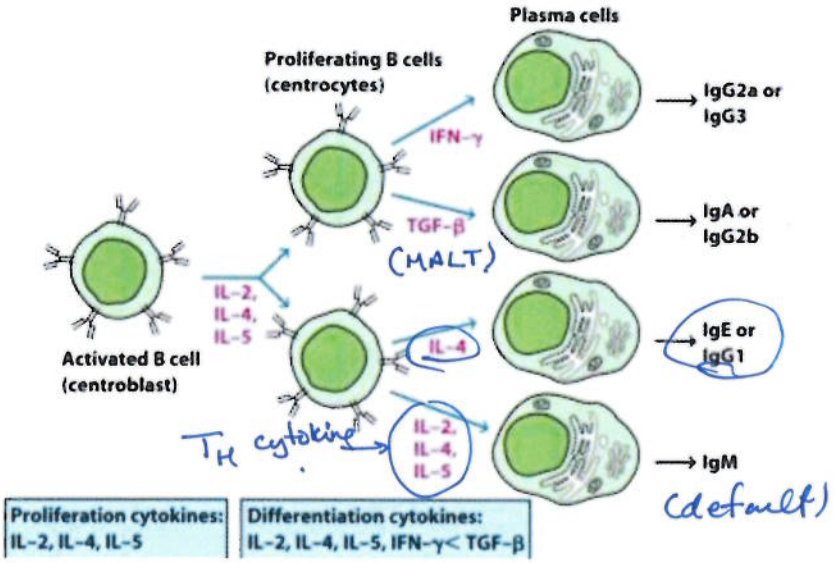
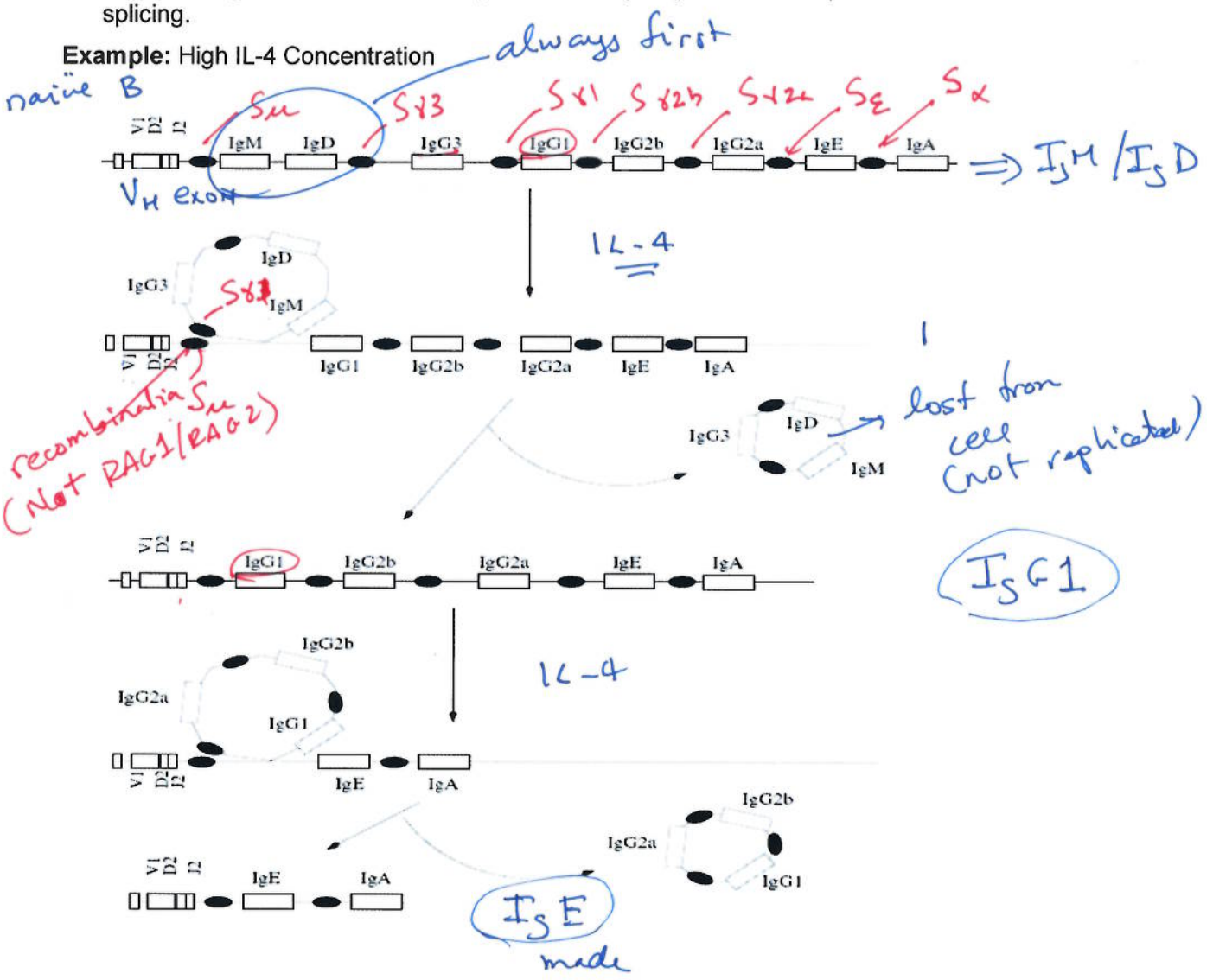
Lecture 10: Class Switching & Affinity Maturation

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.

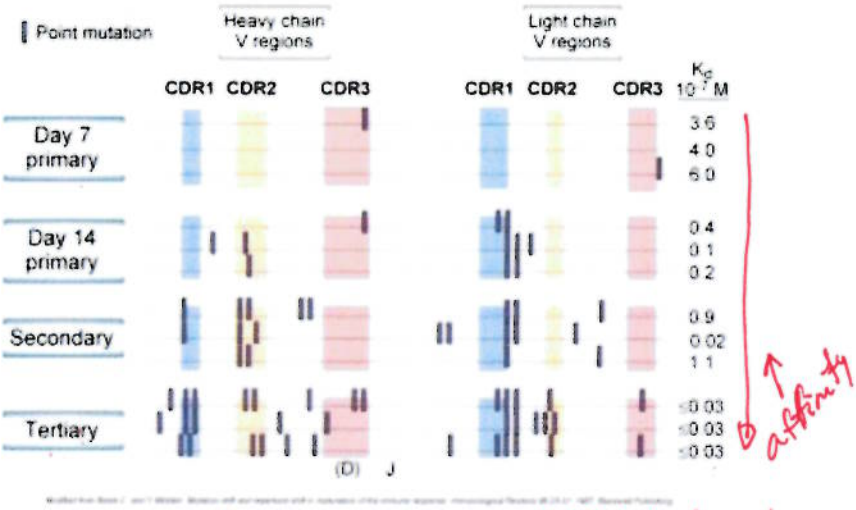


- Homologous DNA segments call switch regions (e.g. Sμ) are found to the 5' side of exons for each type of heavy chain, with the exception of between IgM and IgD.
- Homologous recombination occurs between switch regions.
- Particular switch regions that are used is controlled by the local cytokines after 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.

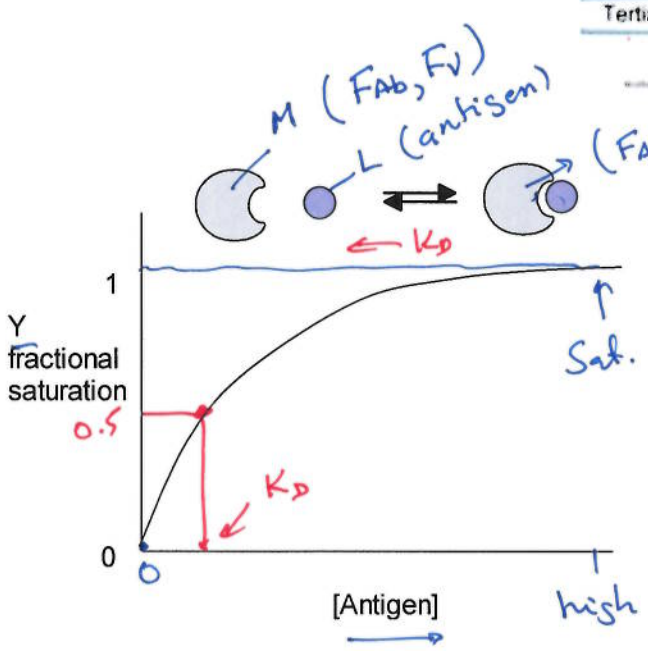
Example: High IL-4 Concentration



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:



$$K_D = \frac{(Ab)(Antigen)}{(Ab\text{-}antigen)}$$

The affinity, or association, constant for binding is $K_A=k_{on}/k_{off}$; where k_{on} is the forward rate constant while k_{off} is the reverse rate constant, the rate at which the antibody-antigen complex dissociates. Differences in binding affinities are usually due to differences in k_{off}.

The dissociation constant is also used: $K_D=1/K_A$. The K_D is the ligand concentration to half-saturate the sites.

The fractional saturation, Y, is defined as:

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

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.

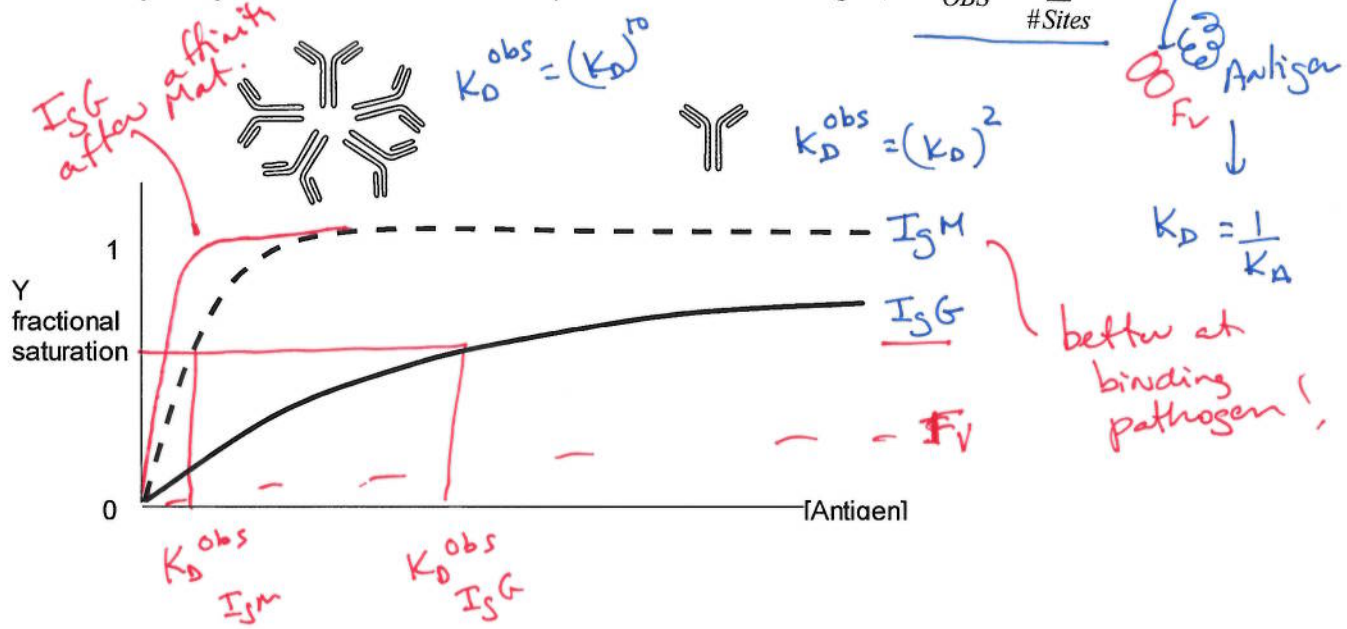
High K_A or Low K_D = high affinity.

Avidity versus Affinity:

Affinity = e.g. interaction between one F_V region and antigen, $\Delta G° = -RT \ln K_{EQ}$.

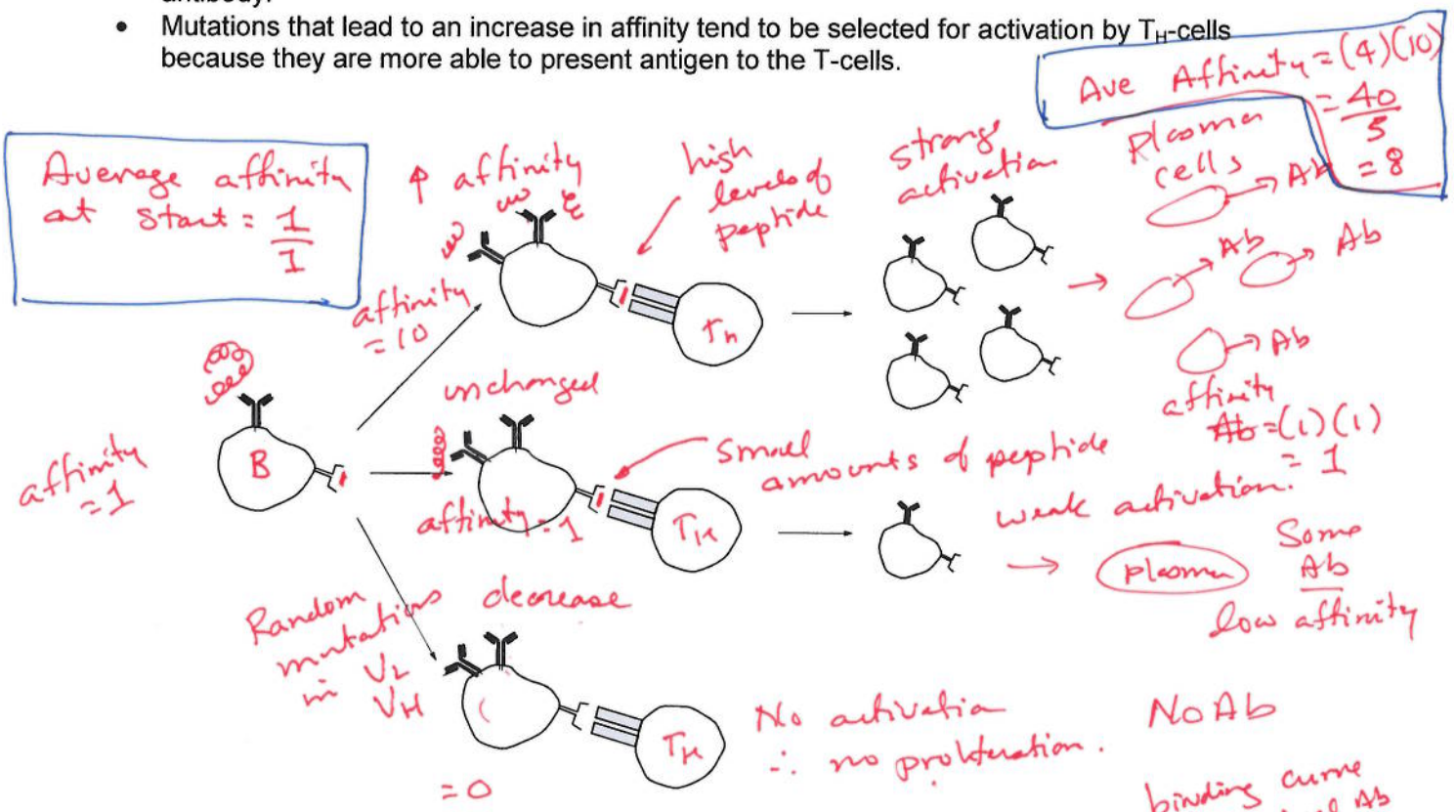
Avidity = e.g. interaction between multiple antibodies and antigen, $\Delta G^o_{OBS} = \sum_{\#Sites} \Delta G^o$

$\Delta G^\circ = -RT \ln \frac{1}{K_D} = -RT \ln K_A$



Affinity Maturation Process:

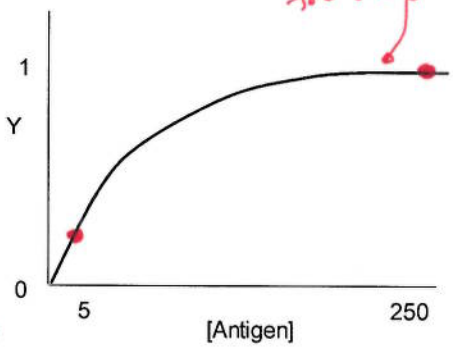
- Somatic cell mutations restricted to heavy and light chain V regions, this is possible because they are in separate exons, removed from the constant regions (which do not accumulate mutations).
- Mutations can cause increased binding, decreased binding, or even make a non-functional antibody.
- Mutations that lead to an increase in affinity tend to be selected for activation by T_H-cells because they are more able to present antigen to the T-cells.



Effect of Antigen concentration on Affinity Mateation

Antigen Concentration	K_D of Antibodies μM		
	1 week	2 weeks	8 weeks
5 mg/mouse	5.0	1.0	0.01
250 mg/mouse	5.0	5.0	6.0

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_H 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.