Immunology 2011
Lecture 7
Cellular Basis of Antibody Diversity
30 September
• Read ahead
• *Work out study questions & problem sets*
• *Review exams immediately*
• *Work in study groups*
HANDOUTS

#4: Problem Set 2

TODAY

Cellular Basis of Diversity, Chapter 7
Genetic basis of Diversity, Chap 8, App. 9
ANTIGEN-SPECIFIC TRIGGERING

Proliferation

Differentiation

T-CELL FUNCTIONS

T-CELL FUNCTION: T-cell killing: transplants, tumor cells

Mixed Lymphocyte Reaction (MLR)

Delayed type hypersensitivity, (DTH); e.g. tuberculin reaction

Suppression/Regulation (Tolerance)

COMPLEMENT

Killing of bacteria

Inflammation

Inactivation of viruses

Allergy

AUTOIMMUNITY

THREE "LIMBS" OF THE IMMUNE RESPONSE
Review of Ab Structure/Function

• $H_2L_2$ subunits, total of 1, 2, 3, or 5
• $\geq 2$ Ag-combining sites – $V_H + V_L$
• Isotypes – H-chains: G1-4, A1-2, M, D, E
  L-chains: $\kappa, \lambda$1-4
• Allotypes – allelic variants of $C_H$ and $C_L$
• Idiotypes – unique to combining site
• Assays – Ouchterlony, ELISA, Equilibrium Dialysis, Complement Fixation

What is the cellular basis for Ab production and diversity?
Outline: *Clonal Selection*

*Definition and principles*

*Experimental illustrations:*
  - Ada & Byrt (hot Ag suicide)
  - Mishell & Dutton (\(^{3}\text{H}-\text{TdR}\) suicide)

*Implications for:*
  - Tolerance
  - Memory
  - Affinity maturation
  - “Natural Antibodies”
HOW CAN WE ACCOUNT FOR ANTIBODY DIVERSITY?

Experimentally established principles by the mid 1950’s:

1) There are many possible Ab’s
2) One cell produces only one kind of Ab
3) Primary structure (amino acid sequence) of Ab determines its specificity
1) many Abs
2) 1 cell makes 1 Ab
3) a.a. sequence determines specificity

*Ab-Forming cell precursor (B cell)
Clonal Selection

1) AFCPs are precommitted to a particular antigen.
2) AFCPs bear Ag-specific receptors (=Ab!)
3) Antigen triggers differentiation and proliferation (Clonal Selection/Expansion)
   - Development of the Ab repertoire is Ag-independent. and produces a large, random library.
   - Any Ag will select a family of AFCPs with receptors to which it happens to fit.
Experimental Tests of AFCP
Clonal Precommitment

- Ada & Byrt (*hot Ag suicide*)
- Mishell & Dutton (*$^3$H-TdR suicide*)
Hot Antigen Suicide
Ada & Byrt, 1968

NORMAL SPLEEN DONOR

SPLEEN CELLS

ANTIGEN

900R

IRRADIATED RECIPIENT

TEST SPLEEN FOR Ab-FORMING CELLS AT DAY 7

ADOPTIVE TRANSFER
Hot Antigen Suicide
Ada & Byrt, 1968

Incubate cells with radiolabelled antigen, wash & transfer

ADOPTIVE TRANSFER

TEST SPLEEN FOR Ab-FORMING CELLS AT DAY 7
<table>
<thead>
<tr>
<th>treat spleen cells</th>
<th>immunize recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag used for preincubation</td>
<td>Ag transferred with cells</td>
</tr>
<tr>
<td>1) none</td>
<td>none</td>
</tr>
<tr>
<td>2) none</td>
<td>Fla</td>
</tr>
<tr>
<td>3) $^{125}$I-Fla</td>
<td>Fla</td>
</tr>
<tr>
<td>4) $^{125}$I-Fla</td>
<td>Fla + BGG</td>
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* Negative control – no background response  
** Positive control – adoptive transfer works  
*** Result – hot antigen eliminates $\alpha$Fla response  
**** Specificity control – effect is antigen-specific
1) many Abs
2) 1 cell makes 1 Ab
3) a.a. sequence determines specificity

INSTRUCTION

SELECTION

“hot” Ag “D”
$^3$H-TdR Suicide
*Mishell & Dutton, 1966*

*In vitro* Primary Antibody Response

![Diagram](image)
Ab RESPONSE at DAY 5 (PFCs)

1) 0-24 hr
   Positive control

2) 0-24 hr
   No effect

3) 24-48 hr
   Kill response

4) \(\alpha_{SRBC}\)
   Specificity

\(\alpha_{BRBC}\)

\(\text{Period of }^3\text{H-TdR treatment}\)

\(^3\text{H-TdR SUICIDE OF ANTIGEN-SPECIFIC AFC PRECURSORS}\)
1) many Abs
2) 1 cell makes 1 Ab
3) a.a. sequence determines specificity
B-cells are highly diverse and precommitted

- They bear on their surface a sample of the Ab they can produce
- They proliferate and differentiate* in response to Ag stimulation

*...into Ab-secreting plasma cells, and into memory B-cells
The Immune system as a defence organization

1. Its function is selective destruction.
2. It is large, complicated and elaborate.
3. It is expensive.
4. It is wasteful.
5. It has distinct components performing apparently identical functions.
6. It is slow to react.
7. It is prepared for events that never happen.
8. It fights today’s threats with the solutions of past problems.
9. It is susceptible to corruption.
10. It can destroy that which it protects.

Peter Parham, 1990
Paul Ehrlich, Side Chain Theory, 1900
CLONAL SELECTION AS IT RELATES TO:

- TOLERANCE
- MEMORY
- AFFINITY MATURATION
- “NATURAL” ANTIBODIES
CLONAL SELECTION AND TOLERANCE

Tolerance: _Ag-specific non-responsiveness_

_Clonal abortion_ – elimination of precommitted self-reactive B-cells early in development

This is an important mechanism, although not by itself sufficient to explain natural tolerance (later lecture)

**Question:** Can the experimental approaches we’ve examined be used to induce tolerance _in vivo_?

*Experimental animals, yes
Humans...not yet*
CLONAL SELECTION AND MEMORY

Clonal expansion results in increased numbers of memory B-cells specific for the Ag

Increased responsiveness of memory cells and Isotype Switching will be discussed later (next hour & week)
PRIMARY AND SECONDARY HUMORAL RESPONSES

**PRIMARY RESPONSE**
- SLOW
- LOW Ab LEVELS
- SHORT-LIVED
- MAINLY IgM
- LOW AFFINITY

**SECONDARY RESPONSE**
- FAST
- HIGH Ab LEVELS
- LONG-LIVED
- MAINLY IgG
- HIGH AFFINITY

(kinetics of clonal selection)
(Ig structure)
AFFINITY MATURATION BY ANTIGEN-DRIVEN SELECTION

Question: What about a low initial dose of Ag...?
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*Peter Parham, 1990*
CLONAL SELECTION AND “NATURAL ANTIBODIES”

Much of our serum Ig is not the product of known antigenic challenges. “Natural” antibodies??

1) Ag-specific immune responses can drive proliferation of “bystander” cells (diffusion of cytokines...). The increase in serum Ig seen during an immune response is greater than that contributed just by the specific Ab.

2) Abs to previously recognized Ags will tend to predominate (due to prior clonal expansions), even those resulting from non-specific triggering of AFCP’s
Clonal selection

- Generation of the Ab repertoire is random and Ag-independent
- B-cells are precommitted and bear Ig receptors on their surface
- B-cells respond to antigenic challenge by proliferation and differentiation
- Underlying basis for tolerance and memory