Immunology 2011
Lecture 5
Complement
27 September

TODAY
Complement, Chapter 5, App. 8
Immunoglobulin Genetics, Chap. 6, App. 3, 4

Handout on course web site:
Problem Set 1 Answer Key

Cardinal Signs of Inflammation

<table>
<thead>
<tr>
<th>CALOR</th>
<th>Heat</th>
<th>C3a, C5a</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUBOR</td>
<td>Redness</td>
<td>C3a, C5a</td>
</tr>
<tr>
<td>TUMOR</td>
<td>Swelling</td>
<td>Accumulation of fluid &amp; cells, C3a, C5a, C5b67</td>
</tr>
<tr>
<td>DOLOR</td>
<td>Pain</td>
<td>Swelling and tissue damage</td>
</tr>
</tbody>
</table>

+ Loss of function

May be initiated by...
- Infection (bacteria, viruses et al.)
- Sunburn, allergens...
- Trauma...
- Mediated by immune & coagulation systems, etc.

1894 – Pfeiffer, cholera vibrios are lysed in the peritoneum of immune guinea pigs
1896 – Bordet, lysis of bacteria by immune sera requires two separate components...

Lysis of Vibrio cholera by immune serum
- Fresh normal serum: no lysis
- Fresh immune serum: lysis
- Heated immune serum: no lysis
- Heated immune serum + fresh normal serum: LYSIS

Normal serum “complements” the ability of antibodies in immune serum to lyse targets

How is complement-mediated damage targeted?

Antibodies:
- Classical pathway (mostly)

Unique structures on microbes:
- Alternate & MBlectin Pathways

1) Classical Pathway
- Initiated by AgAb complexes
- Protein/protein interactions
- Proteolytic cleavages and conformational changes...
- ...which produce new enzymatic and biological activities

Structure of Mouse IgG2
Harris et al., Nature 360:369-72, 1992

To bind C1q:
- Ab must be bound to Fc
- Two Fc’s in close proximity (CHE)
2) Alternate Pathway
- Initiated by microbial cell walls et al...

3) MBLectin Pathway
- Initiated by microbial carbohydrates…

The "innate immune system" comprises many molecules that can recognize particular microbial targets.

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Table of Contents

1. The Immune System as a Defense Organization
2. Biological Activities of Complement
3. Biological Significance of Complement Activity

1) Cytolysis: disruption of cell membrane ("hemolysis").
2) Anaphylotoxin activity: ("vasoactive", "phlogistic"), increased capillary permeability, leakage of fluid.
3) Chemotaxis: attraction of PMNs.
4) Opsonization: phagocytosis by macrophages & PMNs.
5) Tissue damage: effects of lytic complex and accumulation of PMNs (Arthus reaction, Immune Complex Disease).

Inflammation: heat, redness, swelling, pain, loss of function...
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Peter Parham, 1990
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IMMUNE COMPLEX DISEASE

Circulating Ag/Ab complexes may result from:

- Passive immunization (self-limiting)
- Drug reactions (penicillin, sulfa...)
- Autoimmune disease (autoantibodies)
- Chronic viral infection

- Immune complexes bind to basement membrane of kidney glomeruli, vasculature, skin...
- Complement is fixed
- Inflammation and tissue damage ensues

The doctor asked for some further tests (Fig. 20.1) but in the meantime gave her some antihistamines with the warning that if these were not helpful she would need a course of corticosteroids. He diagnosed a drug allergy. Her symptoms did not improve and she was started on oral prednisone. A renal biopsy was considered but not done because all the symptoms cleared following a course of corticosteroids. These weeks later, Yvonne went for a check-up and all her tests had returned to normal.

COMPLEMENT DEFIENCIES

C5-9 (MAC): Limited susceptibility to infections (!)
C3, P, B: More severe infections
MBL: Mainly childhood infections

Supportive therapy: treat infections, fresh-frozen plasma to replenish complement levels.

C1,4: Increased risk of developing autoimmune disease
Active in soluble phase, important in normal clearance of trace amounts of immune complexes. C4b receptors (CR1) on RBC transport ICs to liver where they are removed.

Measuring Antibody/Antigen Reactions

1) Precipitation: Ouchterlony
2) Solid-state binding, labelled antibody: RIA/ELISA
3) Liquid-phase binding: Equilibrium Dialysis
4) Binding of complement: Complement Fixation (hemolysis)

Towards the end of a holiday abroad, at the age of 28, Yvonne developed acute cystitis with difficulty of micturition and some urinary frequency. When she got home she went to see her doctor, who gave her an antibiotic, trimethoprim, which she was to take for 7 days. She was, of course, not given penicillin.

She finished all the tablets and 3 days later developed a headache and some itchy bumps on her skin. The next day she had aching and swollen joints, mainly of the wrists and knees, although her hands were affected as well. She did not think that these symptoms had anything to do with the drug as she had already stopped taking it. She went to her doctor who confirmed that she had urticaria, but she also had a raised temperature and swollen glands in her neck. Examination of her urine showed evidence of protein.

Different Routes to Immune Complex Reactions

Intravenous: Drugs, anti-toxins & anti-venins, therapeutic mAbs
Subcutaneous: Vaccination (tetanus) (classic Arthus skin reaction...)
Inhaled: “Farmer’s Lung” (actinomycetes et al.)
May also be autoimmune (e.g. SLE, RA)

Complement Fixation Assay

We want to detect Abs by their ability to fix complement...

...but like Ab/Ag binding, complement binding is invisible.
**Complement Fixation Assay**

- Standard Ab (e.g. anti-viral)
- Standard Complement
- "Sensitized" erythrocytes - EA

Test unknown samples for **antigen**

*EA - visible indicator of the presence of complement

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**Complement Fixation Assay: Example**

<table>
<thead>
<tr>
<th>Tube</th>
<th>Sample</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>saline + C + EA</td>
<td>lysis</td>
<td>neg control</td>
</tr>
<tr>
<td>2)</td>
<td>Ab + saline + C + EA</td>
<td>lysis</td>
<td>neg control</td>
</tr>
<tr>
<td>3)</td>
<td>Ab + VA + C + EA</td>
<td>no lysis</td>
<td>pos control</td>
</tr>
<tr>
<td>4)</td>
<td>Ab + and No. 1 + C + EA</td>
<td>lysis</td>
<td>no VA present</td>
</tr>
<tr>
<td>5)</td>
<td>Ab + and No. 2 + C + EA</td>
<td>no lysis</td>
<td>OA present</td>
</tr>
<tr>
<td>6)</td>
<td>under No. 2 + C + EA</td>
<td>lysis</td>
<td>anti-complement</td>
</tr>
<tr>
<td>7)</td>
<td>Ab + and No. 3 + C + EA</td>
<td>no lysis</td>
<td>no anti-complement activity</td>
</tr>
</tbody>
</table>

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**Basis for Complement Fixation Assay**

| Ab + C + EA | Lysis |
| Ab + Ag + C | Ab/Ag + C + EA | No Lysis |

Presence of antigen is made visible by inhibition of lysis.

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**Core Notes, Appendix 8**

**Antigen-specific triggering**

- Proliferation
- Differentiation
- T-cell "help"

- T-cell killing: virus-infected cells, transplants

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

- Mixed Lymphocyte Reaction (MLR)

- Tolerance, suppression

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**T-Cell Functions**

- Inactivation of viruses
- Allergy
- Complement

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**Autoimmunity**

**Three "Lims" of the immune response**

- **Afferent**
  - Antigen processing (dendritic cells, MΦ et al.)
  - Antigen "presentation"
- **Central**
  - Antigen presentation
  - Inflammation
  - Killing of bacteria
- **Efferent**
  - Silent Lymphocyte Function (SLF)
  - Cellular and humoral immunity, (CT, P) adaptive reaction
  - Immune suppression