UNIVERSITY OF YORK
BSc Stage 3 Degree Examinations 2017-18

Department:
BIOLOGY

Title of Exam:
Advanced Topics in Immunology

Time allowed: 2 hours
Total marks available for this paper: 100

This paper has two parts:

Section A: Short Answer / Problem / Experimental Design questions (50 marks)
- Answer all questions in the spaces provided on the examination paper

Section B: Essay question (marked out of 100, weighted 50 marks)
- Answer either question A or question B or question C
- Write your answer on the separate paper provided and attach it to the back of the question paper using the treasury tag provided
- The marks available for each question are indicated on the paper

For marker use only:

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SECTION A: Short Answer / Problem / Experimental Design questions

Answer all questions in the spaces provided
Mark total for this section: 50

FEEDBACK after exam (in blue):
General comment where Qs asked to name two (or three) examples or reasons for something: If more than two (or three) answers were given, only the first two (or three) were considered by marker.

1. Compare and contrast dendritic cells and macrophages by giving three examples each of:

   [LO1]

   a) Their similarities: (3 marks)

   1) 

   2)

   3)

   Any 3 of the below:
   - Both are cells of the innate immune system
   - Both have dual origin: 1) yolk sac-derived and 2) from hematopoietic stem cells
   - Both are very heterogeneous populations
   - Both express similar 'pathogen-sensing machinery' in the form of pattern-recognition receptors (PRRs)
   - Both express similar 'pathogen uptake machinery' in the form of scavenger receptors, mannose receptors, and opsonizing receptors (e.g. C' receptors, FcR)
   - Both are able to take up pathogens by endocytosis
   - Both acquire distinct functional properties influenced by the tissue and the immunological microenvironment

   b) Their differences: (3 marks)

   1) 

   2) 

   3)

   Any 3 of the below:
   - Macrophages are superior to DCs in taking up and killing pathogens, the latter thanks to microbicidal activity (like anti-microbial peptides, enzymes, ROS) upon activation
- DCs are superior to macs in taking up and presenting Ag to naïve T cells
- Activation enhances phagocytosis in macs, whereas DCs become poorer at endocytosis/phagocytosis upon activation
- Following activation, DCs loose CCR5 and up-regulate CCR7, allowing migration to secondary lymphoid organs (e.g. LN) to initiate adaptive response
- DCs can cross-present exogenous Ag on MHC-I
- Macrophages can be generated from blood monocytes by culture in M-CSF, DCs can be generated by culture in GM-CSF + IL-4

This was an easy Q with many possible answers for both parts a and b, and most students answered the Q very well. For part b, if cell surface markers were listed as a difference, a mark was only given if an example of such a marker was given.

2. T-cell activation is dependent on signal 1 and signal 2. Describe the importance of these two signals in immune tolerance. (4 marks) [LO2]

Signal 1 = MHC:peptide; signal 2 = co-stimulation
Signal 1 without signal 2 renders naive T cell unresponsive (anergic) to subsequent stimulation, even if Ag is subsequently presented by a professional APC. (2 mark)
This prevents immune response to self Ag, as many self Ag will be presented by tissue cells that lack co-stimulatory molecules. (2 mark)

Most students had responded that signal 1 in the absence of signal 2 leads to anergy, but to get full marks it was necessary to add “even if Ag is subsequently presented to the T cell by a professional APC expressing both signal 1 and 2”. The Q asked for the importance of signal 1 and signal 2 in immune tolerance, so no marks were given for the explanation of what signal 1 and 2 are.

3. a) IgA is the dominant immunoglobulin class at mucosal surfaces. List three ways by which mucosal IgA participates in host defense in the intestine. (3 marks) [LO2,3]

Answers from:
- It prevents adherence of microorganisms to the epithelial layer
- It neutralizes toxins and enzymes in the gut lumen
- It neutralizes toxins (e.g. bacterial LPS) that has penetrated the epithelial cells
- It exports toxins and pathogens from the lamina propria while being secreted

b) State two properties of mucosal IgA that make it more suitable than IgG in mucosal immune responses. (2 marks)

Answers from:
- IgA dimers can be transported across the epithelium by binding via the J chain to the poly-Ig receptor. (IgG does not form dimer/multimer and thus have no J chain.)
- Via secretory component, mucosal slgA binds to the intestinal mucus layer where it can mediate some of its effects. (There is no ‘secretory IgG’.)
- IgA is inefficient in activating the classical pathway of complement. (IgG can activate complement.)
Some students answered that mucosal IgA is more suitable than IgG in mucosal immune responses because it has secretory component – in order to receive a mark some more explanation was required, including that IgA is dimeric with J chain, enabling it to bind to poly-IgR and be transported to the luminal side, whereas IgG is monomeric and does therefore not have J chain, meaning it cannot be transported to the luminal side in the first place. Half a mark was given if mentioning that IgA cannot induce inflammation; a full mark was given if in addition the example was given that IgA cannot activate complement or act as an opsonin. Note, the J chain does not make the antibody more resistant to proteolytic cleavage by pathogen enzymes.

4. Describe the two proposed models by which AIRE induces transcription of tissue-specific antigens. (4 marks) [LO3]

Model one is the classical transcription model (1 mark) where Aire binds DNA through sequence-specific interaction with promoter elements within TRA genes. Gene expression is activated in these genes (1 mark).
Model two is the random transcription model (1 mark). In this model Aire contributes to random activation of genes by loosening up the chromatin structure to increase the general accessibility of TRA genes. (1 mark)

Practically all students answered this question correctly. Some students lost marks for not stating the classical and random transcription model.

5. Explain the concept of epitope spreading in autoimmunity. (5 marks) [LO1,2,4]

Infection with pathogen (virus/bacteria) triggers the innate immune system to produce interferons (1 mark). The innate response/ interferons promotes minor damage to infected/uninfected host tissue leading to release of host cell cryptic epitopes (1 mark). These cryptic epitopes, distinct from pathogen epitopes, are picked up by dendritic cells (1 mark). These dendritic cells activate autoreactive T cells that have receptors for the cryptic epitopes (1 mark). The activated autoreactive T cells migrate back to site of infection and kill host tissue (1 mark).

The majority of students did very well in answering this question. A mark was removed for not mentioning IFN. A few students mixed this concept up with molecular mimicry.

6. Immunotherapies are proving promising in the treatment of autoimmune diseases. [LO2,5]

a) Discuss the immunological concept that abatacept therapy manipulates. (3 marks)

Abatacept is a CTLA-4-Ig fusion protein (1 mark). CTLA-4 is a negative co-stimulatory molecule that binds CD80/86 on APC (1 mark) involved in switching off activated T cells by blocking signal 2 (1 mark).
The majority of students answered this question correctly. Many stated it was a monoclonal antibody but then described it as a fusion protein, so no marks removed.

b) Explain the three principles of human clinical trials that should be adopted into immunotherapy testing in pre-clinical models. (3 marks).

Studies should be randomised in selection of which animal receives the placebo versus test therapy (1 mark), trial should be performed blind where the person analysing the data should not be aware which sample is from the placebo or test therapy group (1 mark) to prevent cognitive bias. Data analysis should be stratified to determine if therapy is successful in a subgroup instead of making conclusions on the whole cohort (1 mark).

Almost all students performed badly on this question. Although the majority correctly stated perform blind or double blind with an appropriate explanation, other principles routinely given were 'increase numbers, controls, treat with respect'. All animal experimentation is conducted with respect and care for animals, and ethical approval must be in place before any experiment can be performed. Moreover, there are strict rules and regulations that must be followed to ensure that all animal experiments are designed to contain appropriate controls as well as sufficient numbers of animals to be able to perform proper statistical analysis, and therefore draw appropriate conclusions. Thus, animal numbers should not be a limiting factor with animal experimentation. Some students did correctly state randomise and stratify but did not give the appropriate explanation why, a mark was deducted for that.

7. The figure below shows the results of an in vitro assay in which three different populations of ex vivo-isolated CD25+CD4+ cells (A, B, and C) have been tested for their ability to suppress the proliferation of a responder T-cell clone X. As illustrated in the figure, different ratios of T-cell clone X and cells A-C cells have been used.

[LO5,6]

a) What are the immunological principles of an in vitro suppressor assay, i.e. what considerations would you have to take into account when
designing an assay as described above? (5 marks)

A suppressor assay examines if one cell population (‘Treg cells’) can suppress the proliferation/activation of another T responder cell population. (1 mark)
As such, the choice of Ag(s) for the assay is important, as you need to activate both responder cells and the Treg cells via their TCR (1 mark) in order to induce
i) responder cell proliferation (1 mark), and ii) Treg cells to become suppressive. (1 mark)
The responder cell population and the Treg cells need to be cultured together (cannot be separated by membrane), as cell-cell contact is important for in vitro suppression. (1 mark)

b) Briefly describe the main findings of the experiment shown in the figure, and explain how you came to your conclusions. (3 marks)

When T-cell clone X is stimulated with Ag in the absence of CD25+CD4+ cells, T-cell clone X proliferates (left bar).
To examine the ability of the CD25+CD4+ cells to suppress this proliferation, co-cultures are set up in which T-cell clone X is mixed with the CD25+CD4+ cells (at different ratios as indicated in the fig).
Cells A are very efficient in suppressing T-cell X. At a 1:1 ratio between X and cells A, there is almost no proliferation detected. (1 mark)
Cells B are suppressing T-cell X, but are not as efficient as cells A, as you need 10 times more cells B to reach the same suppression as for cells A. (1 mark)
Cells C cannot suppress T-cell X, because proliferation in co-cultures is identical to that of T-cell clone X cultured alone. (1 mark)

c) Give two reasons that could explain the results with T-cell clone X plus cells C. (2 marks)

i) Cells C do not recognize/respond to the Ag used in the assay; thus, cells C will not be activated, and therefore not suppress the proliferation of T-cell clone X. (1 mark)

ii) Cells C are activated by the Ag used in the assay; however, they are not able to suppress, and are thus not true Treg cells. (They could be activated CD4+ T cells, expressing CD25+ as a result of in vivo activation.) (1 mark)
8. You wish to investigate whether an immunotherapy that targets follicular T helper cells will prevent type 1 diabetes in humans. You decide to test the immunotherapy in an animal model in which mice have been populated with human immune cells from a type 1 diabetic patient. [LO2,3,4,5]

a) Of the four animal models shown below, which is the most appropriate for your studies? Explain your reasoning. (5 marks)

[Images of four different mouse models: NOD mice, NOD mice expressing a transgene encoding HLA-DQ8, NSG-SGM3 mice, NSG-SGM3 mice expressing a transgene encoding HLA-DQ8]

The most appropriate animal model is the NSG-SGM3 mouse expressing a transgene encoding HLA-DQ8 (1 mark). NSG-SGM3 mice are genetically modified to prevent the mouse immune system from developing (1 mark). These mice are good recipients for human immune cells as the human cells will not be rejected by the murine immune system (1 mark). The HLA-DQ8 MHC molecule is essential for human follicular T helper cells to promote type 1 diabetes (1 mark). NOD-HLA-DQ8 mice are not appropriate as the murine immune system and human immune system would fight each other leading to graft versus host disease (1 mark).

The students did quite well in this question. Most correctly identified the NSG-SGM3 strain as being good for engraftment of human cells, with clear description why. It varied as to whether they felt the transgene was important (it is). Others selected NOD mice due to its known T1D prevalence (but sadly this would reject human cells) and the transgene. For those students, marks were given for picking the transgene and explanation why even if the strain was wrong.

b) An ELISA was performed assessing IL-21 concentrations in serum of animals receiving the placebo or the immunotherapy.
Looking at the data below, explain the rationale for selecting IL-21 as a readout for evaluating the efficacy of the immunotherapy and discuss whether the immunotherapy was successful. (4 marks)

IL-21 is a cytokine produced by follicular T helper cells (1 mark) and increased levels of IL-21 in the serum is associated with enhanced follicular T helper cell numbers (1 mark). Type 1 diabetes is associated with abnormally high IL-21 levels and numbers of follicular T helper cells in the blood (1 mark). The therapy decreases IL-21 therefore likely decreases follicular T helper cells numbers and is successful (1 mark).

Almost all students answered this question correctly. Marks were deducted for not stating enhanced IL-21 and Tfh are linked to T1D in patients.

c) As an additional readout for the success of the immunotherapy, you perform immunohistochemical analysis of the pancreas. Considering the data above, what anatomical feature would you expect to be different between the placebo-treated and immunotherapy-treated animals? (1 mark)

Placebo group should contain ectopic germinal centres, whereas the immunotherapy group should not. (1 mark)

The majority of students answered this question correctly. However, there were a few students that stated the placebo group would have fewer insulin producing beta cells. This is actually true and so although not the expected answer, the students were given full marks. Some student stated less ectopic GC in treated group without actually defining which treated group-read the question carefully. Marks were deducted for that.
SECTION B: Essay question

Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A or B

Mark total for this section: 50

EITHER

A) Discuss why immune responses generated at mucosal sites are distinct from those stimulated elsewhere in the body.

The mucosa-associated immune system (MALT) comprises:
- Nasal-Associated Lymphoid Tissue (NALT) – lining of the nose
- Bronchus-Associated Lymphoid Tissue (BALT) – upper respiratory tract
- Gut-Associated Lymphoid Tissue (GALT) – GI tract

The immune system at mucosal sites has its own characteristic features. Taking the GALT as an example, immune responses in this tissue are distinct from those generated after an immunization or when antigen enters the blood stream; this is due to the GALT having its own contents of lymphoid cells, hormones, and other immunomodulatory factors. In the GALT, host-microbiota mutualism is established postnatally => microbes and host live in harmony in healthy individuals.

"Immunosuppressive" milieu of MALT:
- DCs "conditioned" to favour Treg cell induction
- TGF-beta produced by steady-state intestine
- Retinoic acid
- Steady-state macrophages non-responsive to TLR stimulation (secrete IL-10)
- Intestinal homeostasis (ratio between T effector and Treg cells)

Ways of limiting commensal and pathogen access to mucosal immune system:
- Intestinal epithelial-cell barrier: tight junctions, Goblet cells secreting mucins, Paneth cells secreting antimicrobial peptides
- Mucosal IgA: dimeric, transported across epithelium, neutralizing, non-opsonizing, does not fix complement, limit access of microbes to mucosal surfaces without risking inflammatory damage to tissue, important role in symbiotic relationship between host and their commensal bacteria, helping to restrict microbes to gut lumen

Commensals don’t activate the innate immune system:
- Localization of PPRs: TLR4 expressed deep down in crypts, TLR5 expressed basolateral => only invading pathogens will activate these TLRs
- Virulence factors in pathogens: Type III secretion systems in pathogens trigger intracellular inflammasome
- Commensals avoid PPR activation:
  - changes in flagellin sequence => TLR5 hyporesponsive
  - suppress I kB degradation (not done by pathogens)
  - promote nuclear export of NFkB (not done by pathogens)
In steady-state, the mucosal immune system is exposed to Ag following ‘silent’ uptake of harmless Ag (food Ag, commensal Ag), e.g. via M cells or via CX3CR1+ phagocytes extending dendrites into lumen to capture Ag and thereafter handing Ag over to CD103+ DCs that migrate to draining LN. This leads to induction of Treg cell responses, examples being Th3 responses to oral Ag, and iTreg/Tr1 responses to commensals.

Harmless Ags (food Ag, intestinal flora Ag) tolerated
After pathogen exposure:
Recruitment of different DC subsets (steady-state vs. recruited DCs)
Recruitment of monocytes that differentiate into inflammatory macrophages
Thus, ‘active infection’ by pathogen leads to Teffector (Th1/Th17) response.

OR

B) Discuss the developmental pathway for B cells and explain how B cells contribute to allergic responses.
B cell development occurs in two organs: bone marrow and spleen.
In bone marrow, distinct pathway, initiating from HSC that develop to common lymphoid progenitors (CLPs).
CLPs; three fates: T cell, NK cell, B cell. Stromal signals in BM allow CLPs to develop down B cell pathway.
Series of transcription factors push B cell development; E2A initiates, acts on CLPs but they still retain T and NK potential; EBF decreases other lineage potential needs E2A to work. Pax5 complete commitment.
Pax5 pushes cells into pro B cell stage
Pre-pro B first rearrangements of DJ in receptor, followed by VDJ at pro-B cell stage. Enables IgM heavy chain to go to cell surface, stabilised by a surrogate light chain composed of VpreB and lambda 5 molecules. Needs VpreB transcription factor to stabilise interaction. This stage first checkpoint; failure to rearrange and express IgM heavy chain results in death of developing B cell.
Pro-B move to pre B cell, start rearrangement of light chain V-J. Successful rearrangement it then complexes with heavy chain and surrogate light chain is removed. B cell progresses to immature B cell stage. Undergoes second quality control step where receptor affinity for self antigens is assessed. High affinity; receptor editing where new recombination of receptor heavy chain subunits occur. If not successful, B cell dies.
Immature B cells passing second quality control step move to spleen for completion of development. Immature B cells transition through three phases, in third phase they receive signals through B cell to tell them to differentiate down the marginal zone (weak signals) or follicular (normal to strong signals) route. MZ B cells stay in the red pulp of spleen whereas FO B cells populate follicles in the spleen or recirculate.
For allergy, students will be expected to discuss role of allergens and IgE, mast cells, histamine, cytokines, hygiene hypothesis, genetic susceptibility, asthma and new therapies to target allergies.

OR
C) Discuss the function and role of T regulatory cells and how they can be used in therapeutic approaches against autoimmunity.

Treg cells are part of the body's peripheral tolerance mechanisms (Ignorance, Anergy, Deletion, Immune suppression by Treg cells), which complement the central tolerance mechanisms (pos/neg selection of T-cells in thymus). Outcome when tolerance breaks down = autoimmunity.

Treg cells' role and function:
- Different types of Treg cells:
  - nTreg, iTreg, Th3, and Tr1 or for Foxp3+ cells new nomenclature: Treg, pTreg, iTreg
- Characteristic features of Treg cells (anergic, need to be activated to suppress, expression of Foxp3, CD25, CTLA-4, cytokines IL-10 and TGF-b) and how they are induced (thymus-derived versus induced in the periphery).
- Mechanisms by which Treg regulate immunity:
  - Inhibitory cytokines IL-10, TGF-beta, IL-35
  - Cytolysis (killing) via granzyme, perforin
  - Metabolic disruption of target cell via IL-2 consumption (depleting IL-2), or by inhibiting target cells through adenosine secretion, or cAMP
  - Targeting DCs via CTLA-4:
    - Degradation of CD80/CD86
    - Inhibition of IL-6 and TNF-a production by DCs
    - Expression of IDO

How Treg cells can be used in therapeutic approaches against autoimmunity:

Link between mutations in CD25 that impact on Treg numbers or function in autoimmunity; the high-affinity IL-2R (including CD25, the alpha chain) important for IL-2 signals for Treg proliferation and survival.

Link between the decrease of, or impairment of, Treg numbers/function in named autoimmune conditions.

Strategies to mitigate autoimmunity based on Treg therapeutic approaches:

- Isolation of Tregs from patient, in vitro expansion with anti-CD3/anti-CD28Abs, then reimplantation into patients

- Anti-CD3 mAb therapy, humanised mAb that causes increase in Tregs in vivo in patients with T1D and modifies disease resolution

- Low dose IL-2 therapy to selectively increase Tregs over effector T cells

- Tolerogenic DCs approaches: ablation of costimulation or incubation with immunoregulatory cytokine that decreases the power of DCs to promote effector T-cell responses, instead drive Treg suppression.