USE OF ANTIBODIES AS THERAPEUTICS

CRITICAL FACTS

1. One of the most rapidly expanding areas of pharmacology is the use of humanized monoclonal antibodies as therapeutic agents. In particular, they are receiving considerable attention as “personalized” cancer therapies, because of their specificity and the large number of potential targets.

2. In addition to their specificity, one advantage of monoclonal antibodies is their long half-life. Their major drawback is hypersensitivity reactions, in particular human antimouse antibody (“HAMA”) reactions.

IMPORTANT MATERIAL FROM OTHER LECTURES

1. Antibodies (HHD, Summer 2006).
2. Immunosuppressive drugs (Dr. Regal, HHD, Summer 2006).

OBJECTIVES

1. Understand and be able to apply the rules for naming therapeutic antibodies (do NOT attempt to memorize all of the specific names of the antibodies and their antigens that are used in this part of the lecture).

2. Be able to list the advantages of using antibodies as therapeutic agents.

3. Understand the theory behind the design of mouse/human antibodies, particularly the difference between humanized and chimeric antibodies.

4. Know the common routes of administration, pharmacokinetics and common side effects of antibodies used as drugs.
One of the most rapidly expanding areas of pharmacology is the use of humanized monoclonal antibodies as therapeutic agents. In particular, they are receiving considerable attention as “personalized” cancer therapies, because of their specificity and the large number of potential targets.

### MONOCLONAL ANTIBODY NOMENCLATURE

from Ballow (2005) Am. Acad. Allergy Asthma and Immunology

**AS WITH MOST PHARMACOLOGY NAMING RULES, THERE ARE EXCEPTIONS!!!**

<table>
<thead>
<tr>
<th>Source Identifiers</th>
<th>General Disease or Target</th>
<th>Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i.e., species that the product made in)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>u</strong> = human</td>
<td><strong>vir</strong> = viral</td>
<td><strong>col</strong> = colon</td>
</tr>
<tr>
<td><strong>o</strong> = mouse</td>
<td><strong>bac</strong> = bacterial</td>
<td><strong>mel</strong> = melanoma</td>
</tr>
<tr>
<td><strong>a</strong> = rat</td>
<td><strong>lim</strong> = immune</td>
<td><strong>mar</strong> = mammary</td>
</tr>
<tr>
<td><strong>e</strong> = hamster</td>
<td><strong>les</strong> = infectious lesions</td>
<td><strong>got</strong> = testis</td>
</tr>
<tr>
<td><strong>i</strong> = primate</td>
<td><strong>cir</strong> = cardiovascular</td>
<td><strong>gov</strong> = ovary</td>
</tr>
<tr>
<td><strong>xi</strong> = chimera</td>
<td></td>
<td><strong>pr(o)</strong> = prostate</td>
</tr>
<tr>
<td><strong>zu</strong> = humanized</td>
<td></td>
<td><strong>tum</strong> = misc</td>
</tr>
</tbody>
</table>

| | e.g., **TRASTUZUMAB** is a humanized monoclonal antibody | e.g., **ABCIXIMAB** is anti-platelet |
| | **ABCFIXIMAB** is chimeric | **PALIVIZUMAB** is anti-viral |
| | **IBRITUMOMAB** is a mouse (not humanized, not chimeric) antibody | **ADALIMUMAB** is a rheumatoid arthritis treatment |
| | e.g., **ALEMTUZUMAB** is a CLL treatment | e.g., **EDRECOLOMAB** is used to treat colon cancer |
Advantages of Antibody Therapy

1. **SPECIFICITY**

2. **NUMBER OF POTENTIAL TARGETS**

3. Long-term benefit to short-term therapy

4. Definition of disease process

### Characteristics of an Ideal Therapeutic Antibody

<table>
<thead>
<tr>
<th></th>
<th>LYTIC EFFECTS</th>
<th>NON-LYTIC EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Opsinization</td>
<td>Receptor blockade (and/or internalization)</td>
</tr>
<tr>
<td></td>
<td>Complement activation</td>
<td>Aggregation and precipitation</td>
</tr>
</tbody>
</table>

- **LYTIC EFFECTS**
  - Opsinization
  - Receptor blockade (and/or internalization)
- **NON-LYTIC EFFECTS**
  - Complement activation
  - Aggregation and precipitation

1. High degree of affinity and specificity
2. Reduced immunogenicity (↓ HAMA human anti-mouse antibody response)
3. Long half-life
4. Adequate recruitment of effector functions (can be increased by coupling to cytotoxic agent, e.g. **IBRITUMOMAB**)

### Pitfalls in Generating Pure Human Antibodies

1. Ethical considerations of human experimentation
2. B cells for mAb production need to be derived from the bone marrow (painful) or the spleen (unacceptable)
3. 'Immunological tolerance' mechanisms to 'self-antigens' prevent the expansion of B cell clones

### Construction

- most are chimeric or humanized mouse/human monoclonal antibodies that are generated using molecular biological techniques – fully human antibodies are in development
Monoclonal Antibody Production

Immunization of mouse

To stimulate antibody production

Antibody-forming cells isolated from spleen

Tumor cells are grown in tissue culture

Antibody-forming cells are fused with cultivated tumor cells to form hybridomas

Hybridomas screened for antibody production

Antibody-producing hybridomas cloned

Monoclonal antibodies isolated for cultivation
<table>
<thead>
<tr>
<th>DRUG</th>
<th>TARGET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTERFERE WITH IMMUNE SYSTEM FUNCTION</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Asthma treatment</strong></td>
<td><strong>OMALIZUMAB</strong></td>
</tr>
<tr>
<td><strong>Crohn’s Disease</strong></td>
<td><strong>INFLIXIMAB</strong></td>
</tr>
<tr>
<td><strong>Multiple sclerosis</strong></td>
<td><strong>ALEMTUZUMAB</strong></td>
</tr>
<tr>
<td><strong>Multiple sclerosis</strong></td>
<td><strong>NATALIZUMAB</strong></td>
</tr>
<tr>
<td><strong>Organ transplantation</strong></td>
<td><strong>BASILIXIMAB</strong></td>
</tr>
<tr>
<td><strong>Psoraisis</strong></td>
<td><strong>ABATACEPT</strong></td>
</tr>
<tr>
<td><strong>Psoraisis</strong></td>
<td><strong>EFALIZUMAB</strong></td>
</tr>
<tr>
<td><strong>Rheumatoid arthritis</strong></td>
<td><strong>ABATACEPT</strong></td>
</tr>
<tr>
<td><strong>Rheumatoid arthritis</strong></td>
<td><strong>ADALIMUMAB</strong></td>
</tr>
<tr>
<td><strong>Systemic Lupus Erythematosus</strong></td>
<td><strong>ETANERCEPT</strong></td>
</tr>
<tr>
<td><strong>Systemic Lupus Erythematosus</strong></td>
<td><strong>Ruplizumab</strong></td>
</tr>
<tr>
<td><strong>PROMOTE IMMUNE SYSTEM FUNCTION??</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Coronary heart disease and stroke</strong></td>
<td><strong>ABCIXIMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>ALEMTUZUMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>BEVACIZUMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>CETUXIMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>EDRECOLOMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>EPRATUZUMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>GEMTUZUMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>IBRITUMOMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>RITUXIMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>TOSITUMOMAB</strong></td>
</tr>
<tr>
<td><strong>Antineoplastics</strong></td>
<td><strong>TRASTUZUMAB</strong></td>
</tr>
<tr>
<td><strong>Respiratory syncytial virus</strong></td>
<td><strong>PALIVIZUMAB</strong></td>
</tr>
</tbody>
</table>
**Administration**

1. IV

2. Extremely long half-lives (detectable in serum 3-6 months after completion of treatment)

**Common Side Effects**

1. Hypersensitivity reactions – fever, headache, anaphylaxis

2. HAMA response can result in kidney damage

3. Infections

4. Unknown effects on immunization, carcinogenesis, mutagenesis, impairment of fertility, pregnancy, nursing infants (human IgG is secreted in milk)
ANTICOAGULANT, THROMBOLYTIC, 
and ANTI-PLATELET DRUGS (reprise) 
Katzung (9th ed.) Chapter 34

CRITICAL FACTS

1. Antiplatelet drugs are used for prophylactic and/or long-term anticlotting treatment. As with thrombolytic and anticoagulant drugs, their major side effect is **BLEEDING**.

2. Major mechanisms of action of antiplatelet drugs include inhibition of prostaglandin synthesis, blockade of either the glycoprotein IIb/IIIa or ADP-induced activation of the GPIIb/IIIa receptor and phosphodiesterase inhibition.

3. Antiplatelet drugs are often used in combination with other agents: **ABCIXIMAB** and **EPTIFIAZIDE** with **HEPARIN** or **ASA**; **DIPYRIDAMOLE** with either **WARFARIN** or **ASPIRIN**.

IMPORTANT MATERIAL FROM OTHER LECTURES

1. Intracellular signaling and prostaglandin synthesis (esp. cyclic nucleotide phosphodiesterase – Principles, 2005).

2. Anti-inflammatory drugs (Dr. Regal, Hematopoiesis, Spring 2006)

3. Anticoagulants and thrombolytics (Dr. Fitzakerley, Hematopoiesis, Spring 2006)
DRUGS YOU NEED TO KNOW

ABCIXIMAB (Centocor)
ACETYLSALICYLIC ACID (Aspirin)
CLOPIDOGREL (Plavix)
DIPYRIDAMOLE (Persantine)
EPTIFIBATIDE (Integrilin)
TICLOPIDINE (Ticlid)
TIROFIBAN (Aggrastat)

OBJECTIVES

1. Be able to describe with the biochemical mechanisms of action and adverse effects of the anti-platelet agents listed above.
2. Know the specific instances where anti-platelet drugs are used in conjunction with other anti-clotting agents.

General Mechanisms of Action

Inhibit platelet adhesion and aggregation by:

1. Inhibiting cyclooxygenase: e.g. ASA
2. Blocking glycoprotein IIb/IIIa receptor: e.g. ABCIXIMAB, EPTIFIBATIDE, TIROFIBAN
3. Inhibiting the binding of fibrinogen to activated platelets: e.g. TICLOPIDINE and CLOPIDOGREL
4. Inhibiting cyclic nucleotide phosphodiesterase: e.g. DIPYRIDAMOLE

Antiplatelet drugs are used for prophylactic and/or long-term anticlotting treatment. As with thrombolytic and anticoagulant drugs, their major side effect is BLEEDING.
1. PROSTAGLANDIN SYNTHESIS INHIBITORS

**Mechanism of Action**
- thromboxane A2 causes platelets to change shape, release granules and aggregate
- **ASA** causes irreversible acetylation of cyclo-oxygenase, which results in a decrease in prostaglandin synthesis
- other salicylates and NSAIDS have a shorter duration of action (due to reversible actions) → less effective for long term use

**Pharmacokinetics**
- short drug half-life (15 min) but the effect may last for 4-7 days because platelets lack the machinery for protein synthesis → effects persist until new platelets are formed.

**Therapeutic uses**
- prevent further infarcts in people who have 1 or more MIs
- prevent transient ischemic attacks (TIAs), ischemic stroke and other thrombotic events

**Adverse Effects**
- bleeding
- GI and CNS effects (see Dr. Regal’s notes)

2. GLYCOPROTEIN IIb/IIIa INHIBITORS

**Mechanism of Action**
- bind to glycoprotein IIb/IIIa receptor on platelet surface and prevent binding of adhesive glycoproteins (particularly fibrinogen) to activated platelets
  - **ABCIXIMAB** is a humanized monoclonal antibody directed against the IIb/IIIa complex
  - **EPTIFIBATIDE** is a fibrinogen analogue i.e., a competitive inhibitor
  - **TIROFIBAN** is a non-peptide, competitive inhibitor
Therapeutic Uses

- give IV in conjunction with percutaneous transluminal coronary angioplasty to prevent abrupt closure of vessel
- acute coronary syndromes (e.g., unstable angina and acute MI)

Adverse Effects

- bleeding
- thrombocytopenia with chronic use

3. ADP RECEPTOR ANTAGONISTS

Mechanism of Action

- **TICLOPIDINE** and **CLOPIDOGREL** are irreversible ADP receptor antagonists that inhibit ADP-induced platelet aggregation by preventing activation of IIb/IIIa receptor
- No effect on prostaglandin metabolism

Pharmacokinetics

- Ineffective *in vitro*
- Maximal effect observed only after several days of treatment – prolonged bleeding times persist for several days after therapy.

Therapeutic Uses

- recommended for patients that don’t tolerate aspirin
- standard practice calls for administration in patients receiving a stent

Adverse Effects

- **CLOPIDOGREL** has fewer side effects than **TICLOPIDINE**
- 10-50% of patients experience bleeding, nausea, diarrhea, rash
- 1% severe leukopenia; very rarely thrombotic thrombocytopenic purpura (TTP)
4. DIPYRIDAMOLE

- vasodilator that:
  1) inhibits cyclic nucleotide phosphodiesterase 3
     a. **cAMP inhibits** degranulation
     b. **PDE3 degrades cAMP** (decreasing intracellular cAMP concentrations) resulting in **increased degranulation**, i.e., PDE inhibits the inhibition of degranulation
     c. **DIPYRIDAMOLE inhibits** PDE3 (increasing intracellular cAMP) resulting in **decreased degranulation**; i.e., DIPYRIDAMOLE inhibits the inhibition of the inhibition of degranulation
  2) inhibits adenosine uptake via A₂ receptors
     - negligible clinical value when given alone
     - does not influence prothrombin time or activity (does **NOT** increase bleeding)
     - negligible side effects (dizziness, headache, rash)
     - in combination with **WARFARIN**: effectively prevents embolization from prosthetic heart valves
     - in combination with **ASPIRIN**: prolongs platelet survival in thrombotic diseases

---

Antiplatelet drugs are often used in combination with other agents:
- **ABCIXIMAB** and **EPTIFIAITIDE** with **HEPARIN** or **ASA**;
- **DIPYRIDAMOLE** with either **WARFARIN** or **ASPIRIN**.
ACROSS

8. Anticoagulant that causes thrombocytopenia in 25% of patients.
11. Low molecular weight fragment of heparin.
12. This anticoagulant is rapidly absorbed and nearly completely bound to albumin.
16. Inhibits binding of fibrinogen to activated platelets.
17. Thrombolytic drug with fewer hypersensitivity reactions than streptokinase, perhaps because it is synthesized in the human kidney?

DOWN
1. Unmodified human tissue plasminogen activator.
2. Vitamin K₁.
4. An anticoagulant that has the same mechanism of action as heparin, but greater bioavailability and a longer half-life.
5. Derivative of the saliva of the medicinal leech; thrombin inhibitor.
6. Drug that is of negligible clinical value when given alone, but is often co-administered with warfarin or aspirin.
7. Anticoagulant that inhibits epoxide hydrase.
10. Activates plasminogen by inducing a conformational change.
14. Drug approved for use in patients with HIT.
15. Monoclonal antibody that binds to the glycoprotein IIb/IIIa receptor.