

## DEFINING THE KINETICS OF TRANSCRIPTION FACTOR AND INTERFERON-GAMMA EXPRESSION IN EFFECTOR T CELLS

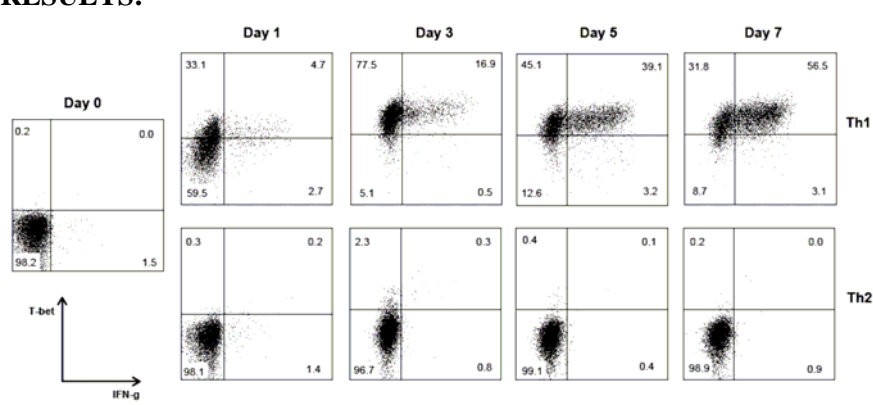
Gökmen M R, Lord G M

Guy's Hospital and St. Thomas' and King's College London

**INTRODUCTION:** Despite the success of renal transplantation as a treatment for end-stage renal failure, allograft rejection is still the major problem causing graft loss. The major cause of this graft loss is pathogenic effector CD4<sup>+</sup> T cells, whose lineage and function is determined by specific transcription factors. T-bet is a transcription factor preferentially expressed in Th1 CD4<sup>+</sup> T cells, which transactivates interferon-gamma production (IFN- $\gamma$ )<sup>1</sup>. Th1 cells are responsible for renal allograft rejection and many forms of autoimmune renal disease. The time course of T-bet expression and subsequent IFN- $\gamma$  production, the process by which naive T cells become effector Th1 cells following activation, has not yet been fully determined.

**METHODS:** CD4 positive, CD25 negative T cells were prepared from the peripheral lymph nodes of balb/c mice using magnetic bead separation. The cells were activated using plate-bound 2 $\mu$ g/ml anti-CD3 and 2 $\mu$ g/ml anti-CD28, and cultured in conditions favouring skewing towards a Th1 or Th2 phenotype (interleukin (IL) -12, anti-IL-4 and IL-4, anti-IFN- $\gamma$  respectively). A sample of cells from each condition was reactivated with phorbol 12-myristate 13-acetate (PMA) and ionomycin at various time intervals during the culture; cells were subsequently stained for cell surface markers, intracytoplasmic cytokines and intranuclear transcription factors.

### RESULTS:



No significant T-bet expression was detected by intranuclear staining at any stage in the Th2 condition. In contrast, in the presence of Th1 polarising cytokines, the proportion of cells expressing T-bet was seen to increase up to day 3, after which cells remained T-bet positive. Among these T-bet positive cells, the proportion which also expressed IFN- $\gamma$  increased during the course of the culture. Interestingly, however, there remained a significant number of T-bet positive, IFN- $\gamma$  negative cells throughout the experiment; this phenomenon has not previously been identified.

**CONCLUSIONS:** Although the role of T-bet in Th1 cell lineage commitment and IFN- $\gamma$  transactivation is well established, these data demonstrate that there is a possible further element of control in determining whether cells acquire a Th1 effector phenotype, as characterised by IFN- $\gamma$  production. Further experiments are underway to determine the possible underlying mechanisms. A more complete understanding of these fundamental aspects of T cell biology promises to open up novel diagnostic and therapeutic possibilities.

1. Kaplan B. Overcoming barriers to long-term graft survival. *American Journal of Kidney Diseases*. 2006; 52-64
2. Szabo SJ *et al*. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell*. 2000; 100: 655-669

