Problem solving exercise – atherosclerosis and a pig problem

Answer Sheet

Part I:
A typical **intimal fibrofatty atherosclerotic plaque**, demonstrates a fibrocellular cap and a lipid core (containing macrophages and an extracellular lipid pool with cholesterol crystals). There is movement of circulating monocytes into the tunica intima to form tissue macrophages, where the macrophages phagocytose LDL and oxidised LDL to form foam cells. Smooth muscle cells migrate from the tunica media into the tunica intima, proliferate, change phenotype to secretory myofibroblasts, and secrete extracellular matrix including fibrous tissue. Atherosclerosis is primarily an intimal process affecting large-medium sized muscular arteries (e.g. coronary & carotid arteries) & large elastic arteries (e.g. aorta), although there may be thinning of the tunica media underneath a plaque.

QA At the earliest stages of plaque development, what influences lipid circulating in the blood, in the form of Low Density Lipoprotein (LDL), to enter the tunica intima of the artery wall?

A  The **response to injury hypothesis** postulates repeated injury to endothelium caused by the four “major” risk factors: smoking, hypertension, diabetes, hyperlipidaemia (high LDL & low HDL). Endothelial dysfunction following injury, plays a crucial, initiating role in plaque formation. This allows lipids to pass more readily from the bloodstream into the tunica intima as a consequence of the combination of hyperlipidaemia and endothelial dysfunction/injury.

QB What stimulates blood monocytes to enter the arterial wall (becoming tissue macrophages) at the same site as the LDL?

B  Endothelial dysfunction/injury also triggers increased adherence of monocytes and platelets. Macrophages enter the arterial wall at this site. Both macrophages and platelets, when activated can release cytokines.

QC If the macrophages can phagocytose LDL forming “foam cells” (containing intracellular lipid), how does the extracellular lipid pool form and grow larger? (seen as cholesterol crystals and lipid gruel in your section).

C  Macrophages within the intimal plaque imbibe lipid to become “foam cells”. If the lipid remains intracellular at this early stage then only “fatty streaks or dots” are seen (usually in children/young adults). A causal relationship between atherosclerosis and fatty streaks/dots in children is suspected but not proved. Native and oxidised LDL can activate macrophages. Macrophage activation by the LDL, leads to free radical (ROS) production. The ROS oxidise LDL. However, oxidised LDL is toxic to macrophages inducing death by both necrosis and apoptosis, causing release of lipid to form extracellular lipid pools with necrotic cell debris (“gruel”). Positive feedback occurs and there is a process with features of chronic inflammation in the vessel wall.

QD What triggers the smooth muscle cells in the tunica media to migrate to the tunica intima, proliferate and secrete extracellular matrix?

D  Macrophages become activated by oxidised LDL and secrete factors (as well as platelet release of PDGF) that encourage the recruitment of smooth muscle cells from the tunica media into the tunica intima, which proliferate and secrete
collagen and extracellular matrix; macrophages also secrete enzymes & cytokines, etc that propagate injury.

QE Can you assign the four major risk factors (smoking, hypertension, diabetes mellitus & hyperlipidaemia) to pathogenic events in atherosclerosis?

E All 4 “major” risk factors: smoking, hypertension, diabetes, hyperlipidaemia (high LDL & low HDL), can cause endothelial dysfunction and injury, the key initiating role in plaque formation. The “major” risk factors: diabetes & hyperlipidaemia can alter blood lipid levels, in particular by increasing LDL & reducing HDL levels. Entry of LDL (resulting from high circulating levels of LDL) into the arterial wall at a site of endothelial dysfunction / injury is the important early event in atherosclerotic plaque formation.

QF What makes an atherosclerotic plaque rupture? Why is this an important event?

F Rupture or ulceration of a plaque, by definition, implies loss of the overlying endothelium. Rupture of an atheromatous plaque is usually associated with a breach of the fibrocellular cap and, hence, exposure of connective tissue collagen and the lipid core to the bloodstream. The latter is important as it exposes additional thrombogenic substances to the blood and also allows for discharge of debris (release of cholesterol emboli) into the bloodstream. Plaque rupture is probably caused by a combination of several factors. The overlying endothelium and fibrocellular cap may be mechanically weakened externally by turbulent blood flow (generated by partial occlusion of the blood vessel by neighbouring plaques and the plaque itself) and/or internally by a haematoma (from e.g. the thin walled capillaries vascularising the plaque) growing within the plaque. Calcification of the fibrocellular cap reduces its mechanical compliance and further predisposes to rupture. It is suggested that proteolytic enzymes released by ‘intraplaque’ macrophages and other leucocytes may disrupt and weaken the fibrocellular cap. Finally, while endothelial injury may be the initiating step of atherosclerosis, exposure to oxidised LDLs, cytokines and proteolytic enzymes generated within the plaque may further damage and disrupt the overlying endothelium and cap.

This is an important event because plaque rupture leads to thrombosis! Rupture of a plaque exposes subendothelial tissue and the lipid core and hence, as mentioned above, thrombogenic substances to the bloodstream (eg. von Willebrand’s factor, tissue factor, collagen). Thrombosis of a coronary or carotid arterial plaque has obvious potentially fatal consequences!

Part II

Q1 Describe these appearances, using accurate, professionally appropriate terminology, and interpret the relationship between A and B.

1 Figure A shows large, crumbling, thrombotic vegetations destroying the aortic valve.

Q2 What is the relationship between the bacteriological and histological findings?

2 Figure B shows a slice of lung with prominent pulmonary oedema, appearing as a frothy fluid, filling the airspaces. The pulmonary oedema is a result of left heart failure, caused by aortic incompetence. The positive culture implies the
existence of on-going, chronic bacterial infection. This is producing tissue destruction, with resulting thrombosis and organisation within the valve.

Q3 What would you expect to see on higher power examination of the regions marked 3.1, 3.2 and 3.3? (Explain why).

3 At 3.1, the densely haematoxyphilic aggregates represent colonies of bacteria. In 3.2, the endocardium is replaced by alternating layers of platelets and fibrin, indicating the deposition of thrombus from rapidly flowing blood. At 3.3, higher power shows proliferating fibroblasts and macrophages, probably with intervening neutrophil polymorphs: i.e. the components of granulation tissue. This is the beginnings of a repair reaction, underlying the more acute, superficial reactions of tissue destruction and thrombosis.

Q4 In a flow diagram, show the relationships between as many of the features in the story as possible. How might the postmortem appearance in small intestine (Figure E) and myocardium (Figure F) fit into this diagram?

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Overcrowding

Aggressive behaviour ear-biting

Local infection

Transient septicaemia

Aortic valve colonised

Valve destruction

Thrombotic vegetations

Infected thrombotic emboli

Infectious microthrombi + thrombosis

Widespread microthrombi + haemolysis

Anaemia

Reticulocytosis

Multiple systemic embolic thrombi e.g. Kidney, gut (Fig E)

Pyaemia e.g. Heart (Fig F)

Infarction
Reticulocytosis* = finding of reticulocytes (an immature form of red blood cells) on the blood film. This indicates increased haematopoeisis with increased formation of both fully formed red blood cells and the immature forms (reticulocytes) which are both released into the blood.

**Q5** What would his advice about butchering have been?

5. Because of the pyaemia (globules of pus in the blood), this pig’s tissues are infected and unsuitable for sale.

**Q6** Explain why these animals should be susceptible, and why the right side of the heart should be particularly vulnerable? What sort of organisms might be cultured on this occasion? This condition is difficult to treat, even in human beings. Can you suggest why?

6. The source of infection in the puerperal cow is usually faecal organisms from the immediate environment transmitted into the vagina. Infection is at a high level and colonises the first valves it comes to ie the tricuspid. In the pig, by contrast, colonisation by small blood-borne inocula of *Erisipelothrix* favours the aortic valve: the infection takes hold in the aortic valve because it is prone to damage through minor trauma sustained during opening and closure in high pressure gradients. A right-sided bias similar to the bovine endocarditis, and for the same reason, is found in intravenous drug users employing non-sterile inocula. In dogs, the condition is not common but when it does occur is found most frequently on the left atrio-ventricular valves. The condition is difficult to treat because the infection occurs in relatively avascular tissues (the heart valves) and is overlain by thrombus. Penetration of antibiotics is therefore relatively poor and liable to be delayed until after permanent damage to the valves has occurred. The patient is then vulnerable to additional pathology such as embolism and infarction.