Immune Tolerance Laboratory

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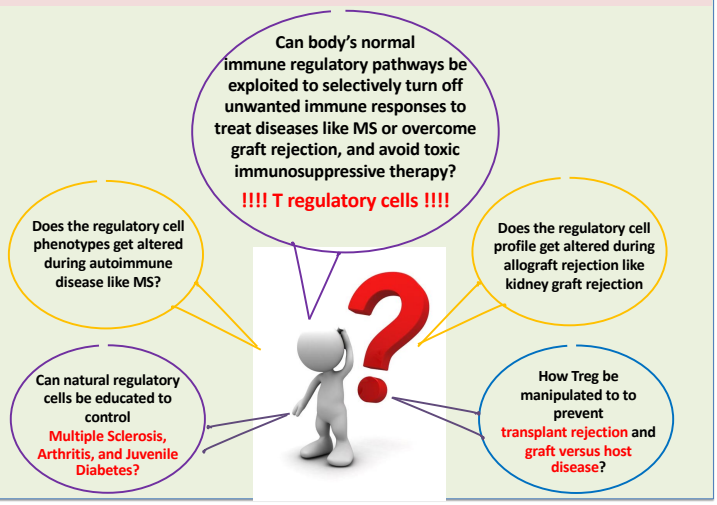
Immune Tolerance Laboratory focuses on T regulatory cells examining how to enhance the natural immune regulation in transplant rejection and autoimmunity.



T Regulatory Cells (Treg)

T regulatory cells are T cells with capacity to regulate immune response. These are derived from thymus (tTreg) and constitute 5-10% of CD4 expressing T cells. These were first identified as $CD4^+CD25^+$T cells in rat transplantation model by our group (Hall, 1990), and later in mice models of autoimmunity. In rodents and humans, these **tTreg** express Foxp3. Our group focuses on **alloantigen-specific Treg,** the main mediators of transplant tolerance. Human Treg are defined as **$CD4^+CD25^+CD127^{lo}FoxP3^+$Treg** .

Treg from rodents can be activated along two pathways. **With Th1 cytokine IL-2**, and antigen they are activated and express receptors for late Th1 cytokines (Interferon-gamma receptor **IFNGR**) and are termed as **Ts1 Treg**. **With Th2 cytokine IL-4**, and antigen , they express the receptor for late Th2 cytokine Interleukin-5 **(IL-5R-alpha)**, and are known as **Ts2 Treg** (Verma et al., 2009). Ts1 and Ts2 Treg delay cardiac allograft rejection, reduce autoimmunity (Ts2), are antigen specific and more potent than **naïve thymic Treg (tTreg).**

Further activation of Ts1 and Ts2 with antigen and late Th1 or Th2 cytokines generate **Th1-like or Th2-like Treg** respectively. Both have 100-1000-fold enhanced suppressive ability compared to tTreg. Th1-like Treg delay allograft rejection with no other immunosuppression (Verma et al. 2014). Current clinical trials with tTreg therapy use polyclonally expanded tTreg, which are non-specific, suppress at 1:1 ratio and require large numbers. Potent Ts1/Ts2 or Th1-like/Th2-like Treg have enormous therapeutic potential as fewer numbers are required.

Figure 1. Ts1 pathway

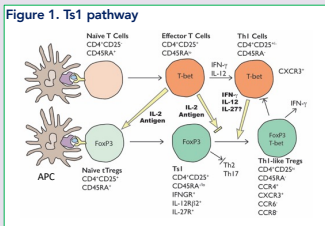
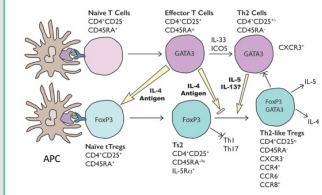
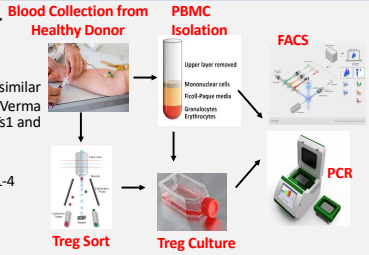


Figure 2. Ts2 pathway



tTreg activation with antigen and either IL-2 (Fig 1) or IL-4 (Fig 2) ***in vitro*** *generate Ts1 or Ts2 respectively. Ts1 cells express IFNGR and Ts2 cells express IL-5R-a.* ***Both Ts1 and Ts2 showed in vivo antigen specific suppression of rejection in a rat model*** *of cardiac allograft at 1:100 compared to tTreg that suppress at 1:1 ratio.*

Theme 1: Characterisation of Human T Regulatory Cells



We aim to examine if human Treg can be activated along similar pathways that we have described in rats (Verma 2009; Verma 2014) with antigen and Th1 or Th2 cytokines to generate Ts1 and Ts2 cells.

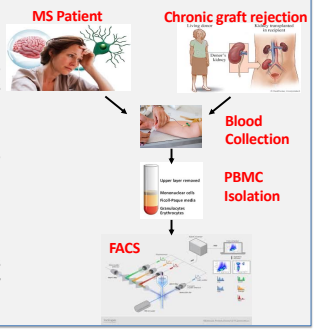
Aims:

1. Activation of human Treg with alloantigen and IL-2 or IL-4
2. Phenotypic characterisation of activated Treg
3. Functional characterisation of activated Treg

Significance:

Characterisation of activated human Treg will establish if similar pathways of activation exist in human. Activation protocols will establish methods to generate potent antigen specific Treg that can have major therapeutic applications in transplantation and autoimmunity. These studies will help make clinical trials with Treg more feasible as opposed to current practices that require large number of Tregs because of their low suppressive ability.

Theme 2: Activated Treg monitoring in Blood of MS Patients or Kidney Transplant Patients



We have examined T regulatory cells in healthy individuals and MS patients to monitor activated Treg and the Chemokine Receptors they expressed. Activated Treg profile was altered in MS patients.

Aims:

1. Monitor activated T regulatory cell profile in patients with MS or kidney transplant recipients
2. Compare the profile with healthy human subjects
3. Assess the effect of therapy on Treg profile

Significance:

Examination of Treg phenotypes in patients with multiple sclerosis or chronic allograft rejection will help assess the effect of Therapy on Treg. This can serve as a biomarker for the response to therapy.

Theme 3: Antigen-Specific T Regulatory Cells in Transplant Tolerance

Projects aim to selective propagation of existing alloantigen-specific T regulatory cells within natural thymic Tregs and characterize them phenotypically and functionally.

Aims:

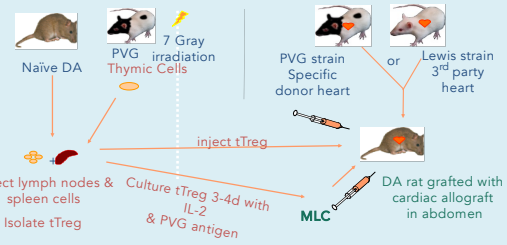
To characterize Ts1 and Ts2 cells phenotypically and functionally.

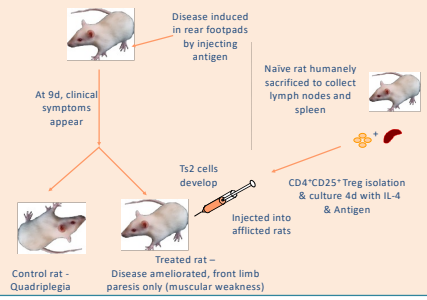
1. Activation of rat Treg with alloantigen and either IL-2 or IL-4
2. Phenotypic characterisation of activated Treg by FACS and PCR
3. Functional characterisation of activated Treg by suppression assay

Significance:

Characterisation of methods to generate potent antigen specific T regulatory cells that can have major therapeutic applications in transplant rejection. These studies could help make clinical trials with Treg more feasible as opposed to current practices that require large number of tTregs because of their low suppressive ability.

Cardiac Allograft Rejection Model





EAE Model- a rat model of Multiple Sclerosis in humans

tTreg isolated from lymph nodes and spleen of Lewis rats were cultured with antigen-primed Lewis thymic cells for 4d to generate antigen-specific Ts2 cells. These Ts2 were then injected into rats that have EAE disease induced 9-days prior. Disease severity was examined using weight and clinical scoring system.

Theme 4: Antigen-Specific Treg in Autoimmunity in EAE Model

In our animal model of MS, treatment with Ts2 and Th2-like Tregs significantly reduced clinical symptoms of autoimmunity. Further characterisation of these is necessary to understand the mechanisms further.

Aims:

To characterize Ts1/Ts2 and Th1/Th2-like Treg phenotypically and functionally.

1. Generation of Ts1 and Ts2 cells by activation of rat Treg with autoantigen and either IL-2 or IL-4
2. Generation of Th1-like and Th2-like Treg by activation of rat Ts1 and Ts2 respectively
3. Characterization and Functional assessment of Ts1/Ts2 and Th1-like/Th2-like Treg in rat models of autoimmune disease

Significance:

Highly potent antigen-specific Treg may control autoimmune injury and re-establish tolerance. Such cells would have massive potential for a variety of autoimmune diseases where the auto-antigen is known. This will help devise methods to generate potent antigen-specific Treg that can be applied in clinic for therapeutic trials.

Techniques to Learn

- T regulatory cell isolation from human blood
- Multicolour Flow Cytometry
- Cell Sorting using flow cytometry
- Magnetic bead-based cell separation
- Cell proliferation assays
- Suppression Assays
- Antibody Assay
- RNA extraction and cDNA synthesis, RT-PCR
- T regulatory cell isolation from rat tissues
- Cell culture
- Histology and immunohistochemistry
- Animal models of autoimmunity for Multiple sclerosis or Guillain barre syndrome
- Animal model of cardiac allograft rejection

Contact Information

Projects can be allocated on any of the themes to suit the student's interest. To find out more information about any of the themes above, and to discuss your area of interest, pls contact us.

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