

CD4⁺CD25^{HIGH+} REGULATORY T CELLS IN MINIATURE SWINE

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(Background) In a mouse, there has been increasing evidence that *FOXP3* expressing CD4⁺CD25⁺ regulatory T cells (Tregs) inhibit potentially reactive T cells to allo-antigens and play an important role in transplant (Tx) tolerance. Similarly, human CD4⁺CD25^{high+} T cells represent Tregs, characterized by anergic and antigen-specific suppressive properties. Despite the significance of miniature swines as a pre-clinical model, only limited data are available on whether the porcine counterpart to the murine or human Tregs exists. **(Methods)** From peripheral blood of five adult miniature swines, CD4⁺ cells (CD4⁺), CD4⁺CD25^{high+} cells (CD25^{high+}), CD4⁺CD25^{low+} cells (CD25^{low+}), and CD4⁺CD25⁻ (CD25⁻) cells were isolated with a cell sorter. Expression of the homologous gene to murine *FOXP3* was quantified by RT-PCR in isolated fractions. The data was normalized to *HPRT*. Proliferation of CD25^{high+} and suppressive property of CD25^{high+} to CD4⁺ responder cells upon allo-geneic stimulation was evaluated by MLR and dilution assay, respectively, after isolation and 9 day culture with allo-geneic cells in the presence of IL-2. **(Results)** CD25^{high+} expressed *FOXP3* at 10times higher level, compared to that of CD25^{low+} (p<0.05). CD25⁻ cells expressed virtually no *FOXP3*. CD25^{high+} was anergic and suppressed the proliferation of responder CD4⁺ upon allo-geneic stimulation after isolation. After culture, CD25^{high+} cells remained anergic and exerted more suppressive property to cells used for culture than to 3rd party cells. **(Conclusion)** 1) CD25^{high+} in miniature swine peripheral blood expressed *FOXP3* at an extremely high level. 2) This fraction was anergic and exerted suppressive property, which became antigen-specific after the exposure to allo-antigen. 3) We found for the first time that miniature swine CD25^{high+} represents the homologous population to the murine and the human Tregs. Therefore, miniature swine Tx models may provide opportunities for pre-clinical evaluation of innovative Tregs-based tolerance strategy.

VALUE OF FOXP3 EXPRESSION IN PERIPHERAL BLOOD AS REJECTION MARKER AFTER MINIATURE SWINE LUNG TRANSPLANTATION

(Background) The outcome of highly immunogenic lung-transplantation (Tx) remains unsatisfactory, despite the development of potent immunosuppressants (IS). This may result from the lack of a non-or minimal-invasive method to detect early rejection. There is emerging evidence that the expression of *FOXP3* (a specific marker for regulatory T cells) is paradoxically up-regulated within rejecting graft in clinics. **(Methods)** Orthotopic lung-Tx was performed, using miniature swines without IS. Chest radiography and open lung biopsy were performed to monitor rejection. The expressions of *FOXP3*, *perforin*, *Fas-L*, and *IP-10* in the peripheral blood and the graft were quantified at transcriptional level. Additionally, steroid pulse + 6 day-tacrolimus were administered to recipients from post-operative day (POD) 4 as a rescue therapy. **(Findings)** Early rejection was detected by open lung biopsy, but misdiagnosed by chest radiography on POD 4. The expression of *FOXP3* in the peripheral blood reached its highest value as early as POD 4, before an abrupt decline. Neither *perforin*, *Fas-L* nor *IP-10* in the peripheral blood exhibited significant increase or decrease during rejection. There was a definite correlation between the expressions of *FOXP3* in the peripheral blood and within the graft during rejection (p<0.0001, R = 0.961). A rescue IS therapy from POD4, when the peak of *FOXP3* was seen, prolonged graft survival (23.5 days vs. 9.1 days without IS p<0.001). **(Interpretation)** *FOXP3* in the peripheral blood may be a more promising candidate for a minimal invasive method to detect early rejection after lung-Tx, compared with chest radiography or conventional rejection markers.