Antigen – Antibody Interaction #4 8/7/2020

Ag - Ab bonds

① Hydrogen bond : Ag OH.... Ab O

② Ionic bond

③ Hydrophobic Interactions

④ Van der Waal forces due to particles' movements

The antigen's amino acids must be compatible with the antibody's amino acids for the binding to take place. Antibodies have different binding sites but they can change their heavy chains to change from one class of antibody to another. (Example : IgG to IgM). However, they continue binding to the older antigen. This is called a class switch and depends on the immune system's needs. This helps the antibodies to switch functions.

Interaction shapes :

① Pockets : antigen fits in the antibody's binding site that is shaped like a pocket.

② Groove :

③ Extended surface : both antigens and antibody's binding sites are flat.

Cross Reactivity (Multispecificity):

1 antibody can bind to more than 1 antigen in the presence of a shared epitope. This means that 2 antigens can be similar and this allows them both to bind to the antibody's binding site.

Drug allergy:

Penicillin has a β lactam ring. Due to its small size, it is classified as a hapten. It does not trigger an immuno activation But if penicillin + carrier proteins = immunogen → IgE release by immune system

If penicillin cannot be given, give tetracyclin.

Elisa: To check for the presence of the antigen/antibody.

Aplate with wells (96 wells).
Positive control: To make sure the test was accurate.
It contains HIV and we are sure of it.
Put the HIV proteins in the plate. If it binds

They take the patient's serum and put it on the plate. Incubate it and check for interactions. Antibody ... Antigen. Wash the unbinded off.

To check for interaction:

Get an antibody against the FC fragment of the antibody. It is called a secondary / conjugated antibody. This secondary antibody is conjugated with (HRP) horseradish peroxidase. If the secondary antibody binds with the FC fragment, it gives off a colour.

Negative control:

X primary antibody --- antigen interaction = X secondary antibody - primary antibody attachment = X colour change

↑ antibody in serum = ↑ in colour change

- Colourless → colour change takes place at the last step.
- Cross reactivity can influence Elisa. That is why +ve and -ve controls are important.
- Can determine disease stage using Elisa.
- Used to check for Sexually transmitted diseases in blood donors.
- Fluorescent dyes can be used to check Elisa's result. It is not used primary due to it being time-consuming, costly and needing fluorescent microscopes.

Lateral flow test :

Example : Pregnancy Test
Checks for the presence of (hCG) in urine.

Site 1: urine is applied here, highly absorbant
contains anti-hCG bead complexes
● ------- anti-hCG (so, not human antibodies)
bead

Mouse anti-hCG antibody : line 1
positive

Sheep anti-hCG antibody: used as control. Attaches to mouse

Step 1: urine on anti-hCG → +ve : hCG attaches to antibody
or
→ -ve : nothing attaches to antibody

Step 2: diffuses and takes the antibody along with it
Step 3: Line 1 → +ve : hCG binds to mouse antibody
or
→ -ve : nothing attaches

Step 4 : Line 2 → +ve : hCG binds to sheep anti-hCG

Why do we use mouse anti-hCG?
human -- human antibody antigen interactions cannot happen
Only a different species can attack another's antigens.

Why do we use sheep anti-mouse anti-hCG?
Same reason. Mouse -- Mouse antibody antigen interactions cannot happen.

Immunofluorescence:

We conjugate antibodies which are attached to fluorochromes (give fluorescense under fluorescent machines / microscopes).

FITC (a dye) ---------- attaches to ---------- Amino Acids
|
Proteins

= green colour under UV light

Antibody against a bacteria ---------- attaches to ---------- bacteria
|
FITC

= ✓ bacteria = green
✗ bacteria = nothing

X cost effective
depends on tissue instead of serum

(a) direct (b) indirect

. 1 step
. 1 antibody against the antigen

FACs:

used to diagnose cancer
depends on fluorescent dye
we use antibodies against specific cells to sort them out
different antibodies are conjugated with different colours

antibody —attaches to— specific cell
|
fluorescent dye + UV light = colour change if true

This allows us to count cells whether they be cancerous or not. Every cell (dyed or not) is counted and seperated into different tubes.