Tolerance - 2.
Regulatory T cells; why tolerance fails

Abul K. Abbas
UCSF
Lecture outline

• Regulatory T cells: functions and clinical relevance

• Pathogenesis of autoimmunity: why self-tolerance fails

• Therapeutic approaches for immunological diseases
Regulatory T cells

Thymus

Lymph node

Recognition of self antigen in thymus

Recognition of antigen in secondary lymphoid tissues

Inhibition of T cell responses

Inhibition of other cells

DC

Naive T cell

Effector T cells

NK cell

B cell
Properties of regulatory T cells

- **Phenotype:** CD4+, high IL-2 receptor (CD25), low IL-7 receptor, Foxp3 transcription factor; other markers

- **Essential features** of stable Tregs:
  - Foxp3 expression: requires demethylated non-coding CNS2 sequence in promoter
  - CD25 (IL-2Rα) expression: IL-2 is a necessary survival factor
  - CTLA-4 expression: required for suppressive function of most Tregs
  - (Inability to produce IL-2)

*Take home messages*
The significance of Foxp3+ Tregs

- **Genetic evidence:** Foxp3 mutations $\rightarrow$ autoimmune disease (IPEX); in mice, disease can be corrected by providing normal Foxp3+ cells

- Do defects in Foxp3+ Tregs or resistance to Treg-mediated suppression contribute to common autoimmune diseases?
  - Inconsistent and variable data
Populations of Tregs

• **Thymic (natural)**
  - Induced by self antigen recognition during T cell maturation

• **Peripheral (adaptive)**
  - In response to antigen exposure in the periphery; contribution to preventing inflammatory disease?

• **Induced (in vitro; sometimes called Tr1)**
  - Culture with TGFβ + IL-2; therapeutic options

• There are no reliable markers for distinguishing these Tregs in a “bulk” population
Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation
  - Genetic deletion of CTLA-4 in Foxp3+ cells results in severe systemic autoimmunity and lymphoproliferation
  - Commonly used assay for Treg function in humans bypasses the need for B7 and cannot measure this activity
Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation

- Inhibitory cytokines produced by Tregs (TGF-β, IL-10, others?) suppress immune responses (DCs, Macs, T cells)
  - IL-10 deletion in Foxp3+ cells results in colitis
  - IL-10 is also produced by Foxp3- cells

- Consumption of IL-2

- Many others reported
The balance of effector and regulatory T cells
Role of Tregs in fetal tolerance

• In evolution, placentation developed at the same time as the ability to generate FoxP3+ peripheral Tregs
• Paternal antigens expressed in the fetus induce long-lived antigen-specific Tregs
• Elimination of fetal antigen-specific Tregs in mice results in fetal resorption

• Anatomic restriction of immune regulation?
• Role in humans? Are defects in regulatory memory the basis of recurrent fetal loss?
Tregs and the microbiome

• Commensal bacteria promote development and accumulation of Tregs in the colon and skin

• Intestine:
  - *Clostridia*
  - May be related to production of short-chain fatty acids (e.g. butyrate) which induce TGF-β and epigenetic changes in Foxp3 locus

• Skin:
  - Wave of Treg accumulation occurs in neonates, may coincide with commensalism
  - Suppress subsequent inflammatory reactions to commensal bacteria
"Non-immune" functions of tissue Tregs

- Tregs in adipose tissue regulate lipid metabolism
- Tregs in muscle and other tissues produce growth factors that promote repair (trauma, infections, degenerative diseases)
- Tregs in skin stimulate cycling and differentiation of hair follicle stem cells
- Do Tregs adapt to their environment, or do distinct subsets exist that populate different tissues?
Regulatory T cells

• Explosion of information about the generation, properties, functions and significance of these cells

• Will cellular therapy with ex vivo expanded Treg become a reality?

• Therapeutic goal: induction or activation of Treg in immune diseases
The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses
  - Grow up patient’s Tregs ex vivo
  - Ongoing clinical trials in graft rejection, T1D show it is safe
  - Very little efficacy data
The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses

- **Challenges:**
  - Technically difficult, individualized

- Administer antigen or antigen mimic in ways that preferentially induce Tregs?
  - Weak stimulus (peptide antigen, anti-CD3); + IL-2?
Functions of Interleukin-2: the dogma

Interleukin-2 (IL-2, T-cell growth factor)

- APC
- Helper T lymphocyte
- Autocrine action of IL-2
- B lymphocyte
- NK cells
- Proliferation, survival and differentiation of T cells
- Effector and memory T cells
The unexpected biology of IL-2

• Interleukin-2 is the prototypic T cell growth factor (TCGF), required for initiating clonal expansion of T cells in response to antigen

• Prediction: what will be the consequence of eliminating IL-2 or the IL-2 receptor?
The unexpected biology of IL-2

• Interleukin-2 is the prototypic T cell growth factor (TCGF), required for initiating clonal expansion of T cells in response to antigen

• **BUT:** knockout of IL-2 or the $\alpha$ or $\beta$ chain of the IL-2R results not in immune deficiency but in systemic autoimmunity and lymphoproliferation
Dual roles of IL-2 in T cell responses

**Induction of immune response**
- APC
- Costimulator (B7)
- CD28
- Resting (naive) T cell
- IL-2
- Expansion and differentiation: effector T cells

**Control of immune response**
- Self-reactive T cell in thymus or periphery
- IL-2
- Regulatory T cells

**Take home messages**

*Surprising conclusion from knockout mice: the non-redundant function of IL-2 is in controlling immune responses*
IL-2 dependent activation of regulatory T cells suppresses effector responses

Other beneficial actions of IL-2 in autoimmune diseases

- IL-2 inhibits the development of Th17 cells
  - Does STAT5 (IL-2-induced) compete with STAT3 (required for Th17 development)?

- IL-2 inhibits development of follicular helper T (Tfh) cells
  - Reduced autoantibody production
  - May partly account for success in SLE
Therapeutic potential of IL-2: a revision

• IL-2 was originally used to boost immune responses in cancer, HIV infection (promoting effector and memory T cells)
  • Inconsistent clinical results
Therapeutic potential of IL-2: a revision

• IL-2 was originally used to boost immune responses in cancer, HIV infection

• IL-2 treatment can increase number and functional activity of Tregs
  • Low-dose IL-2 to treat steroid-resistant chronic GVHD, vasculitis
  • More recent clinical trials ongoing in type 1 diabetes, SLE, graft rejection
Therapeutic potential of IL-2: a revision

- IL-2 was originally used to boost immune responses in cancer, HIV infection
- IL-2 treatment can increase number and functional activity of Tregs

The challenge: IL-2 activates both effector and regulatory T cells
- Low-dose IL-2 acts preferentially on Foxp3+ Tregs
- Mutant forms of IL-2 that preferentially activate Tregs
- IL-2-antibody complexes that target cytokine preferentially to IL-2Rα or IL-2Rβ

Take home messages
Role of dendritic cells in self-tolerance

- **Mature DCs** (activated during innate immune responses to PAMPs and DAMPs) express costimulators, secrete cytokines, and initiate immune responses.

- **Immature (“resting”) DCs** live in tissues, display self antigens and maintain tolerance (by inducing anergy, deletion, and/or Tregs).
The potential of “tolerogenic antigen-presenting cells”

- Exploiting antigen-pulsed DCs to induce tolerance
  - Phase 1 trial of DCs pulsed with citrullinated peptides in RA published in 2015
  - Maintaining DCs in tolerogenic state?

- Can DCs be modified to make them tolerogenic?
  - Expression of costimulator antagonists, immunosuppressive cytokines, other inhibitors: being tried in animal models of graft rejection
Regulating immune responses: where are we?

- Elucidating the mechanisms of immune regulation is one of the dominant themes of modern Immunology; obvious relevance to immune-mediated inflammatory diseases, therapeutics, vaccines

- Already leading to new therapeutic strategies

- Continuing challenge is to establish the importance of control mechanisms in the development of inflammatory diseases
Autoimmunity

- **Definition:** immune response against self (auto-) antigen, by implication pathologic

- Much of our knowledge of immunological disorders is based on mouse models
- Elucidating the causes of these diseases has been a challenge
  - Initiating triggers generally unknown
  - Complex interactions between genes and environment
  - Unclear which mechanisms of tolerance fail in any disease
Pathogenesis of autoimmunity

Genetic susceptibility

- Susceptibility genes
- Failure of self-tolerance
- Self-reactive lymphocytes

Reaction to environmental stimuli

- Tissue injury and inflammation
- Activation of tissue APCs
- Activation of self-reactive lymphocytes
- Self-reactive effector lymphocytes
- Tissue injury: autoimmune disease
Genetic basis of autoimmunity

• Multiple genes are associated with autoimmunity
  - Most human autoimmune diseases are multigenic
  - Single gene defects reveal pathways of self-tolerance and why it fails (e.g. AIRE, Fas, Foxp3, many others) but are not involved in most, common autoimmune diseases

• Genes include HLA, many others
  - Each gene individually makes a small contribution
Genetics of autoimmunity: challenges

- Relating complex genotypes to phenotypic and functional abnormalities, to better understand pathogenesis
  - Complex interactions between genes and environment, often difficult to define

- Predictive value of genetic polymorphisms
  - Unlikely because of low odds ratios

- Using polymorphisms to identify therapeutic targets
  - Difficult because any one gene makes a small contribution
Infections and autoimmunity

• Infections trigger autoimmune reactions
  - Clinical prodromes, animal models
  - IBD is dependent on gut commensals

• Some autoimmune diseases are prevented by infections (type 1 diabetes, multiple sclerosis, others? -- increasing incidence in developed countries): mechanism unknown
  - The “hygiene hypothesis”

• The role of the microbiome?
Therapy of immune disorders: rational approaches target lymphocyte activation and subsequent inflammation
Molecularly targeted therapies for immunological diseases: the rational approach

• Target the molecular basis of lymphocyte activation and effector functions: rationally designed therapies
  – Based on understanding of lymphocyte biology
  – Risks -- reactivation of infections

• Induce antigen-specific immunological tolerance: requires identification of target antigens
  – Being tried in MS, type 1 diabetes (in which the major autoantigens are known)
  – Based on successes in allergic diseases
Autoimmune diseases

- Experimental models are revealing pathways of immune regulation
  - But experimental animals are often inadequate models of human diseases

- Improving technologies for human studies are enabling analysis of patients
  - Need for studying antigen-specific cells

- Challenges:
  - Defining which mechanisms of immune tolerance fail in different autoimmune diseases
  - Using this knowledge to develop therapies