Lecture 10: Class Switching & Affinity Maturation

Class Switching: Do not get class switching confused with VDJ joining. Although both involve DNA rearrangements of the heavy chain locus, VDJ joining occurs in the bone marrow and generates a functional V_{H} exon. Class switching occurs in mature B cells after activation and changes the heavy chain constant exons from one type to another.

- Homologous DNA segments call switch regions (e.g., S_{mu}) are found to the 5' side of exons for each type of heavy chain, with the exception of between IgM and IgD.
- Homologous recombination occurs between switch regions.
- Particular switch regions that are used is controlled by the local cytokines after activation.
- Recombination is initiated in response to hyper-mutation of the switch regions (DNA repair process).
- DNA (exons coding for heavy chains) are removed during recombination is usually lost from the B-cell.
- Constant region closest to the complete V-exon (VDJ) is the one expressed due to mRNA splicing.

Example: High IL-4 Concentration
Affinity Maturation (also called Somatic Hypermutation): During the immune response, the average affinity of antibodies can increase 100-1000 fold as a consequence of random mutations in both the $V_{H}$ and $V_{L}$ exons. The increase in affinity allows antibody to binding to pathogens at low concentration. Note that the constant exons are spared from this mutagenesis.

Ligand Binding Review:

\[ Y = \frac{[Ab-L]}{[Ab]+[Ab-L]} = \frac{K_A[L]}{1 + K_A[L]} = \frac{K_D}{1 + K_D} \]

Where $[Ab-L]$ is the antibody–antigen complex, $[Ab]$ is the free antibody and $[L]$ is the ligand (antigen).

The fractional saturation ranges from 0 ($[L]=0$) to 1 ($[L] >> K_D$). The $K_D$ is [L] that gives $Y=0.5$.

High $K_A$ or Low $K_D$ = high affinity.

Avidity versus Affinity:

Affinity = e.g. interaction between one $F_v$ region and antigen, $\Delta G^0 = -RT \ln K_{eq}$.

Avidity = e.g. interaction between multiple antibodies and antigen, $\Delta G_{oBS}^{0} = \sum \Delta G^0$.

\[ K_D^{obs} = K_D \]

\[ K_D = \frac{1}{K_A} \]
Affinity Maturation Process:
- Somatic cell mutations restricted to heavy and light chain V regions, this is possible because they are in separate exons, removed from the constant regions (which do not accumulate mutations).
- Mutations can cause increased binding, decreased binding, or even make a non-functional antibody.
- Mutations that lead to an increase in affinity tend to be selected for activation by T<sub>H</sub>-cells because they are more able to present antigen to the T-cells.

Effect of Antigen concentration on Affinity Maturation

<table>
<thead>
<tr>
<th>Antigen Concentration</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; of Antibodies μM</th>
<th>1 week</th>
<th>2 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg/mouse</td>
<td>5.0</td>
<td>1.0</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>250 mg/mouse</td>
<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Eisen and Siskind (1964) Biochemistry 3, 1966.

at 250 mg/mouse all Ab are sat regardless of mutation, all B-cells are stimulated by same amount; no selective pressure, no affinity maturation.

Role of cytosine deaminase in affinity maturation & class switching: A cytosine deaminase is induced in B-cells during activation by T<sub>H</sub> cells. This Activation induced deaminase (AID) coverts cytosine to uridine in DNA, causing direct mutation to the V-regions and initiation of DNA repair-recombination at the switch regions.