Lymphoid System: cells of the immune system

Answer Sheet

Q1 Which areas of the lymph node have most CD3 staining?

A1 Most CD3 staining is present in the paracortex (T cell areas). This is towards the outside of the lymph node, between the lymphoid follicles.

Q2 Are there any CD3+ cells in the germinal centres? If so, why are these CD3+ cells present here?

A2 Occasional CD3+ T lymphocytes are present within germinal centres. These T lymphocytes are interacting with B lymphocytes, to provide the “help” necessary for the B lymphocytes to become activated, proliferate and switch the class of antibody that they secrete.

Q3 At high power, which parts of the cells seem to express CD3 (nucleus or cytoplasm/membrane)?

A3 CD3 is present on the plasma membrane. However, with the resolution of light microscopy, it is only possible to say that immunostaining for CD3 cannot be seen in the nucleus.

Q4 Which areas of the lymph node have most CD20 immunostaining?

A4 CD20 immunostaining of B cells is seen in lymphoid follicles / germinal centres.

Q5 Where do you see CD68 positive cells in the lymph node?

A5 CD68+ cells are present at the centres of lymphoid follicles, these are macrophages. There are scattered CD68+ cells throughout the paracortex (T cell areas) and the sinuses of the lymph node, these are dendritic cells.

Q6 Associated with B cell areas, specialised macrophages are present. Where can you see these?

A6 CD68+ macrophages are present in the germinal centres of lymphoid follicles.

Q7 What is the main function of these specialised macrophages?

A7 These macrophages phagocytose dying (apoptotic) B lymphocytes that have not survived the process of affinity maturation (somatic hypermutation followed by selection for B lymphocytes with high affinity immunoglobulin receptors for antigen).

Q8 In T cell areas, CD68+ cells are present. What are these likely to be? What is their main function?

A8 These are dendritic cells, a specialised type of antigen presenting cells, able to activate naïve T lymphocytes (i.e. T lymphocytes which have never previously responded to antigen).

Q9 At high power, can you see any differences in morphology (shape) between the CD68+ cells in T cell areas and those elsewhere in the lymph node? Explain any differences you see?

A9 In the paracortex, CD68+ cells have long dendritic (branch-like) processes, which maximise their surface area for contact with T lymphocytes. The CD68+ cells at the centres of lymphoid follicles are round or oval, without dendritic processes. These macrophages are specialised for phagocytosis and do not need to maximise their surface area.

Q10 Some CD68+ cells are present in the sinuses of the lymph node. Where have these come from and what is their function?

A10 The CD68+ cells in the lymph node sinuses have come from peripheral tissues or other lymph nodes in a chain of nodes. They are in the process of entering the lymph node from peripheral tissues and are bringing antigen into the lymph node in order to present it to T lymphocytes.

Q11 What process takes place in the cortex? Which immunostain supports this?

A11 Positive selection (mediated mainly by thymic epithelium identified by the cytokeratin immunostain) is believed to be the main process occurring in the cortex. Although thymic epithelium is present both in the cortex and in the medulla, it is believed to carry out its main function, that of positive selection, in the cortex.

Q12 What process takes place in the medulla? Which immunostain supports this?

A12 Negative selection (mediated mainly by dendritic cells) is believed to be the main process occurring in the medulla. The dendritic cells stain positively for CD68+ and the majority of these cells are present in the medulla, where they are thought to mediate negative selection (some can be seen in the cortex as well). CD3+ cells are present in both the cortex and medulla, as CD3 and the T cell receptor are

expressed throughout the processes of positive and negative selection. This is because T cells are selected on the basis of the affinity of their T cell receptor and CD3 helps carry signals from the T cell receptor into the T lymphocyte.

Q13 What are the different functions of mature CD4+ T cells and CD8+ T cells? How could you distinguish between them in tissues?

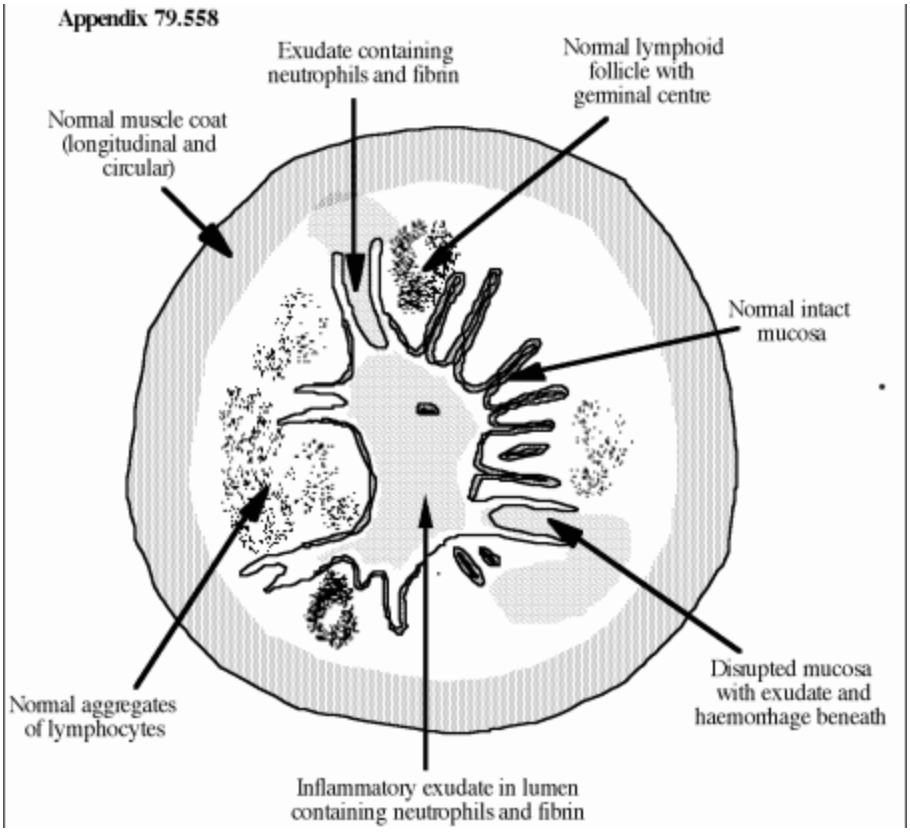
A13 CD3+ mature T cells can be classified into CD4+ (helper) T cells and CD8+ (cytotoxic) T cells by immunostaining with antibodies against CD4 & CD8.

Appendix: abnormal - 79.558

This slide shows a transverse section of a vermiform appendix, which is much larger than normal. The lumen contains a fibrino-purulent exudate, containing many neutrophils, which appear to stream from ulcerated areas in the lining mucosa. Neutrophils infiltrate the submucosa, spreading into the muscle where fluid exudate separates individual muscle fibres. Dilated blood vessels surrounded by inflammatory cells are seen under the serosa.

Image Map: A_AI_AC_AP_30

The pathological process is acute inflammation of the appendix or acute appendicitis.



Acute Inflammation

Q14 What are the other cell types?

A14 A. Basophils, B. Neutrophils, C. Eosinophils, D. Lymphocytes, E. Monocytes, F. Red Blood Cells. You may also identify small fragments amongst the red blood cells which are the platelets.

Q15 Where in the body are neutrophils formed? What route did the neutrophils take to reach the lumen of the bronchus?

A15 Neutrophils are formed in the bone marrow. Some reach the lumen of the bronchus via the blood vessels, mostly post-capilliary venules, in the sub-mucosal zone of the bronchial wall. Others have migrated from the capillaries of the alveolar walls into the alveoli, forming the pneumonic neutrophilic exudate, and a small proportion of these may be coughed up or forced upwards into the bronchus.

Q16 How long do leukocytes remain in the blood? What happens to them eventually?

A16 The time for which the main leukocytes involved in acute inflammation remain in the blood depends on the cell type. Broadly the times are as follows:

B. Monocytes: approx. 1 day half-life in the circulation (long-lived as tissues, with a half-life measured in months).

Most leukocytes are recruited to sites of injury or where there is a stimulus produced by an immune response to an exogenous agent such as micro-organisms, parasites, or allergens such as pollen. There they are involved in inflammatory reactions by phagocytosing micro-organisms and killing them. After exhaustion of their supply of killing enzymes and membrane (for phagocytosis), they die mostly by necrosis and some by apoptosis (unused neutrophils also die by apoptosis) and are phagocytosed by macrophages. Macrophages (derived from the circulating monocytes) may re-enter the circulation, possible via lymphatics. Thereafter, they would be removed along with other effete (i.e. old, worn out) or damaged blood cells by phagocytic cells in liver and spleen.

Q17 What would their future have been, had the tissue not been harvested?

A17 apoptosis (see (16) above).

Q18 Are there deleterious as well as beneficial consequences of this degree of neutrophil extravasation and activation?

A18 Neutrophil activation results in the production and release of reactive oxygen species (ROS) and catabolic enzymes, the evolved purpose of which is to inactivate micro-organisms. These substances may, however, also damage normal tissue - a so-called bystander effect.

Q19 How do you suppose a viral infection (such as influenza) of the bronchial epithelium may increase susceptibility to bacterial bronchopneumonia?

A19 The viral infection of the bronchial epithelium will have led to epithelial cell injury and death, allowing entry of bacteria into the bronchial walls and alveolar walls, causing the bacterial bronchopneumonia.

(1) Destruction of bronchial mucosal epithelium.

(2) Further destruction of bronchial mucosal epithelium, with release of bacterial toxins; production of mediators of inflammation, e.g. histamine.

(3) Fibrinous exudate and haemorrhage, resulting from vasodilatation & increased permeability of alveolar capillaries, enter the alveoli, bronchioles and bronchi [**vascular response**].

(4) Leukocytes, largely neutrophils, enter alveoli and bronchioles [**cellular response**].

(5) Exudate (neutrophils & fibrin) in alveoli impairs gas exchange.

(6) Cytokines, IL-1, IL-6, TNF-α, IFN-γ are released by leukocytes, circulate and trigger the hypothalamus to reset the body thermostat

(7) Reduced blood oxygen and increased blood carbon dioxide concentrations stimulate respiratory centres. **Chronic inflammation and repair – Answers**

Chronic Inflammation

Q20 What is the special name given to this combination of fibroblast cells and new blood vessels?

A20 Granulation tissue.

Q21 What would be the effect on lung function of permanent thickening of the alveolar walls by fibrous tissue?

A21 Reduced efficiency of gaseous exchange due to thickening by fibrosis, which may lead to breathlessness and respiratory failure in severely affected cases.

Q22 What is the central cell co-ordinating the process of organisation of angiogenesis with fibrosis?

A22 The macrophage.

Q23 Where is the repair taking place? What cells types are involved in REPAIR?

A23 Repair is taking place in the dermis (under and around the wound closure site) with endothelial cells forming new capillaries and fibroblasts secreting collagenous fibrous tissue.

Q24 Where is the regeneration taking place? What cells types are involved in regeneration?

A24 Regeneration is taking place at the epithelial surface by the squamous epithelial cells, which are proliferating to replace the missing epithelial cells at the site of the wound closure.

Q25 What is the origin of plasma cells and what is their function? What are the other main cell types involved in chronic inflammation?

A25 Plasma cells are mature B lymphocytes emerging from the germinal centres of lymphoid tissue. Each plasma cell makes one specific antibody (in large amounts).The other cell types in chronic inflammation are macrophages, lymphocytes and occasional eosinophils.

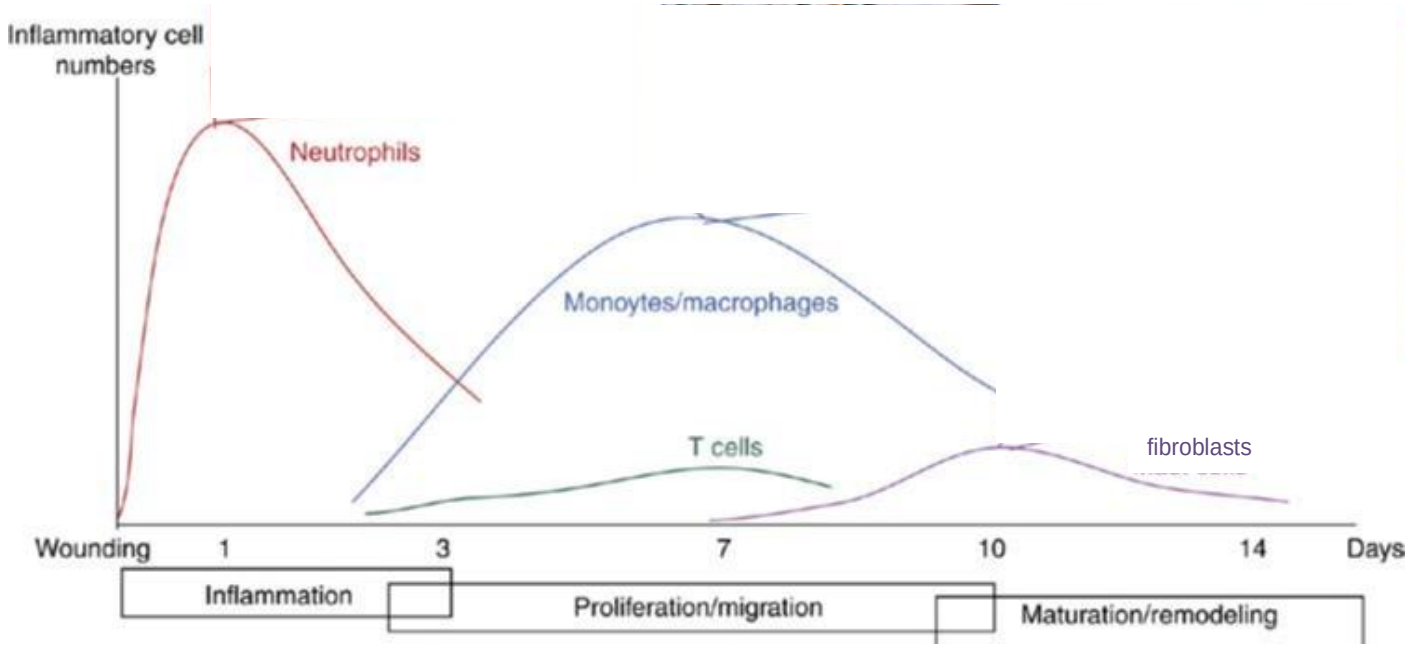
Q26 What is granulation tissue? What are the two main cell types present?

A26 The two cell types seen in granulation tissue are capillaries (endothelium) and fibroblasts. Granulation tissue makes fibrous tissue (mostly collagen fibres).

Q27 What chemical mediators trigger the onset of the repair process? Which cell secretes these mediators?

A27 Macrophages are the central cell recruiting endothelia with FGF & VEGF, and recruiting and stimulating fibroblasts with FGF & TGF-beta

Q28 Draw a graph showing the numbers of neutrophils, macrophages, lymphocytes, plasma cells and fibroblasts as time passes in a focus of tissue injury. What factors contribute to the changes in cellular composition that your graph shows?



Q29 What is the difference between repair and regeneration?

A29 Repair involves synthesis of fibrous tissue (to fill the space left by injured tissue) by granulation tissue. Regeneration involves replacement of lost cells by the same cell type (e.g. epithelial cells proliferate and migrate across a wound to fill the space and recreate the epithelial surface as it was before).

Q30 Which cells of chronic inflammation and/or repair are most prevalent in this case?

A30 Plasma cells are the most prevalent cells with only small numbers of macrophages and lymphoid cells and some background granulation tissue endothelial cells and fibroblasts.