



Identification of an expanded population of activated CD4⁺ CD25⁺ T cells defined by CD45RO and IL-7R α in kidney transplant recipients



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INTRODUCTION

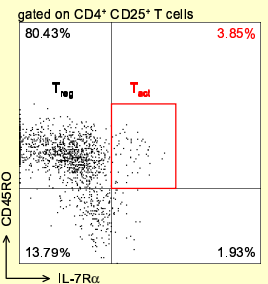
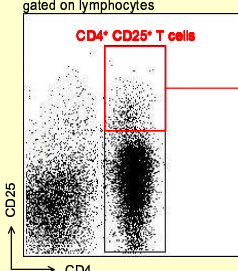
The **antiallograft immune response**, and in particular the T cell immunity, remains an important cause of acute rejection and late graft dysfunction in solid organ transplantation. However, there is no marker of cell-mediated immunity that is sensitive and specific enough to be used routinely in the monitoring of transplant recipients.

Recent studies (1, 2) have shown that T lymphocytes expressing the surface markers **CD4** and **CD25** belong to two functionally very different subsets of T lymphocytes, that may be distinguished by the expression of another surface marker, the interleukin (IL-) 7 receptor α chain (**IL-7R α** , CD127), and by the intracellular expression of the transcription factor **FoxP3**.

CD4⁺ CD25⁺ T cells

Regulatory T cells (T _{reg})	Activated T cells (T _{act})
CD45RO ⁻ IL-7R α ^{low} FoxP3 ⁺	CD45RO ⁺ IL-7R α ^{high} FoxP3 ⁻
<ul style="list-style-type: none"> induce and maintain peripheral tolerance to self-antigens modulate immune responses to a variety of allo-antigens may suppress allograft rejection <i>in vivo</i> (reviewed in 3), allowing for the development of a state of tolerance towards the graft 	<ul style="list-style-type: none"> trigger adaptive immune responses could be implicated in the transplantation rejection process

Illustration from a healthy individual gated on lymphocytes



OBJECTIVE

Investigate the presence and the function of the CD4⁺ CD25⁺ CD45RO⁺ IL-7R α ^{high} activated T cell population (T_{act}) in kidney transplant recipients.

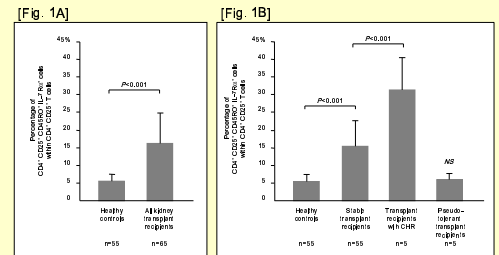
PATIENTS

- 55 healthy controls
- 65 kidney transplant recipients, of whom:
 - 55 had a **stable graft function**, defined by a stable serum creatinine level below 150 μ mol/l, a 24h-proteinuria inferior to 0.5 g/day and no circulating donor-specific anti-HLA antibodies, under standard immunosuppression (calcineurin inhibitor + MMF \pm prednisone);
 - 5 had **biopsy-proven diagnosis of chronic humoral rejection (CHR)**, with circulating donor-specific anti-HLA antibodies and capillary C4d deposits;
 - 5 were **pseudo-tolerant patients**, i.e. on NO or MINIMAL immunosuppression (prednisone alone, MMF alone, or azathioprine \pm prednisone) and with a stable graft function.

RESULTS

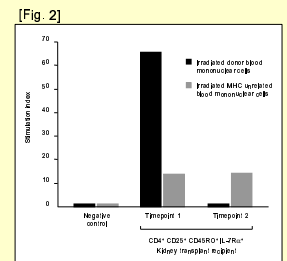
The frequency of the T_{act} population correlates with the clinical status of the kidney transplant recipients

Overall, the T_{act} population was found to be significantly increased in 83% (54/65) of the transplant recipients (mean: 16.11 \pm 8.64% of CD4⁺ CD25⁺ T cells) compared to healthy controls (mean: 5.37 \pm 2.07%; $P < 0.001$) [Fig. 1A]. In the five patients with CHR, this T_{act} population was highly expanded (31.33 \pm 9.30%; $P < 0.001$), whereas it was comparable to healthy controls in the five pseudo-tolerant recipients (5.99 \pm 1.65%; $P = 0.26$). Intermediate levels (15.48 \pm 7.03%; $P < 0.001$) were found in the 55 stable recipients [Fig. 1B].



The T_{act} population contains allospecific T cells

In a kidney transplant recipient at the time of CHR diagnosis, the proliferative response of the T_{act} population was found to be 5-fold higher when stimulated by irradiated PBMC from the living donor as compared to a stimulation by irradiated PBMC from a MHC-unrelated subject (timepoint 1). However, when we repeated the analysis two months after completion of a course of high-dose IVIg therapy (total: 2g/kg), no allospecific response was detected anymore (timepoint 2) [Fig. 2].

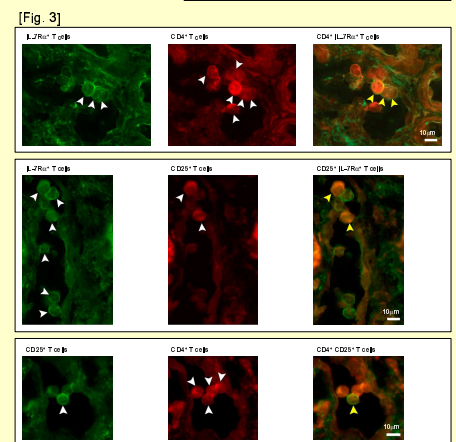


The T_{act} population secretes pro-inflammatory cytokines

We could show that after polyclonal stimulation a substantial percentage of the T_{act} population secreted pro-inflammatory cytokines such as IFN γ (3.61 \pm 1.99%) and TNF α (21.56 \pm 6.06%) [data not shown].

The T_{act} population infiltrates the kidney allograft of transplant recipients with CHR

We investigated the phenotype of the cells infiltrating the kidney allografts of the five patients with biopsy-proven CHR. About 20% of infiltrating CD4 T cells were CD25⁺ and all of these cells co-expressed IL-7R α . Overall, 50% of CD4 T cells were IL-7R α ⁺. However, no expression of FoxP3 could be detected. This result indicate that the T_{act} population is the predominant CD4⁺ CD25⁺ T cell population infiltrating the kidney allograft in the case of CHR [Fig. 3].



CONCLUSION

After kidney transplantation, an expanded circulating CD4⁺ CD25⁺ T cell population characterized by the expression of CD45RO and IL-7R α was found in most recipients, but it was particularly expanded in those with CHR. In stable recipients with NO or MINIMAL immunosuppression, the frequency of this T_{act} population was similar to the one found in healthy controls. This expanded population contained allospecific CD4 T cells and secreted effector cytokines such as IFN γ and TNF α , thus potentially contributing to the chronic rejection process. Moreover, this population was found to infiltrate the allograft of patients with a documented diagnosis of CHR. Taken together, these results indicate that monitoring the percentage of the circulating T_{act} may represent a useful tool to monitor CD4 T cell immune responses after kidney transplantation.

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