Immune system
Immune system

Immune system protects us from a diverse world of pathogens (viruses, bacteria) that are still evolving.
Innate (nonspecific) immune system

Physical barriers

skin

mucous membranes

(cover body cavities with exterior openings)
Innate (nonspecific) immune system

Response to damaged tissues (inflammation)

Injured tissues release chemical signals

Increased leakiness of local blood vessels (swelling)

Phagocytes consume bacteria and tissue debris; tissue heals

Cells of innate immune system express receptors that recognize molecules that are broadly shared by pathogens.

Many pathogens have evolved to escape this recognition!
Excess fluid containing tissue debris and pathogens is flushed through the lymphatic system. Here this fluid gets cleaned and checked for pathogens before it is returned to blood stream.

Lymph nodes contain many lymphocytes (B and T cells) that check for pathogens.
Different B cells and T cells have different receptors and only those that are specific for pathogens get activated.
Activated adaptive immune system

Activated T cells produce multiple copies with identical receptors in order to quickly kill other infected cells.

Activated B cells start replicating and at the same time they also mutate their receptors in order to produce B cells whose receptors bind even more strongly to pathogens!
Memory of past infection

After infection is cleared, some B and T cells are converted to memory cells.

**Vaccination**
Inject certain virus markers to prepare immune system for a fight against real virus.
Adaptive immunity in health and disease

Combating infectious disease-causing agents

Mis-regulation leads to autoimmune diseases

The challenge: develop principles that govern the emergence of a systemic immune or autoimmune response and design rules for therapies/vaccines

Outline
1. Mechanisms for T cell specificity for foreign peptides
2. Implications for the influence of host genetics on HIV control
Diversity of T cell receptors

In adults there are $\sim 10^{12}$ T cells in total ($\sim 10^{10}$ T cells in blood) and there are $\sim 10^8$ distinct T cells.

Diverse T cell receptors are generated with VDJ recombination.

V segments  D segments  J segments  constant region

Note: VDJ recombination is also responsible for huge diversity of B cell receptors.
T cell recognition of foreign peptides is very sensitive

T cells can recognize 3-5 foreign peptides in a sea of ~30,000 self peptides

Recognition: strong binding of T cell receptors to foreign peptides
AIRE (autoimmune regulator) causes transcription of a wide selection of genes/proteins in the thymus.

T cells scan thymus gland for 4-5 days, where they are selected against \(\sim 10^3-10^4\) self-peptides.

Thymic selection processes may tune the T cell repertoire. Huseby et al. (50) discovered that T cells that develop in genetically altered mice that express only one type of self-pMHC in the thymus can be stimulated, with relatively high probability, by point mutants of pathogen-derived pMHCs that they recognize. In contrast, for T cells that develop in mice with many types of pMHC (\(\sim 10^3-10^4\)) in the thymus, most such point mutations abrogate recognition (50).

To consider this difference in recognition properties due to the number of self-pMHCs present in the thymus during T cell development, we present the following simple model (62, 63). The pMHCs are divided into two parts, as shown in Figure 3b. TCRs comprise three loops. The CDR1 and CDR2 loops largely make contact with MHC amino acids, whereas the CDR3 loop contains almost all the peptide contact residues of the TCR. Therefore, the TCR is also divided into two parts (Figure 3b). To assess which kinds of T cells survive thymic selection and their properties vis-à-vis recognition of pathogen-derived pMHCs, one needs a way to estimate the free energies of TCR-pMHC binding. Huseby et al. (49, 50) used inbred mice in their experiments, so the MHC proteins were all the same. Although the CDR1 and CDR2 loops vary from one TCR to another, the CDR3 loops remain largely invariant.
T cell recognition of foreign peptide is both degenerate and specific

degeneracy - each TCR can be activated by many different foreign peptides

specifcity - most single point amino acid mutations of the foreign peptide are not recognized by the same TCR

How does the thymus gland design T cell receptors that are self-tolerant and degenerate/specific for foreign peptides?
Thymic selection against one or many types of self peptides

E. Huseby et al., Cell 122, 247 (2005)

Compare properties of T cells developed in normal mouse and in engineered mouse that express only one type of self peptide in thymus.

Find T cells that recognize a particular foreign peptide. Check whether T cells can recognize point amino acid mutations of the peptide.

**Normal mouse** - selection against many peptides
selected T cells are **specific** to point mutations

**Engineered mouse** - selection against one peptide type
selected T cells are **cross-reactive** to point mutations
Thymic selection against one or many types of self peptides

E. Huseby et al., Cell 122, 247 (2005)
Model

TCR-peptide-MHC interaction free energy:

\[ E_{\text{int}}(\vec{t}, \vec{s}) = E_c + \sum_{i=1}^{N} J(t_i, s_i) \]

Miyazawa-Jernigan

TCR-peptide contacts: \( N \approx 5 \)

collection of \( M \approx 10^3 - 10^4 \) random peptides

randomly generated TCR sequences, where amino acids are chosen with probability \( f_a \) with which they appear in human proteome.

A. Košmrlj et al., PNAS 105, 16671 (2008)
A. Košmrlj et al., PRL 103, 068103 (2009)
Miyazawa-Jernigan matrix describing interactions between amino acids

values of matrix $J(a,b)$ in units of $k_B T$

Miyazawa-Jernigan matrix was obtained by fitting the free energy values of folded proteins.

S. Miyazawa and R.L. Jernigan, 
Model recapitulates the specificity/cross-reactivity results from mice experiments

Challenge selected TCRs with foreign peptide and check how good are they at recognizing mutations of foreign peptide.

imported contact: more than half of 19 amino acid mutations prevents recognition from the same TCR.

Model recapitulates the specificity/cross-reactivity results from mice experiments
Surviving T cells: $|E_{\text{int}}| < |E_n|$ for all peptides; $|E_{\text{int}}| > |E_p|$ for at least one peptide

Selection condition is equivalent to the choice of the Extreme Value
**Extreme value distribution**

**Selection condition for TCR $\vec{t}$**

$$E_n < \min_{\vec{s} \in M} \left\{ E_{\text{int}} \left( \vec{t}, \vec{s} \right) \right\} < E_p$$

$$E_{\text{int}} \left( \vec{t}, \vec{s} \right) = E_c + \sum_{i=1}^{N} J(t_i, s_i)$$

$$\rho(E_{\text{int}} | \vec{t})$$

**Probability of TCR selection:**

$$P_{\text{sel}} \left( \vec{t} \right) = \int_{E_n}^{E_p} \Pi \left( x | \vec{t} \right) dx$$

$$\Pi \left( x | \vec{t} \right) = M \rho \left( x | \vec{t} \right) \left( 1 - P \left( E < x | \vec{t} \right) \right)^{M-1}$$

**Properties of $\Pi(x | \vec{t})$**

**mean value:**

$$E_0(\vec{t}) = E_c + \sum_{i=1}^{N} \mathcal{E}(t_i) - \sqrt{2 \ln M \sum_{i=1}^{N} \mathcal{V}(t_i)}$$

**standard deviation:**

$$\Sigma_0(\vec{t}) = \sqrt{\pi^2 \sum_{i=1}^{N} \mathcal{V}(t_i) / 12 \ln M}$$

**Increasing $M$ (self-peptides)**

**TCRs with weaker amino acids**

- **negative selection**
- **positive selection**
- **apoptosis**

**Extreme value distribution**

A. Košmrlj et al., PRL 103, 068103 (2009)
The limit of large peptides (N)

Scaling in the large peptide (N) limit: \[ \{E_c, E_p, E_n, \ln M\} \propto N \]

Mean value:
\[ E_0(\vec{t}) = E_c + \sum_{i=1}^{N} \mathcal{E}(t_i) - \sqrt{2 \ln M \sum_{i=1}^{N} \mathcal{V}(t_i)} \]

Standard deviation:
\[ \Sigma_0(\vec{t}) = \sqrt{\pi^2 \sum_{i=1}^{N} \mathcal{V}(t_i)/12 \ln M} \]

Selection condition for TCR \( \vec{t} \)

\[ E_n < E_0(\vec{t}) < E_p \]

like micro-canonical constraint in Statistical Physics

Probability of selection:
\[ p(\vec{t}) \propto \exp \left[ -\beta E_0(\vec{t}) \right] \]

Amino acid composition of selected TCRs:

\[ f_{a}^{(sel)} = \frac{f_a \exp \left[ -\beta (\mathcal{E}(a) - \gamma \mathcal{V}(a)) \right]}{\sum_{b=1}^{20} f_b \exp \left[ -\beta (\mathcal{E}(b) - \gamma \mathcal{V}(b)) \right]} \]

\[ \beta > 0 \] STRONG AA

\[ \beta < 0 \] WEAK AA

A. Košmrlj et al., PRL 103, 068103 (2009)
The image is a phase diagram illustrating the relationship between the number of self peptides and the binding to MHC. The diagram shows a phase transition between strong and weak AA (amino acids) based on the binding energy $(E_n - E_p)/N = 0.5$. The threshold for negative selection is indicated by the line $\beta[(k_B T)^{-1}]$. The diagram is color-coded to represent the energy levels, with stronger binding indicated by more intense colors. The axes are labeled as follows:

- Number of self peptides
- Binding to MHC
- $(E_n - E_C)/N$
- Threshold for negative selection

The phase diagram is used to understand the dynamics of peptide binding and the selection process in the immune system.
How good is analytical result for short peptides (N=5)?

\[
f_{a}^{(\text{sel})} = \frac{f_a \exp \left[ - \beta (\mathcal{E}(a) - \gamma \mathcal{V}(a)) \right]}{\sum_{b=1}^{20} f_b \exp \left[ - \beta (\mathcal{E}(b) - \gamma \mathcal{V}(b)) \right]} \]

\[
\beta = -0.37 (k_B T)^{-1} \quad \gamma = 0.83 (k_B T)^{-1}
\]

analytical result

simulation

\( N = 5, \ M = 1000 \)

selected TCRs
Selected TCRs are enriched with weak amino acids

analyzed 18 crystal structures

(E. Shakhnovich et al., PLoS, 2007)

A. Košmrlij et al., PNAS 105, 16671 (2008)
TCR recognition of foreign peptide is both specific and degenerate

“Weak” peptide contact residues on TCR must bind a sufficient number of its stronger complementary amino acids for recognition via multiple moderate interactions

Specificity:

<table>
<thead>
<tr>
<th>TCR</th>
<th>foreign peptide</th>
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</table>

Point mutations abrogate recognition

Degeneracy:

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Many mutations sustain recognition

Selection against one peptide - only few important contacts

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Number of important contacts for specific/cross-reactive T cells

E. Huseby et al., Cell **122**, 247 (2005)

<table>
<thead>
<tr>
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<th>P-I</th>
<th>P2</th>
<th>P3</th>
<th>P5</th>
<th>P8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALQKY</td>
<td>-2.0</td>
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<td></td>
<td></td>
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**Thymic selection against**

- **many self peptides** (normal mouse)
- **one self peptide** (engineered mouse)

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specific T cells: many important contacts where mutations abrogate recognition

cross-reactive T cells: only mutations at the few important contacts abrogate recognition

change of binding free energy between TCRs and peptides with single amino acid change

Calculated binding energies were calculated from surface plasmon resonance (peptide mutants) or TCR multimer staining (I-Aβ1). The data collectively indicated that the cross-reactive TCRs were less specific than the specific TCRs but were also less sensitive to mutations. This suggests that the cross-reactive TCRs may be more likely to cross-react with other peptides than the specific TCRs.
TCR specificity ~ statistical scan of a “bar code”

Enzyme and substrate fit together like a lock and key.

TCR like a bar code scanner - “counts” the number of moderate interactions

[Image of enzyme and substrate fit together]

[Image of TCR like a bar code scanner]

27
The HIV/AIDS epidemic

HIV

Adult HIV prevalence %

- >15
- 10 - 15
- 5 - 10
- 2 - 5
- 1 - 2
- 0.5 - 1.0
- 0.1 - 0.5
- <0.1
- No data

An AIDS patient lies in bed while hospital workers remove the body of a victim of AIDS in Chiradzulu Hospital in Malawi.
HIV hurts immune system by infecting and killing T cells.

Highly mutating HIV may quickly produce mutants, that cannot be detected by immune system.

High production rate of new HIV virions (~$10^{10}$ per day)
Current drug therapies can prevent infection of new T cells, but they cannot remove already infected T cells. When therapy is stopped, virus can become active again.
Typical time course of HIV infection

- Primary infection
- Acute HIV syndrome: Wide dissemination of virus, Seeding of lymphoid organs
- Clinical latency
- Opportunistic diseases
- Constitutional symptoms
- Symptoms of AIDS: Death

CD4+ Lymphocyte Count (cells/mm³)

HIV RNA Copies per mL plasma

(from Wikipedia)
that complement experiments have recently taken steps toward the goal of answering how thymic selection processes may tune the T cell repertoire. Huseby et al. (50) discovered that T cells that develop in genetically altered mice that express only one type of self-pMHC in the thymus can be stimulated, with relatively high probability, by point mutants of pathogen-derived pMHCs that they recognize. In contrast, for T cells that develop in mice with many types of pMHC (≈10^3 – 10^4) in the thymus, most such point mutations abrogate recognition (50).

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Certain MHC types appear more frequently in HIV elite controllers: HLA-B57.
HLA-B57 binds fewer types of self peptides

• Generate all possible peptides from the human proteome and use predictive algorithms to determine how many types of peptides bind to each HLA-B type

• HLA-B57 binds ~ 2-3 times fewer types of self peptides derived from human proteome than a typical HLA-B type and ~5-6 times fewer than HLA-B types that are associated with faster progression to AIDS.

• rhesus macaques: Mamu-B*17 allele (protective for SIV) binds ~ 4-10 times fewer types of peptides than other Mamu alleles

T cell repertoire in a HLA-B57 individual is more cross-reactive to mutants of targeted viral epitopes

HLA-B57 individuals infected with HIV seem to have more cross-reactive TCRs


**Prediction:** people expressing MHC molecules that bind fewer (more) types of self peptides are better (worse) at controlling the HIV infection?
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### Figure 3

**a**
- Apoptosis
- Thymus
- Thymocyte
- Mature T cell
- Negative selection
- Positive selection
- Thymic APC presenting self p-MHCs

**b**
- Strong amino acid
- Weak amino acid
- Amino acids
- Output frequency / input frequency
- A few self-peptides
- Many self-peptides

**c**
- Strong
- Weak
- Amino acids

**d**
- Specificity
- Degeneracy
- TCR
- Peptide

**e**
- Free energy
- En – Ec
- ln(M)/N

---

Summary

• How can TCR recognition of foreign peptides be both degenerate and specific?

• TCRs are enriched with weakly interacting amino acids. TCR recognition of foreign peptide occurs via multiple moderate contacts, each of which has a significant contribution. Breaking any contact by mutating the peptide may prevent recognition (specificity), but there are several different peptides that can be recognized (degeneracy).

• Certain MHC types (HLA-B57) appear more frequently in HIV elite controllers. These MHC types bind fewer types of self peptides. T cells developed against fewer self peptides are better at recognizing mutations of foreign peptides.
Autoimmune diseases

What goes wrong that immune system starts attacking host tissues?

Multiple Sclerosis

Central nervous system (brain and spinal cord)

- normal nerve cell
- nerve cell affected by multiple sclerosis

Type I Diabetes

- Insulin-producing cells
- Insulin-producing cells destroyed
- Insulin secreted into bloodstream
- blood capillary
- pancreas
Autoimmune diseases

What happens if some self peptides are not presented during the development in thymus?
How different should these missing peptides be from other self peptides, so that immune system treats them as foreign?

Why certain viral infections evolve the immune system to a state that is more prone for autoimmunity? Are these viral peptides correlated with self peptides from attacked tissues?

Certain MHC types are associated with higher risk for autoimmune diseases. How these particular MHC types affect the development in thymus and evolution in response to viral infections?
Questions about final projects?

Due Tuesday, Jan 12, in paper or electronic form.

I am traveling from Dec 24-Jan 5

During that period you can reach me via

email: andrej@princeton.edu
skype: akosmrlj