Deliverables

2 One-page and 1 Two-page papers on a single study we discuss in class.
- Focus on:
  - Who is the author?
  - What was the question/problem the study addressed?
  - What was known before the study?
  - What did the study add to that knowledge?
  - What technological advances made the study possible?

10-15 min presentation on a technique on the Timeline of Technology.
- Focus on:
  - Who developed the technique?
  - What problem does the technique solve?
  - What other technologies does it build on?
  - What is the newest iteration of the technique?
Paper Due Dates:
- 1st One Page Paper: 4/24 (Friday)
- 2nd One Page Paper: 5/15 (Friday)
- Two Page Paper: 6/3 (Wednesday)

Presentations:
- Sumedha 4/6 (Gel) Electrophoresis
- Christian 4/13 Cloning
- Andrew 4/20 Southern/Northern/Western
- Charles 4/27 PCR
- Paul 5/4 DNA Sequencing
- Susan 5/11 FACS
• 1796:
• Edward Jenner:
  • Used Cowpox to vaccinate against Smallpox
Luis Pasteur:

- Molecular Chirality
- Fermentation → Pasteurisation
- Germ Theory of Disease (1864)
- Anthrax and Rabies Vaccines
Robert Koch:

- Identified and Isolated causative agents for:
  Anthrax (Bacillus Anthraxis)
  Cholera (Vibrio Cholera)
  Tuberculosis (Mycobacterium Tuberculosis)

- Developed Pure Culture

- Solid Agar Plates for Isolation of clonal Cultures

Koch Postulates:
The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
The microorganism must be isolated from a diseased organism and grown in pure culture.
The cultured microorganism should cause disease when introduced into a healthy organism.
The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
Phagocyte Theory of immunity (ca. 1885)

One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defence of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts.

I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of blood-vessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done. There was a small garden to our dwelling, in which we had a few days previously organised a 'Christmas tree' for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star-fish larvae as transparent as water. I was too excited to sleep that night in the expectation of the result of my experiment, and very early the next morning I ascertained that it had fully succeeded. That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life.
Vaccine effects are mediated by components in the plasma

Emil von Behring (Nobelprize in 1901) (and Kitasato):

• Immune sera can protect naïve recipients

=> Humoral Theory gains the upper hand. Antibodies are recognized as mediators of immunity
Antibody Genetics

Paul Ehrlich:

Sidechain Theory (1897)
- Cells have preformed Receptors (sidechains)
- These get randomly bound by toxins
- Cells make more receptors
- Sidechains get secreted

⇒ Problems:
⇒ Thinks side-chains bind antigens absolutely specific and covalently
⇒ Too many antigens to be feasible
Karl Landsteiner:
- Discovers ABO blood groups (1900)
- Experiments with haptens (1918)
  - Antibodies can be formed against a large number of haptens

We did not think it was possible to explain the workings of normal and immune serum on innumerable cell types by the supposition of innumerable different antibodies and, by analogy, a similar fantastic number of receptors on each cell. We held, ... , that a simpler concept was quite adequate, that an antibody can react with a variety of related but not necessarily identical antigens ... The specificity of serum reactions appears to be expression of grades of affinity which reach a maximum in certain combinations -- those of antigen and homologous antibody ... while the Ehrlich theory admits of only a single absolute specificity
# Cross-specificity

## Table II

2 drops of immune serum were added to 0.2 cc. of the diluted antigens. Readings taken after 1 hour at room temperature and after standing overnight in the ice box.

<table>
<thead>
<tr>
<th>Immune sera for azoproteins made from</th>
<th>Readings taken after</th>
<th>( p )-Aminoazalicylic acid</th>
<th>( p )-Aminosalicylic acid</th>
<th>( p )-Aminosalicin acid</th>
<th>( p )-Aminoquinolinic acid</th>
<th>( p )-Aminophenylactic acid</th>
<th>( p )-Aminobenzoic acid</th>
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</table>

* \( B \)- represents \( \text{NH}_3\text{C}_6\text{H}_4\text{NHCO.}\)

† \( A \)- represents \( \text{NH}_3\text{C}_6\text{H}_4\).
Absolute specificity

Antibody Genetics

ABO Blood Reactions

Blood type

A  B  AB  O

Anti-A

Anti-B

Legend

- Red blood cell
- Galactose
- N acetyl-galactosamine
- N acetyl-glucosamine
- Fucose

Chemical structures:

1. [Chemical structure 1]
2. [Chemical structure 2]
3. [Chemical structure 3]
4. [Chemical structure 4]
Antigen Incorporation Theories

Buchner - 1893
Toxalbumin and antitoxin should, by their nature be, very closely related, and even substances of the same kind, or perhaps they may even be the different modifications of one and the same substance.

Hertzfeld and Klinger - 1918
We shall explain, in what follows, that the essential in all immunization events depends upon the antigen being split up to a certain degree in the organism, from whose origin composite, yet still specific breakdown products are absorbed on the surface of appropriate colloidal proteins, and this form represent 'antibody'.
What are antibodies?

• 1930s: Antibodies are proteins
• Information for all possible antibody can’t possibly fit in a genome!

Kabat, 1938
Instruction Theories

Fig. 4.1. Pauling’s direct template scheme of antibody formation. (From ref. 4.)

Pauling, 1940
• Niels Jerne (1955): Natural Selection Theory

Antibodies are present before antigen is ever present. Antibody binds antigen, is taken up by cell able to reproduce antibody somehow.
The Clonal Selection Theory

- Burnet, Talmage, and Lederberg (1950s):
  - Natural Antibody on cell surface. Binding antigen activates cell (clonal expansion) and makes more antibody
  - Antibody diversity caused by randomly mutating DNA
  - Specificity due to Aminoacid sequence
## Antibody Genetics

### One B cell – One Antibody

<table>
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<tr>
<th>No. of cells in drop</th>
<th>No. of drops inhibitory</th>
<th>No. of drops tested</th>
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<td>6*</td>
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<td>6–10</td>
<td>17</td>
<td>33</td>
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<tr>
<td>First tested versus <em>S. typhi</em></td>
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<tr>
<td>1</td>
<td>3*</td>
<td>18</td>
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<tr>
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<td>8</td>
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<tr>
<td>6–10</td>
<td>22</td>
<td>42</td>
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</tbody>
</table>

Lymph node cells from rats presensitized to *S. adelaide* plus *S. typhi* were dispensed in micro droplets and incubated for 4 hr. They were then tested by the introduction of motile bacteria.

* These droplets were also tested for activity against the alternative serotype and were negative.
Reading

Antibody Production by Single Cells
G.J.V. Nossal & Joshua Lederberg
1958
Nature

Why did they do their experiment?
What did they do?
What was their interpretation of the results?