How antibody diversity is established

Hans-Martin Jäck • Erlangen
Humoral Immunity – The players

The World of Antigens

The Ig receptors recognize:

- Proteins
- Lipids
- Nucleic acids
- Carbohydrates
- Organic molecules or Haptens (Half-Ag)
- Metals
- Plastic

But only proteins are good T cell-dependent antigens

Clonal Expansion

Differentiation

Short-lived
Plasma cells

Long-lived
plasma cells

Memory
B cell

IgM

Ag

IgM

IgD

Naive
B cells

+/-TH

+TH

+TH

IgG, IgA, IgE

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Adaptive Humoral Immunity

Discovery
DISCOVERY - Humoral Immunity (Berlin 1890)

1901: Von Behring
1st Nobel prize for serum therapy

- Soluble (humoral), inducible and specific immunity against a pathogen (diptheria or tetanus) → antitoxin
- Naive individual can be protected by transferring serum from an immunized individual (serum therapy)
- Serum therapy to treat Diphtheria and Tetanus infections
Antitoxins are not only induced by pathogens but also by red blood cells and non-pathogenic organic molecules (haptens).

'ANTIKÖRPER' replaces 'ANTITOXIN'
'ANTIGEN' (für Antikörper generierend) replaces 'TOXIN'
Antibody structure

1962 Quaternary Structure (H₂L₂)
1972 Nobel prize in Medicine
1965 Discovery of V regions

G. Edelman
N. Hilschmann
L. Craig

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Antibody - Function

Antigen binding sites
= Paratop
"Magic Part"

3 loops (fingers) from each V region form the antigen binding site (paratop)
CDRs = complementary determining regions 1-3

Effector sites
"Bullet Part"

- Tissue distribution
- Serum halflife
- Complement
- Phagocytosis
- Recruiting of cells

Antibodies are bifunctional (Paul Ehrlich‘s Magic Bullets)
Antibodies are bifunctional (Paul Ehrlich’s Magic Bullets)
Antibody Repertoire

Early Models
Paul Ehrlich

- **Side-Chain (Seitenketten) Theory**
  - Explained inducibility and specificity
  - Nobel prize 1908
- Founder of chemotherapy
  - Salvarsan against syphilis

**Side-Chain Theory (1900)**

- Soluble side-chains (anti-toxins = antibodies)
  - Selection model
Instruction model (Breinl and Haurowitz, 1931; Pauling, 1940)

- Only one or few antibodies are produced.
- Antigen serves as folding template, which forms antigen binding site at the antibody.
- Theory was disproven in the 60ies by Edgar Haber.

Aus: Golub & Green: Immunology - A Synthesis, 2nd edition
Natural Selection Theory (N. Jerne 1956)

- Geringe Mengen spezifischer Ak im Serum bereits vor Infektion vorhanden (natural antibodies)
- Ag bildet mit korrespondierendem Ak1 einen Komplex, der von Zellen phagozytiert wird
- Phagozytose induziert nur die Synthese von Ak1
- Problem: Ak-produzierende Plasmazellen phagozytieren nicht
Clonal Selection Theories (1956-58)

Burnet Talmage

B cell repertoire
(~ $10^{11}$ specificies)

Clonal Selection & Expansion

Antigen

B cell receptor

B cell clone

Differentiation

- $T_H$

Short-lived plasma cell

“Memory” plasma cell

Memory B cell

+ $T_H$
Size of the antibody repertoire?

How many different antibodies are needed?

20^6 = 6 \times 10^7 \text{ linear peptide epitopes}

\rightarrow 6 \times 10^7 \text{ different antibodies}
Antibody Repertoire

Genetic Models
Genetic Models

Germline Theory (e.g., Niels Jerne)

Somatic Variation Theory (e.g., Lederberg)

Recombination Theory [Dreyer and Bennett Modell (1965)]
1. Information for billions of antibodies can not be stored in the human genome
   • 20 amino acids and epitope with 6 amino acids yielis in about $20^6 = 6 \times 10^7$ linear epitopes
   • L chain: ~ 600 bases; H chain: minimal ~ 1200 bases
     → together ~ 2000 bases
   • Storage space for $6 \times 10^7$ antibodies
     $6 \times 10^7 \times 2000 = 1.2 \times 10^{11}$ bases
   However, human haploid genome consists of about $3 \times 10^9$ bases

2. How is transcription of a single antibody gene regulated?
3. How does affinity maturation work?
Somatic Recombination → The Key Experiment

The experiment

Liver DNA  
6kb  
4kb  
Probe (radiolabelled L chain mRNA)

Myeloma DNA  
8kb

The explanation

Germline

Myeloma


S. Tonegawa  
Nobel Price 1987  
Basel Institute of Immunology

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VDJ Recombination

Genetics and Biochemistry
VDJ Recombination

S. Tonegawa
Nobel Price 1987

V segments    D segments    J segments

ca. 2.5 Mb (mouse)

HC locus

Recombination

Transcription Translation

V(D)J recombination generates antibody diversity

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V(D)J-Recombination generates CDR3

Sequences encoding the 3. hypervariable region (CDR3) of a L und H chain are formed during VDJ recombination

CDRs form the antigen binding site (Paratop)
Genetic Mechanism (Looping-out and deletion)

Recombination signal sequences

Looping-out & deletion
RAG1/2 + DNA repair

V(D)J recombination generates antibody diversity

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**RSS – Recombination Signal Sequences**

Recombination signal sequences (RSS)

- RSS: 7-23-9 bzw. 9-12-7 motives
- Hepatmer (7) und nonamer (9) as well as length (but not sequence) of spacer are evolutionary conserved
- Essential for V(D)J recombination
- Direct intrachromosomal recombination
- 12/23 rule:

  Recombination occurs preferentially between a DNA segments with a 12bp spacer and a DNA segment with a 23bp spacer
Recombination through Looping-out & Deletion

RAG-dependent phase
(only in lymphocytes)

RAG1/2

1. Recognition
2. Cleavage

Paired complex
Cleaved Signal complex

Non-homologous end-joining (NHEJ) phase
(ds DNA break repair system - in all cells)

Ku70/80

3. Ligation
• XRCC4
• Ligase IV

Signal Joint

3. Processing
• DNA-PK
• Artemis
• TdT

4. Ligation
• XRCC4
• Ligase IV

Coding Joints
Recombination-Activating Genes (RAG1/RAG2)

- Products of the Recombination Activating Genes RAG1 and RAG2 (Schatz, Oettinger and Baltimore, Cell 1989; Science 1990)
  - Coding sequence is not interrupted by introns (procaryotic origin?)

- Only expressed in developing thymocytes and B lymphoid cells
- Both RAGs are essential for V(D)J joining at the Ig AND TCR loci
- RAG deficiency result in SCID (no B and T cells)
- Biochemical activities
  - Recognize recombination signal sequences (RSS)
  - Generate single-strand cut
  - Catalyze formation of hairpins at coding sequences
Mechanism of RAG-mediated cleavage of RSS

1. Recognition of RSS
2. Formation of synapse (12/23 rule)
3. Formation of a hairpin at coding end
4. Formation of blunt end at signal end

- Synapse formation
- Endonucleolytic single strand cut
- Formation of synapse (12/23 rule)
- Recognition of RSS
Establishment of primary V repertoire

- ~134 V_H
- 13 D_H
- 4 J_H
- 5 C_H

V_H regions (ca. 6760)

Recombinatorial diversity

- ~85 V_κ
- 4 J_κ
- 1 C_κ

V_κ regions (340)

Recombinatorial diversität

~2.3x10^7 Abs

V(D)J recombination generates antibody diversity

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Size of the antibody repertoire?

How many different antibodies are needed?

Number of amino acids

Minimal site of a peptide epitope

\[ 20^6 = 6 \times 10^7 \text{ linear peptide epitopes} \]

\[ \rightarrow 6 \times 10^7 \text{ different antibodies} \]
The world of B cell antigens

Ig receptors recognize:
- Proteins
- Lipids
- Nuclei acids
- Carbohydrates
- Organig molecules or Haptens (Half-Ag)
- Metals
- Plastic

But only proteins are good T cell-dependent antigens
Antibody Repertoire

Junctional Diversity
Random processing of hairpin and insertion of non-templated nucleotides

Junctional diversity increases antibody repertoire

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Establishment of primary V repertoire (mouse)

- \( \sim 134 \, V_H \)
- 13 \( D_H \)
- 4 \( J_H \)
- 5 \( C_H \)

\( V_H \) regions (ca. 6760)

\( \sim 85 \, V_\kappa \)
- 4 \( J_\kappa \)
- 1 \( C_\kappa \)

\( V_\kappa \) regions (340)

Recombinatorial diversity

Combinatorial Diversity

~ 2.3 \( \times 10^7 \) Abs

Junctional diversity

10\(^9\) - 10\(^{12}\) Abs

(reperire, lat. wiederfinden)

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Summary: Preimmune Repertoire

Recombinatorial diversity
• Random assembly from V, D & J

Combinatorial diversity
• Random pairing of H & L chains

Junctional diversity
• Unprecise V(D)J joining
• Nucleotide (N) addition (TdT)
• Usage of three RF in D segments

ca. $10^7$ antibodies

$10^9$-$10^{12}$ antibodies
Antibody Repertoire

Central B cell maturation
CENTRAL B CELL MATURATION

V exons are generated through somatic DNA rearrangements

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Products of V-D-J Recombination

- **Productive VDJ**
  - *pairing*
  - *non-pairing*
  - *autoreactive*
  - full-length \( \mu H \) chain

- **Non-productive VDJ**
  - (2/3 of all rearrangement)
  - TAA
  - truncated \( \mu H \) chain
  - no BCR

- **Degradation**
  - NMD
  - (nonsense mediated mRNA decay)
B Cell Maturation

Checkpoints
Central Checkpoints

Checkpoints

Pairing Ig chains?

µHCfct?

Stem cell

Pro-B

Early Pre-B

Late Pre-B

Immature B

Mature B

V_{H} \rightarrow D \rightarrow J_{H}

V_{L} \rightarrow J_{L}

Central Tolerance

- Deletion
- Anergy
- Receptor editing

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**Ig Receptors**

**Pre-BCR**
- **Unique tails**
- **Surrogate L chain**
- **VpreB**
- **VH**
- **Cμ1**
- **Cμ2**
- **Cμ3**
- **Cμ4**
- **μH chain**
- **Igα/Igβ**
- **ITAMs**

**BCR**
- **L chain**
- **VH**
- **Cμ1**
- **Cμ2**
- **Cμ3**
- **Cμ4**
- **μH chain**
- **Igα/Igβ**
- **ITAMs**

• Vettermann et al., Sem. Immunol 2006
• Vettermann & Jäck, Trends Immunol. 2010

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**Function of the \( \lambda 5 \) tail**

- **Autoreactive** lambda 5 tail binds to **self ligands**, e.g., DNA, heparan sulfates, galectin1 (Bradl et al., 2001, 2003; Gauthier et al., 2003)

- Lambda 5 tail enhances pre-BCR-induced **proliferative signals** (Vettermann et al., 2008; 2010)

Autoreactivity can temporarily be explored to expand antibody repertoire

*From Bradl et.al, Signal Transduction 2007*
The Pre-BCR Checkpoint

The Pre-BCR: A passport for pre-B cell differentiation and expansion

- Igα KO
- Igβ KO
- µHC_{mem} KO
- µHC_{dys}

Stem cell → Early Pro-B → Late Pro-B → Early Pre-B → Late Pre-B → Immature B cell

LC ?
Function of the pre-B cell receptor (1)

- Survival and differentiation
  \[ \text{miRNA-mediated repression of Bim} \]

Pre-BCR

Late Pro-B → Early pre-B → Late pre-B

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Pre-BCR-Mediated Control of Cell Survival

RNA Interference by small non-coding RNAs

- miRNA
  - Pol II/III
  - Drosha
  - Exportin 5
  - NPC

mRNA 3’UTR

- Ribosome
- RISC

- Mature miRNA
- passenger strand
- leading strand

miR-17~92 cluster

Ventura et al. 2008; Xiao et al. 2008; Koralov et al., 2008)

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Function of the Pre-B Cell Receptor (2)

- Survival and differentiation
  → miRNA-mediated repression of Bim

- Retargeting of V(D)J recombinase from the IgH to the IgL locus?
  → Allelic exclusion ???
  → Opening of the IgL locus

Late Pro-B → Early pre-B → Late pre-B

Pre-BCR

VpreB

$V_H$

$\lambda 5$

$\beta 8$

$\mu H$-Kette

$C_{\mu 1}$

$C_{\mu 2}$

$C_{\mu 3}$

$C_{\mu 4}$

$Ig\alpha/Ig\beta$

unique tails

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Allelic Exclusion

Allelic exclusion  
→ *Product of one allele is produced*

Feedback model (F. Alt)

IgH-Kette & mRNA !!!

IgH gene

Allele 1

Allele 2

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Function of the Pre-B Cell Receptor (3)

- Survival and differentiation
  → miRNA-mediated repression of Bim

- Retargeting of V(D)J recombinase from the IgH to the IgL locus?
  → Allelic exclusion ???
  → Opening of the IgL locus

- Proliferative clonal expansion
  → Multiplication of pre-B cells with functional μH chains
  (Hess et al., PNAS 2001)
Function of the Pre-B Cell Receptor (3)

Pre-B cell

Clonal expansion

Expanded pre-B clone

\[ V_L \rightarrow J_L \]

Immature B cells

Increased combinatorial diversity

→ Autoreactivity can temporarily be explored to expand Ab repertoire
Mechanism of Pre-BCR Signaling

- Herzog et al, Nat. Immunol 2009

Pre-BCR-induced proliferation and differentiation are incompatible !!!!
VDJ Recombination

Central B Cell Tolerance
Checkpoint 3: B Cell Tolerance

Soluble antigen

Immature B cell

Anergy

+Ag

Deletion

Cell-bound antigen

Bone marrow Stroma cell

Receptor editing

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Checkpoint 3: B Cell tolerance

Central mechanisms
(immature B cells)
- Deletion
- Anergy
- Receptor editing

Peripheral mechanisms
(transtional B cells)
- Anergy
- Competition

Receptor editing

Autoreactive receptor

Edited receptor
B Cell Maturation

Peripheral Maturation
PERIPHERAL B CELL MATURATION

Bone marrow

- Stem cell
- Immature B
- V(D)J rearrangement
- Cell expansion
- Selection of functional BCR
- $1^0$ negative selection
- ~ $2 \times 10^7$ pro-B/day

Blood

- ~ $2 \times 10^6$ cells in T1B pool

Peritoneal cavity

- BAFF April
- $B_1$

Spleen

- IgM
- MZ
- FO
- T1 FO T2
- IgM

Transitional subsets

- T1
- T2

Mature B subsets

- ~ $8 \times 10^5$ cells/day in mature B cell pool

Naive B cell pool

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Allelic Exclusion

Allelic Exclusion
Allelic Exclusion: Definition

A mature B cell expresses on the cell surface only one kind of antigen receptor (or B cell receptor)

- Sheldon Dray in rabbit (Immunology, 1964) → anti-V und anti-C allootypic sera
- Benvenuto Pernis in rabbit (JEM, 1965) → anti-V und anti-C allootypic sera
- Eberhardt Weiler in mouse (PNAS, 1965) → anti-C-allotypic mAb
Allelic Exclusion: Rationale

Why do we need allelic exclusion?

- Required for clonal selection of monospecific B cells
- Avoids deletion of a B cell with a ‘good’ antibody
- Prevents waste of undesired antibody production
- Prevents appearance of a “bispecific“ B cell with low surface abundance of an autoreactive B cell receptor (escapes tolerance checkpoints)
Allelic Exclusion: Models

**Feedback model**
(F. Alt)

- IgH-Kette
- mRNA
- IgH gene
- V_{Hr}Exon
- C_{Hr}Exons
- Allel 1
- Allel 2

- Genetic regulation

**Cellular selection model**
(Wabl & Steinberg)

- Proliferation and differentiation
  - No genetic regulation
  - Pre-B cells with two H chains have a disadvantage in proliferation and/or differentiation

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Allelic Exclusion: Exp. Approaches

Feedback model
(F. Alt)

Mice with transgenic µH genes

Authors' conclusions

► µH chain signals allelic exclusion

Equation by other investigators

► µH chain stops IgH gene rearrangement

- translated H chain
- (stable) H chain mRNA,
- UTR of the mRNA

• Genetic regulation
Allelic Exclusion: Layers of Control

- H chain protein
- H chain mRNA

?  ?  ?
How can a pro-B cells sense a productive VDJ rearrangement in the absence of a H chain protein?
Allelic Exclusion: RNA Model

Sense μHC mRNA from a productive VDJ rearrangement accumulates and is translated.

Nonsense μHC mRNA from a non-productive VDJ rearrangement is rapidly degraded by the nonsense-mediated mRNA decay (NMD) machinery.

Jäck et al. EJI ; Baumann et al. EMBO J. ; Wittmann et al., MCB 2005

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Stable μHC mRNA could be a sensor for a productive VDJ rearrangement and thus temporarily suppress differentiation and/or rearrangement of the second IgH allele.
Allelic Exclusion: Experiment

**HYPOTHESIS:** Pro-B cells can sense VDJ recombination at the mRNA level

**EXPERIMENT**

**EXPECTATION**

Late Pro-B → Early pre-B → Late pre-B
Pro-B cells sense H chain mRNA
Conclusion

• Stable H mRNA alone inhibits ...........

  ➢ pro-B cells (cellular selection)
  ➢ V-to-DJ recombination (genetic feed-back)

• However, both would contribute to allelic exclusion