### Antigen processing

<table>
<thead>
<tr>
<th>Cytosolic pathogens</th>
<th>Intravesicular pathogens</th>
<th>Extracellular pathogens and toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>any cell</td>
<td>macrophage</td>
<td>B cell</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Degraded in</th>
<th>Cytosol</th>
<th>Endocytic vesicles (low pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptides bind to</td>
<td>MHC class I</td>
<td>MHC class II</td>
</tr>
<tr>
<td>Presented to</td>
<td>CD8 T cells</td>
<td>CD4 T cells</td>
</tr>
<tr>
<td>Effect on presenting cell</td>
<td>Cell death</td>
<td>Activation to kill intravesicular bacteria and parasites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins</td>
</tr>
</tbody>
</table>

*Figure 5-2 Immunobiology, 6/e. (© Garland Science 2005)*
Class I Ag processing

- TAP = transporters associated with antigen processing
- Transport peptides into ER
Proteosome degrades cytosolic proteins

- Large, multi-subunit complex
- Degrades foreign and self protein
- LMP2, LMP7 and MECL-1 induced by γ-Interferon
  - Preferential proteases for class I peptides
  - Cleaves after basic, hydrophobic amino acids
MHC class I
Ag processing pathway

Partly folded MHC class I α chains bind to calnexin until β2-microglobulin binds

MHC class I α:β2m complex is released from calnexin, binds a complex of chaperone proteins (calreticulin, Erp57) and binds to TAP via tapasin

Cytosolic proteins are degraded to peptide fragments by the proteosome, a large multicatalytic protease

TAP delivers a peptide that binds to the MHC class I molecule and completes its folding. The fully folded MHC class I molecule is released from the TAP complex and exported

Figure 5-6 Immunobiology, 6/e. (© Garland Science 2005)
Pathogen evasion of class I Ag presentation

- Herpes simplex
  - Inhibition of antigen transport by TAP
- Adenovirus, Cytomegalovirus
  - Decreased MHC class I expression by retaining class I in ER
- Plasmodium
  - No Ag presentation since no MHC expression on RBCs
MHC class II Ag processing

Antigen is taken up into intracellular vesicles

In early endosomes of neutral pH endosomal proteases are inactive

Acidification of vesicles activates proteases to degrade antigen into peptide fragments

Vesicles containing peptides fuse with vesicles containing MHC class II molecules

Figure 5-7 Immunobiology, 6/e. (© Garland Science 2005)
MHC class II

- **Invariant chain=**
  - Binds in class II groove in endoplasmic reticulum

- **CLIP peptide=**
  - Stabilizes class II
  - Prevents loading of peptide until fusion with phagolysozome
Cleavage of Invariant chain

- Invariant chain (li) binds in the groove of MHC class II molecule
- li is cleaved initially to leave a fragment bound to the class II molecule and to the membrane
- Further cleavage leaves a short peptide fragment, CLIP, bound to the class II molecule
Class II Ag processing pathway

- Invariant chain (li) forms a complex with MHC class II molecule, blocking the binding of peptides and misfolded proteins.
- li is cleaved in an acidified endosome, leaving a short peptide fragment, CLIP, still bound to the MHC class II molecule.
- Endocytosed antigens are degraded to peptides in endosomes, but the CLIP peptide blocks the binding of peptides to MHC class II molecules.
- HLA-DM binds to the MHC class II molecule, releasing CLIP and allowing other peptides to bind. The MHC class II molecule then travels to the cell surface.

Figure 5.10: Immunobiology 6/e. (© Garland Science 2005)
MHC and peptide stability

• Very stable binding
• Peptide not lost or gained by diffusion into groove

⇒ Allows rare peptides to be presented
⇒ MHC only present at surface with peptide in cleft
MHC locus
A diversity of peptides can be presented by the MHC

- Polygeny
- Codominant expression of MHC genes
- Polymorphism across population
Polygeny

Gene structure of the human MHC

Gene structure of the mouse MHC

Figure 5-11 Immunobiology, 6/e. (© Garland Science 2005)
Polygeny

• Class I=
  – 3 $\alpha$ chains: HLA-A, HLA-B, HLA-C
  – $\alpha$ chain pairs with $\beta_2$ microglobulin

• Class II=
  – 3 $\alpha$, $\beta$ pairs= HLA-DR, HLA- DP, HLA- DQ
  – Extra $\beta$ chain in HLA-DR

⇒ Total= 3 class I, 4 class II molecules encoded on each chromosome
Codominant expression

- 2 parents x 3 class I = 6 class I
- 2 parents x 4 class II = 8 class II
- Can mix $\alpha,\beta$ from parents
Polymorphism = different $\alpha, \beta$ alleles in population
Class I variability
Class II variability
Importance of MHC diversity

• Within population, allows presentation of some antigenic peptides from pathogen

• Problems with MHC homogeneity
  – Epstein-Barr in Papua, New Guinea
  – HIV immune evasion
  – Inbred mice
Mouse immunology vocabulary

• Isogenic = genetically identical at all loci
  – C57BL/6, BalbC

• Congenic = genetically identical at all loci except one
• **Transgenic**=
  – extra copy of gene that may be normal, altered, or different allele
  – can have tissue-specific expression

• **Knock-out**=
  – specific gene is deleted by homologous recombination

• **Knock-in**=
  – specific gene is replaced by altered form by homologous recombination

• **Knock-down**=
  – specific mRNA is decreased by RNA interference (RNAi)
  – can have tissue-specific expression
RNA interference (RNAi)

http://www.life.uiuc.edu/shapiro/RNAiApps.html
Gene deletion by homologous recombination

Locus of interest in the genome

Introduction of a foreign sequence and deletion of a part of the gene

Rearranged locus: the gene is no more expressed

Homologous recombination

http://www.genoway.com/
Gene insertion by homologous recombination

Locus of interest in the genome

Introduction of a transgene in the locus of interest

Homologous recombination

Rearranged locus: the transgene B is expressed
DNA pronuclear injection

1. Ovocytes → DNA micro-injection → Transplantation in a surrogate mother → Screening of newborn mice for transgene detection → Founders breeding

2. Transgene
ES based technologies: generation of gene targeted mice

Genetically modified ES cells → Blastocyst injection → Transplantation in a surrogate mother → Birth of chimeric mice → Generation of Heterozygous and Homozygous mice

http://www.genoway.com/
Transgenic mice

Female mouse is injected with follicle-stimulating hormone and chorionic gonadotropin to induce superovulation, and then mated.

Injected eggs are transferred into uterus of pseudopregnant female.

Some offspring will have incorporated the injected $E_\alpha$ gene (transgene).

Fertilized eggs are removed from female. DNA containing the $E_\alpha$ gene is injected into the male pronucleus.

Mate transgenic animal to $E_\alpha^-$ C57BL/6 mice to produce a strain expressing the $E_\alpha$ transgene.

Figure A-42 Immunobiology, 6/e. (© Garland Science 2005)
Creating a gene knockout

- **neo** =
  - resistance to drug G418

- **HSV-tk** =
  - expression in presence of ganciclovir is lethal

Figure A-44 Immunobiology, 6/e. (© Garland Science 2005)
Knockout mice
L. monocytogenes infection

• Mutant mice with a defined genetic defect in the beta 2-microglobulin or the H2-I-A beta chain
• Virtually devoid of functional CD8 or CD4 alpha beta T cells
• The lethal dose of L. monocytogenes was lower, but were able to resolve low dose infection
• The course of disease was exacerbated and clearance was markedly delayed
• Granuloma infection increased
George Snell’s experiment
MHC restriction
Superantigens: Disrupting MHC restriction

• Bacterial or viral in origin
  – Bacterial: *Staphylococcus, Streptococcus, Mycoplasma*
  – Viral: MMTV

• Bind to Vβ of TCR
  – Has specificity for certain Vβ genes
  – Away from CDRs

• Binds to outer portion of MHC
  – Binds to many different MHC molecules
  – Away from peptide groove
Superantigens

**Bacterial superantigen**
e.g., SE, TSST-1

- Antigen-presenting cell
- MHC class II
- TCR
- T cell

**Viral superantigen**

- Antigen-presenting cell
- MHC
- TCR
- T cell

**Figure 5-19 Immunobiology, 6/e. (© Garland Science 2005)**
The effect of superantigens

- Stimulates 2-20% of all T cells
- Overexpression of T cell cytokines
  - IL-2: T cell proliferation
  - TNF-α: You should know!

⇒ Can lead to shock when too many cytokines are expressed
⇒ Can lead to immunosuppression
  - Deletion by apoptosis?
  - T cells anergy?
  - Bone marrow suppression?
⇒ Can lead to immune system diversion
The problem with transplant rejection: T cell alloreactivity
Minor histocompatibility antigens

![Diagram showing skin graft survival rates and rejection times based on MHC compatibility between donors and recipients.](Figure 13-36 Immunobiology, 6/e. (© Garland Science 2005))
Recipient APC present minor histocompatibility Ag

Polymorphic self proteins that differ in amino acid sequence between individuals give rise to minor H antigen differences between donor and recipient.
APCs cause T cell proliferation
Transplant rejection

• Initial damage due to CTL response

• Helper T cell cytokines cause long-term inflammatory response
Hyperacute rejection

- Complement mediated rejection
- Preformed Ab to blood group, MHC molecules
- Problem with xenografting (α-Gal)
Preventing transplant rejection

• Match MHC alleles
• Immunosuppression
Donor matching

• Blood type matching
• Microcytotoxicity test
• Mixed lymphocyte reaction
Microcytotoxicity testing

(a) HLA-A allele 2
Donor cell
Antibody to HLA-A allele 2
Complement
Cells become leaky
Dye (trypan blue or eosin Y)
Dye taken up

HLA-A allele 1
Recipient cell
No lysis

(b) Antibody to different HLA-A antigens

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<td>Donor 1</td>
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<td>Donor 2</td>
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</tbody>
</table>
T cell proliferation assay

1. Immunize with antigen A
2. After 5–10 days, remove cells from lymph node
3. Culture cells with antigens A or B
   - no antigen
   - antigen A
   - antigen B
4. Measure T-cell proliferation

Figure A-36 Immunobiology, 6/e. (© Garland Science 2005)
CTL assay

Label target cells with \( \text{Na}_2^{51}\text{CrO}_4 \)

Add cytotoxic T cells to labeled target cells

Killed cells release radioactive chromium
Mixed lymphocyte reaction

- Mix MHC\(^{a}\) T cells and irradiated MHC\(^{b}\) non-T cells as antigen-presenting cells

- Measure proliferation of T cells by incorporation of \(^{3}\)H-thymidine
  - \(T\)-cell proliferation depends largely on differences in MHC class II alleles

- Measure killing of \(^{51}\)Cr-labeled target cells to detect activated cytotoxic T cells
  - Generation of cytotoxic T cells depends largely on differences in MHC class I alleles
Immunosuppressive drugs

Prednisone
increases IκB production

Azathioprine/MMF
Az: purine analog that inhibits DNA synthesis
MMF: binds enzyme to prevent purine synthesis

Cyclosporin, Tacrolimus:
interferes with T cell proliferation by binding calcineurin

Rapamcyin:
interferes with serine threonine kinase, limiting costimulatory response

![Diagram showing mechanism of action of calcineurin inhibitors](image)
Immune Therapy Could Treat Leukemias, Autoimmune Diseases, Transplant Rejection

DURHAM, N.C. -- In studies with mice, treatment with a new monoclonal antibody that targets immune system B cells has shown considerable promise for treating leukemias, autoimmune diseases and transplant rejection, according to immunologists at Duke University Medical Center.
Types of antisera

• Polyclonal=
  – Collection of antibodies from the serum
  – Multiple binding specificities to many epitopes of one or more antigens

• Monoclonal=
  – Homogeneous preparation of antibody
  – Single binding specificity to one epitope on a single antigen
Polyclonal antibody

Small antigen, 2 epitopes: antigen valence = 2

Intermediate antigen, 6 epitopes: antigen valence = 4

Large antigen, 10 epitopes: antigen valence = 8

Figure A-10 Immunobiology, 6/e. (© Garland Science 2005)
Monoclonal antibody production

Spleen cells producing antibody from mouse immunized with antigen A

Myeloma cells (immortal) lacking antibody secretion and the enzyme HGPRT

Mix and fuse cells with PEG

Transfer to HAT medium

Immortal hybridomas proliferate; mortal spleen cells and unfused HGPRT- myeloma cells die

Select hybridoma that makes antibody specific for antigen A

Clone selected hybridoma

HGPRT= hypoxanthine:guanine phosphoribosyl transferase

HAT= hypoxanthine-aminopterin-thymidine medium; lethal to cells in absence of HGPRT enzyme
Humanized monoclonal antibodies

- Variable region = murine
- Constant region = human
- CDRs from mouse cloned into human Ig
- Treatment:
  - Cancer
  - Transplantation
  - Autoimmune diseases
  - Inflammatory/allergic
Phage display

1. Isolate population of genes encoding antibody variable regions.
2. Construct fusion protein of V region with a bacteriophage coat protein.
3. Cloning a random population of variable regions gives rise to a mixture of bacteriophage—a phage-display library.
4. Select phage with desired V regions by specific binding to antigen.

Figure A-15 Immunobiology, 6/e. (© Garland Science 2005)
Monoclonals as therapy

- **Tumor-specific antibody** binds to the tumor cell.
- **Tumor-specific antibody conjugated to toxin** results in conjugates being internalized and killing the cell.
- **Tumor-specific antibody conjugated to radioisotope** causes radiation to kill the tumor cell and neighboring tumor cells.

*Figure 14-17 Immunobiology, 6/e. (© Garland Science 2005)*
Her2 overexpression associated with some breast cancers

**HER2 Preclinical Studies**

Indicators of Increased HER2 Production:

1. Increased Gene Copy Number
2. Increased Transcription
3. Increased Protein Synthesis

**In vitro studies demonstrated that a murine anti-HER2 monoclonal antibody, 4D5, inhibited the growth of the HER2-positive tumor cells.**

- Pietras, et al.
Normal Cell
In normal breast tissue cells, the HER2 gene produces a protein receptor on the cell surface. These growth factor-like receptors are thought to play a role in normal cell growth by signaling the cell to divide and multiply.

HER2 Overexpressing Cancer Cell
Cancerous breast tissue cells that overexpress (or overproduce) the HER2 gene produce extra protein receptors on the cell surface. The higher density of receptors triggers the cell to divide and multiply at an accelerated rate, thus contributing to tumor growth. Approximately 25-30% of all women with metastatic breast cancer overexpress the HER2 protein.

Herceptin® (Trastuzumab)
It is thought that Herceptin (a HER2 antibody) binds to numerous HER2 receptor sites found on the cell surface, blocking the receptor sites and possibly preventing further growth by interrupting the growth signal. As a result, the HER2 antibody may slow progression of the disease.
Herceptin increased efficiency of chemotherapy

*Chemotherapy = either doxorubicin or epirubicin plus cyclophosphamide, or paclitaxel.
Targeting radiation therapy

• **Zevalin®, Bexxar®**
  – Binds to CD20 molecule on mature B cells (and lymphomas)
  – Zevalin conjugated to either indium-111 (111In) or yttrium-90 (90Y)
  – Bexxar conjugated to iodine-131 (131I)
  – Usually used in combination with Rituxan that also binds to CD20
Humanized Ab neutralize proteins of immune response
Multiple uses for a single antibody

Daclizumab (anti-CD25)²

HuZAF™
(Anti-Gamma Interferon)

Nuvion® (anti-CD3)

Anti-αβ¹ Integrin

Anti-αβ¹ Integrin Fab

Anti-IL-12

Daclizumab is an immunosuppressive IgG₁ monoclonal antibody that binds specifically to the alpha subunit (p55 alpha, CD25 or Tac subunit) of the human high-affinity interleukin-2 (IL-2) receptor that is expressed on the surface of activated lymphocytes. It functions as an IL-2 receptor antagonist and inhibits IL-2-mediated activation of lymphocytes, a critical pathway in the cellular immune response involved in transplant rejection and potentially in autoimmune diseases.

Clinical development by Roche in transplantation; PDL assumed development in autoimmunity in October 1999.

* Marketed as Zenapax® by Hoffmann-La Roche in kidney transplantation.
Targeting TNF-α

- Humira (adalimumab) = binds TNF-α
- Remicade (infliximab) = binds TNF-α
- Etanercept = soluble decoy receptor for TNF-α (not an antibody)
mAb to prevent transplant rejection

OKT3
- interferes with T cell proliferation by binding to CD3

Xenapax/Simulect
- interferes with T cell proliferation by binding to IL-2 receptor (α) subunit
- Daclizumab (Xenapax) is the humanized mAb and basiliximab (Simulect) is the chimeric mAb

![Diagram of IL-2 receptor antagonists](image-url)
Belatacept: Costimulation blockade

- soluble fusion protein of the B7-binding domain of CTLA4 and an Ig tail
- inhibits lymphocyte co-stimulation through CD28 for the potential treatment of solid organ transplant rejection.
- Belatacept is currently undergoing phase III clinical trials (Aug 2005)
Transplant survival rates

<table>
<thead>
<tr>
<th>Tissue transplanted</th>
<th>5-year graft survival*</th>
<th>No. of grafts in USA (2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>65–75%</td>
<td>15,680</td>
</tr>
<tr>
<td>Liver</td>
<td>65–75%</td>
<td>5594</td>
</tr>
<tr>
<td>Heart</td>
<td>68%</td>
<td>2231</td>
</tr>
<tr>
<td>Pancreas</td>
<td>30%/80%#</td>
<td>1492</td>
</tr>
<tr>
<td>Lung</td>
<td>40%</td>
<td>1077</td>
</tr>
<tr>
<td>Cornea</td>
<td>~70%</td>
<td>~40,000†</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>40%/60%</td>
<td>15,000‡</td>
</tr>
</tbody>
</table>

*Survival rates at 5 years post-transplantation.

†Approximate number.

‡Approximate number.
Fetal tolerance

- Placenta
  - Does not exhibit classical MHC molecules
  - Expresses HLA-G that inhibits NK cells

- Maternal induced T cell tolerance to paternal alloantigens
  - Responsiveness returns after pregnancy

- Placental secretion of cytokines (IL-4, TGF-β, IL-10) that promote less harmful TH2 response

- Placental tryptophan starvation suppresses T cell activation
  - Indoleamine 3-dioxygenase catabolizes tryptophan