A Primer on

Humoral Immunity, Antibody Constructs, and Applications to Cancer Immunotherapy

For
The International Society for Biological Therapy of Cancer

San Francisco CA
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University of Wisconsin
Madison
Humoral Immunity, Antibody Constructs and Applications to Cancer Immunotherapy

- What is Antibody (Ab)?
- Why do we have it?
- How and when is it made?
- How does it work?
- **CAN IT BE USED AGAINST CANCER?**
Ehrlich’s side chain theory

Roitt et al. 1985

Antigen

Aby

Aby

Aby
Immunoglobulins (Antibodies)

- Proteins found in plasma of all vertebrates
- Bind with high specificity to their molecular targets (antigens)
- Each individual has a broad spectrum of Ab to many, many antigens
- Provide protection against pathogens
- Demonstrate memory (better protection upon second exposure)
Ig heavy and light chain gene recombination and transcription

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-8a
Ig heavy and light chain protein expression

A. **μ Heavy chain**

- Primary RNA transcript
- Messenger RNA (mRNA)
- Nascent polypeptide
- Mature polypeptide
- Assembled Ig molecule

B. **κ Light chain**

- RNA processing (splicing)
- Translation
- Processing, glycosylation of protein

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-8b
IgG binding regions and domains

Antigen Binding

J. Schlom: Biologic Ther. Of Cancer 95
Immunoglobulins

- Multimeric proteins, made of heavy and light chains
- Formed by clonally distributed ($\sim 10^9$) patterns of somatic gene rearrangements of V, D, J region genes

- HOW DO THEY BIND TO ANTIGEN?
Amino acid variability is greatest in CDR, hypervariable, regions

Abbas and Lichtman: 2003
CDR regions correspond to antigen binding
High Affinity Antibody: strong attractive and weak repulsive forces

Roitt et al. 1985
Phases of the humoral immune response

Abbas and Lichtman:2003
Antibody mediated opsonization and phagocytosis of microbes

Abbas and Lichtman:2003
Antibody Dependent Cell-mediated Cytotoxicity (ADCC)

Abbas and Lichtman:2003
Early steps in Complement activation
Late steps in complement activation:
formation of the membrane attack complex (MAC), resulting in osmotic lysis
Abbas and Lichtman:2003

Making Monoclonal Antibody (mAb)

Isolate spleen cells from mouse immunized with antigen X

Mixture of spleen cells, including some producing anti-X antibody

Fusion

Mutant myeloma line; unable to grow in HAT selection medium; does not produce antibody

In vitro selection in HAT medium

Only fused cells (hybridomas) grow

“Clone” cells (so each well contains the progeny of one cell)

Screen supernatants for presence of anti-X antibody and expand positive clones

Hybridomas producing monoclonal anti-X antibody
Affinity of polyclonal vs high affinity monoclonal antibody

Roitt et al. 1985
Clinically Relevant mAb target antigens

<table>
<thead>
<tr>
<th>LEUKEMIA</th>
<th>SOLID TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-20</td>
<td>B</td>
</tr>
<tr>
<td>CD-19</td>
<td>B</td>
</tr>
<tr>
<td>CD-5</td>
<td>T</td>
</tr>
<tr>
<td>GD-2</td>
<td>NBL/Mel</td>
</tr>
<tr>
<td>Her2</td>
<td>Breast</td>
</tr>
<tr>
<td>EpCAM</td>
<td>AdenoCA</td>
</tr>
</tbody>
</table>
Mechanisms of mAb mediated anti-tumor effects

Delivery of Toxic Agent

Toxin, Drug, Radionuclide, etc

Tumor

Death
$^{131}$I-3F8 binding to melanoma

**CDC**

C'1-9 Cascade → Tumor → Osmotic Lysis

**ADCC**

NK → FcR → Tumor → Necrosis & Apoptosis
HARVESTED BONE MARROW
- CFU-Mega
- CFU-GE-MM
- Stem
- BFU-E
- CFU-C
- CFU-E

CD19
CD10
CD20

Anti-CD19
Anti-CD20
Anti-CD10

Antigenic Heterogeneity

RE-INFUSED BONE MARROW
- CFU-Mega
- CFU-GE-MM
- Stem
- BFU-E
- CFU-C
- CFU-E

Lymphoma Cell Depletion
1. Complement
2. Immunotoxin
3. Magnetic Beads

ABMT for B-cell NHL: Infusion of PCR+ vs. PCR- marrow

Gribben et al.
Biol Ther. Of Cancer, 1995
CD59 and S protein inhibit MAC formation.

**CD59** inhibits poly-C9 assembly.

**S protein** inhibits membrane insertion of C5b-C7.

Abbas and Lichtman: 2003
CD59, but not CD55 or CD46, regulates Complement mediated killing of NHL lines by Rituxan in vitro

### Table: Expression of complement regulatory proteins on CD20 expressing multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) B-cell lines, and cell line sensitivity to rituximab-mediated complement lysis

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Type</th>
<th>CD59</th>
<th>CD55</th>
<th>CD46</th>
<th>CD20</th>
<th>Viability (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rituxan</td>
</tr>
<tr>
<td>ARH-77</td>
<td>MM</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>90.6</td>
</tr>
<tr>
<td>DHL10</td>
<td>NHL</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>96.0</td>
</tr>
<tr>
<td>NAWALMA</td>
<td>NHL</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>98.7</td>
</tr>
<tr>
<td>IM9</td>
<td>MM</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>88.0</td>
</tr>
<tr>
<td>DHL4</td>
<td>NHL</td>
<td>±</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>100.0</td>
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<tr>
<td>HS SULTAN</td>
<td>MM</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>84.0</td>
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<tr>
<td>MM-AS</td>
<td>MM</td>
<td>0</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>82.7</td>
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<tr>
<td>MM-SV</td>
<td>MM</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>96.0</td>
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</tbody>
</table>

*Myeloma and NHL B-cell lines were evaluated by single-color flow cytometry for expression of complement regulatory protein expression (CD46, CD55, and CD59) and CD20. Intensity of staining is denoted as follows: 0, no expression; ±, dim; +, moderate; ++, bright; and ++++, very bright. Viabilities were assessed by trypan blue staining and represent means of triplicate samples.*

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*SP Treon et al. J. Immunother. 24:263, 2001*
ADCC is regulated by Inhibitory:Activating FcR.
In Vivo IL-2/Rituximab Trial

- IL-2 (day 0) + Rituximab (wk 4)
- IL-2 (wk 4) + Rituximab (wk 4)
- Rituximab (wk 4)
- PBS

Days Post-Reconstitution

Percent Survival

M.A. Caligiuri 05/02
# Efficacy of FcR influences in vivo Rituxan Effects

**AA #158 of FcRIII**

- **V** → Higher Affinity for IgG
- **F** → Lower Affinity

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>In Vitro</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/V</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>V/F</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>F/F</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

In Vivo to Rituxan

Receptor Blockade

EGF

EGFR

Tumor

Stasis

Signal Activation

Agonistic anti-fas

Tumor

Necrosis & Apoptosis
Albertini et al 1998
Some anti-id antibodies can inhibit antigen binding

antibody to non-binding site idiotope

antibody to combining site idiotope

hapten

binding inhibited

Roitt et al. 1985
Patient Anti-id Antibody Inhibits Binding of hu14.18 to Tumor cells (Flow Cytometry Assay)
Inhibition of hu14.18 mAb binding to GD2 on cells or in ELISA by post Rx pt. sera

<table>
<thead>
<tr>
<th></th>
<th>Patient Pre</th>
<th>Patient D15</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Inhibition</td>
<td>0%</td>
<td>99%</td>
</tr>
<tr>
<td>Flow MFI</td>
<td>357</td>
<td>16</td>
</tr>
<tr>
<td>Flow inhibition</td>
<td>0%</td>
<td>96%</td>
</tr>
</tbody>
</table>

Hank et al, unpublished
Mimicry of a TAA determinant by anti-id antibodies
IMMUNOTHERAPY?

Potential for Intervention

Tumor Size

1. Oncogenesis
2. Undetected Growth
3. Diagnosis
4. Remission
5. Regrowth
6. Relapse
Ch14.18 mAb does not penetrate well into measurable tumors

Kendra K et al.  
J. Of Immunother.  
22:423, 1999
Anti-GD2+IL2 Immunocytokine

GD2 Antigen

Tumor Cell

hu14.18-IL2

IL-2 Receptor

IL-2

T Cell or NK Cell
Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases


**PBS Control**

123 ± 69

**ch14.18 + II-2**

34 ± 21

**ch14.18-II-2**

0 ± 0
Hu14.18-IL2 Efficacy:
Dependence on Tumor Establishment

hu14.18-IL2 (10ug/d) for 5 days starting on day 5, 7, 9, or 11 following $5 \times 10^5$ NXS2 cells injected on day 0, and harvested on day 28.
Single chain IgG

J. Schlom
Biol Ther. Of Cancer, 1995
Single chain scFv

$V_H$

Linker

$V_L$

Ag

J. Schlom: Biologic Ther. Of Cancer 95
Potential uses of scFv

• Smaller molecule, penetrates better
• Link to toxins
• Link to TCR or FcR signaling components to provide mAb mediated specificity to T or NK cells (“T cell bodies”, or “artificial receptors”)

Bifunctional mAb: Heteroconjugate vs. Quadroma

1) heteroconjugate
   complete immunoglobulins, chemically cross-linked, multimeric form, multivalent

   TARGET
   \( \text{anti-Tac} \)
   \( \text{anti-CD16 or CD3} \)

   EFFECTOR
   \( \text{CD16 or CD3} \)

2) bispecific
   hemi globulins, native disulfide linkage, (hybrid hybridomas or disulfide exchange)
   monomeric form, bivalent

   TARGET
   \( \text{anti-Tac} \)
   \( \text{anti-CD16 or CD3} \)

   EFFECTOR
   \( \text{CD16 or CD3} \)

R. P. Junghans et al, 1996
Anti-tumor applications of mAb

1. Naked MAb
2. Immunocytokine
3. Multistep targeting
4. Receptor blockade
5. Signal transduction

Adapted from N-K Cheung 2003
# Clinically Approved MoAb for Cancer-Rx-2004

<table>
<thead>
<tr>
<th>Generic</th>
<th>Brand</th>
<th>Target</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Rituxan</td>
<td>CD20</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>Trastutumab</td>
<td>Herceptin</td>
<td>HER-2</td>
<td>HER-2 Breast CA</td>
</tr>
<tr>
<td>Gemtuzumab</td>
<td>Mylotarg</td>
<td>CD33</td>
<td>AML (mAb-toxin)</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Campath</td>
<td>CD52</td>
<td>B-CLL, CTCL</td>
</tr>
<tr>
<td>Ibritumomab/Tosifumomab</td>
<td>Zevalin/Bexxar</td>
<td>CD2</td>
<td>Refractory B NHL (Radiolabeled mAb)</td>
</tr>
<tr>
<td>Basiliximab/Daclizumab</td>
<td>Anti-TAC</td>
<td>CD25</td>
<td>Anti-Graft Rejection/GVH</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Avastin</td>
<td>VEGF</td>
<td>GI Malignancies</td>
</tr>
<tr>
<td>Edrecolomab</td>
<td>17-1A</td>
<td>EpCam</td>
<td>GI Malignancies</td>
</tr>
</tbody>
</table>
Collaborators in UWCCC  
Immunocytokine Research - 2004

- UWCCC
  - J Hank
  - M Albertini
  - J Gan
  - A Rakhmilevich
  - I Buhtoiarov
  - H Lum
  - J Yang
  - H Schalch
  - K Osenga
  - J Schiller
  - D Mahvi
  - KM Kim
  - J Eickhoff
  - A Sternberg

- C.O.G and N.A.N.T.
  - Many Pediatric Oncologists

- Lexigen
  - S Gillies

- EMD
  - B Clements

- Scripps
  - R Reisfeld