Learning Outcomes

- Revise the immunological basis of the 4 types of hypersensitivity

- To use histology to reinforce learning concerning the different mechanisms of hypersensitivity for Type I and Type IV hypersensitivity

- To specifically appreciate the clinical features and histology of Tuberculosis and the use of different techniques for making a diagnosis

- Appreciate the aims and importance of the “One Health Initiative” using Mycobacterial disease as a critical example
Hypersensitivity

Immune response is designed to protect

However, immune response against innocuous antigen or an excessive immune response may cause harm and this is termed “HYPERSENSITIVITY”

Look at lecture notes for revision
<table>
<thead>
<tr>
<th>I</th>
<th>Antibody mediated (IgE)</th>
<th>2-30 minutes</th>
<th>Antigen crosslinks IgE antibody pre-bound via FcERI on mast cells</th>
<th>Asthma, Systemic anaphylaxis, Hayfever</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Antibody mediated (IgM/IgG)</td>
<td>5-8 hours</td>
<td>Antibody bound to cell surface antigen mediates cell-destruction via ADCC</td>
<td>Blood transfusion reaction, Haemolytic disease of the newborn</td>
</tr>
<tr>
<td>III</td>
<td>Immune Complex mediated (IgA/IgM/IgG)</td>
<td>2-8 hours</td>
<td>Immune complexes deposited in various tissues bind FcGRIII on mast cells with degranulation and neutrophil degranulation</td>
<td>Systemic Lupus Erythematosus (SLE), Arthus Reaction</td>
</tr>
<tr>
<td>IV</td>
<td>Cell-mediated</td>
<td>24-72 hours</td>
<td>Memory Th1 cells release cytokines which recruit and activate macrophages</td>
<td>Contact dermatitis, Tuberculosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mycobacterial disease</td>
</tr>
</tbody>
</table>
Type 1 Hypersensitivity
Consider the following questions before you look at the sections

1. What cell types are you expecting to see in the histological sections? (Revise inflammatory cell appearances in appendix 1)

2. What other changes might you expect to see? (Clue: think about the various cells and mediators involved in Type I Hypersensitivity and their potential effects on tissues).
Type I Hypersensitivity - Nasal Polyps

Look at slides of a nasal polyp. NDP images 89.758 and 04.17c; image maps A_NP_PQ_NO_05_A_NP_PQ_NO_06.

Polyp is a term referring to an outgrowth from a mucosal surface.

Some are due to hypersensitivity type I reactions such as this nasal polyp, whereas others may be inflammatory (non-hypersensitivity) in origin.

Now look at the toluidine blue sections (89.758 and 04.17c). Many details are shown less well than in the H&E section, but one feature is conspicuous: there are scattered cells with prominent purple granules in their cytoplasm. These are mast cells and are difficult to identify in the H&E section.
Type I Hypersensitivity - Asthma

Look at images of asthma (83.41)

Consider the following questions

• What changes do you notice in the bronchus?
• How might these relate to the symptoms of asthma?
• What inflammatory cells can you identify in the section?
• Can you identify mast cells in the H&E section?
Type I Hypersensitivity

Normal Bronchus

- Cartilage
- Smooth muscle
  - Contracted in asthmatic bronchus
- Sub-mucosal mucous glands
  - Hypertrophied and hyperplastic in asthmatic bronchus
  - Mucous plugging
- Basement Membrane
  - Thickened in asthmatic bronchus
- Reduced luminal diameter

Asthmatic Bronchus

- Cartilage
- Smooth muscle
  - Contracted in asthmatic bronchus
- Sub-mucosal mucous glands
  - Hypertrophied and hyperplastic in asthmatic bronchus
  - Mucous plugging
- Basement Membrane
  - Thickened in asthmatic bronchus
- Reduced luminal diameter
Type I Hypersensitivity Issues to consider – discuss with a demonstrator

Where are the IgE antibodies in these examples?

What are the potential allergens in these settings?

What chemotactic and vasoactive mediators are released?

How does the oedema fluid develop in the nasal polyp?

What would you expect the symptoms to be?
Group Review Timepoint 1
Type IV Hypersensitivity

We will now consider Type IV Hypersensitivity using TB as an example.
So…….

TB looks identical histologically in humans and animals.

TB can be a zoonosis.

Humans can infect animals.

Animals can infect each other across species.

Animals can infect humans.

Controlling infection in different populations requires strategies across more than one species.

Many species → one disease → ONE HEALTH

Learn more here and here.
Intertwining of human and animal health –
the concept of “One Health”

One world one medicine one health.

The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations
and communications in all aspects of health care for humans, animals and the environment.

The One Health concept recognizes that the health of people is connected to the health of
animals and the environment.

A One Health approach is important because 6 out of every 10 infectious diseases in humans
are spread from animals.
Tuberculosis – invader vs host

It may help to think of DTH in this case being the interplay between organism, immune response and tissue damage.
What is a granuloma?

Collection of macrophages

Recruited by immune system as an attempt to “wall-off” organisms/substances it cannot eliminate

Morphologically variable in different settings

- Naked or with surrounding lymphocytes
- Multinucleate (Langhans) giant cells
- Asteroid bodies
- Central necrosis
- Central neutrophils

Read more about granulomas
Tuberculosis – activating the immune response

Antigen brought to hilar lymph node by alveolar macrophages is processed and presented to T-cells.

Immune environment leads to a cell mediated immune response dominated by CD4+ Th1 lymphocytes.

Th1 cells release cytokines, including IFN-γ, that recruit and activate macrophages.
In lymph node, response dominated by **Th1/macrophage** cooperation (leading to macrophages aggregating to form granulomas)

T cells, sensitized in the lymph node, reach the Ghon focus via blood and form granulomas there

Macrophages are activated appearing enlarged with much pink cytoplasm – called “epithelioid” macrophages because they resemble epithelial cells

Some macrophages fuse to form “multinucleate giant cells” with U-shaped nuclei (“Langhans’ cell”)

Centre contains soft dead material resembling **cream cheese** (caseous necrosis), due to destruction of normal tissues by released enzymes.

Granulomas may coalesce together as they enlarge.

Fibroblasts start to lay down fibrous tissue around the granuloma in an attempt at healing
Consider the following questions before you look at the sections

1. What cell types are you expecting to see in these granulomas?

2. Relate what you see back to the illustration on the previous slide – can you identify all these components in the tissue section
Type IV Hypersensitivity Practical Exercise (1)

Look at the slides and describe what you see

- Tuberculosis (Post-primary)
  - NDP Images: 9.4: 73.1120; 66.0255 & 80.0281
  - Image Map: A_TB_GN_LN_04
- Post-Primary Tuberculosis (please handle these sections with care) –
  - Large Glass Slides: 9.5: 57.110 (H&E); 9.6: 57.110 (ZN)
  - Image Maps: A_TB_FS_LU_02; A_TB_FS_LU_03; A_TB_FS_LU_10; A_TB_FS_LU_11
Look at the sections from the right upper lobe of a lung which was removed from a woman who had been suffering from tuberculosis for seven years and was losing weight.

Why is there cavitation?

What are the potential consequences of such cavitation?

- Lung: fibro-caseous TB – NDP Images: 60.1158 & 50.0470
- Lung: TB bronchopneumonia – NDP Image: 57.0350
Questions to discuss with a demonstrator

Why do granulomas form in TB infection?

Think of some other conditions where you might expect granuloma formation

What cell types are present in the TB granulomas?

Which class of T-cells are activated in the immune response to TB?

How is the tissue damage caused?

Where is TB infection first presented to the immune system? Where might you predict that granulomas will be seen in primary TB?
About Mycobacteria

Long-lived bacteria which proliferate slowly but are protected by their thick, waxy outer coat.

Stimulate a type IV delayed type hypersensivity response.

They are difficult for phagocytes to kill, and although initially a few neutrophils may be involved; very soon macrophages with surviving intracellular bacteria dominate the response.

Mycobacteria have a cell wall which contains a wax-like substance, mycolic acid. This does not take up the stains generally used for tissue and bacteria, so in order to reveal them; the ZIEHL-NEELSEN STAIN is used. The specimen is exposed to a hot solution of basic dye (carbol-fuchsin) which is able to penetrate the wax. The binding of the dye is so strong that subsequent treatment with solvents such as acid and alcohol does not remove it.

Mycobacteria which cause tuberculosis are often termed acid & alcohol-fast bacilli (or AAFB). Some other types of Mycobacteria are acid-fast only.
Practical exercise: Slide review


Image Map: A_TB_ML_LU_04

18/12 † died from tuberculous meningitis secondary to military TB.

Note: basic structure of granulomata is exactly like those you have seen in the lymph node, but are widely separated from each other.

Ziehl-Neelsen - NDP Images: 9.8: 68.808 /Glass Slide: 9.8: 68.808

Renal transplant patient.

Look for fuscia-staining organisms visible with the x 40 objectives. (There may be only one granuloma near the edge of your section containing numerous bacilli; ask a demonstrator if you cannot find any).
TB – Pathway of infection

1. Primary infection may be in lungs (from inhalation or tonsil or Peyer’s patches from ingestion).

2. Primary infection in the lungs is typically peripheral. The peripheral lesions termed the Ghon focus.

3. During primary infection, organisms are engulfed by alveolar macrophages with the associated immune response forming the primary focus.

4. Therefore, the response is at two separate sites:
   - Site of entry usually the periphery of the lung (Ghon focus)
   - Draining lymph nodes.

   This pattern is known as the primary Ghon complex (Ghon focus + local lymph node involvement).
TB – Outcomes of infection

- **Primary TB**
  - Previously unexposed host
  - 5-10% clinically significant disease

- **Latent lesions**

- **Progressive primary TB**
  - Widespread consolidation of middle/lower lobes

- **Miliary TB**
  - Erosion into blood vessels
  - Infective material enters blood stream
  - Disease disseminates to multiple organs
  - May see tuberculous meningitis
  - Abundant small lesions like millet seed

- **Secondary disease**
  - Reactivation
  - Re-infection
  - Often apical
  - Heals by scarring in many
  - May progress especially if immune deficient

- **Cavitating disease**
  - Abundant caseating necrosis
  - Often apical as higher [O₂]
  - Erodes into bronchi
  - Risk of coughing infected material
Group Review Timepoint 2
Macroscopic Pathology Review (1)

- **Rabbits**: injected with mycobacteria: differential susceptibility - R 61.284 & R 61.285. Bovine M. tuberculosis (0.04 mg) given intravenously. It died four weeks later. Note numerous miliary lesions, especially in the lungs.

- **GUINEA PIG**: M. tuberculosis - R61.287. Intramuscular human M. tuberculosis was given into the right thigh and the animal killed 6 weeks later. Note the enlarged draining lymph nodes, scattered lesions in the lungs, liver and spleen. Much of the pallor of the liver is due to fatty change. The lesions resemble a human primary complex with miliary spread.

- **Lung**: Primary complex and miliary spread - P47.088. An 11 year old girl had a three week history of persistent vomiting. On admission there were signs of meningitis and tubercle bacilli were found in the cerebro-spinal fluid. She died eight days later and was found to have miliary tuberculosis with meningitis. In the lung there is a peripheral yellow nodule (GHON focus - the site of primary infection) and enlarged hilar lymph nodes, which together constitute the PRIMARY COMPLEX. The tiny yellow tubercles seen in other parts of the lung are the result of blood-borne (miliary) spread of the organisms.

- **Spleen**: Miliary Tuberculosis - 21.95. Multiple pale nodules throughout the spleen.

- **Millet seed**: Milium - R65.033 & 90.1. Miliary TB derives from the observation that the granulomas form structures so large that they are visible to the naked eye and mimic the appearance of millet seed.

- **Lymph nodes**: Tuberculosis - 28.267. Extensive destruction and enlargement of lymph nodes by tuberculosis. The friable necrotic ("caseous") material has been partially lost from one of them. From the neck of a 20 year old woman.

- **Lung and small intestine**: post-primary TB - P61.667. Thick, fibrotic pleura with cavitating tuberculosis at the apices and extensive tuberculous bronchopneumonia. Swallowed infected sputum has resulted in spread to the Peyer's patches of the ileum. A 48 year old man had suffered night sweats and loss of appetite for 4 months with recent weight loss. Extremely ill on admission to hospital, he died 4 days later with massive lung destruction and ulceration of the larynx and small intestine.

- **Kidney**: TB - R83.1000A. A kidney with advanced caseous post-primary tuberculosis.

- **Spine**: TB - 00.164. Destruction of vertebral bodies with subsequent collapse may result in extreme angulation of the spine (a gibbus). Not only does this cause severe deformity but the spinal cord may be damaged. The arrow indicates the remains of a vertebral body. The other side shows caseation tracking posteriorly to form a 'cold abscess'.

- **Lung**: Miliary TB - 41.65. Discrete tuberculous granulomas are scattered throughout this lung. An 18 year old girl, ill for two weeks with cough and headache gradually became comatose and died. She had meningitis, miliary tuberculosis and a caseating lesion in the other lung, which was the source of her widespread disease.

- **Lymph nodes**: Tuberculosis - S56.3743. Enlarged nodes, present about nine months in the neck of an 18 year old girl, contain irregular areas of tuberculous ("caseous") necrosis. No other evidence of infection was found. Where might the primary site have been?

- **Lung**: Post-primary TB - R64.310. From a 53 year old man with cough and weight loss. There is cavitating apical tuberculosis of both lungs and tuberculous bronchopneumonia. The pleura shows fibrotic thickening.

- **Kidney**: TB - P64.706. A kidney almost destroyed by advanced caseous post primary tuberculosis.

- **Spine**: TB - 45.293. There is destruction of an intervertebral disc and bilateral extension into the psoas sheath. Downward spread within the latter may result in a 'cold abscess' in the inguinal region.
Independent work: Use the institutional registration* to look at the e-pathpots cases relevant to this session (or self-register if you wish)

• User name: CAMPATH
• Password: tenniscourtroad

* By kind agreement of Dr Jeremy Rashbass, National Lead for Cancer Registration.
Unknown slide reporting tips

Looking at:

- Start at low power (possibly even naked eye) and look at the distribution of abnormality
- Work through the expected structures – is anything missing or is there something additional which does not belong there?

Writing about:

- “Say what you see”
- Illustrations should be summary maps not artistic creations which belong in the Tate
- Describe and then make a definite conclusion
- Use common sense
1:1 teaching – review your report with a demonstrator

The ability to describe accurately and succinctly what you see is a critical skill for any medic, vet or scientist (and many other situations as well).

Looking at and reporting these sections is a practical exercise allowing you to develop this skill-set.

And if you are lucky enough to be a pathologist then you can do it every day until retirement.
Unknown case for report writing

Review this section of spleen (55.24) from a post mortem on a 76 year old lady who presented with “PUO” (Pyrexia of Unknown Origin)
Personal Review

Write down 5 sentences summarising what you have learned in this practical

Write down 1 question that you want to investigate further
TB infection – One Health in action

Screening and diagnosis now and then
The following material is designed to make you think and extend your knowledge around the global issues both human and animal in TB infection
Discuss with the demonstrators if you have time or come and discuss in the Moodle Fora
TB has always been challenging to culture and diagnose.

Using Guinea Pigs to culture TB
- Culture is difficult and takes many weeks
- Molecular techniques may be used, including identification of multi-drug resistant strains

Community based screening programmes were used. Queueing for screening Lung Function tests for TB identification

Image credit: CDC/ Merle J. Selin 1227

Image credit: CDC 1438
Mantoux Test
Screening tool for TB Diagnosis
Solution of Tuberculin is injected intra-dermally
Induration is measured – different degrees of reaction are of significance in different situations.
Interested to learn more?
IFN-g release assays also used for screening.
Interested to learn more?
Image credit: Greg Knobloch 6806

Many different methods have been tried in developing countries for identifying cases.
Spit station for screening in Kyrgyzstan with staff member in full PPE (Personal Protective Equipment) awaiting sample
Image credit: CDC/ Dilyara Nabirova, Kyrgyzstan 19651
Whilst treating a multi-drug resistant TB patient in India, prevention of infection of others is critical.
- Appropriate Personal Protective Equipment (PPE) is required
- Note that the patient has a mask on also

One method of disease prevention is BCG vaccination
Bacillus Calmette-Guerin, derived from a weakened M. Bovis
Delivered intra-dermally
Efficacy levels vary in different populations

Interested to learn more?

Image credit: CDC/ Tony Fredrick, India. 19724

Image credit: CDC/ Donald Kopanoff 3752
Badgers, cattle, raw milk and man
Raw Milk

Unpasteurised milk

1935 – Pasteurisation of milk begins in England with reduction of transmission of Bovine TB to humans

Raw milk sales in Scotland banned since 1983 due to spate of deaths

Legal in England and Wales but only sold direct to consumer and not to shops

Must have a warning label "this product has not been heat-treated and may contain organisms harmful to health"

Health agencies advise against consumption due to risks from infectious agents

Advocates claim health benefits, improved nutritional value and immune system benefits
As well as screening human populations, veterinary screening techniques are important. Veterinary Surgeon (1932) examining Cow’s udder to look for evidence of Tuberculous mastitis.

Image credit: CDC/ Minnesota Department of Health, R.N. Barr Library; Librarians Melissa Rethlefsen and Marie Jones. 8554

Normal udder on left; Tuberculous udder on right with arrows highlighting caseating material

Image credit: CDC/ Minnesota Department of Health, R.N. Barr Library; Librarians Melissa Rethlefsen and Marie Jones 8550
Spread of Bovine TB 1986 vs 2009

Badger Numbers
- ↑24% in badger social groups to around 50,000.
- ↑43% badger setts to approximately 248,000.
- ↑77% number of badgers to between 300,000 and 400,000.

Randomised Badger Culling Trial:
- Suspended due to 27%↑ in bovine TB outbreaks compared to areas without culling.
- Preliminary Analysis: proactive culling of most badgers in an area ↓19% bovine TB within cull area, but ↑29% up to 2 km outside cull area.
- Apparently contradictory findings explained in follow up study which showed culling of badgers disrupts territorial behaviour with badgers roaming further afield and different groups of badgers mixing, resulting in an increased spread of the disease.

2010 Coalition Government
A culling strategy should be a. Sustained (at least annual), undertaken on a regular basis over a period of at least 4 years in order to achieve low local badger populations in high TB incidence areas; b. Over a large area (the evidence suggests a minimum area of 150 km2); c. Conducted where land access is over 70% of the area; d. Effective and humane and conducted by competent operators; and e. Where possible, conducted in areas with boundaries or buffers (such as motorways, conurbations, coast, and substantial rivers) around the culled area to mitigate any risks from the perturbation effect.

Final report 2008:
The overall benefits of proactive culling were modest. Given its high costs and low benefits, badger culling is unlikely to contribute usefully to the control of cattle TB in Britain, and recommend that TB control efforts focus on measures other than badger culling.
Man is a danger to dogs?

Case reports and rare series suggest that MTB can spread from human owners to dogs.

How could you design a study to prove or disprove this theory?
Questions to consider (1) – join the discussion in the Moodle Fora

What makes Mycobacteria different to other bacteria?

Would you advise the Government to support a badger cull?

What other diseases would fit within the “One Health” umbrella?

Why should we consider diseases of humans and animals under “One Health”?
Questions to consider (2) – join the discussion in the Moodle fora

Why is TB a longstanding worldwide problem?

Why was there an upsurge in cases from the 1980’s?

How might we diagnose TB infection?

Can you suggest how we could protect Health Care workers and family members of the patient from contracting infection?

What particular problems are we facing in the fight against TB?
As part of the new CUMEN initiative, we will be running an interactive case next week on the discussion forum on moodle.

The clinical team have prepared a case study related to learning in this practical – this will take you through presentation, diagnosis and treatment over a series of stages.

It is an interactive and iterative learning process so be prepared to comment at each stage of the case – the case will not progress without comments.

It will be a good opportunity to reinforce your understanding of this topic and will be an introduction to the teaching approach that we use for clinical pathology.
Appendix 1
Immune cell appearances

• Acute Inflammatory Cells
  • Neutrophil
  • Eosinophil
  • Mast Cell

• Chronic Inflammatory Cells
  • Macrophage
  • Lymphocyte
  • Plasma cell

Neutrophil
  • Multilobed nucleus and pink cytoplasm (does not appear strikingly granular on H&E stain)
Immune cell appearances

- Acute Inflammatory Cells
  - Neutrophil
  - **Eosinophil**
  - Mast Cell

- Chronic Inflammatory Cells
  - Macrophage
  - Lymphocyte
  - Plasma cell

Eosinophil
- Bilobed nucleus and granular bright fuscia refractile cytoplasm
- “Tomato in aviator sunglasses”
Immune cell appearances

• Acute Inflammatory Cells
  • Neutrophil
  • Eosinophil
  • Mast Cell

• Chronic Inflammatory Cells
  • Macrophage
  • Lymphocyte
  • Plasma cell

Mast Cell
• Single nucleus; metachromatic granules
• “Fried-egg”
Immune cell appearances

• Acute Inflammatory Cells
  • Neutrophil
  • Eosinophil
  • Mast Cell

• Chronic Inflammatory Cells
  • Macrophage
    • Lymphocyte
    • Plasma cell

Monocyte/Macrophage
• Abundant pale pink cytoplasm
• Monocytes – rounded, macrophages may be elongated
• “Kidney bean” shaped nucleus
Immune cell appearances

- Acute Inflammatory Cells
  - Neutrophil
  - Eosinophil
  - Mast Cell

- Chronic Inflammatory Cells
  - Macrophage
  - Lymphocyte
  - Plasma cell

Lymphocyte
- Thin rim of cytoplasm
- Large central monotonous dark nucleus
Immune cell appearances

- **Acute Inflammatory Cells**
  - Neutrophil
  - Eosinophil
  - Mast Cell

- **Chronic Inflammatory Cells**
  - Macrophage
  - Lymphocyte
  - **Plasma cell**

  **Plasma cell**
  - Round nucleus with “clock-face” clumped chromatin
  - Eccentric cytoplasm with a pale perinuclear hoff