Module Code: BIO00051H

Examination Candidate Number: ____________
Desk Number: ____________

BSc / MSc Degree Examinations 2018-9

Department:
Biology

Title of Exam:
Mechanism to Therapies

Time Allowed:
Open assessment

Allocation of Marks:
Marks per questions will be allocated as shown below.

Instructions for Candidates:
Before answering the questions read the following paper (supplied):
van Vollenhoven RF, Fleischmann R, Cohen S, Lee EB, García Meijide JA,
Wagner S, Forejtova S, Zwillich SH, Gruben D, Koncz T, Wallenstein GV,
Krishnaswami S, Bradley JD, Wilkinson B; ORAL Standard Investigators.

In your responses to questions do not exceed the suggested word limits and use appropriate references to support your statements. References do not count towards the word count. Please use The New England Journal of Medicine referencing style.
Question 1

In van Vollenhoven et al., pulmonary tuberculosis was a severe adverse effect observed in patients receiving Tofacitinib (Table 3). Explain the mechanistic basis underpinning this presentation. Your explanation should be supported by evidence from pre-clinical models and human genetics studies.

(10 marks; maximum 150-200 words)

LOs
Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

Critically apply knowledge of the therapeutic approaches to interpret and critically evaluate and discuss data from primary research papers

Answer
Tofacitinib inhibits activation of Stat1 downstream of the interferon receptors (predominantly type II, IFNG, but also type I), which are essential in immunity against TB (evidence from IFGgR/- or IFNaR/- mice should be discussed). (5 marks)

Human genetics studies also demonstrate increased susceptibility to mycobacterial infection in individuals with mutations in the following genes in the type II IFN pathway:

IFNGRI

IFNGRII

STAT1

Generally well-addressed although some students neglected the human genetics part of the question.
Question 2

Development of other autoimmune conditions could be a severe adverse effect of the use of anti-inflammatory biologics for the treatment of Rheumatoid Arthritis or Ulcerative Colitis/Crohn's Disease. Give relevant examples and discuss whether this can also be the case for JAK inhibition with Tofacitinib through the same or different mechanisms.  

(20 marks; 300 – 400 words)

LOs

Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

Explain using examples advantages and disadvantages of different molecular targeting approaches in human disease and rigorously evaluate clinical data sets.

Critically apply knowledge of the therapeutic approaches to interpret and critically evaluate and discuss data from primary research papers

Answer

Lupus and autoimmune demyelination have been observed in patients receiving anti-TNF therapeutics (e.g. infliximab, some indicative references should be given here). (4 marks)

Autoimmune demyelination can be explained (Kemanetzoglou E1,2, Andreadou E3. CNS Demyelination with TNF-α Blockers. Curr Neurol Neurosci Rep. 2017 Apr;17(4):36. doi: 10.1007/s11910-017-0742-1) by TNF blockade leading to:

- Increased ingress of peripheral autoreactive T-cells into the CNS. This in agreement with the failure of anti-TNF therapeutics in reducing demyelination and for their effect on aggravating MS.
- Decreased TNFR2 receptors, which are necessary for the proliferation of immature oligodendrocytes and myelin repair.
- Altered cytokine responses by downregulating interleukin-10 and upregulating interleukin-12 and interferon-γ, creating a profile similar to that of MS patients.
- Anti-TNF therapeutics do not penetrate the BBB. This means that they do not counteract the effects of TNF in the brain and leading to high concentration of TNF in the CNS (“sponge effect”).
- TNF blockers may unmask an underlying infection, which can lead to autoimmune demyelination. (6 marks)

due to Tofacitinib, although he proposed mechanism is different. Here the differential roles of Stats in Th cell development and differential efficacy of Tofacitinib against each Stat is considered as the prime reason. (6 marks)

An additional 4 marks will be awarded for excellent answers demonstrating in-depth engagement with the literature and/or notable originality.

Generally well-addressed although the connection of anti-TNF therapeutics to EAE was not as prominent in the responses and this lead to missing some of the most interesting mechanistic details. Skewing the balance between Th1 and Th2 responses and other reasonable explanations not included in the suggested answer above were accepted and rewarded. Neglecting or oversimplifying the Tofacitinib-specific part of the question resulted in losing marks.

**Question 3**

There are currently four FDA-approved JAK inhibitors and more than 10 in clinical trials. These inhibitors exhibit varying levels of specificity toward the subtypes of JAK proteins.

a) Why is subtype specificity so important in the development of these drugs? 

(10 marks ; 150-200 words)

**LOs**

Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

Describe through examples how different clinical approaches including biologics, small molecules and cell based therapies can be optimally engineered and used to treat human disease.

**Answer**

· Points should include important of different subtypes of JAK in different diseases; JAK1/3 in autoimmunity, inflammation, JAK2 in haematology/malignancy (4 marks). Effects of pan-JAK inhibition on blood/immune cell development, side effects, efficacy, off target effect etc. (4 marks)

· Conclusions must be supported by primary literature. (2 marks)

Generally good answers. Some answers contained a little too much background on JAK/STAT activity rather than use primary pre-clinical/clinical trial data to highlight pathologies of off-target effects.

b) You have designed a small molecule inhibitor *in silico* that you hope will be highly specific to JAK1. Design a series of experiments to test the specificity and
efficacy of this small molecule using \textit{in vitro} (cell lines) and \textit{in vivo} (animal) methods. 

(20 marks ; 300-400 words)

Identify and justify experimental approaches that could be applied to development of novel therapeutic for human disease.

Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

\textbf{Answer}

- Answers can be relatively broad as long as they address the question with suggestions/methods supported by previously published primary data. Answers along the lines of:

\textit{In vitro} - Use JAK-negative cell line (e.g. gamma-2) and express the 4 different forms of JAK (1-3 and Tyk2) and relevant receptor (IFNAR, EPOR, IL6R etc) (could conceivably knock-down specific JAKs in cell lines that express multiple forms – but messier) \textbf{(4 marks)}. Determine effects on JAK activity by monitoring levels of activated STAT (phospho-flow, western or STAT reporter assays) \textbf{(4 marks)}. Students should look to test agent and range of concentrations and with relevant controls (+/- cytokine stimulation etc). Could advance experiments to include primary human or mouse derived cells. \textbf{(2 marks)}

\textit{In vivo} – more difficult. Probably need to compare WT with disease models of RA/inflammation/autoimmunity (TNFa transgenic, K/BxN, Skg) \textbf{(4 marks)}. Marks will be available for experimental design (numbers, controls etc), end point analysis (tissue, blood, plasma etc), ways to monitor specificity in vivo (phospho-flow on isolated cell) etc. \textbf{(6 marks)}

Most students managed to get some marks based around the basic experimental design and how to interpret the data. the higher marks were reserved for those providing more detail in cell line design (different receptor types, cytokines, ways of measuring signaling), robust in vivo modeling and analysis. Some students scored very high marks by covering all of these areas.

\textbf{Question 4}

What physiological parameters might influence the pharmacokinetics of tofacitinib. Explain your answers and give at least 2 examples of how a parameter you have named influenced a drug’s PK. \hfill \textbf{(10 marks ; 100-150 words)}

Degree of metabolism, weight, renal function, hepatic function, nutritional status, interacting drugs, environmental factors \textbf{(6 marks)}. References of primary publications should be included for 2 parameters named. \textbf{(4 marks)}

Answers were variable - most named at least 2 parameters and elaborated with good examples. Some students named 6 parameter and 2 good examples so obtained all marks.

\textbf{LOs}
Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

Critically apply knowledge of the therapeutic approaches to interpret and critically evaluate and discuss data from primary research papers

**Question 5**

What imaging techniques could be used to track the pharmacokinetics (PK) and pharmacodynamics (PD) of i) small molecules and ii) biologics to treat rheumatoid arthritis. Briefly describe the techniques and the requirements and design of the imaging experiments. Provide evidence for your statements.

(30 marks, 400-500 words)

Answer should clearly distinguish between measurement of PK eg by looking at the drug absorption, distribution, metabolism and excretion in the body (mouse or human) and PD, looking at what the drug does to its target (2 marks).

Students should name at least 3 imaging techniques (3 marks) – these may include methods used for tracking PK and PD in other diseases. For example, single photon emission computed tomography (SPECT), positron emission tomography (PET), scintigraphy (gamma imager), magnetic resonance imaging (MRI). Answers should name and provide a brief description of the imaging techniques including how the drug/biologic or host target will be imaged (including if they need to be labelled/generated) (12 marks) and subsequent imaging. For example, PET imaging for measurement of PK requires a positron-emitting radionuclide that is used to label the drug/antibody which can then be detected after injection. Imaging to be done at regular intervals after injection to determine drug clearance (eg 1, 4, 8, 24, 36, 48, 72hrs). PD imaging require labelling of an antibody to the main target or downstream target or labelling of cells that can be tracked by imaging.

Fluorescent compounds can be used with whole body imaging systems and infrared imaging such as IVIS e.g. Osteosense750, a bone targeting agent. Antibodies can be labeled or activity probes can be used (for example to MMP or cathepsin).

Students should also provide examples of how these imaging techniques have been used and how they would be applicable to measuring PK and PD for RA treatments (small molecules and biologics). (8 marks)

Some imaging has been done for RA and student should include examples of imaging in RA e.g. various biologics used for treatment of RA have been tested for imaging to assess PD, eg Conti et al, Eur J. Nucle Med and Mol Imaging, 2012 used radiolabelled infliximab (TNF antagonist) + scintigraphy to assess TNF in joints. PET with F-FDG is used to assess PD by detecting FDG build-up as measure of joint inflammation and drug PD after treatment. (e.g. Irmler Arthritis Res Ther, 2010). Tran (Hum Antibodies, 2011) used radiolabelled CD20 monoclonal antibody to assess CD20 positive B cells in synovium of patients – this imaging could be used to assess PD (5 marks).

Examples of references:

2) Smith-Jones et al (Nat Biotech, 2004) assessed PD of the Hsp90 inhibitor, 17-AAG for Her2 degradation and antitumor activity. They chelated the gamma emitter 111In and the positron emitter 64Cu to the antiHER2 antibody Herceptin to image Her2 expression by PET and gamma imaging before and after treatment.

Use of nanobodies against CD206 (macrophage mannose receptor, MMR) have been used in SPECT imaging to visualize macrophages in carcinoma models in mice – similar approaches could be used in RA (Movahedi, Cancer Res. 2012)

Answers were generally good enough for a pass but often lacked sufficient detail describing the imaging technique and, in particular, design of the imaging experiments e.g. injection, imaging time points, details of what is being detected in case of cell types. Clear examples of imaging in RA to discuss feasibility were sometimes lacking. Few students discussed imaging techniques that could be used and instead focussed on what had been used in RA. Marks were also given for good comparison of imaging techniques between use in biologics vs small molecules.

LOs

Describe through examples how different clinical approaches including biologics, small molecules and cell based therapies can be optimally engineered and used to treat human disease.

Integrate concepts from pathology, physiology, pharmacology and mechanistic biology inputs into the therapeutic discovery process and explain through examples how clinical success can be determined.

Identify and justify experimental approaches that could be applied to development of novel therapeutic for human disease.