Lecture 21 – SPR, TCR Structure, Transgenic Mice

Surface Plasmon Resonance (SPR)

- Measure the binding affinity & kinetic on- and off-rates by detecting presence of mass on a gold surface.
- The antibody can be attached to the surface, and antigen is flowed over the surface.
- Response (RU) of the instrument is proportional to the amount of antigen bound.
- By varying the antigen concentration it is possible to determine the dissociation constant, $K_D$, from the measured RU at equilibrium.
- The initial rate of the RU change after addition of the ligand can be used to measure the kinetic on-rate: $k_{on}^{obs} = k_{on}[antigen]$. $RU(t) = (1 - e^{-k_{off}t})$

- The off-rate can be obtained by measuring the signal after washing out the antigen: $RU(t) = e^{-k_{off}t}$

SPR Data. Increasing amounts of antigen (cyan, blue, magenta, red) were introduced at time=0 to a plate containing antibody. As ligand absorbs to the plate the response increases. At $t=50$ the ligand was removed. The height of the response after equilibrium is reached is proportional to the fractional saturation, $Y$. The rate of signal build-up at the different ligand concentrations can be used to estimate the on-rate. The decay of the signal after ligand is removed provides the off-rate.

SPR Instrumentation. Panel A illustrates only the optical path. Light beams 1, 2, and 3 are refracted by the circular glass (GP) prism and show total internal reflection at the glass (G) water interface, generating an electrical field (E, yellow) that extends several hundred angstroms into the aqueous compartment. Note that the three light beams strike the glass plate at different angles due to the curvature of the prism. Although three light beams are shown, in practice a continuous spread of light is used. In an SPR sample cell (B) a thin layer of gold (Au) is coated on the glass slide. Coupling between the light and the electrons will excite plasmon waves in the metal, leading to absorption of the light. The strength of the coupling depends on the angle of incidence and the refractive index of the aqueous compartment. In this example light beam 2 is absorbed by the gold film. Panel C shows the effect of increasing the mass at the gold surface by the attachment of antibody molecules (Ab). The change in refractive index alters the incidence angle that stimulates plasmon waves. In this example, light beam 3 is now selectively absorbed by the gold after the attachment of antibodies. The binding of ligand to the antibody (not illustrated) would lead to a further change in the angle of incidence that stimulates plasmon waves in the gold. The change in the incidence angle required to stimulate plasmon waves is measured by the instrument.
TCR Structure:

This is a ternary complex consisting of:
- Class I MHC: HLA-A2(A6) (6th allele of HLA-A2 class I HMC)
- HTLV-1 Tax peptide: human T-cell lymphotropic virus.
- αβ-TCR receptor that is specific for this peptide and this allele of HLA-A. Note that the structure of the constant domain of the α-chain is not shown in this structure.

TCR on αβ TCells:
1. αβ-chains (recognize self MHC-I/peptide complex). Each chain consists of:
   i) Variable region (amino terminus), with three hypervariable (also known as complementary determining regions, or CDR1) loops. Similar level of diversity as BCR.
   ii) Constant region (carboxy terminus)
   iii) Membrane anchor
   β = heavy chain (VDJ)
   α = light chain (VJ)

2. Associated with phosphorylation signaling domain composed of γε δε heterodimer and ζζ homodimer. The collection of signaling chains is referred to as CD3, and is recognized by anti-CD3 antibodies.

3. Crystal structure shows the following interactions between the TCR and MHC. 1, 2, and 3 are the hypervariable loops of the individual chains of the TCR.
Testing the biological relevance of this model:

T<sub>H</sub>-cell line was generated that was specific for moth cytochrome c (MCC) residues 88-103 in the context of IE<sup>k</sup>. This T<sub>H</sub> cell line would recognize the MCC peptide when bound to the IE<sup>k</sup> allele of type E class II MHC. Alteration at positions 95, 99, or 102 within the peptide could not activate an immune response.

The model predicted the following:

- Residue 95 (K) should be recognized by MHC (anchor residue)
- Residue 99 (F) should be recognized by the α-chain of the TCR.
- Residue 102 (D) should be recognized by the β-chain of the TCR.

To determine which positions interact with the MHC and which interact with the two different chains of the TCR the following experiments were done:

**Testing MHC Interaction**: MHC binding measured using a RIA approach by immobilizing IE<sup>k</sup> in a plastic well. The following Radioactive MCC was added, along with other non-labeled peptides and the amount of bound radioactivity was measured.

**Conclusion?**

**Peptide (unlabeled)**

None 100% (top right figure)

-K-F-D- (MCC) 50% (middle right figure)

-A-F-D- 100% (bottom right figure)

-K-A-D- 50%

-K-F-A 50%

**Testing TCR Interaction**: Transgenic mice, expressing only the rearranged α-chain of the TCR that recognized MCC-IE<sup>k</sup> were produced. These mice produce T-cells with 90% of the α chain from the introduced transgene. **Why are all the α-chains the same?**

**Peptide**

- K-F-D-(MCC) 100% (control)

- K-A-D- 10%

- K-F-A 100%

- K-F-W 100%

- K-F-R- 100%

Replacement of the middle residue (F to A) prevented activation, therefore the Phe(F) at position 99 is recognized by the α-chain of the TCR.