BSc Degree Examinations 2018-9

Department: BIOLOGY

Title of Exam: Immunology and Infection

Time Allowed: 2.5hrs

Marking Scheme:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper

Instructions:
Answer all questions in the spaces provided on the examination paper

Materials Supplied: Calculator

For marker use only:  

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DO NOT WRITE ON THIS BOOKLET BEFORE THE EXAM BEGINS
DO NOT TURN OVER THIS PAGE UNTIL INSTRUCTED TO DO SO BY AN INVIGILATOR
1) a). In an infection experiment you discover that mice with a specific mutation display significantly lower levels of perforin and granzyme following infection with a virus. Which cells of the innate or the adaptive immune system that are the main producers of these factors might be defective and responsible for this phenotype?  

**Answer:**  
Natural killer cells (innate) or Cytotoxic CD8+ T cells (adaptive) (Cytotoxic T lymphocytes also acceptable) - (2 marks).

**LOs**  
Articulate subject-specific terminology correctly.  
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes.

Most students gave the correct answer. Most common error was to include neutrophils instead of NK cells.

b) Which other key inflammatory cytokine is secreted by both of these cell types?  

**Answer:**  
Both cell types secrete IFNgamma (1 mark).  
Note that as perforin and granzyme are conceptually linked to IFNg (students hear these in the same context), even if a student doesn't remember the names of the cell types in A, they can still answer this question.

**LOs**  
Articulate subject-specific terminology correctly.  
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes.

Most students gave the correct answer.

c) What is the fundamental difference between these two cell types? (2 marks)

**Answer:**  
Natural killer cells have no antigen-specific receptors and do not clonally expand
upon stimulation, CTLs do have a T cell receptor specific for antigen and clonally expand upon stimulation (2 marks).

Note that there are other differences (e.g. which cytokines activate each cell type) but the question is looking for the ability to identify the fundamental difference between the two cell types.

Note that students who do not answer A can still get at least one mark (clonal expansion; as we are comparing innate vs adaptive).

LOs
Articulate subject-specific terminology correctly.
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes.
Employ critical analytical skills to interrogate host-microbial immune responses.

Generally well addressed. Mentioning TCR was essential for full marks. Students who answered that “CTLs are cells of the adaptive system and NK cells are cells of the innate immune system” did not get any marks as this was given in the question.

d) Propose a simple experiment that would help you identify which of the two cell types is predominantly responsible for the observed reduction in perforin and granzyme. Explain how you would interpret the experiment. (6 marks)

Answer:
Measure granzyme and perforin, or IFNgamma (all acceptable, at least one required) at early (2-4 days) and late (8-12 days) timepoints post-infection (4 marks). If the difference is observed early the effect is due to NK cells (innate cells), if the effect is only observed late then the defect is due to CTLs (adaptive cell) (2 marks).

Note that the question can be at least partly answered even if the student does not know the answers to any of the previous sub-questions, as they should spot that we are comparing innate vs adaptive.

LO
Employ critical analytical skills to interrogate host-microbial immune responses.
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes.

Overall this was well addressed with a range of answers. As this was an experimental design question, marks were given to reasonable answers even if these were different to the suggested answer above. These included direct measurement of perforin and granzyme in isolated cells, in vitro assays, knockout models (e.g. IL2, MHC, IFNa/b), reconstitution with WT cells etc. However the question asked for a “simple” experiment, so answers proposing complicated KO models did not get full marks. Issues with removing MHC or IFNa (affect both NK cells and CTLs) were also
taken into account. Note that getting methodological details wrong did not result in loss of marks, as the question was probing principles. An appreciation of the fact that NK deficiencies were likely to happen earlier was positively marked.

2) Explain how type I Interferons function in immune responses. (4 marks)

Answer:
They can inhibit pathogen replication through inhibition of protein translation in the infected cell. (1 mark)
They induce cell-death (apoptosis) of infected and neighbouring cells. (1 mark)
They activate other innate immune cells (e.g. NK cells). (1 mark)
They activate the antigen presentation machinery leading to the initiation of adaptive immunity. (1 mark)

LOs
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes.
Articulate subject-specific terminology correctly.

Performance here was not as good as expected, given that the answer to this question was clearly discussed and presented in lectures. The two most common errors were to discuss type II IFNs or neglecting to address the function of type I IFNs, which was what the question asked.

3. You construct an Escherichia coli strain to examine the expression of one of its adhesins under two different growth conditions. You determine that expression is increased when grown with lactose versus glucose as a carbon source (18 vs 4 units of expression) and increased in stationary phase versus logarithmic growth phase (24 vs 16 units).

a) If the E.coli strain is a pathogen, would this be indicative that the adhesin is a virulence factor? Justify your answer. (3 marks)

Answer should reflect an understanding of what a virulence factor is, and interpret the data in context of the growth conditions in a host. Many virulence factors are indeed upregulated in the absence of glucose. Growth phase dependent regulation is less clear. Marks will be given for a considered answer.

LOs
Articulate subject-specific terminology correctly
Identify features that facilitate microbial transmission, infection establishment and evasion of host anti-microbial response
A range of marks were obtained. The link between the host environment, the data and virulence was made to varying degrees. Points released for good reasoning.

b) Are the data provided sufficiently robust? Briefly explain your answer.  
(1 mark)  
No. a point for showing awareness of lack of information on replicates or identifying no statistics shown

A common error was to discuss whether the conclusion was robust; this was not the question.

c) Give an overview of a possible regulatory mechanism that can cause a difference in expression for one of the conditions mentioned above.  
(6 marks)
A. cAMP-CAP dependent regulation with details about how the import of glucose and/or lactose leads to regulation through a protein phosphorylation cascade. 1 pt for identifying cAMP+CAP, 2 points for linking correctly with gluc and lac concentrations, 3 pts for identifying other key features like protein phosphorylation, cascade, transport

OR
Log/stat: quorum sensing (1); in E coli through AHLs- description of type of molecule made, secreted (2), sensed through a 2 component signaling system, which then phosphorylates a DNA binding protein to activate gene expression (details 3).

OR
Information derived from other modules if applied correctly will be given marks (eg sigma factor-dependent regulation). More detail releases more marks.

LOs
Articulate subject-specific terminology correctly
Identify features that facilitate microbial transmission, infection establishment and evasion of host anti-microbial response

Most answers generated a good amount of marks. Most students chose to focus on lactose and glucose as carbon source. Some excellent, concise answers relating the regulation also to adhesin expression, and identifying cAMP-CAP as global regulator.

The answers relating to stationary phase regulation, invoking quorum sensing and 2-component signaling, were mostly very complete.

4. Briefly describe how a virus can attach to the surface of a cell and summarize the relevance of this adhesion for the virus.  
(5 marks)
Answer should reflect basic structural knowledge of viruses, eg spike proteins. Relevance should include at least some of: tissue tropism and initiate facilitate entry into host cell and possibly more immune related answer eg, stimulate a host
response (signaling). Answers that are more relevant to bacteria or parasites will not be given credit, unless in the answer it was made clear that “cell” was a bacterium etc.

LOs
Articulate subject-specific terminology correctly
Identify features that facilitate microbial transmission, infection establishment and evasion of host anti-microbial response

Many answers generated a good number of marks, with at least some relevant aspects included, using correct terminology and showing good understanding. Most correctly identified the need of adhesion for entry, usually including why entry is vital. Specificity of spike protein to cell receptor, and the resulting tissue/host specificity for infection, was less frequently identified.

5. You are conducting an assay to measure the concentrations of immunoglobulins that are specific for a microbe that has previously infected three patients. The results are tabulated below.

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IgG</td>
<td>-</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>IgA</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgE</td>
<td>-</td>
<td>+</td>
<td>++++</td>
</tr>
</tbody>
</table>

(a) Which patient is vulnerable to recurrent infections with the same microbe? Explain your reasoning. (3 marks)

Patient 1 (1 mark). The patient has a defect in class switching (1 mark) meaning they cannot generate a memory response to the microbe (1 mark).

Almost every student identified Patient 1, the majority gave the rationale for lack of class switching (students that said the cells should switch were also awarded the mark) and the majority stated that there would be no memory.

(b) Which patient could be described as atopic? What type of microbial infection did they have? (3 marks)

Patient 3 (1 mark). The patient has excessive IgE in their serum (1 mark). IgE
Almost all students answered all parts of this question correctly.

LOs
Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes

Describe the structural and functional attributes of the primary and secondary lymphoid organs essential for an effective anti-microbial response

Employ critical analytical skills to interrogate host-microbial immune responses

6. Discuss the peripheral tolerance mechanisms that ensures an immune response to pathogens but not our own tissues.

   (6 marks)
   Immune system restricts recirculation of B and T cells to secondary lymphoid tissue (1 mark)
   T cell activation is dependent on thresholds of TcR-MHC-peptide and secondary costimulation signals (1 mark)
   CD4+ T cells need to be activation to provide help for CD8 T cells for activation via IL-2 (1 mark).
   Negative costimulators switch off activated T cells (CTLA-4) and B cells (FcgRBII) (1 mark).
   T regulatory cells control autoreactive T cells (1 mark).
   The BcR undergoes screening for autoreactivity following SHM in GC. (1 mark)

The majority of students received more than half marks for this question. They showed good reasoning; unfortunately some students confused peripheral tolerance with central tolerance and lost marks. Some students talked about CTLA-4 only in the context of Tregs. However full marks required knowledge that CTLA-4 is employed by all T cells, not just as a mechanism employed by Tregs to control autoreactive cells.

LOS
Articulate subject-specific terminology correctly

Describe the structural and functional attributes of the primary and secondary lymphoid organs essential for an effective anti-microbial response
7. The diagram shows two potential rearrangements for segments in the TcRβ chain. Discuss whether (A) or (B) leads to the greatest diversity in the T cell repertoire.

(A)

(B)

(A) gives greatest diversity (1 mark). The 23-12 rule means only a 23bp segment can recombine with a 12bp segment (1 mark). In B, D would be excluded giving less diversity in the V region of the TcR chain (1 mark).

Almost every student received full marks for this question, readily identifying A, discussed the 12-23 rule really well, and pointed out loss of D in (B) would decrease diversity. Some students lost marks for not pointing out the latter point.

LOs

Articulate subject-specific terminology correctly

Describe the structural and functional attributes of the primary and secondary lymphoid organs essential for an effective anti-microbial response

Employ critical analytical skills to interrogate host-microbial immune responses
8. (a) Explain the molecular mechanisms that promote naive T cell migration from the blood into the T cell areas of lymph nodes. (5 marks)

Mark each up to maximum of 5
- High endothelial venules (HEV) express cell surface glycoproteins called addressins (e.g. PNAd such as CD34) (1 mark)
- T cells express L-selectin (CD62L) that binds PNAd resulting in T cell rolling (1 mark)
- LN stromal cells and HEV produce chemokines CCL19 + CCL21 which bind to luminal surface of HEV (1 mark)
- These bind chemokine receptor CCR7 expressed by naive T cells (1 mark)
- This triggers activation of integrin LFA1 allowing firm adherence to ICAM1 on HEV (1 mark)
- T cells then extravasate across HEV, follow chemokine gradient produced by stromal cells into T cell areas (1 mark)

Many answers demonstrated good depth of knowledge (individual molecules, sequential role) resulting in high number of marks. Some did not make connection between chemokines and activation of integrins necessary for full marks.

b) What changes underpin migration of effector T cells to peripheral sites of inflammation? (3 marks)
- Effector T cells lose expression L-selectin (CD62L) means lack of recirculation through lymph nodes. Also accepted loss of CCR7 (1 mark).
- Effector T cells gain expression of VLA-4. Also accepted increased LFA-1 (1 mark).
- This binds to VCAM-1 expressed by endothelium at sites of inflammation. Also accepted other named correct adhesion molecules (1 mark)

Also answered well, although not as consistently as naive T cell migration to LN in (a). Some only gave answers in terms of changes to T cell or changes to endothelial cell (both required for full marks). Some answers gave increased blood flow but this was not given mark in absence of a mechanism for increased flow of cells to interact with endothelium i.e. increased endothelial cell adhesion molecules.)
Articulate subject-specific terminology correctly

Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes

Describe the structural and functional attributes of the primary and secondary lymphoid organs essential for an effective anti-microbial response

9.
a) Complete the following table. (3 marks)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Where does protein containing antigen originate?</th>
<th>What cell does this pathway activate?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC class I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC class II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-presentation</td>
<td></td>
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</tbody>
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<th>Pathway</th>
<th>Where does protein containing antigen originate?</th>
<th>What cell does this pathway activate?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC class I</td>
<td>Cytosol</td>
<td>CD8 T cell</td>
</tr>
<tr>
<td>MHC class II</td>
<td>Extracellular or vesicular</td>
<td>CD4 T cell</td>
</tr>
<tr>
<td>Cross-presentation</td>
<td></td>
<td>CD8 T cell</td>
</tr>
</tbody>
</table>

(0.5 mark each)
Question was answered well but also caused confusion. Most understood middle column was asking about extracellular vs intracellular. Some gave incorrect answers in terms of pathogens ("bacteria", "virus", "parasite"). Whilst vesicular (or equivalent) was accepted for MHC class II, cross-presentation required knowledge that is presentation of exogenous derived peptide on MHC I. Some thought cross-presentation activated both CD4 and CD8 T cells.

b) Detail the key signalling events in CD4 T cells that lead to Th2 differentiation. (4 marks)

- IL-4 binds to IL-4 receptor on surface of CD4 T cells (1 mark)
- IL-4R signalling activates STAT6 (via JAK phosphorylated); STAT6 dimerises and translocates from cytosol to nucleus (1 mark)
- STAT6 induces expression of transcription factor GATA3, IL-4, IL-5, IL-4 receptor (1 mark)
- GATA3 stabilises expression of IL-4, IL-5, IL-4R, GATA3 (1 mark)

Well answered overall. Some gave an incorrect STAT protein (½ mark). Full marks required articulation that STAT6 causes initial expression of named Th2 associated genes, and that this is stabilised by GATA3.

LOs
Articulate subject-specific terminology correctly

Summarise the cellular and molecular constituents of the innate and adaptive immune systems, and their unique abilities to recognise microbes

Describe the structural and functional attributes of the primary and secondary lymphoid organs essential for an effective anti-microbial response

10. Two experimental vaccines against malaria are being tested in mice. The mice are divided into three groups each with 5 mice:
    Group 1: non immunization control receiving saline.
    Group 2: immunized with vaccine Psp1
    Group 3: immunized with vaccine Psp2.

All of the above mice were later infected with Plasmodium parasites. Mice were checked daily for parasites in blood and when parasites were detected in the blood the animals were euthanized as follows:
    Group 1: 2 mice euthanized 4 days post infection (dpi), 1 mouse 5 dpi and 2 mice 6 dpi
    Group 2: 2 mice euthanized 5 dpi, and 3 mice 7 dpi.
Group 3: 1 mouse euthanized 6 dpi and the rest survive until the experimental endpoint of 12 dpi.

**LOs**

**Articulate subject-specific terminology correctly**

**Illustrate using specific examples how knowledge of complex anti-microbial immune responses promote vaccine development**

**Employ critical analytical skills to interrogate host-microbial immune responses**

a) Use the axes below to draw survival curves for each group mice from the time of infection.

(5 marks)

Students should draw a survival curve as below. A line for each group should be drawn and a legend included to show which line represents which group (1 mark per line x3 = 3 marks). X and Y axes need to have the correct labels and titles (1/2 mark per label set and title x 4 = 2 marks)

Most answers generated a good amount of marks. Some students used curved lines - this suggest that mice are euthanized earlier (curved lines not accepted as correct - in this case they get 1.5 marks for line labels instead of 3 for their correct lines+ labels) - these stair-step graphs were discussed in the workshop and model answer provided to students on VLE. Many had number of live/surviving mice on Y-axis and labels 1-5 - this was also accepted. If Y-axis label did not specify live or surviving mice then ½ mark for label was not given. Use of euthanised mouse number and switched graphs were also accepted.
b) For the above mice blood was taken just before infection with *Plasmodium* to assess the serum antibodies to *Plasmodium* using an ELISA assay. The following graph was generated from the data obtained. The graph shows that both immunization protocols produced an antibody response but only *Plasmodium*-specific IgG3 antibodies above a specific level protects mice against *Plasmodium* infection. Which of the lines below (A and B) represents serum from the Psp1 and Psp2 immunized animals. Explain your answer. (3 marks)

![Graph showing survival curve of infected animals](image)

Line A represents the Psp2 immunized group and line B represents the Psp1 immunized group (1 mark). The linear section of line A (OD 0.5-1.5) indicates that a higher dilution of line A is required for the same OD reading indicating that the antibody level is higher in group A vs group B. If antibodies above a specific level is needed for protection then line A must be the one representing the protected mice immunized with Psp2 and line B thus...
represents mice immunized with Psp1 (2 marks).

Most answers generated a good amount of marks. Some don’t specify what line B shows and are given only ½ mark for A = Psp2 - questions asks for both line A and B. Explanation above (or similar) should be included for all marks.

c) You have also run a set of standards with your ELISA and generated the following standard curve. Use both graphs (from question b and c) to determine the amount of IgG (in µg/mL) that should ensure ~80% protection of mice against a *Plasmodium* infection. Show your workings.

![Standard Curve Graph](image)

Students should use the graph in b to work out 1) the OD value for a particular dilution of serum from Psp2 immunized mice (eg OD of 1.5 for the 1/5000 dilution and OD of 1.0 for the 1/10000 dilution – these are the only two that fall in a linear part of the graph and could be used) (1 mark); 2) use the standard curve to check what antibody concentration relates to those OD values eg for OD = 1 the concentration is 100 ng/mL, for OD = 1.5 the concentration is 200 ng/mL. Therefore in the 1/10 000 dilution sample there is 100 ng/mL and in the 1/5000 dilution there is 200 ng/mL (1 mark). Students should multiply by the dilution factor to get 1000 µg/mL in the undiluted serum (100 ng/mL x 10 000 or 200 ng/mL x 5000 = 1 000 000 ng/mL. (1 mark for the correct concentration in the undiluted serum in ng/mL and 1 mark for correct concentration in µg/mL or 2 marks for going straight to correct concentration in µg/mL)

Most answers generated a good amount of marks. Some get conversion from ng/mL to µg/mL wrong or don’t do it so get only 3 of 4 points. Some try to use 80% of OD=2. Marks may be given for knowing they should multiply dilution factor with concentration even if the concentration they use is wrong.

d) The graphic below represents the ELISA set-up to measure the antibodies in question b. Each number represents a component to allow final detection of the
antibody. Name the reagent/component that is represented by each number and explain your answer. (4 marks)

1 = Plasmodium Antigen or protein (1 mark) – students should point out graph b shows Plasmodium-specific antibodies and the ELISA plate should therefore be coated with a Plasmodium antigen or protein
2 = Undiluted and diluted serum samples- b specifies that IgG3 antibodies are detected in the serum samples of mice (1 mark)
3 = Enzyme-linked (or biotinylated anti-IgG3 antibody – graph b shows IgG3 in serum so the secondary antibody must detect only IgG3 and this should be linked to an enzyme for detection (1 mark - ½ mark for enzyme and ½ for antibody)
4 = Colorimetric substrate – the substrate should be converted by the enzyme in 3 resulting in a colour change for detection by a spectrophotometer at 450 nm (1 mark)

A range of marks were obtained. Many don’t explain their answers; others get components wrong - if components are in right order but shifted then some points are awarded. For 1) students get only ½ mark for plasmodium antigen if no reason is given Most don’t say serum for 2 - IgG antibodies was accepted. Many separate enzyme from antibody. If enzyme is listed in 4 and it is stated that it is linked to the antibody in 3 then marks are awarded. They must still include substrate and it’s colour change in 4. Some use fluorophore, marker and GFP - these are not accepted as correct.

e) What type of ELISA is shown in d. Explain your answer. (2 marks)

It is an indirect ELISA (1 mark) because there are two antibodies between the antigen and detection – the primary antibody (present in the serum sample) and the secondary antibody (anti-IgG3) which is linked to an enzyme for detection (1 mark). A direct ELISA would only have one antibody that is linked to an enzyme to detect antigen

Most get this right.

f) How would you determine whether other plasmodium-specific antibody classes are
produced by the immunization protocol? Give an example of such an antibody. (1 mark)

Students should say they can substitute the secondary antibody (or component 3 in d) for another enzyme-linked antibody that recognizes other classes of antibody (e.g. against IgE, IgM, IgA) (1 mark)

Most get ½ mark for naming a different class of antibody; variable responses about changing secondary antibody (some students get it right). If students say to change antibodies without indication of which then ½ mark for is not given. ½ marks given for other acceptable approaches with specific details.

g) You repeat the ELISA used in b) for a new set of samples. The results show low OD values for all samples suggesting no antibodies. How could you validate the result? (1 mark)

Standards or a positive control should be included in the ELISA to show that the ELISA worked. (1 mark)

Variable, many get it right. 1/2 mark given for other good trouble-shooting suggestions. Giving a reason for failed results but no method for validation is not accepted as correct. Saying to repeat the ELISA or to include a negative control is not accepted as correct - this doesn't show that the ELISA is working. Must include positive control with ELISA repeat.

11. Pathogens are known to avert immune system mechanisms in order to survive.
   a) For two specific pathogens, describe how each averts antibody recognition via antigenic variation. (4 marks)

   i. *T. brucei* - Variable Surface Glycoprotein VSG (1) - recombination/switching, Ab gliding, flagellar pocket uptake, recycling, *T. brucei* surface protein (1)

   ii. *Plasmodium* - var genes encode for PfEMP proteins on surface of RBCs (1) DBL CIDR domains recombine (1)

   All correct answers accepted. Influenza HaNa is another pathogen which utilises antigenic variation.

   b) Describe how *Trypanosoma cruzi* obstructs acquired immunity independent of
antigenic variation. (2 marks)

Uses antibodies to recruit complement to assist trypomastigote binding to host cell receptors (1) and trigger endocytotic uptake into non-phagocytic host cells. (1)

c) Describe the two distinct genetic strategies by which the *Trypanosoma* (*T.brucei* and *T.cruzi*) parasites upregulate levels of their surface antigens. (2 marks)

i. *T.brucei* - RNA Pol I drives surface antigen expression for VSG and Procyclin surface proteins (1).

ii. *T.cruzi* - Gene duplication; surface antigen-encoding genes make up 50% of the *T.cruzi* genome. (1)

12. a) You have developed a novel drug which successfully eradicates 90% of all *Trypanosoma brucei* Stumpy stage parasites. Given the *T.brucei* lifecycle (figure below), what impact upon Human African Trypanosomiasis would you expect to occur? (2 marks)
Successful reduction of ST-cells would reduce HAT Transmission significantly (1), but disease would persist in patients already infected (1)
(Bonus if they guess populations would select for non-stumpy inducing mutants - which kill faster (1)).

You have developed a highly effective vaccine against Amastin protein that specifically targets amastigote stages in both *Leishmania* spp. and *Trypanosoma cruzi*. 
b) What impact upon Leishmaniasis would you expect if the acquired immune response eradicates parasites within 24 hours versus 14 days?  

(4 marks)

Either eradication efficacy would reduce both disease transmission (uptake of amastigotes) as well as disease progression (2). The faster efficacy could interfere with phagocytic cell uptake and manipulation by amastigotes (1). The slower efficacy is less likely to hinder initial infection but should reduce disease outcome and transmission efficacy (1).

c) What impact would you expect this vaccine against Amastin protein to have upon Chagas disease (American Trypanosomiasis)? Explain.  

(2 marks)

The amastigote-targeting host response would have a far reduced impact upon Chagas disease progression as amastigotes remain intracellular in *T.cruzi* (1). Only trypomastigotes are released into the cytoplasm and these are highly infectious to any nucleated cell (1).

d) What distinguishes the impact of targeting amastigotes in these two parasitic diseases?  

(4 marks)

The amastigotes are the only disease-causing parasite stage in Leishmaniasis and only infect phagocytic cells, therefore these remain in the bloodstream longer and risk eradication by acquired immune responses (1). Amastigotes in *T.cruzi* are proliferative, but overwhelmingly intracellular stage - capable of infecting any nucleated cell swiftly should they be exposed to the
bloodstream, but not the primary infectious agent (1). Targeting *T. cruzi* amastigotes will have reduced impact to either infections or transmission (1). Once trypomastigotes invade, they swiftly differentiate into the proliferative amastigote stages and redifferentiate back to trypomastigotes, destroy the host cell and exit (1).