haematopoietic cells in controlling plasma TTV viraemia in four fully myelo-ablated recipients of haplo-identical stem cell transplantation (Maggi et al., 2010).

In order to corroborate such preliminary findings, the present study aimed at detailing the exact blood cell type responsible for maintenance of TTV viraemia using a clean, clinically relevant model of lymphocyte perturbation, namely kidney ± pancreas transplant recipients administered induction immunosuppression when different anti-T-lymphocyte antibodies were used.

METHODS

Seventy patients consecutively receiving a kidney ± pancreas transplant at the Kidney-Pancreas Transplant Centre of the University Hospital of Pisa during 2011–2013 were enrolled in the study after written informed consent (25 receiving a deceased donor kidney transplant alone, 24 living donor kidney transplant, 13 simultaneous kidney and pancreas transplant, 5 pancreas transplant alone and 3 pancreas after kidney transplant). Plasma samples were collected just before administration of induction immunosuppression, and later on days 7, 15 and 30 post-transplant. TTV viraemia was determined by real-time PCR as previously reported (Focosi et al., 2010; Maggi et al., 2008)
et al., 2001, 2010). The absolute lymphocyte count (ALC) in peripheral blood was collected at the same time points using an automated haemocytometer.

Induction immunosuppression consisted of an anti-T-cell agent (either anti-thymocyte globulins (ATG) days 0–6 \((n=23)\) or basiliximab 20 mg days 0–4 \((n=47)\) ) and methylprednisolone 500 mg. Six patients received ATG 0.8 mg kg\(^{-1}\) day\(^{-1}\), while the remaining 17 received a dose of 1 mg kg\(^{-1}\) day\(^{-1}\).

Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil and prednisone. The mean plasma level of tacrolimus was calculated for each recipient across the first month after transplantation.

Differences between means and distributions were evaluated by the two-tailed Student \(t\)-test and Wilcoxon rank sum test, respectively. Associations between variables were determined by applying Spearman rho correlation coefficient.

**RESULTS**

Globally when the data were analysed for the entire study population, the drop in TTV viraemia seen 7 days after transplantation was statistically significant \((P<0.001)\), as well as the rise starting from day 15 (Fig. 1, left panel). Stratifying the kinetics according to type of solid organ transplantation showed no impact (Fig. 1, right panel).

There was no statistically significant difference in either TTV viraemia or ALC at any time point between patients receiving ATG 0.8 mg kg\(^{-1}\) day\(^{-1}\) versus 1 mg kg\(^{-1}\) day\(^{-1}\), suggesting a minimal differential effect between schedules (data not shown). Thereafter the two groups were considered together for subsequent analyses. Fig. 2 shows the impact of different induction regimens on TTV viraemia kinetics after transplantation. Interestingly, the T-lymphocyte-depleting (lysis-inducer) ATG induced higher drops in TTV viraemia than the ‘weaker’ T-lymphocyte-‘paralysing’ anti-IL-2R/CD25 monoclonal antibody, basiliximab. TTV viraemia kinetics during a second transplantation were the same as after the first (data not shown).

In order to confirm the hypothesis that the different effect of the induction regimens on TTV viraemia was related to a different effect on T lymphocytes counts, we plotted the variation in TTV viraemia against that in peripheral blood at days 7, 15 and 30 post-transplantation, obtaining largely overlapping curves (Fig. 3). The ALC curve differed between induction immunosuppressants according to the expected lytic effect of each agent.

The mean plasma tacrolimus level across the first month after transplantation did not correlate with TTV viraemia at month 1 (data not shown).

**CONCLUSIONS**

The impressive drop in TTV viraemia at day 7 post-transplantation may be explained by either assuming removal (lysis) or impairment (functional blockade) of the replication-competent cell population, i.e. T lymphocytes, exerted by induction immunosuppressants. While the anti-IL-2R/CD25 monoclonal antibody is specific for T lymphocytes, one could argue that ATG (a polyclonal immune serum manufactured by immunizing rabbits with human thymus fragments) contains specificities directed against non-T cells. Nevertheless, it has been shown that the vast amount of antibody specificities in ATG is directed against T lymphocytes (Popow et al., 2013), reducing the probability that the variation in TTV viraemia is due to effects of ATG on other cell populations included in the ALC. Similarly, historical immunophenotyping of kidney \(\pm\) pancreas transplantation recipients has shown that maintenance immunosuppression per se accounts for minimal changes in peripheral blood ALC during the first month in the absence of induction immunosuppression, minimizing the chances that the effects on TTV viraemia are due to drugs other than anti-T antibodies.
Interestingly, in contrast to what has been reported in the setting of lung transplantation (Görzer et al., 2014), the mean plasma tacrolimus level across the first month after transplantation did not correlate with TTV viremia at month 1. Longer-term follow-up studies are ongoing to investigate whether there is a time lapse required for immunosuppressants to impair immune surveillance against TTV.

Our current findings are in accordance with previous findings from our group showing similar kinetics of TTV viremia in four haploidentical stem cell transplant recipients showing a decline in TTV viremia, in parallel with elimination of peripheral blood ALC, after a fully myeloablative conditioning regimen based on high-dose ATG (Maggi et al., 2010). The current solid organ transplant model provides a more convincing demonstration because, in contrast to haematological patients, solid organ transplant recipients neither have any baseline perturbation in blood cells nor receive concomitant additional ablative chemotherapy.

In this study, we have demonstrated for the first time that TTV is a T-lymphotropic virus largely affected by anti-T-lymphocyte drugs.

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**References**


