Histology of hypersensitivity including the role in Tuberculosis

1.0. Aims

1. To learn about the four different types of hypersensitivity reaction.

2. To understand that hypersensitivity reactions are the result of the immune system causing disease.

3. To explore nasal polyps, Hashimoto’s thyroiditis, glomerulonephritis in the kidney and tuberculosis as examples of hypersensitivity reactions.

2.0. Introduction

Immune responses enable humans and animals to live in a world occupied by potentially damaging chemicals and organisms. However, sometimes the immune response itself may cause disease. This may occur in a number of ways, and we call these various reactions **hypersensitivity reactions**.

Hypersensitivity reactions can be classified on the basis of the type of immune mechanism that causes the disease (see table below):

<table>
<thead>
<tr>
<th>Hypersensitivity Type</th>
<th>Mechanism</th>
<th>Examples</th>
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</thead>
<tbody>
<tr>
<td>I Anaphylactic type</td>
<td>Allergen (antigen) binds to IgE on mast cells which causes degranulation with the release of chemotactic, vasoactive and spasmogenic mediators.</td>
<td>Nasal polyps</td>
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<td></td>
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<td>Asthma</td>
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<td>Hay fever</td>
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<tr>
<td>II Cytotoxic type</td>
<td>Antigen (endogenous or exogenous) on cell surface is bound by IgG or IgM causing cell dysfunction or cell destruction by phagocytosis or cell lysis.</td>
<td>Hashimoto’s thyroiditis</td>
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<td>Acute rheumatic fever</td>
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<tr>
<td>III Immune complex type</td>
<td>Free-floating antigen is bound by IgG, IgM and IgA forming immune complexes which deposit in various tissues and activate complement initiating an acute inflammatory reaction (local or systemic).</td>
<td>Systemic Lupus Erythematous</td>
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<td></td>
<td>Some types of glomerulonephritis</td>
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<tr>
<td>IV Delayed type (or Cell-mediated type)</td>
<td>Antigen is presented by antigen presenting cells and recognised by sensitised T-cells which recruit macrophages which may form granulomas (in the case of CD4+ T-cells) or kill cells directly (in the case of CD8+ T-cells /CTLs)</td>
<td>Tuberculosis</td>
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<td>Contact dermatitis</td>
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<td>Transplant rejection</td>
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</tbody>
</table>
3.0. Type I – e.g. nasal polyp

NDP Images: 9.1 (H&E): 89.758 & 04.17C
9.2 (Toluidine blue): 89.758 & 04.17C
Glass Slides: 9.1 (H&E): 89.758 & 04.17C
9.2 (Toluidine blue): 89.758 & 04.17C
Image Map: A_NP_PQ_NO_05; A_NP_PQ_NO_06

A polyp is a growth protruding from the mucous lining of an organ such as the nose (or bladder, or intestine), often causing obstruction. Some polyps are due to hypersensitivity type I reactions such as this nasal polyp, whereas others may be inflammatory (non-hypersensitivity) in origin.

Look first at the H&E section and identify the following features:
1. respiratory epithelium (lining outside surface of polyp)
2. small blood vessels
3. oedema fluid in tissue spaces (seen as apparently empty spaces in this section).
4. inflammatory cells in the tissue spaces - plasma cells (eccentric, clock-face nuclei and dusky purple cytoplasm), eosinophils (bi-lobed nuclei and pink/red granular cytoplasm – look like a sunburnt face with sunglasses), lymphocytes (dark, single round nuclei and inconspicuous cytoplasm)

Now look at the toluidine blue section. 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.

Be sure you can answer these questions about the nasal polyp:

Q1 Where are the IgE antibodies located?

Q2 What is the likely allergen (antigen)?

Q3 How does the oedema fluid develop?

Q4 Give examples of the chemotactic and vasoactive mediators that are released?

Q5 What would you expect the symptoms to be? (On the basis of what you see, or perhaps your own experience or that of a colleague who may have Hay Fever.)

An alternative example of type I hypersensitivity is asthma – a scanned image can be viewed on the computer: Lung – bronchial asthma: 83.41. Can you see increased eosinophils in the bronchial wall with thickening of the bronchial wall?
4.0. Type II – e.g. Hashimoto’s thyroiditis

4.1. Normal thyroid – NDP Images: NE6: 58.5  
Glass Slides: NE6: 58.5
Normal thyroid is composed of groups of acini (or follicles); you can think of their appearance in three dimensions as a collection of bubbles. The follicles or acini are lined by thyroid epithelial cells and filled with colloid, a pink-staining proteinaceous material (containing thyroglobulin), from which tri-iodothyronine (T3) and thyroxine (T4) are made. The acini are packed close together and vary in size. Intensely stimulated thyroid tends not to accumulate much colloid at all and the original central spaces become a tiny proportion of the total acinus, but the epithelial cells are tall.

Glass Slides: 9.3: 58.356 & 66.117  
Image Map: A_AL_HU_TH_04; A_AL_HU_TH_05
The acini or follicles have small central lumina with little colloid and the thyroid epithelium is tall. The stroma has been colonised by very large numbers of lymphocytes and plasma cells. This is a violent auto-immune reaction. Try to answer the following from the examination of the section:

Q6  Is there evidence of tissue destruction? Is it a cytotoxic process?

Q7  What type of T lymphocytes would you expect to be involved?

Q8  Is the lymphoid infiltrate organised into lymphoid follicles with germinal centres?

Q9  What is the mature cell produced by lymphoid follicles with germinal centres? Can you see many examples of this mature cell in the section? What is its effect on thyroid epithelium?

Q10 What is likely to be the effect of these changes on plasma levels of thyroxine and tri-iodothyronine? Why do the remaining thyroid acini (or thyroid follicles) have an “over-stimulated” appearance with little colloid and tall epithelium?

5.0. Type III – e.g. Systemic Lupus Erythematosus – NDP Image: 49.464
The scanned images shows kidney glomerulonephritis: Can you see changes (increased cells) in the glomeruli? What are these extra cells? (This is very difficult to see – ask a demonstrator for help)

Think about the following questions regarding type III hypersensitivity in relation to Systemic Lupus Erythematosus (SLE), a disease process in which auto-antibodies are made against DNA or other chromatin components. SLE patients often have skin rashes due to inflammation of blood vessels (vasculitis) in the
skin, kidney disease due to inflammation of the glomeruli (glomerulonephritis),
and joint disease due to inflammation of the synovium (arthritis). Type III
hypersensitivity is caused by immune complexes being deposited.

**Q11** What makes up an ‘immune complex’?

**Q12** Where (in which structures) are immune complexes deposited?

**Q13** How do deposited immune complexes trigger an inflammatory response?

### 6.0. Type IV – e.g. Tuberculosis

An important cause of granulomatous inflammation is infection by *Mycobacteria*. These long-lived bacteria proliferate slowly but are protected by their thick, waxy outer coat. They initiate a type IV delayed type hypersensitivity 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.

**Initial (primary)** infection is usually in the lungs, (but it may be in tonsil or Peyer’s patches of the small intestine with other types of mycobacteria). Alveolar macrophages ingest the bacteria and some enter the interstitium to be carried by the lymph to the draining lymph nodes, usually at the hilum. In the lymph nodes the bacterial antigens are presented, generating a cell mediated immune response dominated by CD4+ T lymphocytes known as Th1 cells. These Th1 cells release cytokines, including IFN-γ, that can recruit and activate macrophages.

Initially, the response is at **two separate sites**; one is the site of entry, usually the periphery of the lung (which is called the **Ghon focus**) and the other is within the draining lymph nodes. This pattern is known as the **primary complex** (Ghon focus + local lymph node involvement).

In the lymph node, the response is dominated by **Th1/macrophage** co-operation (leading to macrophages aggregating to form granulomas) and as soon as any T cells, which have been sensitized in the lymph node, reach the Ghon focus via the bloodstream, granulomas develop there as well. The macrophages in granulomas usually become activated (enlarged with much pink cytoplasm – called “epithelioid” macrophages because they resemble epithelial cells in the large amount of cytoplasm they have), allowing them to secrete more enzymes with greater killing ability. Some macrophages fuse together to form “giant cells” with many nuclei (often forming a ring or U shape in one large cytoplasmic mass) – sometimes called a ‘Langhans’ cell. In most individuals the bacteria are eventually killed and both sites of the primary complex heal by fibrosis, often followed by calcification.

However, sometimes, especially in children, the infection **persists**; more and more macrophages accumulate and die. These macrophages release enzymes.
There is formation of soft dead material resembling *cream cheese* (caseous necrosis), due to destruction of normal tissues by released enzymes. At the same time, the mechanisms of healing are activated and fibroblasts lay down collagen. This state of 'cheesy' tissue destruction surrounded by attempted healing is *fibro-caseous tuberculosis*.

Hilar lymph nodes may enlarge and the destructive process may reach a thin walled pulmonary vein, which allows the soft crumbly material with clumps of infected macrophages to enter the blood and be distributed around the whole body. Where each tiny clump lodges, the same process is repeated. The lymphocytes and macrophages gather, producing granulomata, which reach a size where they are visible to the naked eye.

This blood spread allows infection to reach the meninges and granulomatous inflammation spreads over the surface of the brain (*tuberculous meningitis*). Even with treatment, the patient is likely to die at this stage, and in ancient times, physicians saw tiny white round bodies looking like millet seed (milium) distributed throughout the body after death. This appearance has given rise to the term *miliary tuberculosis*.

Other outcomes than *recovery or early death* occur. Sometimes a chronic state is established, because of inability of the immune system to eradicate the bacilli. *Mycobacterium tuberculosis* itself is not very destructive and yet terrible tissue destruction takes place as the body’s own cells are recruited and themselves die. This process represents *Type IV hypersensitivity*.

6.1. Chronic Tuberculosis (Post-primary TB or Secondary TB)

NDP Images: 9.4: 73.1120; 66.0255 & 80.0281
Glass Slides: 9.4: 73.1120.
Image Map: A_TB_GN_LN_04

Chronic tuberculosis most often occurs in the lungs and it is difficult to know if it is *persistent* from the first infection (perhaps even being *reactivated* many years later) or whether there has been true *re-infection*. Chronic infection also occurs at other sites as a result of blood spread, which lead not to miliary tuberculosis but to persistence in certain favourable environments.

Since many years may elapse before infection at these distant sites is recognized, it suggests that sometimes mycobacteria may be held in check and when the immune system becomes less effective, (due to age, immunosuppression, malnutrition, other disease, etc), the disease progresses once again.

The response to *M. tuberculosis* illustrates how the immune system can *modify the course of an infectious disease*. On *first exposure*, there is *rapid spread* from the portal of entry to the lymph nodes. On *re-exposure*, the circulating long-lived lymphocytes with "memory" of the antigens immediately
recruit other lymphocytes to immobilise and stimulate macrophages with ingested organisms. This means that re-infection may be **localised to the site of entry**.

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.

Note granulomata at different stages, some with and some without central necrosis. As tuberculous granulomata enlarge they coalesce, giving rise to larger areas of necrosis. The bacteria are not visible with this stain.

6.2. Post-Primary Tuberculosis –
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

These are large sections, to be shared. Please be careful not to break them.
In adults, the commonest form of secondary (or post-primary) infection by M. tuberculosis affects the apex of both lungs. In otherwise healthy individuals, this usually heals, but in the debilitated or malnourished, the disease may spread.

6.2.1. Lung: fibro-caseous TB – NDP Images: 60.1158 & 50.0470
6.2.2. Lung: TB bronchopneumonia – NDP Image: 57.0350
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. The cavitation is due to destruction of lung tissue with erosion of a bronchus. Infected necrotic tissue had been coughed up by the patient, with the obvious risk of infecting others and left this cavity in the lung.

Q14 What makes mycobacteria different from other bacteria?

Q15 In a case of lung infection by TB, where are the organism’s antigens first presented to the immune system? How do the antigens get there?

Q16 What sort of T cells are involved in the response?

Q17 Why are HIV infected patients particularly susceptible to mycobacterial infection?

Q18 What is a granuloma?

Q19 What causes the tissue damage (caseation) in TB? Is it the virulence of the organism or the host immune response?

6.3. Miliary Tuberculosis (H&E) – NDP Images: 9.7: 55:274
Glass Slide: 9.7: 55:274
Image Map: A_TB_ML_LU_04
From an eighteen-month old boy who died of miliary tuberculosis and subsequent tuberculous meningitis. The basic structure of the granulomata resulting from blood spread to the lung, is exactly like those you have seen in the lymph node, but they are widely separated from each other.

6.3.1. Miliary tuberculosis (Ziehl-Neelsen)  
NDP Images: 9.8: 68.808  
Glass Slide: 9.8: 68.808
From an immune-suppressed adult, who had received immune suppressing drugs following a renal transplant. A few of the granulomas contain large numbers of red-staining M. tuberculosis, visible with the x 40 (high dry) objectives. (There may be only one granuloma near the edge of your section containing numerous bacilli; ask a demonstrator if you cannot find any).
7.0. Demonstration museum specimens

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.

Museum Specimens

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: miliary - 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: Primary complex with miliary spread - 45.249**
From an 8 year old boy who died of miliary tuberculosis and meningitis. There is a peripheral yellow nodule (Ghon focus - the site of primary infection) in the inferior part of the lower lobe and enlarged hilar lymph nodes, which together constitute the primary complex. Scattered miliary lesions are present, most numerous beneath the pleura.

**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: TB - 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.
8.0. Process identification and report writing

**Spleen: Unknown — NDP Image: 9.9: 55.274**
Glass Slide: 9.9: 55.274

This is a block of spleen from a severely ill old person. Please draw a diagram, write a description and provide an interpretation with identification of the pathological process.

9.0. Some words used

<table>
<thead>
<tr>
<th>Granulum (Latin)</th>
<th>granule</th>
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</tr>
</thead>
<tbody>
<tr>
<td>- oma (Greek)</td>
<td>swelling</td>
<td>granuloma</td>
</tr>
<tr>
<td>Tuber (Latin)</td>
<td>swelling</td>
<td>tubercle, tuberculosi</td>
</tr>
<tr>
<td>Milium (Latin)</td>
<td>millet-seed</td>
<td>miliary</td>
</tr>
<tr>
<td>Caseus (Latin)</td>
<td>cheese</td>
<td>cheesy</td>
</tr>
</tbody>
</table>

*Please make sure the desktop is switched to Pathology Pt1B folder on the PC.*
*Dim and switch off your microscope light.*
*Return the wooden block, if used.*
*Cover the microscope.*
*Push your stool under the bench.*

*Thank you!*