UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualifications:-

B.Sc. *B.Sc. (Intercal)*

Immunology C316: Further Immunology

COURSE CODE	:	**IMMNC316**
UNIT VALUE	:	**0.50**
DATE	:	**14-MAY-04**
TIME	:	**14.30**
TIME ALLOWED	:	**2 Hours 30 Minutes**

TURN OVER

C316 FURTHER IMMUNOLOGY

Candidates must answer **Sections A, B and C**. Please answer each section in a separate book.

The fraction of the total marks allocated to each section is as follows:

Section A: 60/180
(**essay**, 1 out of 3)

Section B: 60/180
(**short answers**, 3 out of 6)

Section C: 60/180
(one question out of one)

TURN OVER

SECTION A

Choose **ONE** question from the list below:

1. Describe stepwise the process of mast cell sensitization and activation.
2. Describe the key molecular and cellular events that occur during B cell development and B cell response to antigen.
3. How do dendritic cells stimulate T cells?

SECTION B

Write short notes on **THREE** of the following:

1. What are 'tetramers' and what are their uses?
2. Influenza virus antigenic drift and shift.
3. Why are lipid rafts important for cell signalling?
4. CD40.
5. The molecular basis of MHC polymorphisms.
6. The role of protein kinases and phosphatases in T cell activation.

CONTINUED

SECTION C

Answer **ALL** the questions, based on data from the paper.

Exonuclease I (ExoI) is an enzyme involved in DNA mismatch repair. This paper examines the role of ExoI in antibody generation.

Figure 1. Legend. Panel (a) FACS analysis of *in vitro* stimulated splenocytes from wild-type (ExoI) or ExoI knockout (ExoI $^{-/-}$) mice. The percentage of splenocytes with surface IgG3 or IgG1 after stimulation for 4 days with lipopolysaccharide (LPS) or LPS plus interleukin-4 (IL-4) is shown. A summary of FACS results using 3 mice per group is shown in Panel (b). Panel (c) [^{3}H]-thymidine incorporation into DNA measured as counts per minute (cpm) after addition of [^{3}H]thymidine for 4 hours by splenic B lymphocytes from wild-type or ExoI knockout mice stimulated for 4 days with LPS or LPS plus IL-4.

Questions

1. Which antibody isotype do the majority of B cells from the spleen express in each culture?

2. What effects do LPS addition, IL-4 addition and ExoI gene deletion have on isotype switching to (a) IgG3 (b) IgG1?

3. What effects do (a) IL-4 (b) ExoI gene deletion have on B cell proliferation?

4. Describe the molecular mechanism(s) that lead to IgG3 and IgG1 expression by the B cells.

Figure 2. Legend. Wild-type or ExoI knockout mice were immunised intraperitoneally with (4-hydroxy-3-nitrophenyl)-acetyl-chicken gammaglobulin (NP-CGG). After 10 days, sera from 6 mice per group were pooled and analysed by (a) IgG1 ELISA (b) IgM ELISA for their ability to bind to plates coated with NP conjugated to bovine serum albumin (BSA) at either 2 NP molecules per BSA molecule (NP_2-BSA) or 17 NP molecules per BSA molecule (NP_{17}-BSA). Serum titres were calculated using the serial dilution closest to ½ the maximal binding.

Questions

5. Which part of the NP-BSA molecule do the antibodies recognise?

6. Which molecule captures only the higher affinity antibodies, NP_2-BSA or NP_{17}-BSA?

CONTINUED

7. What is the effect of ExoI deletion on production of (a) total IgM anti-NP antibodies (b) higher affinity IgM anti-NP antibodies (c) total IgG1 anti-NP antibodies (d) higher affinity IgG1 anti-NP antibodies?

Table 1. Legend. Sequences of the heavy chain V region V186.2 from spleen RNA of wild-type or ExoI knockout mice immunised one or twice with NP-CGG. [a]Mutation frequency is calculated by dividing the total number of point mutations by the number of bases sequenced (276 bp x number of V regions). [c]$P<0.01$ calculated by t-test.

Questions

8. What is the effect of ExoI deletion on the number and type of mutations following 1 or 2 immunisations?

9. How many mutations are there (on average) per V region in the wild-type or ExoI knockout mice after 1 or 2 immunisations?

10. What is the effect of ExoI deletion on the number of B cell clones in the primary response?

11. What mechanism is responsible for the mutations in the V region? Why might there be a bias to G.C mutations in the ExoI knockout mice?

TURN OVER

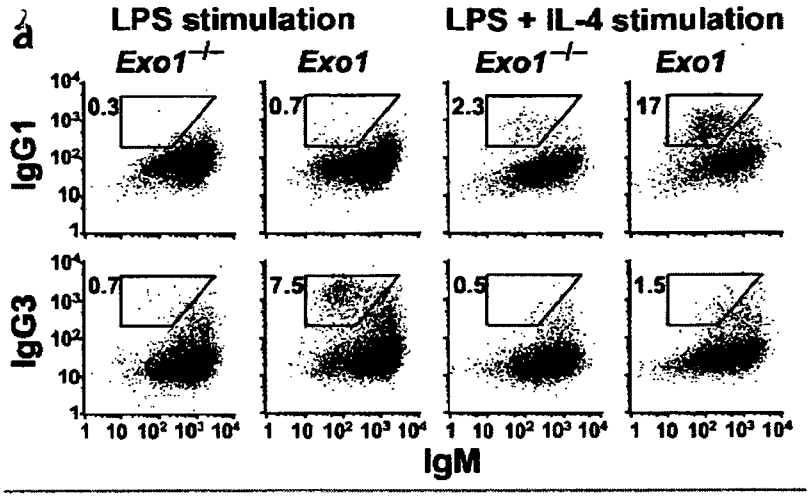
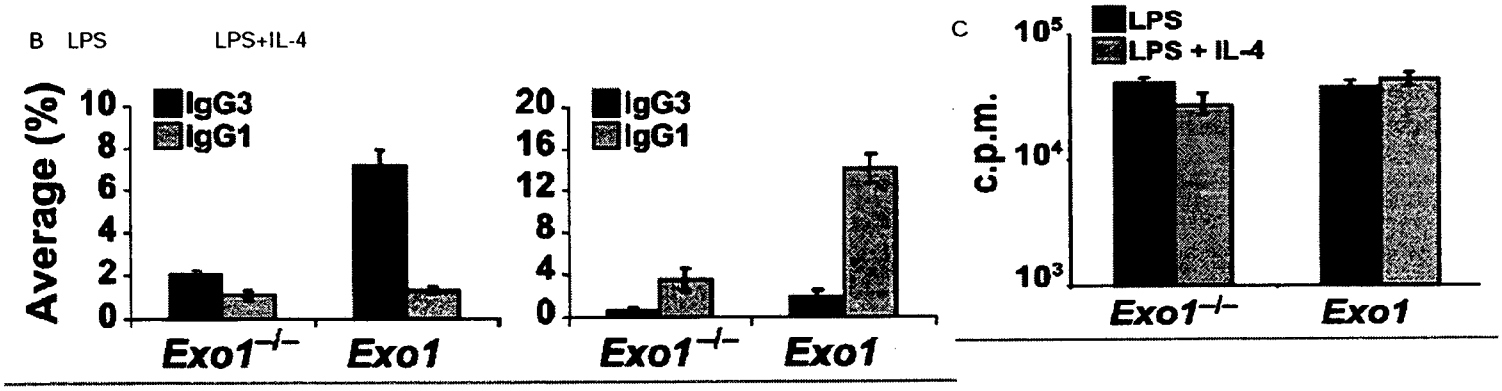


Figure 1. Legend.
Panel (a) FACS analysis of in vitro stimulated splenocytes from wild-type (Exo1) or Exo1 knockout (Exo1 -/-) mice. The percentage of splenocytes with surface IgG3 or IgG1 after stimulation for 4 days with lipopolysaccharide (LPS) or LPS plus interleukin-4 (IL-4) is shown.
A summary of FACS results using 3 mice per group is shown in Panel (b).
Panel (c) [3H]-thymidine incorporation into DNA measured as counts per minute (cpm) after addition of [3H]thymidine for 4 hours by splenic B lymphocytes from wild-type or Exo1 knockout mice stimulated for 4 days with LPS or LPS plus IL-4.



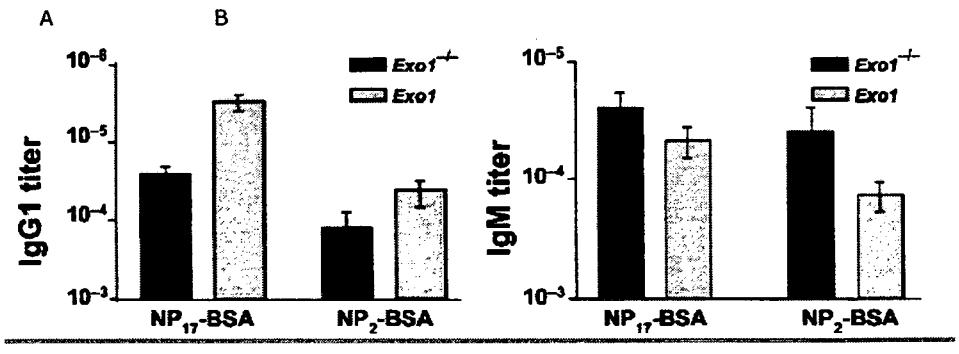


Figure 2 Legend.
Wild-type or Exo1 knockout mice were immunised intraperitoneally with (4-hydroxy-3-nitrophenyl)-acetyl-chicken gammaglobulin (NP-CGG). After 10 days, sera from 6 mice per group were pooled and analysed by (a) IgG1 ELISA (b) IgM ELISA for their ability to bind to plates coated with NP conjugated to bovine serum albumin (BSA) at either 2 NP molecules per BSA molecule (NP2-BSA) or 17 NP molecules per BSA molecule (NP17-BSA). Serum titres were calculated using the serial dilution closest to ½ the maximal binding.

Table 1 Summary analysis of mutation in V186.2

	Primary NP response		Secondary NP response	
	Exo1$^{-/-}$	Exo1	Exo1$^{-/-}$	Exo1
Total point mutations	43	87	168	203
No. of sequences	120	110	42	38
Mutation frequency[a]	2.4×10^{-3}	3.6×10^{-3}	2.0×10^{-2}	2.5×10^{-2}
Mutated V regions (%)	42[b]	61	90	93
CDR3 identities (%)	46[b]	25	46	41
G.C mutations (%)	78	68	94[c]	64

Table 1 Legend.
Sequences of the heavy chain V region V186.2 from spleen RNA of wild-type or Exo1 knockout mice immunised once or twice with NP-CGG. [a]Mutation frequency is calculated by dividing the total number of point mutations by the number of bases sequenced (276 bp x number of V regions). [b]$P<0.01$ calculated by t-test.

END OF PAPER