BI3224 - Introductory Immunology

19/1/22

There exist different levels of organisation in biology

Organelles - macromolecular stable assemblies that create confined
(sub-cellular) spaces which in turn act as interact as units
with emergent properties

Supraorganismal level - ecosystem, evolutionary studies.

In between these two areas, we have the organismal level
— how the individual functions

For billions of years, individual level was the same as
cellular level. But things change when cells assemblages
i.e. multicellular individual behaves as an entity at
a level above.

Natural selection now acts on multicellular individual.
shaping its functional anatomy over ages.

Now, individual level connects cellular level & ecosystem level.

Cells talking to each other (in an individual) over longer
distances introduces complexity and uncertainty,
when functional anatomy is optimised/shaped over a
long time.

Biotic stresses change faster & is bidirectional, as
compared to abiotic stresses. Biotic stress drives
evolutionary arms race.

The immune system is an extraordinary example at
the individual level where uncertainity and complexity
is a way of life.

First half: Historical perspective and nature of evidence in
immunology.

Antibody (Ab)

Its a common motif in immunology. How did we come to know Abs exist?

Immunity against infectious disease

How did we come to think about 'immunity', 'infectious' 'diseases'?

Symptoms — signs of disease/illness

Syndrome — groups of signs & symptoms — a useful category

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Lecture 02

What is disease?

Physiologically, we need a better definition than the semantic one.
Early evidence was experience based.
Symptoms & diseases are important to think about the progression of the illness.
Things that the ill person notices about themself as descriptions of theres disease are <u>symptoms</u>.
What other people see/notice wrong in the person are <u>signs</u>.

<u>Some symptoms occur together</u> — noticing these patterns allows people to <u>group them</u> and give a certain name to each cluster, each disease. (This is empirical)
Then, certain medicine and care for certain disease leads to better outcome. This trial & error method builds a structure with a basis of causality.

The next question is: why do some people & not others get sick?
There emerges a correlation in space and time —
<u>case clustering</u> gives an empirical <u>basis for infectious</u> or <u>transmissible</u> diseases.
We're not thinking about agents of transmission yet.
But we see that some diseases are transmissible, and others are not.
We notice that those who don't catch the disease are those who have had it before — this leads us to the idea of <u>specific immunity</u>.

All the evidence till now is empirical : pre-historical notions.

Contemporary definitions — difference b/w syndrome & disease

Syndrome: A <u>clustering</u> of <u>symptoms</u> that come together reliably and allows us to predict the trajectory, but we <u>don't know</u> what the proximate <u>cause</u> is, is called a syndrome.

If we know the cause, it a disease

30 - 35 mins - missed

<u>Small pox</u> — <u>variola virus</u>

This virus causes clustering of cases in time & space, spreads quickly, infection causes <u>specific symptoms</u> (pox, scarring, loss of hearing & vision) — these features lead to speculate <u>specific causes</u>, because when there's another breakout, those who had gotten it previously weren't infected.

This gives the idea of protection against specific diseases — immunity. Practically, this can be used to recruit nursing people who have had the disease before

Another useful observation : susceptible people exposed to an infected person late in the disease get a mild disease and not a severe one.

This leads to a technological leap : <u>Variolation</u>
We can protect someone from disease by giving them a <u>mild form</u> of disease

This was practiced in China, South east Asia, Africa

Important to keep in mind that this immunity is specific but this notion is overturned later.

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Lecture 3
To variolate someone, the scabs from late stage were dried and ground up and blown through someone's nose to cause a 'mild' if infection, thus giving them specific immunity against this disease

At this point, we didn't know about innate immunity (phagocytosis, complement system etc). The evidence had dragged us towards specific protective adaptive immunity.

1 century English physicians observed that they couldn't variolate some people — the patients didn't get the mild illness.

This was another observational pattern that physicians noticed.

Edward Jenner

Used cow pox to give people protective immunity over small pox

Vaccine comes from latin 'vacca' which means cow.

John Fewster

Physician before Jenner who noticed the same pattern

He also noted that those who couldn't get variolated also didn't catch the disease in the next outbreak.

It seemed they had some sort of 'natural immunity' against small pox.

There was a correlation between those who had this 'natural' immunity and the milking profession. — they had gotten cow pox before

Technological translation → Jenner showed that cow pox vaccination (which carried much less risk than variolation) protected people from small pox

Conceptual conundrum → This is in contradiction to the theory of that time — its against the idea of adaptive inducible specific protective immunity.

It would take substrate from other fields till this could be solved

Before we can study the mechanisms of immunity, we needed to understand the mechanism of transmission

1) How do we arrive at microbes causing disease?

We can find (think about) microbes by seeing them through the microscope (Leeuwenhoek).

2) How do we know microbes are involved in processes?

With experimental set ups, Pasteur established that microbes were involved in fermentation — by using apparatus that could isolate substrate from bacteria.

Students of Pasteur's schools isolated specific bacteria by growing it on solid medium (agar agar).

They also established that microbes caused diseases by injecting titrated amounts to animals and seeing what happens.

Pasteur's ← Another insight : You could weaken the microbe (heat, chemicals) which doesn't give cause infection but gives immunity.

Lecture 4

The evidence we've seen so far led us to adaptive specific immunity, and not innate immunity.

Vaccination (not variolation) challenged the idea of disease specific immunity.

We didn't know the nature of protective immunity because we didn't know enough about what caused the diseases

There were some insights from Pasteur's school.
But, they were studying bacteria, not viruses.
The other facet of unknown was the <u>host body</u> — what was it doing to protect itself?

What do Bacteria do in the body?
There were two hypotheses — bacteria divided rapidly and filled the entire body, killing it.
Other one: Bacteria produce some toxins that kill the body.

<u>Emile Roux</u> & <u>Alexandre Yersin</u> — study of Diphtheria
Corynebacterium diphtheriae affects the throat, causes sores, and which kills a lot of infants.

The scientists had an apparatus that filtered bacteria from the culture. They <u>injected</u> the <u>filtrate</u> to the animal and saw that it still killed animals.

So the disease was being caused by a <u>compound</u> secreted by the bacteria.

Tetanus (<u>Clostridium tetani</u>) is also caused by a toxin produced by bacteria. Behring and Kitasato discovered this in 1890, 2 years after Roux & Yersin.

How do you immunise someone from toxins?
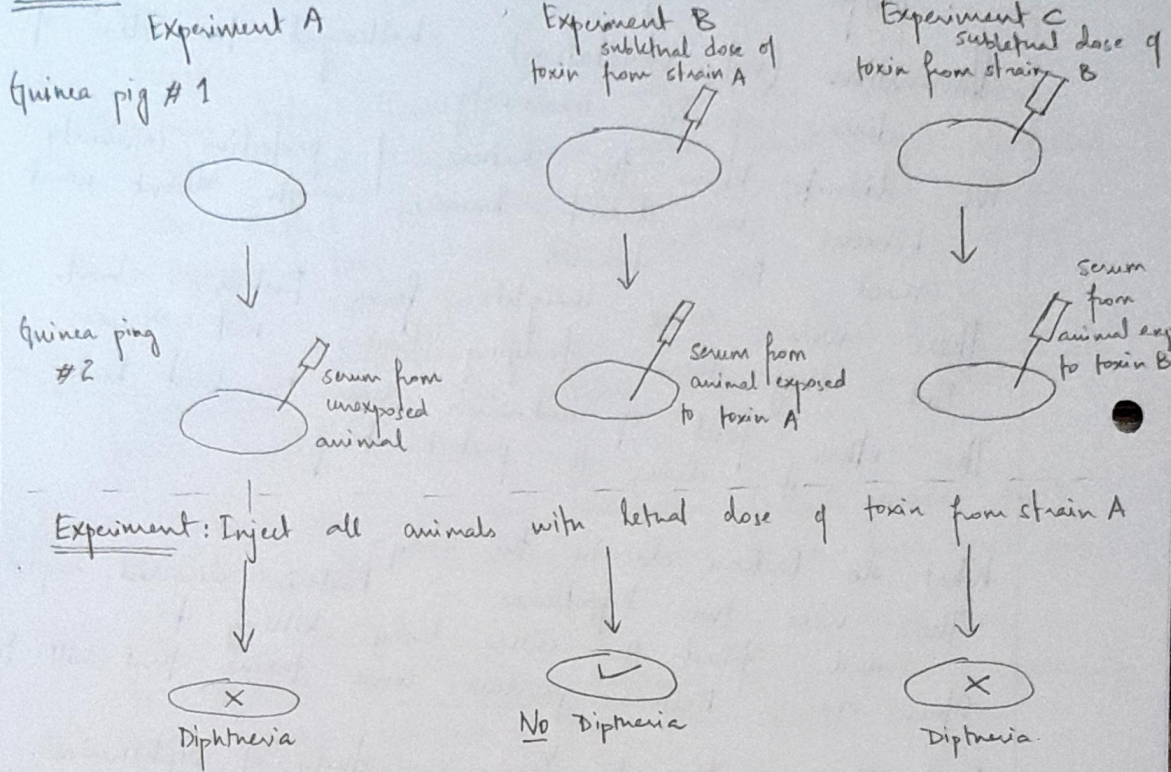With microbes. they were treated with heat, acid or alkali to weaken them, and a titrated amount was administered.

With trial and error, people discovered methods to make the toxins weak — called toxoids.

DPT vaccine — toxoid vaccine

Behring and Kitasato experiment (Robert Koch Institute, Berlin)

Method

	Experiment A	Experiment B	Experiment C
		sublethal dose of toxin from strain A	sublethal dose of toxin from strain B

Guinea pig #1

Guinea pig #2 — serum from unexposed animal / serum from animal exposed to toxin A / serum from animal exposed to toxin B

Experiment: Inject all animals with lethal dose of toxin from strain A

× Diphtheria / No Diphtheria / × Diphtheria

Immune system is present everywhere — if disseminated — but insights can't be gleaned from dissections and the resulting functional anatomy.
Immunity is not tissue specific — the immunogen could be injected in one place, and the bacteria/toxin in another place, and the animal was still protected
This points towards the circulating medium in the body (serum) playing an important role in immunity.

From the experiment, they saw that transferring serum from an immunised animal gave immunity to an unexposed animal. This was a huge step.
Translationally, this made ~~therapi~~ serum therapy possible
Mechanistically, we understand that a cell-free fluid from host (serum) provides protection from the microbes' toxins.
This reduces to a biochemistry problem — this is the trajectory towards discovering antibody ~~problem~~ proteins.

Thomas Gregor Brodie (1897) — worked on the nature (chemist) of Diptheria antitoxin. How the did some experiments and found that its a protein of _globulin_ class.

Lecture 5

Brodie did these experiments to test whether the antitoxin would be destroyed by digestive enzymes, pepsin and trypsin.

He used biochemical methods such as salt fractionation (solubility of protein), molecular filteration, and later experiment with enzymes.

He was still using biological means — as in, whether the guinea pig still got diphtheria or not. Studying the antitoxin would be much easier if the toxin and antitoxin serum could be studied directly

Take toxin A, B and antitoxin A, B in different testtubes and mixed —

	Antitoxin A	Antitoxin B
toxin A	▨	▯
toxin B	▯	▨
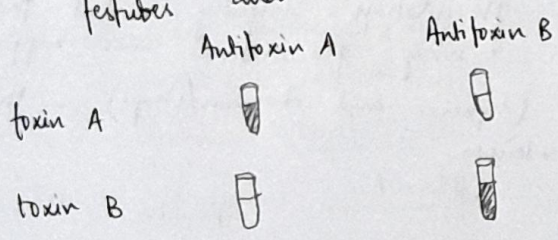
When mixed, contents aggregated / flocculated with the same specificity as that of biological immunity.
So its a good guess that the _flocculating_ entity is also _the protective_ entity.

They tested this by injecting the solution (minus the flocculate) to guinea pig and confirming that removing the precipitate doesn't give immunity.
This is the basic antibody-antigen interaction.

R R Porter (1959)

① Formation of specific inhibitor by Hydrolysis of Rabbit Antiovalbumin.

Any macromolecule elicits a serum antibody response — i.e. mixing the molecule and serum of exposed animal precipitated the contents.

Pasteur's school did same experiments with bacteria — they observed agglutination

Antibodies could be generated to <u>mammalian cells</u> and antiserum was being produced   RBCs — hemagglutination
(discovery of blood type)

Something about adjuvant improving immune response rate

Adjuvant — a substance that enhance's body's immune response to an antigen

Jennerian paradox: immunity can be cross-reactive, the <u>specificity is flexible</u>. —Mechanism?

↗ of all egg albumin
Next set of experiments: Reacting antisera to albumin of different bird eggs — this would have informed a lot about specificity. (Landsteiner 1940)

People were describing flocculation carefully — but that didn't explain why & how flocculation was happening.

Proteases are specific (sequence and domain/shape) — they digest some proteins.

⟶ Something Rod Porter did

papain
First react egg albumin antibody with papine (protease). So now it won't flocculate with egg albumin

So to test this —

he added sera + albumin To this, he added increasing amount of papine — digested antibody.

With increasing addition of digest, the flocculation rate decreased, in a target specific fashion
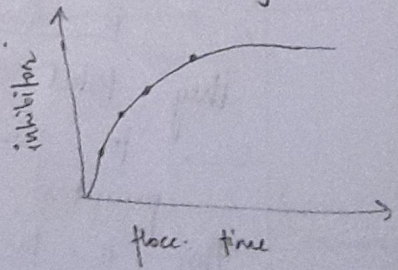
This showed there's a difference between binding (Ab-antigen) and flocculation

⟶ Even after digestion, the antibody binding part is still present, but its not clumping. It was discovered <u>by</u> the <u>inhibition</u> of clumping

inhibition ↑

flocc. time →

* With a part of the sera each protein removed the
precipitating antibodies for itself and for the more weakly
reacting proteins

⑨

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Lecture 6

Landsteiner [1940]

Cross reaction of egg albumin sera (Beidenberg & van der seer)

They mixed hen albumin rega with turkey, guinea
hen and goose. The flocculation intensity
varied based on evolutionary relationship.

Another expt: mix sera with turkey albumin, remove the
flocculate and then mix the rest of it with
other albumin to see whether it cross reacts or
not.

Result*: If reacted with turkey first, it didn't flocculate
with the rest.
But if reacted first with goose, if flocculated with
all other albumin.

Porter's Second Experiment. (1959)

He generated antibodies against the fragments created
by papain. Fragment 1 Ab bound with whole
egg albumin antibody and inhibited flocculation, but
fragment 2 didn't inhibit flocculation. (& didn't bind to
whole egg albumin
antibody)

Fc - fragment crystallizable
Fab - fragment antibody (inhibits flocculation)

Edelmann and Benacerraf (1962)

They found that all Antibodies migrate as single band in
electrophoresis, but after treatment with 8M urea,
they began to travel in 2 bands
This was true for all antibodies, irrespective of the target.
This pointed towards a light chain and heavy chain

Going back to Innate Immunity

In 19th century. people observed that if serum was
added to bacterial culture, then they observed that
bacteria was lysed.

one ← Ehrlich - "complement" mediates lysis   Bordet - many complement

* **Thomson** (1903) - (human serum causes lysis of bacteria as well)
  ↳ serum of normal, vaccinated & vaccinated rabbits. ↓
  lysed bacteria the same   C & C

Chapin and **Cowie** (1907)

①	Normal human serum	Lysis	They started to work
②	Absorbed - at 0°C	No lysis	out the pathway of
③	Heat treated	No lysis	complement reaction
② + ③	solution	Lysis!	

↳ This showed that ② absorption removes a specific target immune component that regulates the process, whereas ③ removes a downstream, general protein, non-target specific.

Evidence was gathered and ultimately interpreted in a prejudicial manner

* **Arthur Wormall** (1925), (1926)
  They began working out the components of complement
  by destroying parts of it with enzyme/ammonia
  and then adding heat treated serum and seeing
  whether complement action occurs or not.

  They are talking about classical complement pathway -
  Something triggered by specific antibodies.
  How did we arrive at non-specific, general reactive
  innate immunity?

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Bal & Rath Resonance (1)
Immune system - has to be present everywhere
                has to be inducible, & otherwise resting
                it undergoes cellular maturation after being triggered.

→ Immune Response involves - Detection and Action [modular]
→ Two models of target recognition; clonally uniform & diverse
• Clonally uniform - identify targets by recognising molecules that are
                    "foreign" by classification category. (eg lipopolysaccharides)
                    Macrophages & polymorphonuclear WBCs. But this
                    is not enough for mammals
• Clonally - Each target will trigger only a subset of immune system
  diverse   but one cell is not enough - cell proliferation is
             important - also immune memory!

Lecture 7

Antibodies act in the serum of blood, so they were called humoral immunity.

How do we arrive at immune cells?

We knew about microbes as cells, and about the cells in the body. But how do we connect these cells to immunity?

Phagocytosis and Immunity (1891) - Elias Metchnikoff
He was studying the body's response to bacteria
How to arrive at the idea that there are processes of non-specific immunity provided by specialised immune cells, are common to all metazoans.

He notes that some microbes are found in the fluids and others inside the cell. He postulates that microbe's passage inside the cell is due to amoeboid movement of the cell or the microbe.

• Microbe going into body cell — rarer occurrence Eg. Malaria
• Cell engulfing a microbe — more common; the amoeboid cells send out pseudopodia to engulf the microbe

He called them phagocytes — fixed (endothelial cells), free (WBCs)

He also clarifies that 'phagocyte' and 'lymphocyte' are not synonymous
He describes 3 types of leucocytes — small, immobile cell (lymphocytes), macrophage & microphage (neutrophils — PMNL)

He connects phagocytosis with immunity: "the more malignant the microorganism, the rarer is its presence in macrophage." In diseases that are rapidly fatal, the bacteria are mostly found in the blood and present as general acute sp septicemia. So, macrophages are functionally efficient at localising infection. but if it gets out of hand. then the body is overwhelmed and phagocytosis is not effective.

(12) How to quantify phagocytosis so it can be reproduced?

\# Metschnikoff's observations were dismissed as opportunistic observations, that immunity was mainly humoral and not cellular. Others also foisked upon him that he was proposing an either/or idea

1. He says that <u>vaccination</u> <u>increases</u> (enhances) the <u>efficiency of local reaction</u> to re-infection i.e. the efficiency of macrophages

(?) 2. Second point — phagocytes act antagonistically to microbes, so it's important for immunity, and this process is enhanced by in vaccinated animals. (now known as inflammatory response).

This finding was disregarded because Behring & Kitasato had showed that specific immunity was transferable by cell-free medium — serum. But also vaccinated individuals show enhanced phagocytic action. What would have resolved these two methods of thinking?

Metschnikoff paper
* Over the course of a disease, bacteria are first found largely in the blood, but around the time of disease resolution, the microbes are found in phagocytes of the spleen.
* Compares phagocytosis to intracellular digestion of amoeba
* Phagocytes show <u>positive</u> chemotaxis to some stimulants and negative chemotaxis to others
* Another kind of opposition : phagocytes only 'include' microbes killed by other means, and not live ones.

3/2/22

Lecture 8
Metschnikoff paper
- When bacteria enter the body, there's an inflammatory response and phagocytes come to the site of invasion
- He compares macrophage phagocytosis to intracellular digestion mechanism of amoeba — evolutionary comparison.
- He also says that <u>cellular microenvironment</u> plays an important role in mediating phagocytosis — because bacteria are detected and act as <u>chemotactic cues</u>.

In vaccinated animals, leucocyte includes bacteria, but not in unvaccinated animals.

→ Major <u>Leishmann</u> 1902 — method of <u>quantitatively</u> <u>estimating</u>
   the phagocytic power of leucocytes

Metschnikoff had anecdotal evidence of phagocytosis. Leishmann
developed stains to study cells.
He counted the number of bacteria phagocytosed by
poly_{"morpho}nuclear leucocytes in vitro as compared to a
control blood sample. the compared the numbers statistically

→ <u>Hamburger</u> 1916 — Research on Phagocytosis
   By this time, they could measure the <u>time</u> for q
   phagocytosis, <u>number</u>, efficiency etc.
   Then they compared these numbers when cell was
   placed in <u>different chemical environments</u>. They
   studied the effect of acid. alkali, salt. N_2 etc on
      the efficiency of phagocytosis.
   They began to substitute bacteria with other particles of
      different shapes and sizes. They used carbon,
   starch granules (rice flour) & other such materials.

→ <u>Eric Ponder</u> 1926 — Theory of Phagocytosis
   They began to study various factors that affect
   phagocytosis and experimenting with them.
      There's also modelling

→ Emily Mudd & Stuart Mudd 1932
   They note that the ability of phagocyte to include
   <u>spread</u> over the <u>surface of particle</u> undergoing ingestion
   is a principal factor in phagocytosis.
   They found that direct observation agrees with deductions
      from theory.

   ——— There was still an unnecessary binary b/w humoral
      and cellular immunity.
   People had studied the lymph, lymph nodes etc, but
      they didn't know the function! They'd also noticed
   that lymph nodes had immune cells. but again no
      idea of their function.

Henry Wright 1852 — Use of Thymus Gland

- lymphatic system was known. lymph turns more opaque after food ingestion — so it was thought that lymphatic circulation has to do with nutrition.
  ↳ because of some kind of lipids

- Embryologists are studying the development of animals. They are studying the growth of organs after birth. Except for all others, the thymus gland shrinks in size as the animal grows.

- The thymus contains, it was observed, mainly one kind of cell, also found in lymph and blood. and they are seemingly inert — they were called lymphocyte.

- Wright wrongly infers that thymus supplies corpuscles to compensate the large nutritional demand. They thought it was a storage organ which shrinks with growth.

Example of how wrong inferences come about while trying to fit known evidence/data into imagined ideas or concepts.
We figured out the function of thymus by studying transplantation

Transplantations mostly didn't work. but one kind transplantation did — skin from one part of person's body could be grafted to another part and that would work.

4/5

Lecture 9
People working on transplantation were not thinking about immunity at all — evidence comes from unexpected sources.

Autografts seemed to work more than other transplants. Another thing was that usually the transplant rotted away, i.e it had no nutritional supply. So, they 'fed it from outside' through a external source s.t the patient's own blood fed the graft — and this seemed to work more than others.

Reverdin 1869

Skin transplants were needed often because of injuries & burns.
Split skin graft - grafting smaller slices of skin (s.t. only
epidermis which doesn't receive nutrition from vasculature)
seemed to work better.

Pollock 1871 - reported that autogenous grafts were successful
but homografts (from one human to another) soon disappeared
on wounds in the same patient.

Schone 1912 - Homografts always failed, later grafts from the
same donor failed more rapidly than the first

"Patient's body rejected the graft adaptively that was
donor specific."

Through the 19th century, people were working out kidney
transplantation, because it has 3 distinct connections.
But the organ was always rejected — and this was because
of surgical methodology because of the thread.

Alexis Carrel — he developed a method of vascular suturing
such that the thread was not in the blood flow.
So the blood wouldn't clot and the arteries & veins
could be stitched

Despite this development, only renal autografts would work,
and not allografts.
Helped differentiate their surgical failure from biological ones

Factors of resistance to heteroplastic tissue grafting : JB Murphy 1914
Chick embryos offered suitable conditions for growth of foreign
implanted tissue. But chick develops resistance to tissue
around the time of hatching. The same resistance can be
supplied to embryo at earlier stage if spleen or bone
marrow are implanted — "induced immunity". They observed
the same inflammatory response that Metschnikoff had
This development in embryology advanced the concepts
in immunology.

~ 38 - 40 mins — missed

Abigail Lathrop

In late 19th century, one of the objects of conspicuous consumption were fancy pets — specialised mice became very popular as gifts. Lathrop bred inbred strains with particular strains.

She began working with L. Leob using pedigrees of mice for biomedical research, with people in Harvard med school. They studied the genetics of tumor biology.

Charles Little — established the Jackson lab, sidelining Lathrop, which maintains and provides particular strains of mice.

Charles Snell 1948 — methods for study of histocompatibility genes.

8/2/22

Lecture 10

Tissue culture came in — tumor cells could be cultured, and stable cancer lines could be put back into mice. They saw that some tumors grew while others didn't.
Tumor transplantation was easier and more common than organ transplantation

Little analysed the genetic susceptibility to transplantable tumors. They observed that tumors grew in the same strain, if one of parents was of same strain and not in other strains.
⇒ Tumor will grow progressively only in mice carrying dominant genes present in the strain of origin. There were called Histocompatibility genes (MHC), and they played a major role in susceptability of to tissue transplantation, and & along the same lines, to organ transplantation.

Rejected skin grafts were inflamed with same kind of WBCs (macrophages & lymphocytes) stuck to it.
So immune response was associated with organ transplant rejection.
Moreover, people noticed that Thymus was very similar to lymph nodes, and must be involved in immune system in some way

Mice weaning - 3-4 weeks   lifespan - 2-3 years   (17)
    young adult - 6 weeks
    Adults - 8 weeks

Jacque Miller 1964

It was already known that mice thymectomized at 2 months have less no. of lymphocyte, but had no defects in immune responses.

When thymectomy was followed by a potentially lethal dose of radiation & bone marrow therapy, the mice failed to respond to skin homografts & sheep erythrocytes, 2 weeks after irradiation.
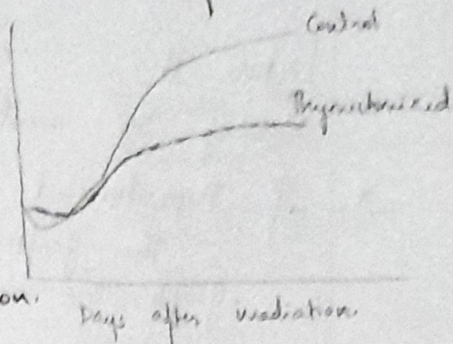
Sham thymectomized control mice had normal immune responses after total body irradiation

∴ Recovery of immune mechanism is thymus dependent.

It was also known that neonatal thymectomy made the animals waste away, because they were susceptible to infections. So they conclude that these animals were actually immunodeficient.

Experimental schedule

Control I	Sham thymectomy	850r + therapy	Immune response ⇒ Rejects skin grafts
Control II	Thymectomy	Sham irradiation	
Expt I	Thymectomy	850r + therapy (normal donor)	cannot reject skin grafts from other strains ∴ They now have lymphocytes from same strain
Expt II	Thymectomy	850r + therapy (neonatal thymectomised donor)	

Then at 14 weeks, they did skin graft and introduced sheep erythrocyte and measured immune response ~ 19 mins. **Results!** (?)

Just removing the thymus doesn't hinder immune response. But if you also remove peripheral cells & put in progenitor cells from * (another strain's) bone marrow, immune reconstitution fails ⇒ Expt II cannot reject skin grafts

Expt I : put in spleen cells → immune reconstitution occurs and skin graft is rejected. ⇒ Thymus plays a role in preparing the lymphocytes so they can reject skin grafts.

* neonatally thymectomised

Control
Thymectomized
Days after irradiation.

Antibody response to sheep erythrocytes

Expt I (which cannot reject skin grafts) can still produce antibodies to some extent, its not fully lost.

In Expt II, the ability is completely lost.

So they begin to understand the role of thymus in immunity

Lecture 11

Redoing the results of Miller paper

* If Thymectomised and irradiated, then bone marrow given, the grafts are not rejected. If not irradiated, then grafts are rejected. ↳ like it becomes like a newborn

* Thymectomised + irradiated + spleen cells — graft rejected
  Thymectomised + irradiated + spleen cells — graft is not rejected
  from neonatally thymectomised mice

Thus they concluded that thymus plays a role in development of mature lymphocytes from progenitor cells

Mean $\log_2$ titer — The dilution of serum at which hemagglutination can be detected. If mean $\log_2$ titer $= 8$, then $\frac{1}{2^8} = \frac{1}{256}$ dilution of serum could show agglutination.
Its a measure of antibody response.

The values were high (6 - 10) in control group & Expt I group, but low (1-2) in thymectomised + irradiated + spleen from neonatally thymectomised mice

→ Function of thymus & bursa system in chicken
    Max Cooper et al (1965) [Robert Good's lab]
Chick embryos had been used for studying transplantation

	Mean no. of lymphocytes	Mean no. of macrophages/PMNL	Skin graft survival >27 days
Control	14.5 k	7k	0/10
Control -irradiated	13.4 k	5.2 k	0/15
Bursectomised + irradiated	13.2 k	9 k	0/15
Thymectomised + irradiated	9 k	6 k	5/12
Thymecto + Bursecto + irradiated	6.8 k ↓ decreases	10 k	

∴ Bursa is not involved in reconstitution of immune competence

Table IV - pg 52 - Graft vs Host reactivity   (19)

They also measured response to diphtheria toxin by injecting it just under the skin, so there's an inflammatory reaction, not mediated by antibodies

	Control	Diphtheria toxoid	
Control + irradiated	1·5	3·2	wattle thickness as measure of inflammation
Thymectomised + irradiated	1·5	1·7	

They also measured antibody response to <u>Brucella abortus</u> and bovine serum albumin (as $\log_2$ titer)

	Brucella	Bovine serum
Control + irradiated	8.10	5.8
Bursectomised	2.75	2.3
Bursectomised + irradiated	—	—

If bursa is removed, there's and animals are irradiated, then there's no antibody response! Whereas inflammatory response is not affected by Bursa, only by Thymus. Thus they recognised that Thymus and Bursa are involved in mediating the development of two kinds of immune cells.

11/2/22

Lecture 12
Methods for Study of Histocompatibility genes - GD Snell (1948)
   Tumors could be reliably transplanted in inbred strains.
Otherwise, the tumors won't grow, or will grow & regress.
   Tumors could be reliably transplanted <u>when strains shared</u> common dominant genes — <u>histocompatibility genes</u>
It was also thought that these genes were involved in tissue rejection as well.

But so far, we've been measuring antibody level to guage immune response. Miller paper showed that thymus dependent lymphocytes are important for antibody response, but Cooper's paper showed that only Bursa dependent lymphocytes were important for Ab response

But combined. it implies that both Thymus & Bursa or lymphocytes are important for Ab response

Also, MHC genes are somehow involved in organ rejection.

Genetic control of Antibody response — Relationship b/w immune response & histocompatibility (McDevitt & Chinitz)

They worked with inbred, mice strains with different haplotypes (MHC gene alleles) to measure antibody response (more quantitative) to pure proteins.

\# There can be several antibodies to the same protein (albumin of birds) e.g.
So, a protein should be simple, & not complex, lesser amino-acid sequence variability.

To homogenise target recognition, they used a protein of polymerised short sequence of amino acid

Strains was were grouped by MHC H-2 haplotype
• They found that the haplotypes could be either low responders or high responders against different antigens. But not a haplotype couldn't be either high or low responsive to all antigens.

• Advanced methods allowed them to study responses in congeneic strains (genetically similar in all but one locus)
   DBA1 — high response   SJL - low response
   They made F1 from these two, & F1 × SJL — backcross
   They kept track of mice that inherited the DBA1 haplotype — and those were high responders.

In Last 5 mins : something about how do T & B cells collaborate? (Next lecture)

1) So, T-cell response was some-how constrained by MHC genes and their gene products — through transplantation

2) Response of B-cells was also controlled by T-cells through an MHC-dependent recognition

T-cells were involved in mediating a lot of immune responses —
* Inflammatory response against skin graft/tumor (independent of B-cells), but constrained by MHC-restricted recognition.

B-cell response is independent of T-cells. but they still need MHC-dependent T-cell mediated help to make antibody responses

Lecture 13

What targets do T-lymphocyte recognise?

So far, we haven't isolated T cells, we're studying it through indirect evidence.

Zinkernagel and Doherty 1974

Advancements came from cancer biology because it was known that T cells mediate/reject tumor transplantation because T cell inflammation was seen around tumors & skin grafts

There was specific adaptive immunity against skin grafts. What was the target?

By then it was known that that viruses cause transmissible diseases. specifically cancer in chickens. The ability to maintain stable cell lines allowed us to grow viruses and study them

People were taking some tumor cells and immune cells and observing if tumor cells were being destroyed. But how was still a question.

MHC-mediated T-lymphocyte response

"H-2 compatibility is essential for sufficiently close association between lymphocyte & target cell."

T-cell would recognises a H-2 gene product and a viral antigen or the viral infection modifies self-components of the cell that T cells recognise. Altered self maybe thought of as changes in H2 antigens.

Remember - MHC haplotypes that are inherited from parents.

→ Either T cells recognise MHC + virus in a combinatorial, separate way, or a combined effect on some proteins is detected

MHC haplotype consists of a set of alleles at several LHC loci. some involved tumor rejection, others in antibody production All of these loci are linked, so the haplotype is usually inherited together in a particular strain of mice

in a way recognised only within H2 compatible system

They are taking F_1 mice (H-$2^{b/k}$) and were remitised with LCMV infection. T-cells from this would recognise virus infected target cells and kill them.

The target cells had either H-2^k or H-2^b.

Prediction 1) T-cells have receptors for $H2^k$ and $H2^b$ on the same cell – homogenous population of T-cells

2) It has subpopulations of T-cells which, in a mutually exclusive way, either recognise $H2^k$ or $H2^b$.

Would T cells kill target cells with either $H2^k$ or $H2^b$ antigens?

Cytotoxic activity of donor T cells in LCM infected irradiated recipients

F_1 mice ~~are immunised to LCMV~~ and T cells are extracted.

Then H-2^b, H-2^k and $H2^{b/k}$ mice strains are irradiated (so they lose all lymphocytes) and injected with ~~immunised~~ T cells. → to immunise

Then, they expose these recipient mice to the virus and after few days, check whether T cells have grown in number. If they've recognised the MHC type, then they would increase in number as a reaction to virus.

↳ ↑% of Cr^{51} ⇒ more cells have been lysed

	Recipient $H2$ type	% Cr released from cells ($H2^k$)	
Expt 1		Infected	Normal
Immune	k/d	50%	12%
	d	16%	12%

So, $H2^k$ cells can be killed only if T cells are incubated in $H2k$ mice.

| Immune | k | 87% | 19% |

This clarifies that the intimacy model (homogenous population of T-cells in F_1 mice with combinatorial receptors) is not correct

This showed that we have different subpopulation of T cells that detect modified MHC proteins directly

The uncertainty of evidence along with sophistication of notion and idea development can be seen here.

[left margin, rotated:] T-cells and virus and incubated in recipient mice so the cells are immunised. Then these cells are released on infected H-2^k lymphocytes, and we see how many of them are lysed

Lecture 14

Zinkernagel & Doherty paper uses a measure of variation - SD or std error of mean. The statistical tests became more frequent and p value went from an additional measure to being a dogma

Since they're not making any statistical tests, they are only looking at major differences, which is sufficient to analyse as evidence for the hypothesis in question.

Assay - quantitative test
Doherty paper had an array table

Because they were discovered using alloantisera
↑
Leukocyte genes
↑

Cantor and Boyse 1975

functional subclasses of T lymphocytes bearing different Ly antigens

Most polymorphic gene locus in an outbred population - MHC gene

If we immunise a mice with cells from donor mice of another strain, then the antibodies will not be made for the common proteins, only for antigen that's foreign to the recipient mice. This is called alloantisera.

Alloantisera was developed with congenic strains — which differ only in one locus & are identical otherwise.

The converse can & also be done — generate alloantisera from 2 strains which have same MHC alleles & are different other wise — we have tools that are allele specific

What is happening? Rewatch ig
Important to remember —
- McDevitt & Chinitz showed that B-cell response was controlled by T-cells through an MHC-dependent recognition.
  This is the idea of helper T cells.
- Zinkernagel & Doherty showed that T-cells lysed infected cells through MHC based recognition — basis for cytotoxic T cells
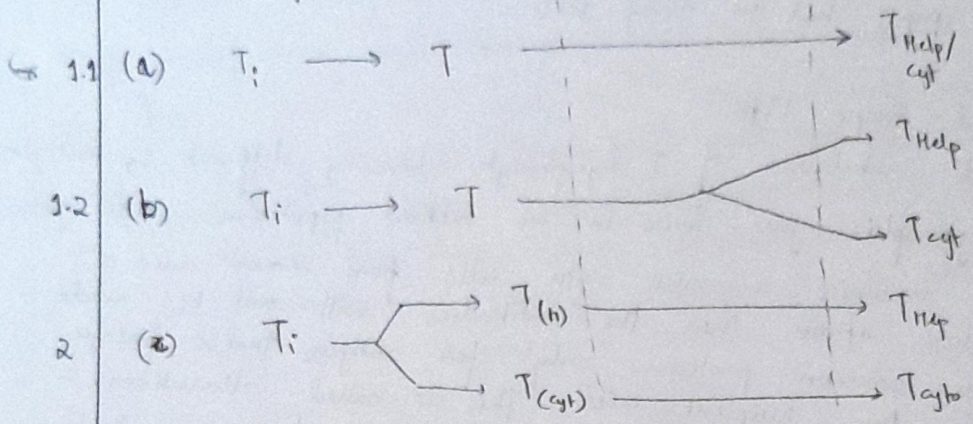
Another observation: Alloantisera were tested by seeing agglutination rates
Some cells would clump because the proteins on them were recognised
But not all cells were clumping — antibodies don't recognise some proteins on some cells.

Is it possible to separate subclasses of T cells from non-immune animals that are already determined to express helper or cytotoxic activity before they encounter the antigen?

- Use alloantisera to remove the subset of leucocytes that clump and then study function of remaining leucocytes
- Cantor & Boyse selectively remove some cells and study their immune function. They found used genes that are exclusively expressed in T cells

How? ~ 44 mins (video)

Possibilities —

| Thymus dependent Differentiation | Antigen stimulation |

1.1 (a) $T_i \longrightarrow T \longrightarrow T_{Help/cyt}$

1.2 (b) $T_i \longrightarrow T \longrightarrow T_{Help}$, T_{cyt}

2 (c) $T_i \longrightarrow T_{(h)} \longrightarrow T_{Help}$, $T_{(cyt)} \longrightarrow T_{cyto}$

21/2/22

Lecture 15 (18th Feb)

Are different functions of T cells (helpers / cytotoxic) mediated by the same cell or different cells? If different, does it occur during immunisation, or the cell lineages inherently different?

This was studied using alloantisera, which recognise proteins on cell surface of T-cells.

Antibody + complement are capable of lysing cells. (Discovery was delayed because complement doesn't lyse one's own cells very well since there are species specific complement inhibitors).

So typical expt : alloantiserum + complement from another species is added to lyse T cells. Sham depleted cells & various alloantisera depleted cells are then transferred to irradiated mice which are then immunised. Post immunisation, cytotoxic & helper assays are done to see effectiveness

If alloantisera destroys one fn, not the other, then it confirms option 2 to be correct (why not option 3 3)

ln (1)

If that's not the case, then we can't say option 2 is not correct

Zinkernagel & Doherty : Either result would give conceptually interesting ideas — either one or immunity model or codification model would be proved correct

Their negative results won't mean much — maybe you just haven't found the right antialloserum which recognises distinct proteins T_H & T_c cells

PFC : plaque forming cells / spleen — how many antibody forming cell foci could be developed per spleen when Ab response was generated in vivo. (in irradiated mice where cells were transferred and immunisation was done)

(direct / developed)

Normal Mouse Serum	1450 / 2160	No depletion ie no allo-antibodies
No cells transferred	11 / 0	— just irradiated mice ie cells
Anti- Thy - 1.2	12 / 10	These 2 specific alloantisera deplete the ability to mount an antibody response
Anti- Ly - 1.2	188 / 35	
——"—— 2.2	1890 / 2940	i.e T_H activity is being depleted
——"—— 3.2	1850 / 2360	→ Don't deplete T_H
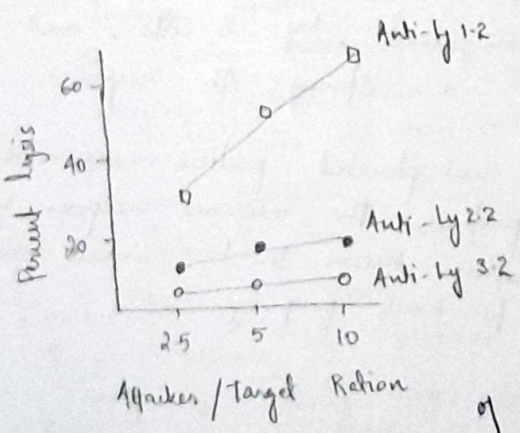
Anti-Ly 2.2 / 3.2 deplete cytotoxic cells but not helper cells

∴ So During thymus-dependent T-lymphocyte differentiation, there are two separate lineages / population of T cells capable of helper or cytotoxic activity prior to any antigenic immunisa-tion

These are CD4 and CD8 T cells.

Percent lysis graph: y-axis "Percent lysis" 20, 40, 60; x-axis "Attacker / Target Ratio" 2.5, 5, 10; lines labelled Anti-Ly 1.2, Anti-Ly 2.2, Anti-Ly 3.2

All of this was dependent on chance that CD4 & CD8 have allelic differences that have segregated in mouse strains. This shows the provisional, uncertain nature of evidence that feeds into conceptualisation

How do T cells help B cells? — Mitchison 1971

Carrier effect in Secondary response to hapten-protein conjugates
Difficult to read but insights are dramatic

When proteins (albumin -bovine /egg) were conjugated with
small molecules → nitrophenol or arsonate, the antibodies generated against them bound to NIP or ARS and not the proteins themselves. But these molecules by themselves didn't produce immune response / antibody response but could bind the antibodies
These small molecules were called haptens.

Carrier effect : when NIP + bovine albumin(1) is injected when animal was immunised with NIP + egg albumin (2).
Antibody response to (1) is lesser than response to (2) if (2) were injected again.

Two explanations —a) local environmental hypothesis — hapten + surrounding protein is recognised by two receptors, one directed to
b) Antigen hapten and other to a determinant on carrier protein

Mitchison established that two second hypothesis was true.
Determinant on carrier protein is recognised by T cells and hapten is recognised by B cells, and there is cross-talk which produces a strong Ab response.

He also showed that when unhaptenated protein was added along with hapten + protein, the immune response was decreased. So, hapten + protein needs to be linked and identified for response to work properly

hapten + protein 1	protein 2	Cells from ① +② were stimulated with →	gives carrier effect potentiation
①	②	hapten + protein 2	

Antigen is recognised by two cells — hapten is recognised by B cell and receptors on T cells recognise the carrier protien. These T$_H$ cells interact with B cells in a target specific fashion through linked presence of hapten and carrier protien, which means that hapten specific B cells must express in turn the MHC-bound ligand that carrier-specific T cells can recognise.

22/2

Lecture 16

Recall: Behring & Kitasato expt — immunising compound is present in the serum.

Guinea pigs have 5g of gamma globulin per 100 ml of blood which includes immunoglobulins & non-immunoglobulins but it was not known to separate them.
When immunised animals were exposed to the same antigen, the total level of gamma globulin in the blood didn't increase! ⟵

People were focusing on how antibody repertoire was produced

→ Paul Ehrlich ~ 1900
↳ Immune system was making new Abs when exposed to antigen. For each immunogen, body would produce proteins with varying 'side chains' that would bind to it (protein structure was not very well known). Gamma globulin is a precursor which is used to make Ab when an immunogen induces it.

→ Linus Pauling ~ 1940
This became a problem in protein structure. The thought of it a changes in tertiary protein structure in response to 'instruction'

Jerne ~ 1954

He changed the then-prevalent idea — he proposed that gamma globulin population present in the body is extraordinarily heterogenous and diverse (not a homogenous subpopulation as thought before).

When immunogen comes in, he said that one or few antibodies are selected, not instructed to be complementary.

But if there was so much heterogeneity, then how did the conc. of specific Abs increase after immunisation.

How did proteins proliferate?

→ Talmage, Burnet (1956-57)

Clonal Selection [Burnet 1957]

Lymphocytes are clonally diverse to start with. Cells can secrete Ab or give rise to other cells that can do so.

A protein and B cell repertoire have many to many relationship.

~~Capacity~~ When antigen - Antibody (on the cell) interaction happens, the lymphocyte will settle down in suitable tissue and proliferate to give rise to a clonal population which can produce the same antibody.

Jerne's selection thus works on cells that express certain (slightly variable) antibodies.

Antibody Production by Single Cells — Nossal & Lederberg (1958)

To verify Burnet's theory, he tested the prediction that each individual cell can only make antibody of one specific type

The methods were published in detail elsewhere, but results were published as a report

Lecture N° 17

Antibody production by single cell

It had been shown that cells (splenic, lymphocyte) from pre-sensitised animals can form antibody in vitro

Their report describes the technique where Ab production by single cells isolated in microdroplets can be detected.

They technique was based on specific immobilization of two types of Salmonella serotypes by anti-flagellar Ab.

Abs against both types are produced in the animal, but single cells only produced one type of Ab.

They found that cells (drops) which inhibited S. adelaide failed to inhibit S. typhii when drop had only 1 cell. When the drop had more than 1 cell, then the possibility they reacted with both (often).

Recall Rod Porter structure of Abs   4/3/22

Nisonoff & Pressman (1958, 1959)
    Each individual rabbits produces unique Abs to the same antigen.
Details? They generate Abs of different affinities to the same antigen.
    Individuals generate somatically different antibodies.
Antiserum produced against haptenated - bovine -γ-globulin (BGG) contained antibodies against hapten & BGG.
Removing BGG Ab didn't decrease anti-hapten Abs, so they are not on the same molecule — heteroligating antibody is absent.

Burnet's predictions are being confirmed

People began to show cells had antibodies attached to them.
    B cell receptors?

We knew that all antibodies were γ-globulins, so how or where were they different? — Hard to figure it out

People tried to separate light chain & heavy chain, and sequence amino acids — but this was messy

But measuring bulk properties was easier —

Barrett et al — 1965 , Dormer et al 1967 — Cabtot

They collected Abs against 4 bacterial carbohydrates and IgG & IgM.

They measured aminoacid composition of these Abs.

It differed between each of these antigen. So there had to be some of sequence variation.

Later study collected Abs from different individuals against same antigen (dextran) and they found a similar result.

This led to speculation that multiple B-cell clones in individuals were being producing Abs, that bound to same antigen but with slightly different affinity and different amino acid sequences

⟹ Antisera consists of polyclonal Ab population — its not a pure mixture, that's why they couldn't be successfully sequenced or crystallised.

Henry Bence Jones — 1847
The answer to these problems came from cancer biology.

4/4/22

Lecture 18 — 25th feb
The huge variety of Abs comes from somatic cells, and not genetic inheritance.

Amino acid sequencing won't be possible with polyclonal Abs.
We need antibodies from single cells (Nossel), but single cells produce v. v. little Abs. so sequencing will be hard

Insight from 1840s
There were reports of tumors associated with bone marrow.
(Bence Jones was a physician & chemical analyst
⎩ This was associated with high levels of protien in the urine.

1901 Boston — Cases of multiple myeloma, which produced a lot of plasma-blasts*

* for* dividing cells that contain a lot of plasma.

An interesting feature of this was proteinuria, along with osteomalacia (thinning of bones)

Robert Willett – 1951 – Review of multiple myeloma
Overgrowth of plasma cells in myeloma is associated with excess protein is hera and in urine → not γ-globulins. They found that these proteins differ in their properties (mobility, mol. wt. solubility etc). Bence Jones proteins are of small mol. wt, not found in serum. They're possibly breakdown products of excess γ-globulins in serum produced by cancerous cell plasma cells. Or they are abnormal paraproteins produced by cancerous cells.

→ Olins and Edelmen 1962
Antigenic structure of polypeptide chains of human γ-globulin
Idea : cancer cells are possibly monoclonal.
Myeloma is cancer of antibody making cells – so this solves the issue with being able to get sufficient protein from single Ab producing cell

Plasma
- cytoma ← Myelomas could be induced in specific inbred strains of mice. From there, cell lines could be created s.t. they secreted antibodies
This led to a whole series of insights.

Elvin Kabat
→ Wu & Kabat 1970 – Analysis of sequencing of B-J & myeloma proteins and their implication.
They had a database of sequences of light chain & other proteins
Early example of bioinformatic analysis.
They compared specificity and complementarity in human and mice light chains.
They characterised 3 regions of high variability (24-34, 50-56, 89-97). They hypothesize that this region contain complementarity-determining residues of light chain.

They were wrong about the source and mechanism of this variability.

Now that there are cancerous monoclonal cell lines — cytoplas plasmacytomas, sarcomas, myelomas — people wanted to see how these cells differ in Ab producing gene loci

Tonegawa et al - 1974, 1976
* Evidence for somatic generation of antibody diversity
They prepared RNA with which contained light chain mRNA and tried to hybridise it with DNA from mouse liver cells
They conclude that genome size is too small to account for diversity of Ab molecules

* Evidence for somatic rearrangement of Ig genes coding for variable and constant regions. ('76)
  · They digested DNA from early embryo stem cells of an inbred mouse strain, and from plasmacytoma cell line using restriction enzyme & did electrophoresis.
  · DNA from embryo hybridised in two components — V & C sequences, whereas tumor DNA showed single component (V+C gene) hybridisation
  ⟹ V & C genes are away in embryo cells but joined together in lymphocytes. (rearranged)

4/3/22

Ratn Lec 19 - 1st March
Two types of insights — mechanistic and technological.

Kohler & Milstein 1975
Continuous cultures of fused cells secreting Ab of predefined specificity
Fusing cells: by making plasma membrane ters porous and centrifuging cells together so they fuse & hope that they express features of both cell types.
They immunised a mice and extracted splenic cells that made antibodies. They fused these cells with continuously growing malignant myeloma cells.

Then they plated these fused cells the way Nossal did and
let them grow. Only those wells with myeloma cell-fuse
would grow — but this could be fused myeloma cell or
unfused cells. Fused cells: hybridomas.
How to identify hybridomas of interest (myeloma + B cell)?
↳ Test the supernatant of these cells for antibodies

They managed to get hybridoma cell lines which monoclonally
produced specific antibodies.
The antibodies so produced by a cell line all bind to specific
epitope on the antigen. This is not very useful
against a microbe that evolves variants quickly.

The next step was to make antigen specific B-cell lines
by fusing by B-cell lymphoma & immunised splenic cells.
Similarly, T-cell hybridomas were also produced.

How to test for target specificity in T cells lines?
By then, it was known that T-cells make cytokines when
stimulated by an antigen.   ↓ Interleukin 2
So the method was to incubate T-cell with an antigen
and see if IL2 was produced, but detecting IL2, which
was cumbersome.

Kappler, and Marrack 1981
Zinkernagel & Doherty expectations — that each T-cell hybridoma
was dependent on target antigen, MHC molecule of the
appropriate haplotype & T-cell receptor was recognising MHC molecules
— were proved with T-cell lines.

Issues with growing malignant cell lines —
1. They don't behave exactly like primary cells behave.
Primary T-cells had individual functions, which were lost
in hybridoma lines

How to grow stable primary T-cell lines?

Schwartz's group (1980)   Coffman's group (1986)

They began to show that primary T cell clone lines showed different functions (that were lost in hybridomas).

Growing & using primary T cell cultures (in adoptive therapy) are further mechanistic insights.

Going back to Burnet's predictions — mechanisms of immune tolerance.

Burnet's argument: there's a random collection of target specific B & T cells, but they shouldn't recognise any targets inside our body (no autoreaction). This requirement for immune tolerance was implicit.

Going back to transplantations; embryo transplantations in Murphy lab

'Actively acquired tolerance' of foreign cells — Billingham, Brent and Medawar 1953

1. When mice & chicken embryo are transplanted homologous tissue, they become tolerant to it when skin grafts are done later in life.

2. This acquired tolerance is immunologically specific — they can still mount immunological reaction against other tissues from different strains.

3. This acquired tolerance is due to specific failure of host's immunological response. Antigenic properties of homograft are not altered & host retains the ability to react against it.

5/3/22

Lecture 20 — 3rd March

Burnet's Theory's predictions

- Every molecule in the biological universe can be recognised by one or more types of B-cells in the body. Receptors for infinite no. antigen can't be coded into genes — its through somatic recombination in lymphocytes.

- How to generate inf. randomised receptors? Somatic mutagenis in a hypervariant segments? But these receptors shouldn't bind to any molecules in the animal's own body.

— So there should occur a second major somatic event during differentiation. As cells expressing receptors are generated, repertoire must undergo somatic selection. Autoreactive cells must somehow be controlled — either by killing them or by making them hyporesponsive.

Both have their own costs.

This is implicit in Burnet's theory.

Zinkernagel and Doherty diagram — how T-cells recognise target?
T-cells are MHC restricted ⇒ they were meeting same receptors on other cells.
T-cells are restricted by one kind of MHC haplotype.
T-cells must also undergo differentiation, but T-cells recognise MHC associated target ⇒ these receptors have different structural constraints. So gene loci are different but mechanism is the same.
T-cell receptors are also generated randomly — the variation in antigen receptor neednot be constrained by

~29 mins MHC receptor.
(Because T-cell receptors repertoire is being generated randomly.
    receptors) x
→ Mouse with pure MHC-k haplotype will have T-cells that recognise target on MHC-b & not MHC-k because T-cell repertoire is being generated randomly.
T-cell repertoire is being generated randomly.

→ Possibility 1: T-cell receptors locus is in linkage disequilibrium with MHC alleles.
But MHC genes are extremely polymorphic in a population.
There's no linkage b/w MHC genes & T-cell receptor genes.
T-cell receptor loci are not MHC allele dependent/specific.

This means → The T-cell receptor repertoire generated will be the same despite the MHC halpotype. This means MHC-k mouse would make T-cell which recognises targets on MHC-b, not MHC-k. This is a mark of sequences. given how many haplotypes there are.

This is a problem of MHC-restricted cell surface recognition of T.lymphocytes

This is the background for immune cell selection.
 Going back to Billingham, Brent & Medawar (1953).
- Murphy first observed induced immune tolerance, but didn't recognised it. No one was thinking about it that way.

Medawar group - Pg. 34
Acquired tolerance is like adaptive immunity — target specific.
They test the specificity through skin grafts in adult life.
 Are the targets on the skin graft somehow being changed?
They remove the skin graft from embryo-transferred individual to another individual of same haplotype and see if it is accepted or rejected
⇒ Target properties are not being modified, the immune system is modified — at a particular developmental stage   5/3/22

Lecture 21 - 4th March
 Immune tolerance comes about by negative selection of B-cells and T-cells during development.
Recap of the problem
 How does allele-specific T-cell receptor repertoire get generated?
MHC genes and TCR genes are not linked.

Bevan 1977
 In a radiation chimaera, host H2 antigens determine immune responsiveness of donor cytotoxic cells.
If not constrained, the body will make TCRs that recognise targets based on any number of MHC haplotypes.
But why? Where will the information about all these other haplotypes come from?
→ These T-cells won't be negatively selected because they are not autoreactive, they're just taking up space. doing anything — they're not autoreactive, they're just taking
This is another somatic problem

Zinkernagel & Doherty — T-cells recognise targets through H2k or H2b, but not both.

What determines the allelic specificity of target recognition? the repertoire?

Is the T-cell allowed to develop if the specificity of its repertoire matches the MHC genes it is expressing?

This is a positive selection.

Does this selection work based on MHC expression of T-cells or through the environmental cues?

Bevan 1977 paper

Bevan is making chimaeras using 2 strains of mice — BALB/c (H2d) and BALB.B (H2b). They are MHC congenic, except for MHC haplotype, all other genes are identical b/w them.

The mice are immunised with minor histocompatibility targets

Minor histocompatibility recognition is MHC restricted i.e. minor hc is recognised only if MHC's match

This was done by injecting F_1 (B10 × B10.D2) spleen cells
                                                    H2b   H2d
non congenic strains

First, BALB/c and BALB.B mice are irradiated. They are reconstituted with progenitor cells from F_1 (C × C.B) mice.
                                                                          H2b H2d

These cells are allowed to mature and then immunised with minor hc targets that can be recognised by both H2b and H2d strains

Do the T-cell repertoire generated (in BALB mice) have target recognition capacity for both H2b and H2d?

If yes, then target recognition is driven by T-cell genetics.

Or is it the MHC of body in which the T-cell is present that is shaping the repertoire?

(38) Once the mice are immunised, he's extracting lymphocytes from them and seeing if he can detect these cells recognising both H2b & H2d minor hc targets.

Answer: Body determines the shape of the repertoire, T-cell genetics don't.

The microenvironment of developing T-cell determines the selection.

Part 2 - Dr. Vineeta Bal   22/3

Lecture 1

What is immunology about?

- Understanding how we survive from microbial attack
- Vaccines : specific adaptive immunity.
- Infections (viral, bacterial, parasitic and fungal)
- Autoimmune diseases : when immune responses turn against the host.
- Allergies : undesirable extreme response to mild antigen
- Transplantations : they work only when all MHC genes match.
  Exceptions — placenta is essentially a transplant growing in
              the uterus.   of half allo foetus
              Cornea transplantation also works because it's a
              non-vascular superficial layer

Studying biology at different levels of organisation — immunology
           integrates all of this.

· Molecules : MHC molecule & diversity act as immune identity
· Intramolecular : Phosphatases/Kinases mediated signaling cascade
· Cell & intracellular : T-cell and phagocytic cell interactions
· Cell & molecular : Antibody mediated target cell killing
· Inter-organ : Bone marrow & spleen ; liver (cholesterol metabolic defects)
              and autoimmune diseases
· Organism-ecosystem crosstalk : gut immune system. (gut microbiome)

What does the immune system do?
· Making sense in a complex, chaotic environment
· Identifying danger among non-dangerous entities
· Weeding out the danger in the best possible way
· Remembering the encounter with danger to prevent further damage

Immune system is peculiar —

1. It is _not localised_ — it's present everywhere in the body.

2. It's resting and can be _induced_ to act.

3. Even after being triggered to function, it needs to undergo _cellular maturation_ before it can be effective

4. Apart from reproductive system, probably the only system where cellular _DNA_ is rearranged or shed as a necessary step during development & maturation.

Beginnings — Jenner put together that cowpox gave protection against smallpox

Smallpox

It has been eradicated by smallpox vaccination. ~ 1979

Observations leading to principles of immune response

- Koch's postulates : microorganism cause diseases (~19th cent).

Lecture 2

1. Koch's postulates

They apply for all microbial diseases

- If you _isolate a pathogen_ from a sick person and transfer it to a healthy person, and the person gets the same symptoms, then that microbe is the _cause of the disease._

- But it's unethical to give diseases to healthy humans now.

2. Microscopes allowed the recognition of many microbes Most pathogen are invaders, unlike microbiome bacteria

3. Antibodies in the serum provide protection against specific diseases — Behring & Kitasato 1890
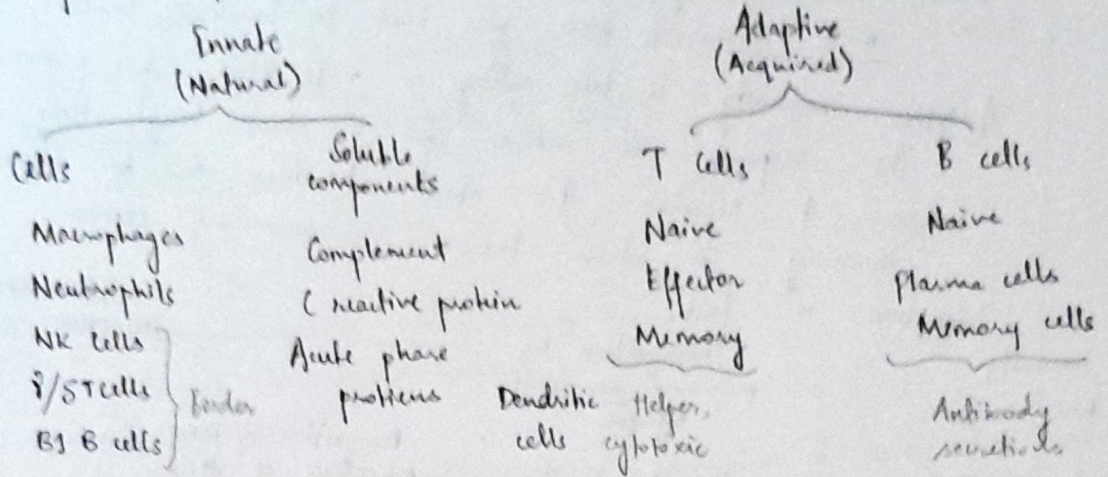
Why do we need immune system?

- Functional _specialisation_ in multicellular organism
- Nutrient rich environment in the body is an invitation to parasites
- _Accumulation_ of mutations over time → loss of regulatory control → malignancy
- Necessity for _surveillance_ against invasions and maintainance of homeostasis.

Severe Combined Immune Deficiency (SCID)

David Vetter - Absence immune system - no B or T cells.

Components of Mammalian immune system

Innate (Natural)		Adaptive (Acquired)	
Cells	Soluble components	T cells	B cells
Macrophages	Complement	Naive	Naive
Neutrophils	C reactive protein	Effector	Plasma cells
NK cells	Acute phase	Memory	Memory cells
γ/δ T cells } border	proteins	Dendritic Helper,	Antibody
B1 B cells }		cells cytotoxic	secretions

Organs

- Primary lymphoid organs : Bone marrow
  Produces immune cells   Thymus
                               Liver & Yolk sac - in early developmental stages

- Secondary lymphoid organs : Spleen
  Mounts immune response   Lymph nodes

- Connections : Arteries & veins } Responsible for lymphatic
               Lymphatics.   recirculation

Lymph node & Spleen

Afferent · Blood supply · Efferent
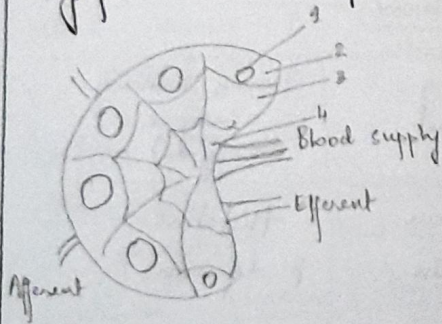
Pathogen from site of infection reach lymph nodes via lymphatics, where there's a repository of leucocytes

1. Germinal center
2. Primary/ lymphoid follicle (mostly B cells)
   (second)
3. Paracortical area (mostly T cells)
4. Medullary cords & medullary sinus

* Primary lymphoid ⇒ no germinal centre
* Primary lymphoid ⇒ no germinal centre
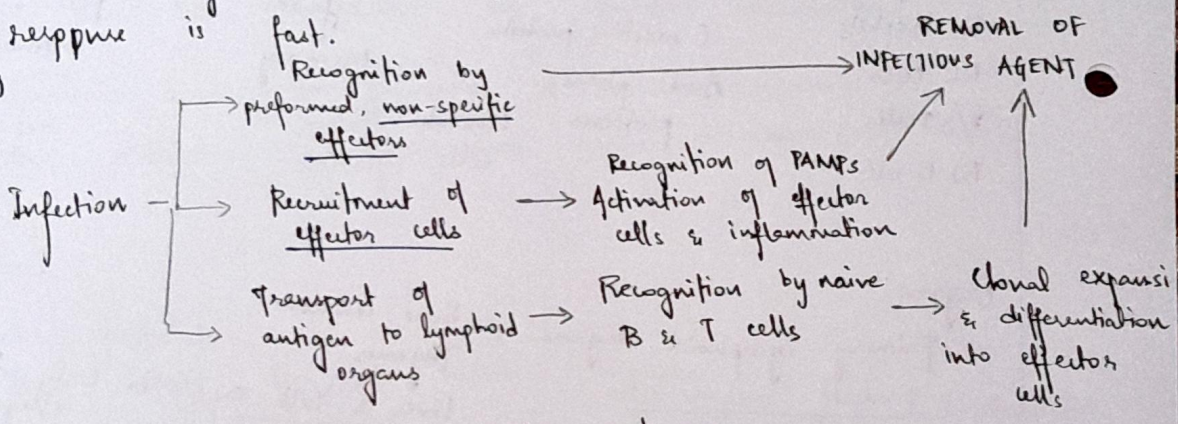
Time scale of immune response
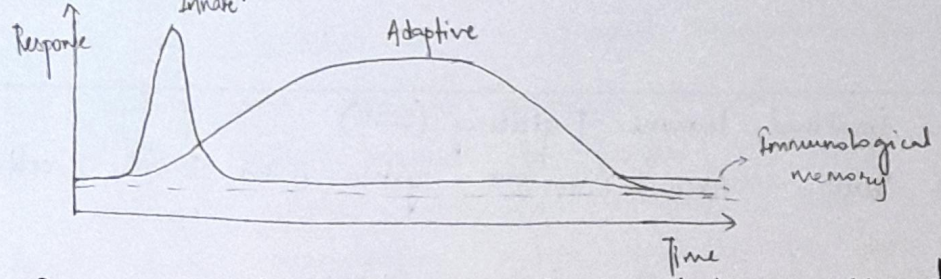
- Innate immunity gets activated immediately & responds within 0-4 hours; it soon goes back to baseline levels
- Adaptive immunity is late acting — >96 hours for primary exposure. The response is active for a much longer time and when it recedes. it's above baseline due to immunological memory. So when there's a secondary exposure, the response is fast.

Early immunity 0-4 hrs		Recognition by preformed, non-specific effectors
Early-induced innate response 4-96 hrs	Infection	Recruitment of effector cells → Recognition of PAMPs Activation of effector cells & inflammation
Adaptive imm. response >96 hrs		Transport of antigen to lymphoid organs → Recognition by naive B & T cells → Clonal expansi & differentiation into effector cells

Recognition by preformed, non-specific effectors ————————→ REMOVAL OF INFECTIOUS AGENT

Common barriers to invasion by pathogen

	Skin	Gut	Lungs	Eyes/Nose Oral cavity
Mechanical	←—— Epithelial cells joined by tight junctions ——→			
	Longitudinal flow of air of fluid		Movement of mucus by cilia	Tears, Nasal cilia
Microbial	←——————— Normal [microbiome] ——————→			
Chemical	Fatty acids	Low pH Enzyme (pepsin)	Pulmonary surfactant	Tears & salivary enzyme (lysozyme)
	β-defensin Lamellar bodies Cathelicidin	α-defensin Reg III (lectividins) Cathelicidin	α-defensin Cathelicidin	Histatins β-defensins

Cellular elements of immune system

Allo blood cells arise from hematopoietic stem cells in the blood marrow.

WBCs come from <u>lymphoid</u>, <u>myeloid</u> & <u>granular</u> progenitor cells.

Differential leucocyte count (DLC) — numbers of WBCs present in blood

1) — Macrophages and monocytes (2-10%)
- Phagocytosis
- Antigen presentation

2) — Dendritic cells
- Antigen uptake and presentation
- Present in lymph nodes, not blood

3) — <u>Granular cells</u>
- Neutrophils : 40-75% - Phagocytosis & activation of bactericidal mechanism
- Eosinophils : 1-6% - killing Ab coated parasites
- Basophils : <1% - Allergic responses, augmentation of anti-parasitic
- Mast cells : Releasing granules containing histamine etc. immunity

4) — Natural killer cells — Releases lytic granules that kills virus-infected cells

5) — Lymphocytes. (20-50%)

Inflammation
It is an <u>early</u> event in <u>immune response</u> — mainly by innate immune system components.

Five classical signs : Calor, dolor, rubor, tumor & functio laesa

when there's a trauma/abrasion, first there's a <u>blood clot</u> and then migration of WBCs to the site.

25/3

Lecture
<u>Neutrophils</u> and <u>macrophage</u> are first to the site. They phagocytose bacteria and release <u>cytokines</u>.

Cytokines <u>increase the porosity</u> — other WBCs slip through the capillaries and more <u>fluid</u> accumulates which causes swelling and heat.

This will get resolved soon in acute inflammation.

Leucocyte rolling

Endothelial cells of vessels express surface protein called
[selectins] which bind to surface protiens on surface of leucocytes.
They stick to the walls and roll along the walls
looking for spaces to squeeze out of the capillary.
This rolling is only in the veins.
Once they are close to the site of breach, the
WBCs (neutrophils & macrophages) will squeeze between
the cells of capillaries.

Extravasation

Endothelial cells express selectins, chemokines & I-cam
Leucocytes first bind to selectin and then to other
receptors. Then they squeeze through the endothelial
cells and move along the chemokine to reach
the site of infection

Chemotaxis

Phagocytes move along a chemical gradient to catch
bacteria and engulf them.

3rd peculiarity

Even after being triggered, immune cells need to undergo
cellular maturation before it can be effective.

Dendritic cells — they are effective at phagocytosing dying/dead
cells. Immature dendritic cells are there in the
periphery. They move into lymphatic vessels once they
ingest stuff. There mature dendritic cells go to
lymph nodes and activate naive T cells.
Dendritic cells are matured by inflammatory compounds.

Two phases of T cell response : Recognition of target and
expansion to gain effector function.
In a pool of naive T cells, the cell that is activated by
the antigen undergoes rapid proliferation to form
clonal effector cells. This is why adaptive imm response
takes 4 days.

Complement System & Adaptive immune system.

Classical complement activation system. When antibodies bind to bacteria, it activates <u>C proteins</u> which ultimately <u>lyses the bacteria</u> by creating a pore and/or attracting phagocytes.

This is the dominant complement pathway which requires adaptive immune components → it came later, evolutionarily. This pathway is much more active response when it's a re-infection.

<u>Alternate pathway</u> of complement (evolutionarily ancient) is more effective during primary infection.

→ Macrophages express receptors for microbial constituents.
- Toll-like Receptors (<u>TLR</u>) + LPS receptor (<u>CD14</u>)
  - ↳ They detect gram negative bacteria (E.coli, salmonella)
  - - Some TLRs are <u>inside the cell</u> which detect viral DNA/RNA

* { - Mannose receptor
    - Glucan receptor
    - Scavenger receptor
These receptors are not specific to one particular agent — they recognise markers common to many bacteria

---

Invading bugs can be —
Extracellular   -(1) in the blood vessel or interstitium
Intracellular   -(2) sitting in <u>bubbles inside infected cells</u>
                  (3) drilling holes in cells to remain in cytoplasm
1. Pneumonia, meningitis, malaria, sore throat, filaria
2. Typhoid, TB, kala azar, leprosy
3. Dengue, Japanese encephalitis, AIDS (all viral - obligatory intracellular)
→ These bacteria even survive and grow inside vacuoles

1. free molecules (Abs or complement) tag extracellular invaders to be eaten by phagocytes

2. Helper / CD4 T cells signal infected cells to kill intracellular bugs lurking in bubbles / vacuoles.

3. Cytotoxic / CD8 T cells signal cells infected, with intracellular pathogen in cytoplasm, to die.

Lecture   29/3/22

Target recognition strategies in the immune system.

Receptor character	Innate	Adaptive
Specificity of receptors inherited in genome	Yes	No
Expressed by all cells of particular type eg PRRs in macrophages	Yes	No
	Y	No
Trigger immediate response	Y	No
Identify broad classes of pathogens		
Encoded in multiple gene segments		
Requires gene rearrangement	No	Yes
Clonal distribution		
Able to recognise wide variety of molecular receptors		

(left margin, in red) Important differences

(left margin) TLR4 -lipopolysacch (Gram -ve)

(left margin) $10^7 - 10^8$ specific receptors

Role of Pattern Recognition Receptors (PRRs) in clearance of pathogen

PRRs recognise pathogen associated molecular patterns (PAMPs).

Eg: Different TLRs recognise microbial components like lipopolysaccharides & ss & ds DNA

NOD & RNA Helicase CARD domain family recognise viruses in the cytosol.

They send signals to the nucleus to modulate the transcription of certain genes.

→ NK cells : Receptors and ligands

They express three types of receptors : inhibitory (binds to MHC I so as to not kill the cell), activating and co-stimulatory.

Altered or absent MHC I cannot stimulate inhibitory signal.
NK cell is triggered by signals from activating signals.

In virally infected cell, the cell's transcription machinery is hijacked and the proteins expressed on membrane are different/less. So there's no inhibition of NK cells.

✱ NK T cell — a type of cell that occurs at v. less frequency.
It has semi-invariant receptors similar to CD4 & CD8 T cells. So this is an evolutionarily intermediate cell.

NK cells kills by releasing granules.

→ ɣ/δ T cell receptors
These cells have more receptor diversity than NK cells, but much less than conventional T cells.
Ligand recognition is independent of MHC molecules.
They have self and non-self ligands.
They produce wide range of responses - inflammation, B cell help, wound healing, cytotoxicity, antigen presentation. etc.

We don't know enough about these cells, and they occur very less in peripheral blood

→ B1 B cells : Surface IgM acts as receptor
They're called 'natural antibodies' because they're found at birth even when there's no trigger.
These receptors recognise repeating structures like bacterial polysaccharides and secrete IgM (pentamer of Ig) which binds to repeating ligand On recognition, B1B cell converts to B1 cell & secretes IgM. & Plasma cells secrete Abs
& they differ from B1 B cells in surface receptors that are expressed

B cell maturation before activation

B cell receptors are essentially immunoglobulins. Once triggered, the cell becomes plasma cell and begins circulat secreting antibodies.

Both heavy & light chains contribute to the specificity of the receptor.
They are synthesized separately and then come together.

Variable (F$_{ab}$)

Constant (F$_c$)

N terminal

C terminal

Hinge region (disulfide bond)

▨ Heavy
☐ Light

When F$_{ab}$ binds to its ligand, the structure of F$_c$ changes slightly — this change triggers macrophage to clear the pathogen.
Free floating, unbound antibodies don't elicit any response.
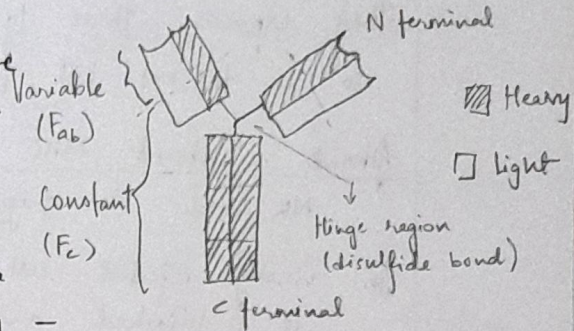
5 types of antibodies —
   Ig G - most common
   IgM - pentameric molecule, very heavy mol. wt, so limited access
   Ig D
   Ig A - commonly found in gut
   Ig E - involved in allergic reaction.

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Lecture
The F$_c$ part of Ig's is also different between
Ig G vs Ig M.

Pentameric IgM is held together by a junctional J chain
The same is true for dimeric IgA.

Receptor-ligand interaction
   The portion of the antigen recognised by an antibody is called an epitope.
It could be a linear epitope or discontinuous epitope.
The antibodies usually don't recognise self targets.
The total antibody repertoire differs from person to person.
Several Abs are generated against the same antigen —
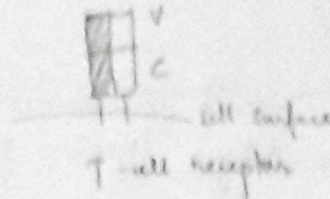So there is polyclonality or redundancy, which is beneficial.
Good!!

Antigens can have multivalent, different epitopes or same repeated epitopes (eg bacterial polysaccharides)
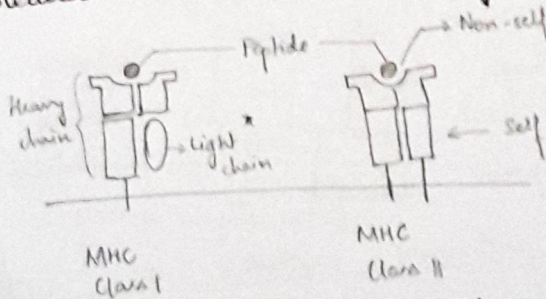3d interactions happen.

B-cell   T-cell receptor

T-cell receptor
- The cytoplasmic tail is very short, but when triggered, they start an intracellular signalling
- There are $\alpha\cdot\beta$ and $\gamma\cdot\delta$ T cell receptors with small differences in receptors
  $\gamma\cdot\delta$ T cells predate $\alpha\cdot\beta$ T cells, but they're less abundant (only 5% in peripheral blood).
- Ligands for T-cell receptors are MHC molecules, which are also membrane bound. → T cell recognition requires two-cell interaction.
- MHC molecules are our immunological identity.

Heavy chain   Peptide   → Non-self   • β_2 microglobulin
→ Light chain   ← Self

MHC Class I   MHC Class II

T-cell receptors recognise non-self peptides on self MHC molecules. target

Why have self & non-self components?
- To spot infected cell as distinct from adjacent uninfected cell
- Distinguishing soluble products of parasite origin as distinct from parasite harbouring cell.
- To be able to distinguish a normal cell from an infected cell from the OUTSIDE, before acting on it.

figure
#
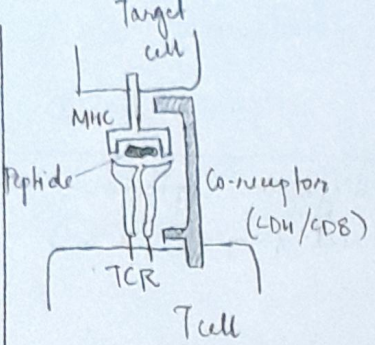
Once altered __MHC__ are bound tentatively by T-cell-receptor (__TCR__), there is another ! co-receptor ! which binds to CD4 TCR and MHC as a confirmatory binding to start the signalling

1/4/22

Lecture

MHC alleles are __expressed__ __co-dominantly__

Human Leucocyte Antigen (HLA) gene has 3 classes –

  Class I – 3 loci : A, B, C   ⎧ DM, DO, DR, DP, DR

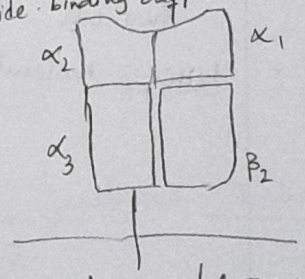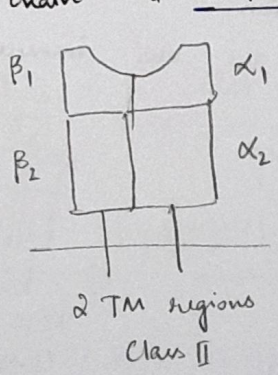 Class II – many loci with families of genes/alleles

  Class III

The __placenta__ helps the survival of the allograft 'baby' by expressing __E, F, G__ class I loci, which is common to most humans.

This class II diversity is linked to failure of peptide-based vaccines

__β_2-microglobulin__ light chain & __HLA-F__ are __monomorphic__ Peptide-binding cleft

Structure :

2 TM regions
Class II

Class I (1 TM region)
More restricted groove

Class II peptide binding cleft is a __groove__ with open __ends__ – so long peptide can bind.

Class I cleft can bind to 7-9 aa long peptide because the groove is more restricted.

The floor of the groove is formed by __β-sheet__, and the surrounding is formed by α-helix – this is a hypervariable region, so MHC can bind to wide range of peptides.

Classical MHC molecules mainly present peptides.

Antigen presenting cells (APCs) put out MHC molecules with bound peptide. T-cell receptors are MHC-allele and peptide-specific. But there can be low affinity interactions.

T-cells get triggered by foreign-MHC bound peptide, even if its low-affinity. This is called <u>alloreactivity</u>.

No MHC is stably always expressed on the cell surface freely — it is bound to some peptide or the other.

MHC I antigen presentation
- The heavy chain and light chain are brought together by peptide loading complex in the ER.
- Proteosome (enzyme) chews up proteins and produces peptides which are <u>transferred to ER</u> and try to bind with nascent MHC.
- Once a peptide binds to MHC stably, this MHC is now sent to the cell membrane to be expressed through the golgi.
- In an uninfected cell, the proteins source is <u>DRiPs</u>, and proteosome cuts it at specific places. When virus machinery takes over, the peptides bound to MHC will change in different ways.

<u>Viruses affect MHC-antigen presenting process in</u> different ways —

Eg. <u>HIV-1</u> downregulates MHC class I and β2m transcription
<u>MCMV</u> redirects MHC class I molecules to lysozyme

Also : see slide. watch movie.

Lecture

Antigen Presenting Cells (APCs)

↳ Dendritic cells, Macrophages and B cells

These cell types express MHC class II on their surface constitutively.

→ Macrophage   → Mature dendritic cells

Antigen uptake is through ~~phagocytosis~~/pinocytosis and through Antigen-specific receptor in B cells

Other features of these cells — co-stimulator delivery, antigen presented and location.

The MHC class II expression gets upregulated during inflammation

MHC II Antigen presentation — Movie

1. MHC II is synthesized in ER and an invariant chain protein binds to it. (a part of it binds to the groove)

2. This is processed into a vesicle (acidic) where the chain of the invariant protein is lysed, while

   ?? CLIP remains bound to the groove   → degraded foreign

3. Then these vesicles are filled with peptides from the outside. Meanwhile HLADM (an MHC-like molecule) catalyses the release of CLIP peptide

4. One of the 'outside' peptides then binds to MHC class II and this is expressed on the cell surface

Cell	Antigen	Molecules per cell
T cell	TCR	100,000
	CD3	124,000
CD4 + T cell	CD4	100,000
CD8 + T cell	CD8	90,000
B cell	HLA-DR	85,000
	CD21	210,000
Dendritic cell		

⋮

- MHC Class I and Class II are expressed comparably in APCs.

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Useful T cells make a choice b/w self and non-self

- T-cell recognition is <u>very sensitive</u>; small numbers of peptide-MHC molecules are sufficient for recognition

- Abundance of self-peptide loaded MHCs on the surface of cells. Even during infection, MHC-foreign peptide complex getting expressed would be only <u>1-10%</u>. Polyclonal responses have buffering capacity.

Developmental trajectories in the immune system

All blood cells arise from pluripotent stem cells in the bone marrow.

Picture: Developmental trajectories of different blood cells

→ Embryonic macrophage development

- In fetus, yolk-sac haematopoiesis leads to primitive <u>yolk-sac derived</u> macrophages.

- Another line of development : liver receives of some cells from yolk sac and produces <u>definitive</u> (fetal-liver-derived) <u>monocytes</u>. Monocytes differentiate into tissue macrophages — they're found in skin and other tissue

- Yolk-sac derived macrophages become <u>microglial cells</u> in the brain. (They don't produce inflammatory response).

- Y-s derived primitive macrophages are also found in skin.

→ B-cell Development

B-cell develop in <u>bone marrow</u> and migrate to peripheral lymphoid organs, where they can be activated.

There is also <u>negative selection</u> in bone marrow — those cells that are activated by self targets are removed

B cell development proceeds through several stages marked by rearrangement and expression of Ig genes.

	Stem cell	Early/late Pro/Pre-B cell	Immature Mature B cell	→ Naive B cell
		Pro B cell / Pre B cell		
H-chain genes	Germline	V-DJ rearrgant / V-DJ rearranged	VDJ rearrangement	
L-chain genes	Germline	Germline	V-J rearranging	VJ rearrangement
Surface Ig	Absent	Absent	Intracellular μ chain	IgD & IgM expressed on cell surface.

1. Heavy chain VDJ rearrangement occurs first.
2. Once heavy chain is synthesized, it is <u>transiently expressed</u> on the surface along with a <u>surrogate light chain</u>.
3. Expression of this uniform surrogate L-chain triggers the VJ rearrangement of L-chain.
4. <u>Binding</u> of heavy chain & surrogate light chain is the signal for survival of B cell.

Apart from reproductive system, immune system is the only place where DNA is rearranged before maturation.

7/4

Lecture

Recombination of minigenes V, D and J to form B/T cell receptor. One minigene from each group is ~~chosed~~ chosen and others are extruded.

<u>Recombinant activation genes</u> (<u>RAG1</u> and RAG2) are responsible for gene recombination during development. They are not active in mature cell

Surrogate light chain : <u>VpreB</u> + λ5 chain — no binding sight and common chain for all cells. light chain recombination and expression occurs.

Then light chain recombination

If first V-J combination is <u>nonproductive</u>, it will go for a <u>second</u> recombination and potentially more combinations.

Negative selection

Binding to self-molecules in the bone marrow can lead to the death of immature B cell. There's also the possibility of receptor editing.

Soluble molecule

- Production of <u>anergic cell</u>: the reactive cell is made <u>tolerant</u> ie. it won't react to the self molecule. Anergic cells usually just die off.

- Low affinity non-crosslinking self molecule — migrates to periphery and recognises foreign particles somewhat similar to the self molecule

- Replacement of L-chain by <u>receptor editing can rescue</u> some <u>self-reactive B cells.</u>

T-cell development

T-cells develop in the thymus — the process is parallel to B-cell development but not identical.

Thymus gland degenerates with age
⇒ T-cell repertoire also goes down with age
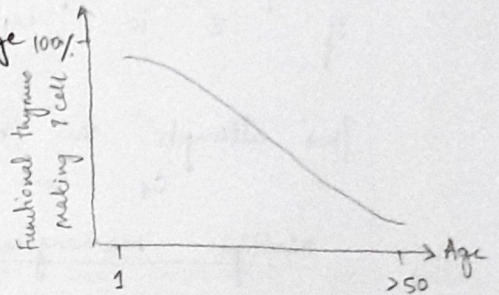
Precursors to T cells move from bone marrow to the thymus and develop there.

Anatomy

- <u>Trabeculae</u>: keeps the cells organised in a lobule

- Cortical & Medullary <u>epithelial</u> cells (thymic origin): they naturally express <u>MHC class II</u>.

- Thymocytes: lots of cells from bone marrow growing from cortex → medulla

- <u>Dendritic cells</u>: present near the <u>medulla</u>, of bone marrow origin
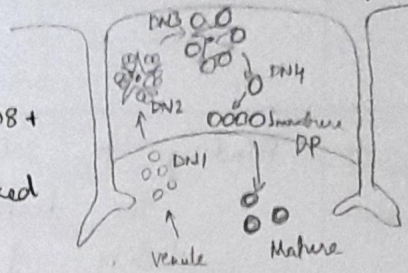
DN : double negative ie. no CD4 - CD8

PP : double positive i.e. both CD4 & CD8

Mature : Single positive — either CD4+ or CD8+

Before mature cells exit, they are checked
for self-reactivity.

- At DN3 & DN4 stage, β chain rearrangement occurs
  and T-cell receptor begins to be expressed
- tharry chain (?) is expressed with surrogate light chain
- VJ recombination occurs ind the DP stage.
  \# T cells either have αβ receptor or γδ receptor.

- First, DJ - VDJ recombination occurs in β or γδ chain ●
  β chain is produced and expressed with surrogate α chain
- CD4 /CD8 induction on cell surface
- α and δ rearrangement & transcription are competing
  processes. If α is expressed, δ genes are extruded.
  If δ is expressed, αβ may not develop further.

Two attempts can be made to produce β chain with
              C_1 and C_2 region.
   Multiple rearrangements possible to produce α chain. ●

Double negative T cells can have αβ or γδ —

Signals through γδ TCR   Signals through pre TCR shuts
(strong Erk signal) shuts off   off γ & δ gene rearrangement
   β chain rearrangement   & commit cell to
& commit cell to γδ lineage   αβ lineage

         ↓   ↓

Cells move to epithelium /   Single positive thymocyte
mucus linining in the periphery   exported to periphery

* Interaction with cortical cells is important for cell development. If they can defect MHC I, they're given a maturation signal, then if they can defect MHC II, they're given survival signal.

\# If they don't recognise MHC, they undergo apoptosis

* Then they move to the medulla, where they encounter APCs and ~~and~~ medullary cells. If they recognise self-proteins, they are destroyed. Other mature T cells are exported to the periphery.

in cortical cells : if present, → cells are kept

For a given cell, positive selection (TCR interaction) occurs first and then negative selection (central tolerance [cell death] and peripheral tolerance [anergy])).

if present, cells are destroyed

1
2   14 15   100

Theoretically possible 14-mers ~ 400
Practically possible (because of enzymes) ~ 70
Actually binding 14-mers ~ 12-15

There are a lot of proteins that are associated with cells. These peptides wouldn't be present in the thymus.
⇒ Some autoreactive T-cells could pass through and destroy self-cells. This is a problem.

Solution: Auto Immune Regulator (AIRE) → primary regulator

There's a different transcriptional pattern in medullary epithelial cells because of AIRE. MECs can randomly transcribe any protein in the genome. So, T-cells that react to other peptides of the body ~~and~~ are removed

→ Positive selection of α·β T cells in cortical epithelium
     Strong or moderate binding of TCR with MHC-peptide complex : T cell is allowed to live. If there's weak or no binding, cells are destroyed

→ Negative selection by dendritic cells, macrophages etc.
     If the binding here is too tight, then too cell is removed.

Regulatory T cell
- They're capable of regulating response. - cells with -ve feedback potential.   CD4 subset
- They are towards higher end of acceptable immune affinity spectrum
- They're called 'thymic' or 'natural' T$_{reg}$.

Apoptosis - Cell death
     Involves lysing of cell membrane and breakdown/degradation
        of nucleus.   ^permeability increase

Steps -
1. Cytochrome C is released into the cytosol from mitochondria
2. Orientation of lipid phosphate is not maintained
3. Cell membrane becomes permeable to molecules and there is internal degradation.

Necrosis : cell contents are released after lysing
     ⇒ causes inflammation, not good.

Allorecognition
   A TCR binds to self MHC & foreign peptide complex.
   Such TCR can also bind to just the peptide (peptide dominant binding) associated with non-self MHC, but with lower affinity.

Similarly, TCRs can also bind to just the MHC molecule (MHC-dominant binding) with lower affinity.

This is relevant especially during transplants. — T cells recognize foreign MHC and peptide (allorecognition) with greater frequency but lower affinity.

12/4

T-Cell Responses

T-Cell Activation : Initiation of T-cell-APC interaction

- T cells initially bind APC through low affinity accessory molecules (LFA1 : ICAM1)

- Then, if TCR binds to MHC peptide, then it signals to LFA1

- Conformation of LFA1 changes to increase affinity and prolong interaction, so that signalling can happen

- If TCR doesn't bind, LFA1 disengages and another T cell comes

CD4 TCR LFA1

MHC, ICAM1
class II

Tight binding
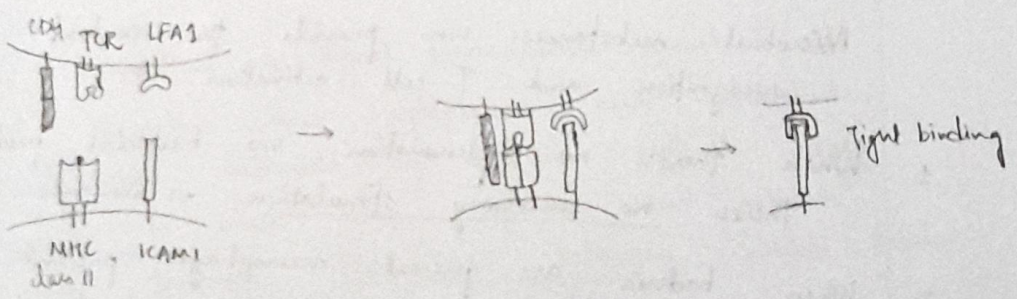
Immunological synapse

The binding region between T-cell and APC where signalling occurs.
There's a signalling zone and secretory zone.

TCR + CD3 mediated signalling is essential for T cell activation

CD3 has ITAM on its cytosolic side. Phosphorylation of ITAMs by CD4 co-receptor LCAK (kinase) is important

for T-cell activation through signalling molecules (lots of them!). It results in transcription of NF kB etc. factors which induce further transcription and cell proliferation.

Requirement of accessory/costimulatory signals for naive T cell activation

(CD80) eg: CD28 - B7 interaction are accessory/secondary signals (which are not via TCR-MHC & CD4)

There are also adhesion molecules (LFA1 : ICAM1) and soluble mediators (cytokines — APC secreted IL-12, IL18 etc.)

Effector T cells are less demanding — just TCR signalling can be sufficient.

Central tolerance — negative selection in the thymus

Peripheral tolerance — inactivation or anergy of T cell through absence of accessory/costimulatory signalling, and the cell is ultimately deleted

19/4/22

Lecture

Microbial substances can provide the context for 'danger' recognition and T cell activation

1 When there's no inflammation, no bacterial proteins present, there's no accessory stimulation ⇒ anergic T cell

2. When bacteria are present, macrophages present antigen and provide a co-stimulatory signal — leads to activation of T cell - differentiation & proliferation

3. When there is both bacterial & non-bacterial material, macrophages present both proteins and co-stimulatory signal. So even if T-cell primarily recognizes non-bacterial pro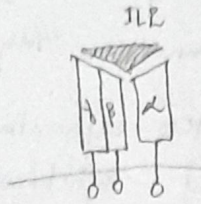tein, it receives a co-stimulatory signal & is activated. This is used for immunisation — called adjuvanticity. Alum is a common adjuvant, that's coated with protein of interest and used for immunisation.

IL-2 mediated proliferation of T-cells

Interleukins are a whole cluster of proteins
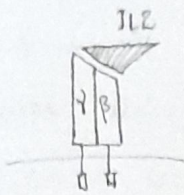
Naive T cell   Activated T cell
Moderate affinity   High affinity

- TCR Activated cells put out α subunit and this IL-2 receptor
  has high affinity and reacts — does its job.
  Bystander effect : If there's a lot of IL2 floating
  around, even naive T cells get moderately activated
  This can also happen when there's co-infection.

  Once activated, T cells express IL2 receptors and also
  starts producing IL2. IL2 induces T-cell proliferation
  which leads to more T cells and more IL2.

- Cytokine interaction & signal transduction events
  Cytokine receptors have cytoplasmic and extracellular
  domains. When activated by cytokine molecules, they
  dimerize to bind with cytokine and activate
  the cell through JAK-STAT pathway. which leads
  to transcription of certain proteins

  JAK — Janus Kinases
  STAT — Transcription factors

  Common functions of cytokines —
  Cytokines have a local radius of action. Lymph nodes closer
  to site of infection is inflamed, not others.

- Induces growth of T cells (IL2)

- Activation of macrophages & upregulation of MHC I, II. costimulatory
  (IL-γ)

- IL4 mainly acts on B cells – activation, growth, IgM → IgE, ↑IgG. IL4 also activates T cells.
- Activation of macrophages and induces NO production – IL3 & Tumor necrosis factor (TNF) ↳ helps kill microbes in vacuoles.
- IL3 acts on hematopoietic cells
- IL 10 and TGFβ – help in restoring homeostasis or by downregulating immune response.

  NK cells, APCs, TCR-expressing cells all secrete interleukins and cytokines

Cell Fate Decision – Differentiation of CD4 & CD8 T cells.

function of CD4 (Helper) T cells –

1. Cytokine secretion
2. Macrophage activation
※ 3. B cell help
4. Memory response
5. 'Regulation' – dampening immune response.

Basic view of CD4 T cell differentiation

T_H1 and T_H2 secrete unique sets of cytokines that promote function

- T_H2 naive cell is activated by IL4 and it activates GATA-3, an important Tx factor. These cells now produce IL4, 5, 10, 13

- T_H1 is activated by IL12 and IL18. Upon activation they produce IFN-γ, IL2 and TNF.

So, cytokine inter environments decide the fate of T_H cells.
Also, if there are more T_H2, it negatively affects T_H1 differentiation.

Another major contributor: Transcription factors – T-bet (T_H1)
Master regulators GATA 3 (T_H2)

There are also other contributors – co-stimulatory molecules, peptide-MHC ligand density, genetic background etc.

Certain master regulators - transcription factors - decide CD4 T cell differentiation into TH1, TH2, TH17, Treg and Tfh

Functions of Thelper cells -

- TH1 - triggers APCs to kill intracellular bacteria
- TH2 - secrete interleukins which acts on eosinophils, mast cells and plasma cells
- TH17 - produces IL-17. It is very pro-inflammatory and plays a role in auto-immune diseases. Its also active in gut immunity
- Tfh - activates B cells (something more?)
- Treg - if inhibits immature dendritic cells & thus prevents T cell activation. Helps dampen immune response.

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Lecture

Tregs : Identification & function

Tregs are selected in thymus from upper spectrum of activity during selection

Function : homeostatic control - control aggressive autoimmune responses.

There's no direct evidence/observation, but if Tregs are absent, then the organism is in a state of perpetual inflammation, immune response is too much.

There are also natural and inducible Tregs. With age, Tregs decrease, so we see an inverse correlation with incidence of auto-immune diseases.

Tregs are also antigen-specific in function - if an autoimmune pathology is triggered by specific antigen, then specific Tregs will be more efficient at controlling it.

Tregs are CD4 cells with markers like <u>CD25+</u>, they produce <u>IL-10, TGFβ</u> & <u>Foxp3+</u>

These cells <u>mop up</u> any <u>extra IL2</u> that would trigger effector cell.

Other mechanisms :

Cytotoxic (CD8+) T cell activation
→ mainly in spleen and lymph nodes.

- Stimulation of naive T cell — <u>TCR binding</u> is the primary signal and then there are co-stimulatory signals from APC surface proteins and ILs. Both signals are required for activation.

- When activated, the naive cell proliferates to form · differentiated effector cells.

- Active CD8 T cells <u>kill virus-infected target cells</u> — mainly epithelial cell. Here, only primary signal is sufficient to kill the cell.

- These active effectors cells are called <u>memory T cells</u>. One cell can kill many target cells.

- Various players in apoptosis
  - <u>Perforin</u> granules : polymer creates a hole in cell membrane, like cytokines. Primary effector.

  - <u>Granzymes</u> (serine proteases), Caspases (cystein proteases)

  - <u>Fas-FasL</u> interaction : membrane ligand-receptor interaction
    <u>TNFR - TNF</u> interaction

  - Mitochondria associated components. — apoptosis inducing factor

Perforin deficient individuals are susceptible to viral infections.

T-cell granule cell release
  CD8 T cells have <u>cytotoxic granules</u> in its cytoplasm.
  If TCR is triggered, the organelles rearrange such that granules are released in the immune synapse to act on the target cell. Golgi also synthesizes and releases <u>IL2, IL?</u> as well.

Caspase activation pathway

Fas-FasL & TNF-TNFR interactions result in death receptor engagement, which further stimulates caspase pathways and ultimately, apoptosis.

Apoptosis doesn't mean cell contents are released, they are sort of digested inside

Perforin — aids in delivering contents of granules into cytoplasm of target cell.

Granzymes — triggers apoptosis.

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Development of Immune Memory

Resonance article illustration

Immune response to secondary exposure is rapid and effective. Once memory cells have proliferated/expanded, it's hard to decrease their numbers. Tregs help keep their action in check.

Immunological memory

It's the ability of immune system to respond more rapidly and effectively* to pathogens that have been encountered previously. It reflects the pre-existence of a clonally expanded population of antigen specific & lymphocytes.

* response of greater magnitude

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Lecture

Phenotypic characteristics of memory T cells —

- CD44, CD45, CD69 status
- Central (CCR7+, CCR CD62L+) and Effector (CCR7-, CD62L-) memory cell

Effector memory cells are the ones soon after infection, they're very active & large in number. Central memory cells are those that remain — they're more quiescent & lesser in no.

Effector cells go everywhere around the body, but central memory cells 'home' to the place of infection. Memory cells have longer activation time, but once activated they behave like effector cell.

Different models of activation and differentiation

Proliferation & differentiation

Proliferation

Memory cell

Immediate effectors

Death

Death and differentiation
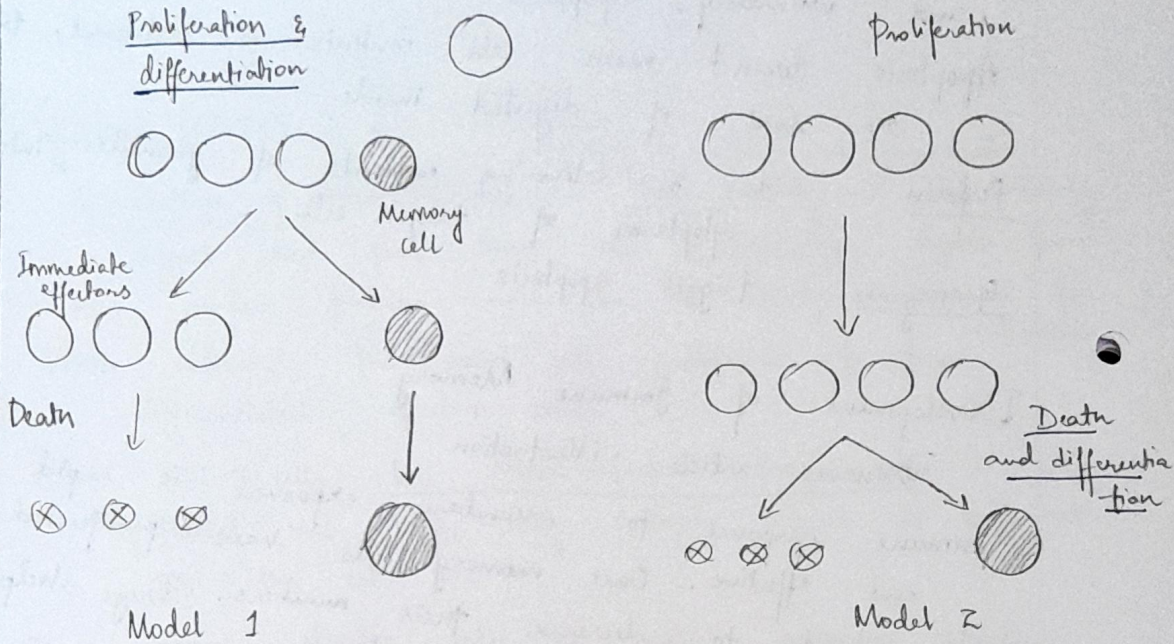
Model 1

Model 2

→ In first model, memory cells are relatively quiescent cells that are produced during proliferation — while effector cells undergo cell death after primary response, while memory cells survive.
This ensures that there is a memory, while in 2nd model, all cells may die off. Another point: minimal mutation in memory cell — but this is not a major problem.
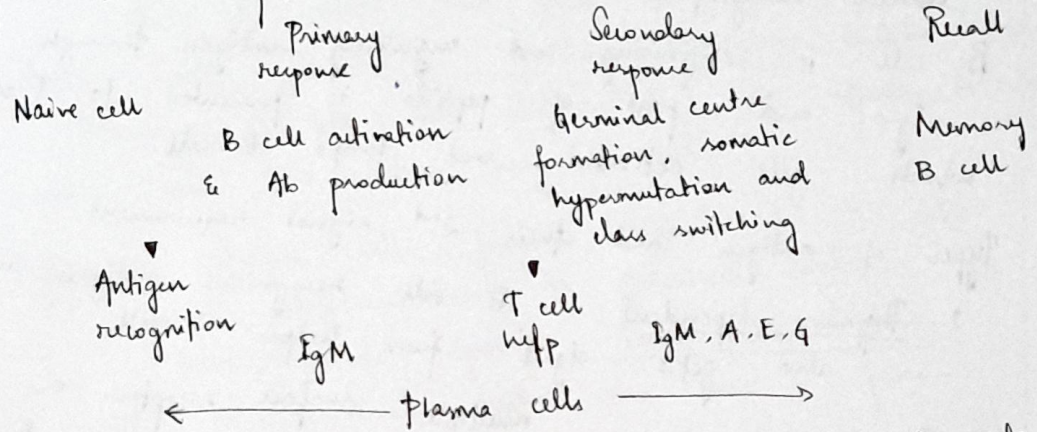
→ In 2nd model : cells proliferate and a few are preserved as memory cells. This seems more reliable — it's important to mount effective response first and think about memory cells later.
But, these memory cells are product of several rounds (2-3) of cell division. It has lesser cell division capability (Hayflick limit / telomere length).

It's better to go for Model 1 during <u>mild infection</u>, and when there is severe infection, survival and proper, effective immune response is more important, so memory cells are picked later.

All of this happens due to <u>local response to antigen load</u> (if more, more T cells are triggered to be effectors), and <u>not centrally regulated</u>

B cell responses
B cell fate decision

	Primary response	Secondary response	Recall
Naive cell	B cell activation & Ab production	germinal centre formation, somatic hypermutation and class switching	Memory B cell

Antigen recognition ▾   IgM   →   T cell help ▾   IgM, A, E, G

←————— Plasma cells —————→

Plasma cell <u>won't lead to memory cell</u>, they die at the end of their lifespan.

B cell receptor complex

Igα Igβ
ITAM

Igα & Igβ are part of receptor complex, like CD4 and CD8
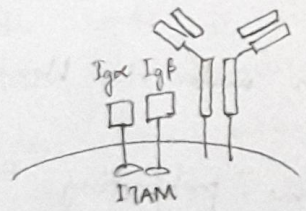
When multiple antibodies on the surface of B cell are activated by repeated antigen (say, bacterial cell wall), then the <u>receptors</u> cross link and there is <u>complex downstream signalling</u>, like T cell signalling.

Naive cell activation requires secondary signal too - complement molecule on soluble antigen (eg. C3d) that triggers the co-receptor on the B cell, so its properly activated

When activated, plasma cells secrete antibodies which have certain immune function -

- Neutralisation - Abs bind to antigen, prevent bacterial adherence and microbe penetration into any cells.
- Opsonisation - this promotes enhanced phagocytosis
- Complement activation - activates complement, which enhances opsonisation and lyse some bacteria.

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Linked recognition

B cell is recognising and engulfing antigen through surface Igs and part of peptide is presented to T cell, which gets activated and helps B cell

Types of antigen and their 2nd signal requirement.

1. Thymus dependent - B cell recognizes antigen and also gets signal from helper T cell

2. Thymus independent - multiple surface receptors are triggered through polyvalent antigen and the 2nd signal is through Toll like receptor (TLR) & Lps. which is not antigen specific This T-independent response only produces IgM.

Anatomy of lymph node

Naive B cells travel to the lymph node via bloodstream and leave via efferent lymph.

B cells recognise antigen and some proliferating B cells migrate into the cortex to form germinal centre. Plasma cells migrate to medullary cords and leave via efferent lymphatics and go to the bone marrow (but not all because niches can get filled).

• MHC II is expressed in endolysosomal compartments and
   then expressed on the surface.

Plasma cells that reach the bone marrow are
   long-lived plasma cells. Short-lived ones are found in
   secondary lymphoid organs.

Germinal centre formation
   Once B cells are activated by CD4 T cells, they proliferate.
   Some B cells move into the follicle and
   undergo more proliferation and somatic hypermutation,
   which modifies the receptors. These B cells
   undergo selection with self antigen.
Some B cells that remain in T cell area secrete Abs
      for a while, and then die off.
Plasma cells have no surface receptors, unlike mature T cells.
   Memory B cell can differentiate into effector B cell,
but not the other way around

T-follicular helper cells provide signals to B cells
      for differentiation in germinal centre.
   T_FH have / secrete specific Transcription factors.
B cell in germinal centre can have 3 fates —
   1. Germinal centre B cell
 * 2. Plasma cell
   3. Memory B cell

B cell binds to virus through viral coat protein. This
   virus is internalised and degraded in endolysosomes #
Peptides from internal proteins of virus are presented
   to the T cell, which in turn helps the B cell.
Covid strain responses — less B cell, but same T cell response

Epitopes — portion of the antigen that is recognised by B cell
      Epitope can be conformational, discontinuous or linear
Often, T cell epitope are buried inside the protein, it has to be

chewed up and prevented with MHC.

Role of carrier protein and in Ab response
Related to Mitchison expt — hapten . carrier effect

- B cell binds bacterial polysaccharide epitope linked to tetanus toxoid proteins
- This antigen (polysaccharide + toxoid) is internalised and processed
- Peptides from protein component (toxoid) are presented to T cell
- Toxoid - recognising T cell helps this B cell produce Antibodies against polysaccharide antigen

Adding alum makes the process more efficient

	Intrinsic properties			Inducible by antigen stimulation		
	Surface Ig	Surface MHC II	High Ig secretion	Growth	Somatic hypermutat^n	Class switch
Resting	High	Yes	No	Yes	Yes	Yes
Plasma	Low	No	Yes	No	No	No

Plasma cells don't express MHC II — they can't trigger T cells and they don't get affected by helper T cells.

Modes of generating diversity in rearranged Ig gene
Two processes: Somatic hypermutation & Class switch

Somatic hypermutation
There are a lot of mutations in the variable region (antigen binding site) of the Ig.

Class switch
Here, the constant region of Ig is changed (to other constant heavy chain), but not mutated

Lecture

Somatic hypermutation

Its a process that <u>creates a lot of B cells</u> with <u>varying</u> receptors to the <u>same antigen</u>. These germinal cells undergo selection again — the cell is killed off if it has low/no affinity, and the cell undergoes proliferation into memory & plasma cells which are again stimulated by T_H cells

Accumulation of Ab diversity and increase in affinity with maturation of B cell response. There is <u>increased</u> mutation, selection & in turn <u>affinity</u>. during primary, secondary & tertiary response over ~21 days. Cells that come out of germinal center has even more affinity. Old memory cells also have to go through the germinal centers again & undergo somatic hypermutation

Movie — <u>Class switch</u>

$I_g A \wedge I_g D$

After VDJ recombination, the V region & C_μ, C_δ regions are transcribed. The constant regions of other isotypes (C_γ, C_α, C_ϵ) are not transcribed

Mechanism of class switch — rewatch video.

Different molecules (interferons, cytokines) trigger the class switch to particular isotypes. (IL4 - $I_g G_1$, $I_g E$) from $IgMs$.

IL4 - IgG_1, IgE

IFN-γ - IgG_3, IgG_{2a} (inhibits IgG_1 & IgE)

TGFβ - IgG_{2b}, IgA

IL5 - augments IgA production

Functional activity and distribution of immunoglobulins.

- Mean serum level — IgE levels are very low, so it's easier to detect IgE increase

- Only IgG is transported across the placenta, which helps fight infection in the fetus.

- IgA (dimer with J junction) is found across all epithelium. The transport is facilitated by the dimerisation. Monomer IgA is found in blood

- Diffusion into extravascular sites — pretty high for all.

Functional activity

- Neutralisation — high in IgG, IgA

- Opsonisation — very high in IgG_1

- Activation of complement system — very high in IgM & IgG_3

- Sensitisation of mast cells (allergic reaction) — IgE

- Sensitisation for killing by NK cells —

IgA secretion & transport

Dimeric IgA binds to receptor on basal layer of epithelial membrane (say, in the gut) and there is receptor-mediated endocytosis of IgA. IgA is then deposited out on the lumen surface.

Dimeric IgA is found largely in the mucosal surfaces — lungs, gut etc. Also breastmilk — IgM, IgG

\# Covid vaccines don't trigger IgA secretion, which would have provided protection at the first border — (cell entry by virus would be blocked → transmission blocking immunity.

Immunity in Health & Disease

Health

- WHO : state of complete physical, mental and social well being and not merely absence of disease or infirmity

- The level of functional & metabolic efficiency of a living organism

Disease

- Disorder of structure or function in a living organism, especially one that produces specific symptoms or affects specific location, and is not a direct result of physical injury

- A condition that impairs normal functioning and is typically manifested by distinguishing signs and symptoms (what patient complains about)
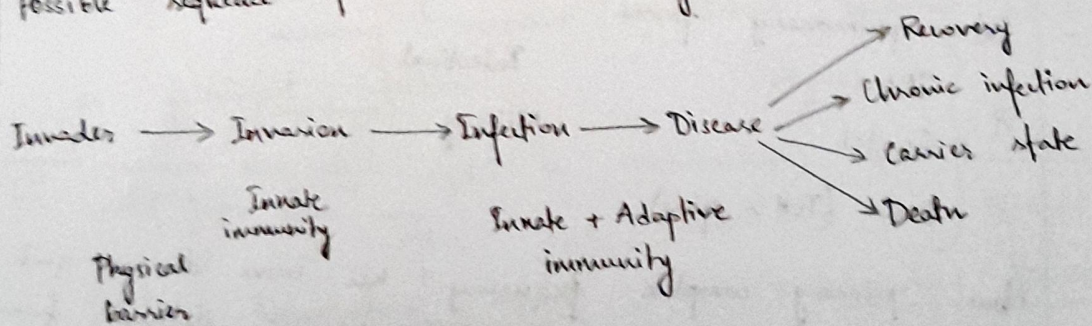
Types of diseases

Infectious   Genetic deficiency

Autoimmune   Malfunctioning of body parts

Allergic   Metabolic

Possible sequence of events encountering an invader

Invader ⟶ Invasion ⟶ Infection ⟶ Disease

                Innate   Innate + Adaptive
                immunity   immunity

Physical
barrier

Disease ⟶ Recovery
Disease ⟶ Chronic infection
Disease ⟶ Carrier state
Disease ⟶ Death

Contributions of immune response

Protective immune response → Complete recovery

Non-protective response

- inadequate in quantity ⎫
- poor in quality   ⎬ Chronic infection
- both   ⎭ Carrier state
                            Death

Journey of invaders in the host

Entry

Journey to the desired niche (eg. neurotropic, liver - Hep A)

Resistance from the host

Infection & clearance of infection

Exit of invader and survival of host

Mycobacterial Infections

Tuberculosis

- Several types of mycobacteria species that cause tuberculosis in various animals. Main bacteria: M. tuberculosis and M. leprii — slow and hard to culture

- Primary complex : seen in the first year of life, where infection affects lungs, feeding & weight gain

- Post primary — adult TB

- Extra pulmonary forms

  ∗ ⎧ TBM meningitis   Intestinal
    ⎨ Genital   Miliary (systemic)
    ⎪   ↳ high death rate because diagnosis
    ⎩ Bone (Pott's spine)   was done very late.

- Now, primary complex frequency has come down quite a bit because of BCG vaccine, derived from M. bovine

- India still has the highest burden of TB — India accounts for 21% of cases.

Lecture

Tuberculosis

TB is found in all countries (doesn't depend on income)

In India, numbers are <u>188 per 100,000</u> people.

Immune response

TB is a facultative intracellular pathogen It is mainly spread through air (aerosols?)

Innate response

1. TLR mediated signalling, <u>complement</u> binding
2. <u>Phagocytosis</u> (by neutrophils & macrophages)
3. <u>M1 response</u> by macrophages — release of pro-inflammatory cytokines, killing of pathogen intracellularly

M2 response?
Bringing response down

4. Proteolytic degradation & antigen presentation on MHC
, T_H1 preferable over T_H2?

Adaptive response

1. Activation & differentiation of <u>T cells</u>, CD4 T cell activation is dominant
   - Release of <u>cytokines</u> (IL4, IL5 etc)
   - T-B cell cooperation, <u>high affinity Ab production</u>
   - killing infected phagocytic cells.

All of this helps in clearing the infection, generation of memory response, or persistent localised infection or fulminant infection.
it could become

For leprosy (also caused by mycobacteria), the dominant response is often T_H2, which is less efficient.
T_H2 is <u>lepromatous leprosy</u> ; T_H1 is <u>tuberculoid leprosy</u>

→ Known problems
- Prolonged treatment duration — weeks to months
- Drug resistance (MDR & XDR strains of TB)

- Association with HIV due to immunosuppression - activation of latent TB?
- Other causes of reactivation of TB
- Good, protective vaccine

BCG Vaccine
- Live attenuated vaccine
- Recommended to be used soon after birth
- Efficiency of vaccine varies with age when vaccine was given. If given after birth, the person would never get pulmonary in developed countries. In India, vaccine protection starts <u>wearing off</u> after 5-10 years ie. immunological memory lasts for life. This is probably because of difference in climatic conditions. (bacteria last longer in the air). which triggers memory cells again and again, so they go beyond Hayflick limit - possibly

Vaccines
They trigger immune memory without producing disease. They must produce long-lasting memory, which should help in killing & clearing the invaders efficiently If we know how natural infection is dealt with by the immune system, vaccine should trigger a similar response (eg. typhoid - cholera vaccine)

Why vaccines fail -
- Don't know <u>what kind of response</u> is needed, & wrong kind is generated
- Immune memory is <u>short lived</u> (type E) in the pathogen (eg. COVID)
- <u>Mutations</u>

Memory recall
- Repeat exposure to original or similar pathogen usually causes expansion of pre-existing T cells to produce same set of cytokines & do other effector function.
- Immune memory cannot be recalled indefinite times.
- Memory exhaustion : frequency of antigenic exposure, genetic factors (?) ; Can memory be replenished?

Lecture

Allergy and Autoimmunity

The proportion of Treg cells go down with age, and autoimmune disease freq goes up — some correlation.

Hygeine Hypothesis

- Excessive hygeine in early childhood decreases exposure to commensals and pathogenic microbes
- Vaccination reduces developing immune system's experience in facing natural infections — debatable, need not be true in every context.
- Overreliance on antibiotics to terminate infections reduces the use of immune system and its ability to discriminate self from foreign pathogenic microbes

This hypothesis says 'unnatural' conditions prevent development of immune system

Allergy

- A number of conditions caused by hypersensitivity of immune system to common things that causes no problems in other people
- Includes : atopic dermatitis, allergic asthma, anaphylaxis (acute, fatal)
- Symptoms : skin rashes, runny nose, breathlessness, swelling
- Incidence of allergy in children is 7-10% in India.

Mast cell activation & degranulation

GI tract : increased fluid secretion, increased peristalsis
⇒ expulsion of content by diarrhea/vomiting

Airways : decreased diameter, increased mucus secretion
expulsion of airway contents by coughing/sneezing

Capillaries : Increased porosity ⇒ blood flow out of capillaries.
Causes swelling

Allergen → DC + TH2 $\xrightarrow{\text{(IL4)}}$ Induction of __IgE__

$\quad\quad\quad\quad\quad\quad$ ↳cytokines $\quad$ Basophil + __Mast cell__

→ Common treatments

· Drugs to decrease symptoms (anti-histaminic agents)
· Identify allergens and _decrease_ exposure
· _Modify_ immune response from TH2 to non-TH2 : immunotherapy

Autoimmune diseases

Mechanisms that contribute to self-tolerance

- _Negative selection_ of B cells and T cells (expression of tissue-specific proteins in thymus)

- _Exclusion_ of lymphocytes from certain tissue (eye, brain, testis)
- Induction of _anergy_ in autoreactive B & T cells
- Suppression of _autoimmune_ responses by __Treg__ cells
$\quad\quad\quad\quad\quad\quad$ just this?

TH cells don't help autoreactive B cells, usually.

Longer the duration of viral infection, higher the chances of development of autoimmune B & T cells.

Types of diseases —

1. _Systemic_ diseases — systemic lupus, sjogren's syndrome

2. _Local_ diseases — alopaecia areata (bald patches)

3. _Organ-specific_ diseases — autoimmune thyroiditis, myasthenia gravis

Treatment

- _Immunosuppression_ (eg. methotrexate)
- _Anti-cytokine antibodies_ (anti-TNFα for rheumatoid arthritis)
- _Replacement therapy_ (thyroxine for autoimmune thyroiditis)
- Drugs (_cholinesterase inhibitor_ for myasthenia gravis)

Animal models for allergy & autoimmune diseases

· Mouse models for both (eg. EAE mice mimics mult. sclerosis)
· Understanding pathogenesis
· Evaluation of drugs.