Tolerance

Positive selection
Negative selection

Central
(Bone marrow, Thymus)

Peripheral
(Spleen, Lymph node etc)

Anergy
Deletion
Exhaustion
Tregs....

Inflammation (Danger, Pathogen.....)
Lecture outline

• What are autoimmune diseases?

• Principles of immune regulation

• Mechanisms of central and peripheral tolerance

• Target antigens: self, cross-reactive, neo-antigens, and beyond...

• Mechanisms of tolerance evasion (i.e., of autoimmunity)

• Inhibitory receptors of T cells and rationale of therapy
Two types of autoimmune disease

organ-specific

- brain
- multiple sclerosis (?)
- thyroid
  - Hashimoto's thyroiditis
  - primary myxoedema
  - thyrotoxicosis
- stomach
  - pernicious anaemia
- adrenal
  - Addison's disease
- pancreas
  - insulin-dependent diabetes mellitus

non-organ-specific

- muscle
  - dermatomyositis
- kidney
  - SLE
- skin
  - scleroderma
  - SLE
- joints
  - rheumatoid arthritis

Frequency: 5% of population
The spectrum of autoimmune diseases

organ-specific

Hashimoto’s thyroiditis
primary myxoedema
thyrotoxicosis
pernicious anaemia
autoimmune atrophic gastritis
Addison’s disease
premature menopause (few cases)
insulin-dependent diabetes mellitus
stiff-man syndrome
Goodpasture’s syndrome
myasthenia gravis
male infertility (few cases)
pemphigus vulgaris
pemphigoid
sympathetic ophthalmia
phacogenic uveitis
multiple sclerosis (?)
autoimmune haemolytic anaemia
idiopathic thrombocytopenic purpura
idiopathic leucopenia
primary biliary cirrhosis
active chronic hepatitis (HBsAg negative)
cryptogenic cirrhosis (some cases)
ulcerative colitis
atherosclerosis (?)
Sjögren’s syndrome
rheumatoid arthritis
dermatomyositis
scleroderma
mixed connective tissue disease
anti-phospholipid syndrome
discoid lupus erythematosus
systemic lupus erythematosus (SLE)

non-organ-specific
Pathogenesis of autoimmunity: the 3 hits

1. Genetic susceptibility
   - Susceptibility genes

2. Failure of self-tolerance
   - Self-reactive lymphocytes

3. Reaction to environmental stimuli
   - Tissue injury and inflammation
   - Activation of tissue APCs
   - Activation of self-reactive lymphocytes
   - Self-reactive effector lymphocytes

PAMPs, DAMPs: Innate Immunity

Adaptive Immunity

Tissue injury: autoimmune disease

by Abul K. Abbas et al.
Texbook 2015 (modified)
Effects of autoimmunity

1) Tissue destruction
Diabetes: CTLs destroy insulin-producing b-cells in pancreas

2) Antibodies block normal function
Myasthenia gravis: Ab binds acetylcholine receptors

3) Antibodies stimulate inappropriate function
Graves’ disease: Ab binds TSH receptor
Mimics thyroid-stimulating hormone
Activates unregulated thyroid hormone production

4) Antigen-antibody complexes affect function
Rheumatoid arthritis:
IgM specific for Fc portion of IgG
IgM-IgG complexes deposited in joints inflammation
GENETIC PREDISPOSITION
- MHC genes
- non MHC genes

AUTOIMMUNITY

Environmental factors
- infection
- drugs
Genetic basis of autoimmunity

• **MHC genes**
  - Major genetic association with autoimmune diseases with particular HLA haplotypes (relative risk of disease)
  - Disease-associated alleles are present in normal individuals

• **Non-MHC genes:**
  - Many loci identified by whole genome association and linkage studies
  - Most are chromosomal locations; actual genes and roles in disease are largely unknown

by Abul K. Abbas et al.
Texbook 2015
**Association** of Human MHC Alleles and Risk for Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Associated HLA Allele</th>
<th>Relative Risk**</th>
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<tbody>
<tr>
<td>Ankylosing Spondylitis*</td>
<td>B27</td>
<td>90</td>
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<tr>
<td>Hereditary Hemochromatosis</td>
<td>A3/B14</td>
<td>90</td>
</tr>
<tr>
<td>Insulin Dependent Diabetes*</td>
<td>DR4/DR3</td>
<td>20</td>
</tr>
<tr>
<td>Multiple Sclerosis*</td>
<td>DR2</td>
<td>5</td>
</tr>
<tr>
<td>Myasthenia Gravis*</td>
<td>DR3</td>
<td>10</td>
</tr>
<tr>
<td>Rheumatoid Arthritis*</td>
<td>DR4</td>
<td>10</td>
</tr>
<tr>
<td>Systemic Lupus Erythromatosis*</td>
<td>DR3</td>
<td>5</td>
</tr>
<tr>
<td>Narcolepsy</td>
<td>DR2</td>
<td>130</td>
</tr>
</tbody>
</table>

* Autoimmune Disease  ** Percent of Patients with Allele Divided by Percent of Non-Affected Persons with this Allele
Association of Human MHC Alleles and Risk for A. Diseases likely due to their capacity to present self-peptides

Figure 8-7
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company
Genetic basis of autoimmunity -- 3

- Genome wide association studies are being done to define genes
  - Crohn’s disease:
    - NOD-2: microbial sensor in intestinal epithelial and other cells
    - IL-23 receptor: involved in TH17 responses
  - Rheumatoid arthritis, others:
    - PTPN-22 (tyrosine phosphatase): may control kinase-dependent lymphocyte activation
  - Multiple sclerosis:
    - CD25 (IL-2 receptor): required for maintenance of regulatory T cells
  - Difficult to define pathways and target them for therapies
Environmental factors in autoimmunity

• Infections
• Cross-reactivity or molecular mimicry
• Release of sequestered Ag
• Smoking can trigger Goodpasture’s syndrome RA
• Smoking damages alveoli, exposes collagen
• Radiation
• Pollution
• Hygiene hypothesis?
The immunological equilibrium: balancing lymphocyte activation and control

Activation
Effector T cells

Tolerance
Regulatory T cells

Reactions against self
Reactions against pathogens
(e.g., <100 species of human bacterial pathogen identified, and exposure to these is generally rare and transient)

No response to self
Controlled response to pathogens
(> 2,000 coevolved bacterial species typically associate as communities inside us — including on or in our skin, guts and mouth. They are relatively resistant to perturbations, such as starvation, and provide us with the metabolic benefit of millions of additional genes and activities)

New point of view on the immune system function:
IS addressed to tolerate rather than to fight invaders
(i.e., hospitality policy rather than intolerance against the invaders taught by IS...)

Inflammatory diseases
The term microbioma is used to describe the microorganisms that are present on and in the human body along with their genetic information and their ecological milieu.

The human body harbours 10 times more microbial cells than human cells and there is increasing acknowledgement that this represents a second genome, which contributes to tissue homeostasis.

Trillions of microorganisms, most of which are bacteria that have co-evolved with the host in a symbiotic relationship.

Microbiota functions: gastrointestinal development (i.e., it is affected in germ-free animals), carbohydrate fermentation, monosaccharides absorption, acid pH on skin and in colon lumen, polyphenols metabolism, water and mineral salts (Fe, Ca, Mg) absorption, increased intestinal transit, resistance to pathogens, isoprenoids and vitamins bio-synthesis, angiogenesis, immune system interaction to improve wanted immune responses....
Intestinal microenvironments and niches

Skin Microbiome: Friend of human body

- atopic dermatitis
- mast cell degranulation and allergic skin inflammation:

Skin Microbiome:
- FLG (filaggrin) mutation
- Asthma or Hay fever

Diagram:
- a Staphylococcus epidermidis

Epidermis
- IL-1α
- T cell

Dermis
- Myeloid cells
- IL-17A, IFNγ
- Immunity against Leishmania major

Leishmania major infection

Nature Reviews | Immunology
Skin Microbiome: from Friend to Enemy of human body

- atopic dermatitis
- mast cell degranulation and allergic skin inflammation:

Nature Reviews | Immunology
Modulation of adaptive immune responses in the gut by \textit{bacteroides fragilis}, segmented filamentous bacterium, clostridia, or other microbiota

\begin{itemize}
  \item \textit{SFB} \textbullet\ Other microbiota
  \item \textbullet\ \textit{Clostridium} cluster IV and XIVA strains
  \item PSA* \textit{Bacteroides fragilis}
  \item Other microbiota
\end{itemize}

\textbf{Gut lumen} \hspace{2cm} \textbf{Epithelial cell}

\textbf{Lamina propria} \hspace{2cm} \textbf{Protection from pathogenic Infections (EC)}

\begin{itemize}
  \item CD70$^+$CD11c$^+$ low DC and CD11b$^+$$^+$$^+$$^+$ macrophage
  \item CD11b$^+$$^+$CD11c$^-$ macrophage
  \item CD103$^+$ DC and TLR5$^+$ DC
  \item CD11b$^+$$^+$$^+$$^+$$^+$ macrophage
\end{itemize}

\textbf{SAA} \hspace{2cm} \textbf{RegIII}$\gamma$

\textbf{IL-1$\beta$, IL-6 and IL-23} \hspace{2cm} \textbf{TGF$\beta$}

\textbf{T$\gamma$,17 cell} \hspace{2cm} \textbf{FOXP3$^+$ T$_{Reg}$ cell} \hspace{2cm} \textbf{Retinoic acid and IL-10}

\textbf{ATP} \hspace{2cm} \textbf{T$_n$,17 cell}

\textbf{B. fragilis} is involved in 90% of anaerobic peritoneal infections and bacteremia associated with intra-abdominal infections, peritonitis and abscesses following rupture of viscus, and subcutaneous abscesses or burns near the anus. Though it is gram negative, it has an altered LPS and does not cause endotoxic shock.

\textit{Nobuhiko Kamada et al. Nat Rev Immunol 2013}
Protective and pathogenic role of the gut microbiota

Nobuhiko Kamada et al. Nat Rev Immunol 2013
Gut microbiota affects extra-intestinal autoimmune diseases

Nature Reviews | Immunology

Nobuhiko Kamada et al. Nat Rev Immunol 2013
Mechanisms by which microbes may promote autoimmunity.

(A) Self-tolerance (anergy)
- "Resting" tissue APC
- T cell
- Self antigen
- Self-tolerance: anergy

(B) Induction of costimulators on APCs
- Microbe
- Activation of APC
- Self-reactive T cell
- Self-antigen
- B7, CD28
- Autoimmunity

(C) Molecular mimicry
- Microbe
- Activation of T cells
- Microbial antigen
- Self-reactive T cell that recognizes microbial peptide
- Self-tissue
- Autoimmunity
Molecular mimicry

• Sharing of epitopes between an infectious agent and its host.

• Antibodies directed against the infectious agents starts reacting with normal self Ag.

• Triggers autoimmunity.
Antiphospholipid syndrome  β2-glycoprotein I  Bacteria, viruses, yeast, and tetanus toxin

Cardiomyopathy (myocarditis)  Cardiac myosin  Coxsackie virus, group A streptococci, chlamydia or Trypanosoma cruzi

Essential mixed cryoglobulinemia  IgG-Fc  HCV

Guillain-Barré syndrome  Peripheral nerve  Campylobacter jejuni
Ag related from hidden location

Many self Ag are found in hidden location eg. C N S, TESTES, EYE (CORNEA)

organ damage

\[ \text{Hidden Ag released} \]

\[ \text{Reaches blood stream} \]

\[ \text{Encounter Ag sensitive cells} \]

\[ \text{Stimulate autoimmunity} \]
HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 peptides during processing.
Central and peripheral tolerance to self

The principal fate of lymphocytes that recognize self antigens in the generative organs is death (deletion), BUT:

Some B cells may change their specificity (called "receptor editing")

Some T cells may differentiate into regulatory (suppressor) T lymphocytes

by Abul K. Abbas et al. Texbook 2015 (modified)
The structure of the thymus

- **Cortex**
  - capsule
  - trabeculae
  - subcapsular epithelium
  - corticomedullary junction

- **Medulla**
  - Hassall's corpuscle
  - cortical epithelial cell (thymic origin)
  - thymocyte (bone marrow origin)
  - medullary epithelial cell (thymic origin)
  - dendritic cell (bone marrow origin)
  - macrophage (bone marrow origin)
Only a small fraction of T cells mature into functional T cells

**Positive selection:**
Occurs in the cortex, requires cortical epithelial cells (MHCI/MHCII positive) & self-peptides
- Less than 5% αβ double-positive Thymocytes can recognize self-MHC.
- First step, (Ca 3% of thymocytes survive!!)
- Selection occurs in 3-4 days!!
Interaction of a double-positive T cell with a self-peptide:self-MHC complex during positive selection determines whether the T cell will become a CD4 or a CD8 T cell.
Negative selection:

Elimination of potentially autoreactive clones.

Requires several cell types besides epithelial cells: For example DC or macrophages
What self antigens are seen in the thymus?

- Ubiquitous cell-associated and soluble self-proteins

- The thymus displays all peripheral tissue antigens in thymic medullary epithelial cells
**AIRE (AutoImmune REgulator) gene**

- The aire gene, identified and characterized by two independent groups [K. Nagamine et al., Nature Genet. 17, 393 (1997); The Finnish-German APECED Consortium, Nature Genet. 17, 399 (1997)], is expressed by epithelial cells in a region of the thymus called the medulla.

- Interest in identifying the aire gene was sparked by a human autosomal recessive disorder called **autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)**. This disease is characterized by autoimmune destruction of endocrine organs, the inability to eliminate Candida yeast infections, and growth of ectodermal dystrophic tissue.

- Researchers surmised that identification of the **genetic mutation associated with APECED** would give important insights into mechanisms of autoimmunity, because this is the **only autoimmune disorder known to be inherited in a Mendelian fashion**.
All tissue-restricted antigens are expressed in the thymus
AIRE contains several domains that are related to those in other transcription factors. Combinatorial interactions between AIRE, unmodified H3K4, DNA topoisomerase 2-alpha, DNA-dependent ProteinKinase, and RNA Polymerase II recruit AIRE to a TRA promoter, and this results in transcription elongation, mRNA processing, and TRA gene expression.
What self antigens are seen in the thymus?

Because the thymus express the universe of tissue-restricted antigens (by AIRE), the related thymocytes recognizing them with high avidity will be deleted in the thymus, whereas those with low avidity will mature and will migrate in the periphery.

As a consequence, all mature T cells are autoreactive (low-avidity) and can potentially be activated by non-self-antigens (high-avidity) by cross-reactivity!!

Take home messages
Clonal Deletion Prunes but Does Not Eliminate Self-Specific αβ CD8+ T Lymphocytes in healthy individuals

Fructose bisphosphate aldolase (FBA) ALSDHHIYL
Keratin (KER) ALLNIKVKL
SMCY (male specific) FIDSYICQV
Preproinsulin (PPI) ALWMLLPL
Glutamic acid decarboxylase 65 (GAD) VMNILQYVV
....not only self-epitopes, but also neo-(self)epitopes can elicit Immunity....

...generally due to post-translational modifications of self-antigens.....

Neo-autoimmunity?
Citrullination or deimination is the conversion of the amino acid arginine in a protein into the amino acid citrulline. Citrulline is not one of the 20 standard amino acids encoded by DNA in the genetic code. Instead, it is the result of a post-translational modification by the peptidylarginine deiminases (PADs) replacing the primary ketimine group (=NH) by a ketone group (=O). This increases the hydrophobicity of the protein, which can lead to changes in protein folding, affecting the structure and function, and inducing immunogenicity of the protein!!

The most common tests for RA diagnosis is the presence in the serum of anti-citrullinated protein antibodies (ACPAs) that are the anti-CCP (cyclic citrullinated peptide) test and the Anti-MCV assay (antibodies against mutated citrullinated Vimentin).

- Anti-hnRNPK: König MF et al, Ann Rheum Dis. 2016...
- Anti-nmMyosin: Girard D et al, Clin Immunol and Immunopathol 1995...
Local immune activation in RA lungs

Malmström V. et al
Nat Rev Immunol, 2017
Involvement of citrullinated epitope in adaptive immunity
Porphyromonas gingivalis-mediated citrullination and induction of anti-citrullinated protein antibodies in rheumatoid arthritis

1. Circulating plasmablasts RA patients preferentially express ACPAs (~20% RA patients vs. 0% healthy controls).

2. The reactivities of RA patient-derived ACPAs are generated by somatic hypermutation.

3. The evolvement of ACPA-encoding B cells in RA patients is an antigen-driven process.

4. RA patient-derived ACPAs, but not non-ACPAs or control antibodies, react with *Porphyromonas Gingivalis* antigens.

5. Anti-*P. Gingivalis* immune responses in RA patients may initiate the generation of ACPAs.

*Take home messages*

Kaihong Su, Ph.D. Lab
Figure 1. The key fitting the lock: deamidation of islet peptides promotes their binding to disease-predisposing HLA class II molecules. Peptide binding to HLA-DQ molecules involves anchor pockets 1, 4, 6, 7 and 9 of the peptide. Amino acids at position 1 and 9 of a binding peptide are crucial in defining peptide binding affinity to HLA-DQ8, whereas amino acids on positions 2, 3, 5 and 8 of the peptide epitope are engaged in recognition by T cell receptors (TCRs) (Top). Active tissue transglutaminase (tTG) can modify glutamines (Q) on positions p1 and p9 of a binding peptide into glutamic acid (E), introducing a more favorable charge to the anchor pockets 1 and 9 (bottom), thereby strongly enhancing the binding affinity of this peptide to HLA-DQ8.

Bart O Roep, Maria JL Kracht, Menno van Lummel, Arnaud Zaldumbide

A roadmap of the generation of neoantigens as targets of the immune system in type 1 diabetes

Current Opinion in Immunology, Volume 43, 2016, 67–73
Insulin-defective ribosomal products (DRiPs) contribute to both CD4+ and CD8+ T cell autoimmunity in type 1 diabetes.
Presentation of $\alpha_{3,135-145}$ pep. of type IV collagen by HLA-DR15 and HLA-DR1 in Goodpasture disease

The peptide binding register of HLA-DR15-$\alpha_{3,135-145}$ is ISLWKGFSS (p1-Ile, p4-Trp, p7-Phe, and p9-Phe)

The peptide binding register of HLA-DR1-$\alpha_{3,135-145}$ is WISLWKGFSS (p1-Trp, p4-Leu, p6-Ly and p9-Ser)

DR15 selects α3135–145-specific Tconv cells but DR1 selects protective Treg cells

If ER peptide trimming is affected, the repertoire of pMHC I complexes is severely modified,… (Blanchard and Shastri, Current Opinion in Immunology, 2008)
Thymic selection of T cells requires recognition of pMHC complexes.

Thymus express all self-antigens of the body (AIRE).

Thymocytes recognizing pMHC on CEC migrate in thymic medulla (about 3%); the majority does not recognize anything and dye (neglect).

MHC II and I dictates the division of double positive CD4/CD8 thymocytes in single positive CD4 or CD8 cells, respectively.

In medulla, T cells recognizing pMHC on DCs with high avidity dye, those recognizing pMHC with low avidity migrate in the periphery.

As a consequence, all mature T cells are autoreactive (low-avidity) and can potentially be activated by non-self-antigens (high-avidity).

Not only self-epitopes, but also neo-epitopes.....

Needs of peripheral tolerance mechanisms avoiding autoimmunity.

Take home messages
Peripheral tolerance mechanisms

• To prevent inappropriate reactions against self antigens (“self-tolerance”)

• To prevent immune responses against harmless environmental antigens, commensal microbes

• To avoid excessive lymphocyte activation and tissue damage during normal protective responses against infections

• Failure of control mechanisms is the underlying cause of immune-mediated inflammatory diseases

by Abul K. Abbas et al.
Textbook 2015
(modified)
Efficient immune responses

Immunogenic antigen (microbe, vaccine)

APC

Antigen (peptide + HLA): signal 1

TCR

Costimulation (signal 2)

Naïve T cell

Effector and memory cells

by Abul K. Abbas et al.
Texbook 2015 (modified)
Human dendritic cells are heterogeneous

Blood

- Myeloid DC
  - BDCA1+ (*CD1c+)
  - BDCA3+ (*CD141+: TM)
  - CD11a+
  - Clec9A+

- Equivalent to CD8+ DC (cross-presentation)??

Skin

- Plasmacytoid DC
  - BDCA4+
  - BDCA2+
  - CD123+ (IL-3R)

- Langherans cells
  - Dermal CD1a+ DC
  - Dermal CD14+ DC

*CD1c antigen is a member of the CD1 family of proteins that are structurally related to MHC class I proteins and mediate the presentation of non-peptide antigens to T cells.
<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
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<th>Human</th>
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<tbody>
<tr>
<td></td>
<td>CD8+</td>
<td>CD8-</td>
<td>pDC</td>
<td>BDCA3+</td>
<td>BDCA1+</td>
<td>pDC</td>
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<td>CD11a</td>
<td>CD1c</td>
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<td></td>
<td>Clec9A</td>
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</tr>
<tr>
<td>Cross-presentation</td>
<td>+</td>
<td>-</td>
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<td>++</td>
<td>+</td>
<td>+</td>
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<td>pH in phagosomes</td>
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<td>nd</td>
<td>neutral</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>nd</td>
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</tr>
<tr>
<td>Cytosol transfer</td>
<td>+</td>
<td>-</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
CD8 T cells specific to cell-associated *Apoptotic* (Self) Epitopes

Which signals dictate divergent fate of cross-presentation: cross-priming vs cross-tolerance?

Physiological turn-over of Tissue cells

Tissue-resident iDC

Apoptotic cell

Cross-presentation

Proteasome

TAP

E.R.

Phagosome

MHC class I
Immature dendritic cells reside in peripheral tissues

Dendritic cells migrate via afferent lymphatics to regional lymph nodes

Immature dendritic cell in the deep cortex

- antigenspecific lymphocyte
- lymphoid follicle
- deep cortex
- medulla

macropinosome

regional lymph node

naive lymphocyte

Cross-tolerance

Figure 1-13 Immunobiology, 6/e. (© Garland Science 2005)
Microbial products, PAMPs, DAMPs, cross-priming
Two subsets of memory T cells with distinct migratory capacity and effector function

Different stimuli on DCs promote T cell differentiation

- iDC
  - CCR1-3, 5, 6
  - CXCR1, 2
  - (CCL2-5)
- mDC
  - PAMPs, DAMPs, Microbial products Adjuvants
  - CD45RA
  - CCR7
  - CD62L
  - IL7R
  - BCL-2
  - Naïve T Cell
  - CCR7
  - CXCR5
  - BCL-6
  - TFH
  - B Cells and antibodies
  - CD45RA
  - CCR7
  - CD62L
  - CD45RO
  - IL-7R, IL-15R
  - T cell areas of lymph nodes and spleen

- iTreg
  - TGF-β
- Th2
  - IL-4, IL-5, IL-13
  - Parasites / Allergy
- Th1
  - IFN-γ
  - TB / Virus / autoimmunity
- Th17
  - IL-17, IL-22
  - Fungi / autoimmunity
- Th22
  - IL-22
  - Skin immunity
- TRM
  - IFN-γ, TNF
  - Tissue immunity

- TGF-β
  - BCL-2
  - IL-4
  - IL-12, IFNs
  - IL-6, IFN-γ, TGF-β
  - IL-6, TNF
  - IL-6, IL-1β
  - IL-16, IL-1β, TGF-β
  - CCR4
  - CCR5
  - CXCR3
  - CCR3
  - CCR10
  - CCR7
  - CCR6
  - CCR1
  - CXCR1, 2
  - CCL2
  - CCL3, 5, 6
  - ELR, SLC
  - MDC
  - SDF-1
  - Tissue immunity
  - BCL-2
  - BCL-6
  - CD69
  - CD103
  - Effector Memory T (T_EM)
  - Peripheral tissues
Efficient immune responses vs. Peripheral tolerance

Immunogenic PAMP/DAMP (microbe, vaccine)
- Mature or Stimulatory DC
- TCR
- Antigen (peptide + HLA): signal 1
- Costimulation (signal 2)
- Naïve T cell
- Effector and memory cells

Tolerogenic antigen (e.g. self)
- Immature or Tolerogenic DC
- Tolerance: functional inactivation or cell death, or sensitive to Treg suppression

by Abul K. Abbas et al.
Texbook 2015
(modified)
Anergy: long-term functional unresponsiveness which occurs when lymphocytes are exposed to the antigen in the absence of proper co-stimulation. In the absence of co-stimulation inhibitory signals dominate.
Under condition in which persisting (self or non-self) antigens cause chronic inflammation, the coinhibitory signals dominate..., avoiding hence excessive, acute, irreversible tissue damage!

This is the most important reason why the diseases related to persisting self- (autoimmunity) or non-self (viral infections...) immune responses are generally chronic!

These mechanisms allow a long-term survival of the host....
The B7:CD28 families

<table>
<thead>
<tr>
<th>Name</th>
<th>B7-1 (CD80)</th>
<th>B7-2 (CD86)</th>
<th>ICOS-L (CD275)</th>
<th>PD-L1 (B7-H1, CD274)</th>
<th>PD-L2 (B7-DC, CD273)</th>
</tr>
</thead>
</table>

Ligands on APCs and other cells

Receptors on T cells

<table>
<thead>
<tr>
<th>Name</th>
<th>CD28</th>
<th>CTLA-4</th>
<th>ICOS</th>
<th>PD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major function</td>
<td>Activation (naive T cells)</td>
<td>Inhibition (mediates suppressive function of regulatory T cells)</td>
<td>Activation (follicular helper T cells in antibody responses)</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

[Diagram showing interactions between ligands and receptors]
CTLA-4 competitively inhibits B7-CD28 engagement

CTLA-4 blocks and removes B7 → lack of costimulation → T cell inhibition

Knockout of CTLA-4 in mice and heterozygous mutation in humans results in immune dysregulation (lymphoproliferation, multi-organ autoimmunity)
Cell-intrinsic factors of CLTA-4 regulation

**Competition**

- **a**
  - APC
  - MHC class I/II
  - TCR
  - CD80 and/or CD86
  - CTLA4
  - T cell
  - CD28

  Competition for CD80 and/or CD86 reduces CD28 co-stimulation and delays onset of disease

**Regulation of signalling components**

**b**

- SHP2 → LAT and ERK dephosphorylation

  Possible inhibition of immune response

**c**

- PP2A → AKT dephosphorylation

- CBL-B: E3 ligase (ubiquitylation pathway)

  Upregulation of CBL-B raises TCR signalling threshold — possible immune regulation?

Rudd, 2008
Cell-intrinsic factors of CLTA-4 regulation

Inhibition of lipid-raft and microcluster formation

- Lipid raft
- TCR ζ-chain
- Downregulation of lipid-raft formation inhibits immune response?

Disruption of ZAP70-containing microcluster formation impairs TCR activation

- LAT, SLP76 and GADS adaptors

Rudd, 2008
The PD-1 inhibitory pathway

- PD-1 recognizes two widely expressed ligands (PD-L1, PD-L2)

- Knockout of PD-1 leads to autoimmune disease (less severe than CTLA-4-KO)
The PD-1 inhibitory pathway: mechanism of action
Actions of PD-1

• PD-1 attenuates TCR signaling in responding T cells leading to the so-defined “T cell exhaustion”

• Greater role in CD8 than in CD4 T cells

• Also expressed on follicular helper T cells; function?
## Functions of immune checkpoints CTLA-4 and PD-1

<table>
<thead>
<tr>
<th></th>
<th><strong>CTLA-4</strong></th>
<th><strong>PD-1</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major site of action</strong></td>
<td>Lymphoid organs</td>
<td>Peripheral tissues</td>
</tr>
<tr>
<td><strong>Main signals inhibited</strong></td>
<td>CD28 costimulation (by reducing B7)</td>
<td>Chronic antigen TCR stimulation</td>
</tr>
<tr>
<td><strong>Cell type suppressed</strong></td>
<td>CD4+ &gt; CD8+</td>
<td>CD8+ &gt; CD4+</td>
</tr>
</tbody>
</table>
Actual and future therapeutic strategies in Autoimmune Diseases (RA, IBD, Psoriasis...)

- Biologicals inhibiting inflammatory cytokines (TNF-α, IL-1, IL-17, IL-6...)
- Immunoregulatory cytokines
- Therapeutic reagents stimulating chek-point molecules on T cells
- Tolerogenic Vaccines
- Reagents improving Treg functions
- etc
The usage of checkpoint inhibitors taught by tumors
T cell responses to tumors

by Abul K. Abbas et al.
Textbook 2015
(modified)
Cancer Immunoediting Model (RD Schreiber modified)

Elimination

Equilibrium* (immune-mediated selection)

Escape

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Elimination</th>
<th>Equilibrium*</th>
<th>Escape</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>++++</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>++++++</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>IL-17</td>
<td>+++</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>IL-4</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>IL-10</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>++</td>
<td>++++</td>
</tr>
</tbody>
</table>

*homeostatic equilibrium persists for several decades establishing a state of chronic low-level inflammation that is instrumental for limiting tumor spread, ultimately leading to long-term survival of the host.
Typically, 20-40% response rates with peak of 60% in melanoma by immune check point molecule combination.

The landscape of T cell activating and inhibitory receptors that can be exploited for new combination strategies in cancer therapy.
Risks of blocking CTLA-4- or PD-1-mediated tolerance in tumors

Autoimmune reactions
- Inflammatory disorders (such as colitis, epiphitis...) in >50% treated patients
- Severity of adverse effects has to be balanced against potential for treating serious cancers

...teaching from these data for the therapy of autoimmune diseases....
Therapeutic potential by stimulating **CTLA-4 or PD-1** in Autoimmunity

Inducing tolerance to prevent graft rejection, treat **autoimmune** and allergic diseases, and prevent immune responses in gene therapy and stem cell transplantation

The usage of recombinant CTLA-4 in human autoimmune diseases (R.A.) provides beneficial effects in a significant percentage of patients
The landscape of T cell activating and inhibitory receptors potentially exploitable in the therapy of autoimmune diseases.
Abatacept (CTLA4-Ig) homodimer

CTLA-4 domain:
Extracellular sequence of human CTLA-4

IgG1 Fc domain:
Hinge, CH2 and CH3 regions of human IgG1 Fc sequence
CTLA4-Ig binds to B7 molecules and inhibits CD28-B7 interactions; suppresses T cell response

by Abul K. Abbas et al.
Texbook 2015
Additional possible mechanisms of action to consider:

B7 engaged by CTLA4-Ig directly signals to dendritic cells

Suppresses T cell response

IDO: Indoleamine 2,3-dioxygenase

Mellor et al. JI 171:1652-5
Boasso et al. Blood 105: 1574-81
Munn et al. JI 172: 4100-10

T cell proliferation is inhibited
Additional possible mechanisms of action to consider:
Potential functions of the IgG1 Fc portion of abatacept

- Binding to Fc receptors
- Antibody-dependent Cellular cytoxicity (ADCC)
- Phagocytosis/clearance of opsonized cells

Resulting in elimination of B7-expressing cells and suppression of T cell response

by Abul K. Abbas et al.
Textbook 2015
The paradox: CTLA4-Ig may disrupt critical pathways required for maintenance of immune tolerance
CD28-B7 interactions are essential for both thymic development of Tregs and for their peripheral homeostasis.

Decrease in Tregs (CD4+CD25+) cells in lymph nodes of wildtype mice treated with CTLA4Ig for 10 days.

Decreased development of Tregs in the thymus in B7 knockout mice.

In certain inbred mouse strains, autoimmune disease is exacerbated in the absence of B7 or CD28 due to loss of Tregs.

Tang et al. JI 171: 3348-52

Williams et al. (not published)
Abatacept (CTLA4-Ig) Mechanism of Action Summary

Mechanisms of action that may function to ameliorate RA:

- Blocking CD28 costimulation
- Induction of suppressor antigen-presenting cells (via IDO)
- Depletion of B7-expressing cells

Mechanisms of action that may exacerbate autoimmunity:

- Inhibition of T regulatory (CD4+CD25+) cell maintenance/development

Caution in using CTLA4-Ig agonists in autoimmunity therapy!?
T regulatory cells are critical components in the maintenance of peripheral tolerance to tissue-specific self-antigens.

In both humans and mice, absence of T regulatory cells is associated with aggressive autoimmunity.
SCURFY MOUSE

X-linked recessive disorder of immune regulation. Affected males have scaly skin, apparent infection, diarrhea, progressive Coombs-positive anemia, thrombocytopenia, gastrointestinal bleeding, hypogonadism, leukocytosis, lymphadenopathy and cachexia. They die within four weeks after birth.

IPEX
recessive X-linked disorder characterized by the neonatal onset of insulin-dependent diabetes mellitus (IDDM), infections, enteropathy, thrombocytopenia and anemia, other endocrinopathy, eczema and cachexia

Tregs, guardians for life


Treg commitment is determined at epigenetic level

From: Regulatory T Cells Stay on Course Hamann, Immunity 2012
Properties of regulatory T cells

• **Phenotype:** CD4+, high IL-2 receptor (CD25), low IL-7 receptor, Foxp3 transcription factor; other markers (Helios, OX40...)

• **Essential features of stable Tregs:**
  - Foxp3 expression: requires demethylated non-coding CNS2 sequence in promoter (TSDR)
  - 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, but ability to steal IL-2

*Take home messages*

by Abul K. Abbas et al.
Texbook 2015
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

- 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

by Abul K. Abbas et al.
Texbook 2015
Future regulatory T cell therapy

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

• **Therapeutic goal:** induction or activation of Tregs in immune diseases
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

Surprising conclusion from knockout mice: the non-redundant function of IL-2 is in controlling immune responses

by Abul K. Abbas et al.
Textbook 2015
(modified)
Tregs induce anergy of autoimmune T cells (Melan-A CD8+ T cells) in healthy individuals.
Tregs induce anergy of autoimmune T cells in healthy individuals

Fig. 4 Detection of low-affinity anergic self-reactive CTLA-4+CCR7+CD8+ T cells in healthy individuals.

Yuka Maeda et al. Science 2014;346:1536-1540
Mechanisms by which autoreactive T cells that escaped the control of p. immulogical tolerance (i.e., infections, pollution...), can establish and maintain autoimmune diseases.....

This remain an open question....