BBS755: Tolerance and Autoimmunity

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Joonsoo Kang, Ph.D.
Department of Pathology
ASC9-1047, 6-2759
A T lymphocyte scans a Dendritic cell

Immunology is a science of self:non-self discrimination

Overview

1. Peripheral Tolerance (contrast to Central Tolerance, will deal mostly with T cells)
2. How to stop T cell activation: Prevent the second signal (Co-stimulation: CD28)
3. Inhibition of Co-stimulation to curb T cell activation (CTLA-4)
4. Inhibition of T cell activation in trans by regulatory T cells
5. Autoimmune diseases are the consequence of failures in T cell tolerance and homeostasis
Tolerance and autoimmunity

• Autoimmunity results from a breakdown of self-tolerance

• Tolerance = lack of response to a particular antigen (T and B cells)

• Possible mechanisms:
  – Clonal deletion (death)
  – Clonal anergy (unresponsiveness, lack of the 2nd signal)
  – Ignorance (lack of sufficient signals from TCR)
  – Suppression (mediated by “regulatory T cells”)
Tolerance can occur at two stages

• During lymphocyte development
  – Clonal deletion

• After development, in mature circulating lymphocytes
  – Clonal anergy
  – Clonal deletion
  – Ignorance
  – Suppression
Why Peripheral Tolerance

• Not all self-reactive T cells are deleted during thymic development
  – Tissue-specific antigens expressed only outside the thymus; **Aire** does not induce all peripheral antigen expression (Aire-deficient: APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy)
  – TCR repertoire is biased towards MHC recognition

• During immune response to foreign antigens self-reactivity can emerge
  – Cross-reactive TCRs that can recognize self at low affinity can be activated by inflammation
  – Inflammation and injury can release normally sequestered self-antigens (proper termination of immune response is critical)
Since tolerance is the absence of activation, understand first lymphocyte activation

Requirements for Adaptive Immune Responses

• Generate robust antigen-specific immune response to relatively low antigen load
• Ensure that the immune response is productive (can clear the pathogen)
• Prevent inappropriate T cell responses to self antigens (tolerance)
T cell activation
Co-stimulation: 2-signal model

T cell activation: Balance between positive and negative signals

CTLA-4 acts in a conventional T cell intrinsic manner

- Activation
- Proliferation
- Effector function

- Inhibition of proliferation
- Altered cytokine secretion
- Anergy
Steps in Adaptive T cell Immune Responses

1. Priming
2. Activation
3. Expansion
4. Development of effector function
5. Homeostasis
6. Contraction of Ag-sp T cell pool
7. Memory formation
The Antigen Receptor is not enough

- MHC/peptide presentation on planar membranes or fixed APC’s do not activate normal naïve T cells
- TCR crosslinking alone does not induce IL-2 production by normal T cells
- TCR engagement alone can lead to T cell non-responsiveness
- Addition of accessory cells could overcome the inability of anti-CD3 crosslinking to stimulate T cells
Peripheral tolerance achieved by regulating costimulation

The activation of naïve T cells requires two signals
- TCR binding MHC/peptide (Signal 1)
- CD28 binding B7 (Signal 2)

B7 is induced only on APCs during inflammation
- IFNg
- TLR signaling

Thus, no inflammation or infection no naïve T cell activation. To achieve tolerance to self, tightly regulate costimulatory signals
Costimulatory molecules: CD28 on T cells, B7 ligands on APCs, and other modulators (CTLA-4 on T cells, a negative regulator of CD28)
Function of Costimulation

- Enhance the magnitude of the T cell response
- Sustain the T cell response
- Regulate the nature of the T cell effector function
- Provide a link between the innate and the adaptive immune systems
- Minimize inappropriate T cell activation by tissue-specific antigens (protect against the consequences of positive thymocyte selection)
Features of Costimulation

- Costimulatory signal is antigen-independent
- Signal does not stimulate T cells in the absence of TCR engagement
- Primarily provided ‘in cis’
- Provides unique signals beyond adhesion
- Supports and augments T cell proliferation, cytokine secretion, and effector function
- Requirement for costimulation is not absolute but the nature of the response and subsequent responses may be altered
CD28

- contains one V-like Ig domain
- constitutively expressed on T cell surface
- binds to B7-1 (CD80) and B7-2 (CD86) (Kd 20-40mM)
- originally proposed to be expressed as a dimer but some evidence for monomers
CD28 crosslinking augments T cell proliferative response to TCR ligation
CD28-mediated increase in IL-2 synthesis
CD28: Prototypic Costimulatory Molecule

- Extracelluar MYPPPY motif --- > ligand binding
- Contains putative SH2 (YMNM) and SH3 domains in the cytoplasmic tail that are proposed to be important for signal transduction
- Expressed on all murine T cells and >80% human T cells
  - Increased % CD28- T cells in chronic activation
- CD28 and ligands are expressed the thymus
  - do not appear to be essential for normal selection
Characteristics of CD28-deficient mice

- Normal thymocyte development
- Reduced T cell responses to mitogens (ConA), peptide antigens, superantigens, some viruses (VSV, but not LCMV)
- Decreased T cell survival following activation
- Decreased cytokine production
- Reduced ability to provide B cell help
- Reduced total serum Ig (20% of normal); decreased IgG1 and IgG2a; normal IgM
Summary of key points

• CD28 is the primary costimulatory molecule for naïve T cells
• CD28-mediated costimulation:
  – increases T cell activation
  – Increases cell metabolism
  – prevents anergy induction
  – prevents apoptosis
  – induces expression of Bcl-xL
  – induces expression of IL-2
  – as a function of optimal activation, induces expression of other costimulatory molecules (e.g. CD40L)
CD28-mediated signal transduction: How does it work?

- Signaling pathway(s) have not been fully elucidated
- Two models proposed:
  - CD28 and TCR signaling pathways are independent, but parallel and synergistic
  - CD28-mediated signaling augments TCR-mediated signaling
  - Not mutually exclusive
Integration of TCR and CD28 Signals

IL-2, Bcl-xL, Myc etc.

CD28  TCR
T cell activation

Acuto and Michel, 2003
CD28 and CTLA-4 signaling

Rudd et al. Imm Rev 2009
What initiates CD28-mediated costimulation?
B7 FAMILY

• B7-1 (CD80): first B7 molecule cloned (Yokochi et al (1982))
• Large family of type 1 transmembrane glycoproteins composed of two groups, each of which has subgroups
• Member of the Immunoglobulin superfamily
• Many family members are expressed in non-lymphoid tissues e.g. brain, heart
• Function of most family members is not known
B7-1 (CD80)

- two extracellular Ig-like domains
- expressed as homodimers on the cell surface
- expressed on APC and activated lymphocytes
- differentially induced upon cell activation and/or inflammation
- 20-40% primary sequence similarity between family members
B7-1 and B7-2 are induced upon APC Activation

- B7-1 is constitutively expressed on DCs, peritoneal MØ, monocytes
- B7-1 expression is induced on activated B cells, eg LPS, IL-4 but not by BCR crosslinking
- B7-2 expressed at low levels on naïve T cells and B cells
- B7-2 is induced on DC, monocytes and activated T cells (mouse)
- LPS upregulates B7-2 on MØ and B cells but most on DC’s
- Kinetics of upregulation of B7-1 and B7-2 are different: B7-2 expression is induced/decreased faster than B7-1
Link between Innate and Adaptive Immune responses

- T cells constitutively express CD28
- Pathogens activate APCs (via Toll receptors, uric acid)
- Activation of APCs upregulates the expression of B7-2 and B7-1 expression
- APC expressing high levels of B7 present antigen to specific T cells thereby providing signal 1 and signal 2
- Activated T cells express CD40L and secrete cytokines --> B cell help
Adjuvants induce costimulatory signals

• Soluble protein antigens are mixed with adjuvants to induce an immune response (expt conditions, vaccine)

• Most adjuvants (Freund’s, Alum) have two components
  – Oil, to prevent rapid clearance of antigen
  – Dead bacteria, to induce B7 expression on phagocytic cells
Phagocytosis and breakdown of bacteria by macrophage induces expression of MHC class II and B7

Macrophage delivers a co-stimulatory signal to T cells recognizing bacterial peptide antigen

Proliferation and differentiation of T cells specific for bacterial protein

Figure 6-13 The Immune System, 2/e (© Garland Science 2005)
Summary of Key Points

- B7-1 and B7-2 are only expressed on professional APC
- B7-1 and 2 are expressed at very low levels on resting APC
- APC activation by inflammation or pathogens induces B7 expression
- B7-2 and B7-1 support CD28-mediated costimulation but are induced by different stimuli and with differing kinetics
What happens when T cells encounter Signal 1 without Signal 2?

- Example: naïve T cell has a TCR specific for an MHC/peptide complex found on a normal liver cell
  - Inactivation: T cell becomes nonresponsive to later stimulation with professional APC (anergy) or is induced to die
  - Ignorance: T cell ignores encounter, and remains responsive to later stimulation with professional APC
Adjuvant Effect!
Activation of T cell clones in the absence of a second signal: Cellular phenomenon of Anergy

- Stimulation of the T cell clone with EDCI-fixed, Ag peptide-pulsed APC resulted in an impaired proliferative response and non-responsiveness upon secondary challenge.
- Unresponsiveness could be reversed with exogenous IL-2 or freshly isolated splenocytes.
- This unresponsive state was termed “Anergy”
Anergy versus Ignorance as mechanisms of T cell Tolerance

- **Anergy/Hyporesponsive**
  - No proliferation
  - No IL-2
  - Non-hematopoietic cells
  - Tumor cells
  - No inflammation

- **Non-Responsive**
  - No proliferation
  - No IL-2
  - “Professional” APC
    (Dendritic cells, etc.)

**Cbl, Itch, Grail E3 ubiquitin ligases**

**TCR**

**CD28**

**Ag/MHC**

**B7**
Anergy versus Ignorance

• Strength of Signal 1 (TCR) in the absence of sufficient Signal 2 (costimulation)
  • Strong TCR signal = anergy
  • Weak TCR signal = ignorance

• T cells are biochemically distinct
  • Anergy - hyporesponsive
  • Ignorant - capable of responding to subsequent antigenic challenge
CD28-mediated costimulation augments the T cell response
- proliferation
- cytokine production
- cell survival

Are these functions unique to CD28 or are there other costimulatory molecules?
BTLA

Modified from Freeman and Sharpe, 2003
How do CD28 and ICOS differ?

- **Expression pattern:** CD28- high constitutive expression
  - ICOS-low constitutive expression, induced upon activation; on Tfh cells

- **Unique ligands:**
  - ICOS does not have the conserved MYPPPY motif; does not bind to CD80/86

- **Signaling:**
  - CD28: 4 Tyr; ICOS: 2 Tyr
  - ICOS is more effective at activating PI3K
  - CD28 but not ICOS- binds to Grb2, activates JNK

- **Effector function:**
  - ICOS does not markedly induce IL-2 and bcl-xL
  - CD28 and ICOS costimulation enhance IFNγ and IL-4 secretion
  - Impt for B cells for making Abs
CD28-mediated costimulation induces expression of other costimulatory molecules
PD-1, activation-induced analog inhibitor of T cell function
TNF/TNFR family members in costimulation of T cell responses

Modified from Watts, T, 2005, ARI
‘Costimulatory’ molecules that function to inhibit T cell responses: Coinhibitors

Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), Programmed Death-1 (PD-1)
B and T lymphocyte attenuator (BTLA),...
CTLA-4 (CD152)

- CD28 homologue (<30% sequence similarity)
- Structural similarities: IgV, MYPPPY
- Shares ligands B7-1 and B7-2
- >100X higher avidity for ligand compared to CD28
- Expressed on CD4 and CD8 T cells
- Expressed on thymocytes
- Unique expression pattern compared to CD28
- Unique function compared to CD28
CTLA-4, conventional T cell-intrinsic and -extrinsic function

• **CTLA-4 is induced upon naïve T cell stimulation**
  – mRNA induced within hour(s); does not require optimal T cell activation
  – basal level of expression in previously activated T cells
• Unique intracellular localization: traffics rapidly to/from the cell surface
• Endocytosed into clathrin-coated pits by AP-50 binding; requires the cytoplasmic tail
• Unique function compare to CD28: inhibitory signal rather than a stimulatory signal

• **CTLA-4 is constitutively expressed at a high level on regulatory T cells**
CTLA-4-deficient mice develop a fatal lymphoproliferative disease

- Normal thymocyte development
- Peripheral T cells become activated leading to fatal lymphoproliferative disorder
- T cell activation apparent as by 4 days post-partum
- Disorder is initiated by CD4$^+$ T cells
- T cell activation requires CD28-mediated costimulation

VERY DIFFERENT PHENOTYPE COMPARED TO Cd28$^{-/-}$ MICE
CTLA-4 crosslinking inhibits T cell proliferation
CTLA-4 crosslinking inhibits naïve T cell activation in response to TCR and CD28 stimulation

<table>
<thead>
<tr>
<th>CTLA-4 X-LINKING:</th>
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<tbody>
<tr>
<td>CD69 induction</td>
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<tr>
<td>CD25 (IL-2Ra) induction</td>
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<tr>
<td>S-phase entry mediators</td>
</tr>
<tr>
<td>IL-2 production</td>
</tr>
<tr>
<td>TGFβ production</td>
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<tr>
<td>NF-AT translocation</td>
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<tr>
<td>Bcl-xL induction</td>
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</tbody>
</table>
CTLA-4 signal transduction

Exact mechanism of Signal transduction is unknown
CD28 and CTLA-4 signaling

Rudd et al. Imm Rev 2009
What is the mechanism(s) mediating CTLA-4 inhibitory signals?

• Several possibilities:
  – Inhibit TCR-mediated signals
  – Secretion of immunosuppressive cytokines
    • TGFβ
  – Inhibit CD28-mediated signals
    • Directly
    • Indirectly - competition for ligand
  – Unique signaling pathway
• CTLA-4 also inhibits stimulation mediated by other costimulatory molecules (ICOS)
  --> not specific to CD28-mediated signals
Autoimmunity can result upon the loss of Inhibitory Signal 2

- Coinhibitors
  CTLA-4 (ligand B7.1/2), PD-1 (PDL1, 2) BTLA (ligand HVEM, herpesvirus-entry mediator).
- Mice deficient in these genes develop autoimmune phenotypes
  - eg. CTLA-4^{-/-}: CD4+ T cell, most severe
  - PD-1^{-/-}: T and B cell
  - BTLA-4^{-/-}: T and B cell

On the flip side, blocking CTLA-4 can enhance T cell response: Ipilimumab (BMS/MEDX) in P3 clinical trials for melanoma treatment
Treatment of metastatic melanoma (Stage IV) using a blocking Ab to CTLA-4 (Ipilimumab® MEDAREX/BMS)

Giao Q. Phan†, James C. Yang†, Richard M. Sherry†, Patrick Hwu†, Suzanne L. Topalian†, Douglas J. Schwartzentruber†, Nicholas P. Restifo†, Leah R. Haworth†, Claudia A. Seipp†, Linda J. Freezer†, Kathleen E. Morton†, Sharon A. Mavroukakis†, Paul H. Duray‡, Seth M. Steinberg§, James P. Allison¶, Thomas A. Davis, and Steven A. Rosenberg†,††
PNAS 2003, 100:8372
53% of patients had an objective response, all with tumor reduction of 80% or more.

Computed Tomographic (CT) Scans of the Chest Showing Tumor Regression in a Patient Who Received the Concurrent Regimen of Nivolumab and Ipilimumab.
Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease

Hironori Ueda¹, Joanna M. M. Howson¹, Laura Esposito¹, Joanne Heward², Hywel Snook¹, Giselle Chamberlain¹, Daniel B. Rainbow¹, Kara M. D. Hunter¹, Annabel N. Smith¹, Gianfranco Di Genova¹, Mathias H. Herr¹, Ingrid Dahlman¹, Felicity Payne¹, Deborah Smyth¹, Christopher Lowe¹, Rebecca C. J. Twells¹, Sarah Howlett¹, Barry Healy¹, Sarah Nutland¹, Helen E. Rance¹, Vin Everett¹, Luc J. Smink¹, Alex C. Lam¹, Heather J. Cordell¹, Neil M. Walker¹, Cristina Bordin¹, John Hulme², Costantino Motzo³, Francesco Cucca³, J. Fred Hess⁴, Michael L. Metzker⁴, Jane Rogers⁵, Simon Gregory⁵, Amit Allahabadia⁶, Ratnasingam Nithiyananthan⁶, Eva Tuomilehto-Wolf⁶, Jaakko Tuomilehto⁶, Polly Bingley⁷, Kathleen M. Gillespie⁷, Dag E. Undlien⁸, Kjersti S. Rønningen⁸, Cristian Guja⁹, Constantin Ionescu-Tirgoviste¹¹, David A. Savage¹², A. Peter Maxwell¹³, Dennis J. Carson¹⁴, Chris C. Patterson¹⁶, Jayne A. Franklyn², David G. Clayton¹, Laurence B. Peterson¹⁶, Linda S. Wicker¹, John A. Todd¹ & Stephen C. L. Gough²
Summary of Key Points

• CTLA-4 shares ligands but has unique function compared to CD28
• CTLA-4 ligation inhibits some TCR- and CD28-mediated signals
• CTLA-4 function has requirements similar to CD28 (Signal 2)
  – requires TCR signals (‘co-’)
  – provided in cis
  – antigen-independent
• T cell activation is regulated by the sum of the signals: stimulatory and inhibitory
Active dampening of autoreactive T cells in trans by regulatory CD4+ T cells

- Recently identified subset of CD4+ T cells called “regulatory T cells” - Foxp3+CD25+ (Treg cells)
- FOXP3 is a Forkhead transcription factor absolutely required for Treg cell function. Inducible by TGFβ.
- Develop in the thymus. Need IL-2 (IL-15) and CD28
- Function to inhibit activation and proliferation of conventional T cells, even when costimulatory signals are present
- When Treg cells are absent (Foxp3 mutants), individuals get autoimmune diseases (IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked)
Regulatory T cells maintain T cell quiescence

Deletional tolerance (recessive)
- Self-reactive T cells are deleted in the thymus. Occasionally, self-reactive T cells may escape deletion
- In the periphery such escaped self-reactive T cells can cause tissue damage

Regulatory tolerance (dominant)
- T cell specific for self antigen becomes a regulatory T cell ($T_{reg}$)
- Cytokines (IL-10 and TGF-β) produced by $T_{reg}$ inhibit other self-reactive T cells

Figure 13-14 Immunobiology, 6/e. (© Garland Science 2005)
CD4+ CD25+ Treg cells inhibit colitis by migrating to the colon and mesenteric lymph nodes, where they interact with dendritic cells and effector T cells.
CD4 CD25 $T_{reg}$ cells proliferate and inhibit the pathogenic effector T cells

After inflammation resolves CD4 CD25 $T_{reg}$ cells remain in clusters with dendritic cell and pathogenic effector T cells
Mechanism of Treg cell-mediated suppression

Multiple, perhaps redundant, pathways are employed

- Immunosuppressive cytokines, TGFβ (acts on T cells and APCs) and IL-10 (on APCs)
- Direct cell killing by Granzymes and Perforin (in a tumor environment)
- Sink for IL-2
- Modifications of APCs so that they are not immunogenic (need CTLA-4?)
- There are also other FOXP3neg “regulatory T cell subsets” (e.g. Tr3 making IL-10)
CTLA-4 is absolutely required for Treg cells to maintain systemic conventional T cell homeostasis

CTLA-4 Control over Foxp3+ Regulatory T Cell Function.
Kajsa Wing et al. (Foxp3-CreTg x Ctl4fx/fx mice succumb to lymphoproliferative, autoimmune disease; delayed by ~5-7 weeks compared to Ctl4-/- mice)
Science 10 October 2008: Vol. 322. no. 5899, pp. 271 - 275

CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance.
Randall H. Friedline et al. (Ctl4-/- T cells are maintained in a quiescent state by CTLA-4+ Treg cells in mice)
Mechanism of Treg action

Suppression of autoreactive T cells by regulatory T cells requires them to interact with the same antigen-presenting cell.

\[ \text{IDO (indolamine 2, 3-dioxygenase)} \]

[Figure 11-21 The Immune System, 2/e (© Garland Science 2005)]
CTLA-4 function

- Naïve T
- Activated T
- FOXP3 Treg

Surface CTLA-4
i.c. CTLA-4
CTLA-4 function

- Naïve T
- Activated T
- FOXP3 Treg

Thymic selection?

TCR repertoire shaping?

Maintenance of naïve T cell tolerance and homeostasis

Contraction of expansion

Fail-safe rerouting of self-reactive T cells?

Friedline et al. JEM 2009
Jain et al. PNAS 2010
CTLA-4, FOXP3 and TGFβ: the tolerance troika

Loss of any one of the troika leads to early onset, fatal lymphoproliferative disease
<table>
<thead>
<tr>
<th>Type of tolerance</th>
<th>Mechanism</th>
<th>Site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central tolerance</td>
<td>Deletion</td>
<td>Thymus, Bone marrow</td>
</tr>
<tr>
<td>Antigen segregation</td>
<td>Physical barrier to self-antigen access to lymphoid system</td>
<td>Peripheral organs (e.g., thyroid, pancreas)</td>
</tr>
<tr>
<td>Peripheral anergy</td>
<td>Cellular inactivation by weak signaling without co-stimulus</td>
<td>Secondary lymphoid tissue</td>
</tr>
<tr>
<td>Regulatory cells</td>
<td>Suppression by cytokines, intercellular signals</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
<tr>
<td>Cytokine deviation</td>
<td>Differentiation to $T_{H2}$ cells, limiting inflammatory cytokine secretion</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
<tr>
<td>Clonal exhaustion</td>
<td>Apoptosis post-activation</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
</tbody>
</table>

Figure 13-16 Immunobiology, 6/e. (© Garland Science 2005)
B cell tolerance (Review)

- Defined as lack of specific antibody secretion to an antigen
- Two reasons B cell tolerance can occur
  - Failure to activate the B cell with the antigen (Signal 1)
  - Absence of T cell help for the B cell (Signal 2)
Ab production requires T cell help

Although most B cell responses require T cell help, still need B cell-specific tolerance

1. T-independent Ag’s
2. Bystander T cell help
3. Somatic hypermutation
<table>
<thead>
<tr>
<th>Multivalent self-antigen</th>
<th>Soluble self-antigen</th>
<th>No self-reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal deletion</td>
<td>Migrates to periphery</td>
<td>Migrates to periphery</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Anergic B cell</td>
<td>Mature B cell</td>
</tr>
</tbody>
</table>

**Immature B cell in bone marrow**

© 2000 Garland Publishing/Elsevier Science
Summary of key points

• Naïve T cells that encounter TCR signal without costimulatory signal can simply not respond and “ignore” the encounter
• Under some circumstances, naïve T cells that encounter a TCR signal without a co-stimulatory signal are functionally inactivated (anergy) or induced to die
• Anergy/deletion vs. ignorance may be determined by the strength of the TCR signal
• T cell activation is determined by a balance of stimulatory and inhibitory signals
• CD28 and CTLA-4 are the most critical costimulatory and coinhibitory receptors on T cells
• Regulatory CD4+ T cells (CD25+FOXP3+) are critical in maintaining self-tolerance and suppressing autoreactive T cells
• CTLA-4, FOXP3 and TGFβ are the key molecules controlling peripheral T cell tolerance
Autoimmunity

• Breakdown of self-tolerance
• Self-reactive T cells are present in the circulating pool, and are inappropriately activated
• Activated (effector) T cells migrate into tissues, where they encounter self-antigen and induce inflammation and tissue destruction

• Classification as for hypersensitivity reactions: according to the mechanism of tissue damage
  – Antibodies to cell surface or matrix proteins
    (Type II, e.g. Myasthenia gravis, Graves’ disease)
  – Immune complex-mediated
    (Type III, e.g. Systemic lupus erythematosus)
  – T cell-mediated
    (Type IV, IDDM, multiple sclerosis, rheumatoid arthritis)
# Types of autoimmunity

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>Autoantigen</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody against cell-surface or matrix antigens (type II)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>Rh blood group antigens, I antigen</td>
<td>Destruction of red blood cells by complement and phagocytes anemia</td>
</tr>
<tr>
<td>Autoimmune thrombocytopenia purpura</td>
<td>Platelet integrin gpIIb:IIIa</td>
<td>Abnormal bleeding</td>
</tr>
<tr>
<td>Goodpasture’s syndrome</td>
<td>Non-collagenous domain of basement membrane collagen type IV</td>
<td>Glomerulonephritis, pulmonary hemorrhage</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>Epidermal cadherin</td>
<td>Blistering of skin</td>
</tr>
<tr>
<td>Acute rheumatic fever</td>
<td>Streptococcal cell wall antigens, Antibodies cross-react with cardiac muscle</td>
<td>Arthritis, myocarditis, late scarring of heart valves</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>Thyroid-stimulating hormone receptor</td>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Acetylcholine receptor</td>
<td>Progressive weakness</td>
</tr>
<tr>
<td>Insulin-resistant diabetes</td>
<td>Insulin receptor (antagonist)</td>
<td>Hyperglycemia, ketoacidosis</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Insulin receptor (agonist)</td>
<td>Hypoglycemia</td>
</tr>
</tbody>
</table>

**Figure 11-1 part 1 of 3** The Immune System, 2/e (© Garland Science 2005)

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>Autoantigen</th>
<th>Consequence</th>
</tr>
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<tbody>
<tr>
<td><strong>Immune-complex disease (type III)</strong></td>
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<tr>
<td>Subacute bacterial endocarditis</td>
<td>Bacterial antigen</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>Mixed essential cryoglobulinemia</td>
<td>Rheumatoid factor IgG complexes (with or without hepatitis C antigens)</td>
<td>Systemic vasculitis</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>DNA, histones, ribosomes, snRNP, scRNP</td>
<td>Glomerulonephritis, vasculitis, arthritis</td>
</tr>
</tbody>
</table>

**Figure 11-1 part 2 of 3** The Immune System, 2/e (© Garland Science 2005)
## Types of autoimmunity

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>Autoantigen</th>
<th>Consequence</th>
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<tbody>
<tr>
<td><strong>T cell-mediated disease (type IV)</strong></td>
<td></td>
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<tr>
<td>Insulin-dependent diabetes mellitus</td>
<td>Pancreatic (\beta)-cell antigen</td>
<td>(\beta)-cell destruction</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Unknown synovial joint antigen</td>
<td>Joint inflammation and destruction</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Myelin basic protein, proteolipid protein</td>
<td>Brain degeneration. Paralysis</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Gluten modified by tissue transglutaminase</td>
<td>Malabsorption of nutrients Atrophy of intestinal villi</td>
</tr>
</tbody>
</table>

*Figure 11-1 part 3 of 3 The Immune System, 2/e (© Garland Science 2005)*
Mechanisms of tissue damage for Type II hypersens.

- Direct mechanisms of tissue damage
  - Autoantibodies directed against a cell surface receptor stimulate or block receptor function
- Indirect mechanisms leading to tissue damage
  - Antibody binding leads to activation of the complement cascade, and thus, to cell lysis
  - Antibody coated cells are cleared by phagocytic cells bearing Fc receptors or complement receptors
Indirect antibody-mediated tissue damage (Type II)

• Autoimmune hemolytic anemia
• Goodpasture’s syndrome
  – Autoantibodies against collagen in basement membranes
  – Antibodies bind to renal glomeruli and cause local activation of complement and an influx of neutrophils leading to tissue damage
Direct antibody-mediated tissue damage (Type II)

- Autoantibodies specific to a receptor can stimulate or block receptor function
- Example: Graves’ disease
  - Autoantibodies to thyroid stimulating receptor on thyroid cells
  - Leads to excessive production of thyroid hormone, leading to hyperthyroid condition
- Example: myasthenia gravis
  - Autoantibodies to acetylcholine receptor present at neuromuscular junctions
  - Antibodies induce receptor internalization and degradation
  - Leads to progressive muscle weakness, and can cause death
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Antigen</th>
<th>Antibody</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graves' disease</td>
<td>Thyroid-stimulating hormone receptor</td>
<td>Agonist</td>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Acetylcholine receptor</td>
<td>Antagonist</td>
<td>Progressive muscle weakness</td>
</tr>
<tr>
<td>Insulin-resistant diabetes</td>
<td>Insulin receptor</td>
<td>Antagonist</td>
<td>Hyperglycemia, ketoacidosis</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Insulin receptor</td>
<td>Agonist</td>
<td>Hypoglycemia</td>
</tr>
</tbody>
</table>
Immune complex-mediated tissue damage

- Type III hypersensitivity reactions
  - IgG antibodies against soluble self antigens
- Example: systemic lupus erythematosus
  - Chronic IgG production against ubiquitous self-antigens, e.g., DNA, histones, RNP complexes
  - Overwhelms normal mechanisms of immune complex clearance
  - Immune complexes present at very high concentrations
  - Complexes deposited in walls of small blood vessels in the kidney, joints and other organs, and on basement membrane in the epidermis
  - Antibody deposition leads to complement fixation and activation of phagocytic cells
T cell-mediated tissue damage

• Type IV hypersensitivity reactions
  – Direct T cell-mediated damage (inflammation)
• Examples: rheumatoid arthritis and multiple sclerosis
  – Affected tissues are infiltrated with activated T cells and macrophages
T cell mediated autoimmunity
Hypersensitivity IV

Mice injected with myelin basic protein and complete Freund's adjuvant develop EAE and are paralyzed

The disease is mediated by myelin basic protein-specific TH₁ cells

Disease can be transmitted by transfer of T cells from affected animal

Figure 13-3 Immunobiology, 6/e. (© Garland Science 2005)
Brain lesions in MS

early

acute

post-treatment

edema
fluid

Figure 28-3  Case Studies in Immunology, 4/e (© Garland Science 2004)
Susceptibility factors for autoimmune diseases

Susceptibility factors
• Genetic
  – Strongest genetic factor is the MHC type of the individual
  – A number of autoimmune diseases, including insulin-dependent diabetes mellitus (IDDM), Grave's disease, Hashimoto's thyroiditis, Addison's disease, rheumatoid arthritis, and multiple sclerosis, have shown genetic linkage to the CTLA-4 locus.

• Environmental
  – History of prior infection with specific infectious agents
Infections can trigger autoimmunity

- Initiating events leading to autoimmune disease not really understood
- Accumulating evidence indicates that infections play an important role
- Example: EAE
  - EAE only induced after injecting self-antigen in adjuvant containing dead bacteria
Established correlation between infection and autoimmunity

<table>
<thead>
<tr>
<th>Infection</th>
<th>HLA association</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Streptococcus</td>
<td>?</td>
<td>Rheumatic fever (carditis, polyarthritis)</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>HLA-B27</td>
<td>Reiter’s syndrome (arthritis)</td>
</tr>
<tr>
<td>Shigella flexneri, <em>Salmonella typhimurium</em>, <em>Salmonella enteritidis</em>,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em>, <em>Campylobacter jejuni</em></td>
<td>HLA-B27</td>
<td>Reactive arthritis</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>HLA-DR2, DR4</td>
<td></td>
</tr>
<tr>
<td>Coxsackie A virus, Coxsackie B virus, echoviruses, rubella</td>
<td>HLA-DQ2, HLA-DQ8, DR4</td>
<td>IDDM</td>
</tr>
</tbody>
</table>

Reactive arthritis

Chronic arthritis in Lyme disease

Figure 11-30 The Immune System, 2/e (© Garland Science 2005)
Mechanisms of autoimmunity

- Self-reactive T cells can be activated during an infection
- Infectious agent may induce co-stimulatory signals on cells expressing low levels of a self peptide
- Self-reactive T cells may cross-react to a pathogen-derived peptide (molecular mimicry)
- Infection may cause tissue damage and disrupt tissue barrier, leading to the release of a normally-sequestered self-antigen and presentation to T cells by professional APCs
“Pathogen-help” leading to autoimmunity (Review)

Figure 6-15 The Immune System, 2/e (© Garland Science 2005)
Molecular mimicry potentially resulting in autoimmunity
Pathogen elicited Ab crossreactivity leading to autoimmunity

Streptococcal cell wall stimulates antibody response

Some antibodies cross-react with heart tissue, causing rheumatic fever

Figure 11-29 The Immune System, 2/e (© Garland Science 2005)
Summary of Key points

• The cause(s) of autoimmunity is not known
• Infection can contribute to the initiation of autoreactivity in genetically susceptible individuals
• Damaged or unhealthy cells can provide stimuli to autoreactive T cells to breach the activational threshold
• Treg cells actively suppress autoreactive T cells
Treatments

- T cell-mediated autoimmune diseases are treated with immunosuppressive and anti-inflammatory drugs (e.g. Cyclosporin, Enbrel<anti-TNFalpha>, Raptiva<anti-CD11a>, Tysabri<alpha4-integrin antagonist>)

- The utility of anti-TNFalpha antibody treatment for many forms of autoimmune diseases
  - TNFalpha is a potent pro-inflammatory cytokine made by TH1 cells
  - Antibody seems effective at reducing pain and joint swelling in RA patients (Also, Rituxan, anti-CD20)

- Modulation of costimulatory signals (CD28 <Orencia, CTLA4Ig for RA>, CTLA-4 agonist, PD-1 agonist, NKG2D blockade)
Six taken ill after drug trials

Six men remain in intensive care after being taken ill during a clinical drugs trial in north-west London.

The six are being treated at Northwick Park hospital. The healthy volunteers were testing an anti-inflammatory drug at a research unit based at Northwick Park Hospital when they suffered a reaction. Relatives are with the patients, who suffered multiple organ failure. Two men are said to be critically ill. An investigation has begun at the unit, run by Parexel, which said it followed recommended guidelines in its trial. The men were being paid to take part in the early stages of a trial for the drug to treat conditions such as rheumatoid arthritis and leukaemia until they were taken ill on Monday within hours of taking it. Eight volunteers were involved, but two were given a placebo at the unit which is on Northwick Park Hospital's grounds but is run independently.

This is an absolutely exceptional occurrence - I cannot remember anything comparable

Richard Ley, Association of the British Pharmaceutical Industry

The hospital's intensive care director Ganesh Suntharalingam said the patients were admitted very quickly and were receiving "close monitoring and appropriate treatment". A Northwick Park Hospital spokesman said two were in a critical condition, while the other four were "serious but stable". Richard Ley, spokesman for the Association of the British Pharmaceutical Industry, said: "This is an absolutely exceptional occurrence. I cannot remember anything comparable."

The Medicines and Healthcare products Regulatory Agency (MHRA) immediately withdrew authorisation for the trial. An international warning has also gone out to prevent it being tested abroad. Its inspectors will visit the research unit and it is in contact with the local strategic health authority, the Department of Health and police about the cases.

Chief executive officer Professor Kent Woods said: "Our immediate priority has been to ensure that no further patients are harmed." We will now undertake an exhaustive investigation to determine the cause and ensure all appropriate actions are taken. "It had approved the trial and the drug had already been tested on animals and in a laboratory. Volunteers are paid up to £150 a day to take part in clinical trials. Healthy volunteers are used to test the safety of the drug in "phase one" of the trial before further tests with people who have the condition to determine whether the drugs work. Parexel, which was running the trial, said it had followed guidelines and such cases were extremely rare. Professor Herman Scholtz, from Parexel, said the clinical research organisation had followed regulatory, medical and clinical research guidelines during the study. He said: "When the adverse drug reaction occurred, the Parexel clinical pharmacology medical team responded swiftly to stop the study procedures immediately." He added: "Such an adverse drug reaction occurs extremely rarely and this is an unfortunate and unusual situation."

Since our unit is located within the hospital, we have immediate access to world-class medical care and we did everything possible to get the patients treated as quickly as possible. BBC LONDON 3/15/2006
Were monocytes responsible for initiating the cytokine storm in the TGN1412 clinical trial tragedy?

Sandilands GP, Wilson M, Huser C, Jolly L, Sands WA, McSharry C.
University Department of Pathology, Western Infirmary, Glasgow, UK. gps1z@clinmed.gla.ac.uk

The precise biological mechanisms that caused the TGN1412 clinical trial tragedy (also known as 'The Elephant Man Clinical Trial') in March 2006 remain a mystery to this day. It is assumed widely that the drug used in this trial (TGN1412) bound to CD28 on T lymphocytes and following activation of these cells, a massive 'cytokine storm' ensued, leading ultimately to multi-organ failure in all recipients. The rapidity of this in vivo response (within 2 h), however, does not fit well with a classical T lymphocyte response, suggesting that other 'faster-acting' cell types may have been involved. In this study we have activated purified human peripheral blood leucocyte populations using various clones of mouse monoclonal anti-CD28 presented to cells in the form of a multimeric array. Cytokines were measured in cell-free supernatants at 2 h, and specific mRNA for tumour necrosis factor (TNF)-α, thought to be the initiator of the cytokine storm, was also measured in cell lysates by reverse transcription-polymerase chain reaction (RT-PCR). Monocytes were the only cell type found to show significant (P < 0·05) up-regulation of TNF-α at 2 h. Eleven other monocyte cytokines were also up-regulated by anti-CD28 within this time-frame. It therefore seems likely that monocytes and not T cells, as widely believed, were probably responsible, at least in part, for initiating the cytokine storm. Furthermore, we propose that a multimeric antibody array may have formed in vivo on the vascular endothelium via an interaction between TGN1412 and CD64 (FcyRI), and we provide some evidence in support of this hypothesis.