

TD-BF04: Selection and tolerance

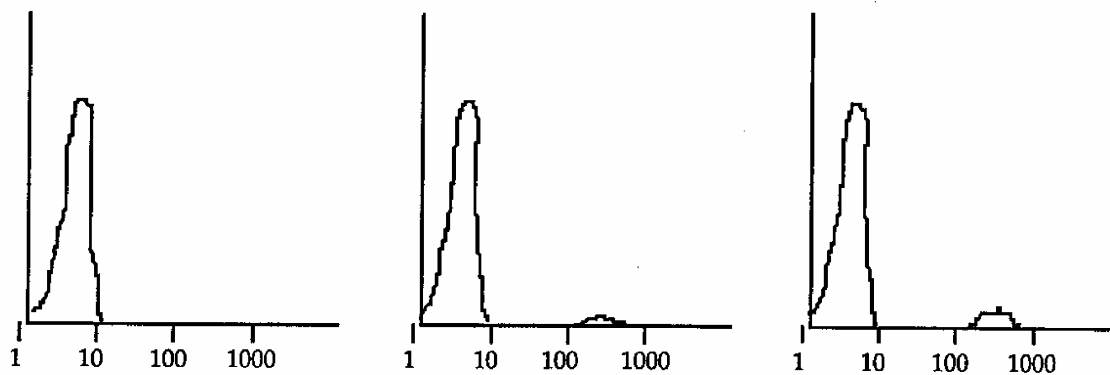
I.

Starting from an MHC H-2^k mouse strain, a transgenic mouse strain was established by incorporating to the mouse genome rearranged TCR α and TCR β genes coding a TCR $\alpha\beta$ specific for a HY peptide presented by IE^k (HY is a protein encoded by a gene located on the Y chromosome; IE^k is a mouse class II MHC molecule). The rearranged transgenes use V α 4 and V β 7 gene segments, respectively.

T lymphocytes, isolated from female transgenic and non-transgenic mice, are first labeled with anti-V α 4 and anti-V β 7 antibodies coupled to fluorochromes. Results of this experiment are presented on Figure 1. The left panel shows the autofluorescence of T lymphocytes incubated in the absence of labeled antibodies.

Figure 1:

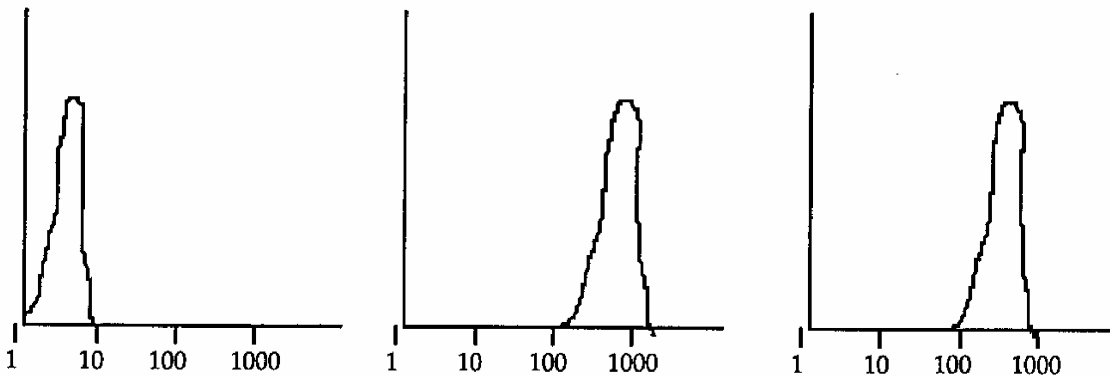
Labeling of non-transgenic female T lymphocytes



Fluorescence in the absence of labeled antibodies

Anti-V β 7 staining

Labeling of transgenic female T lymphocytes



Fluorescence in the absence of labeled antibodies

Anti-V α 4 staining

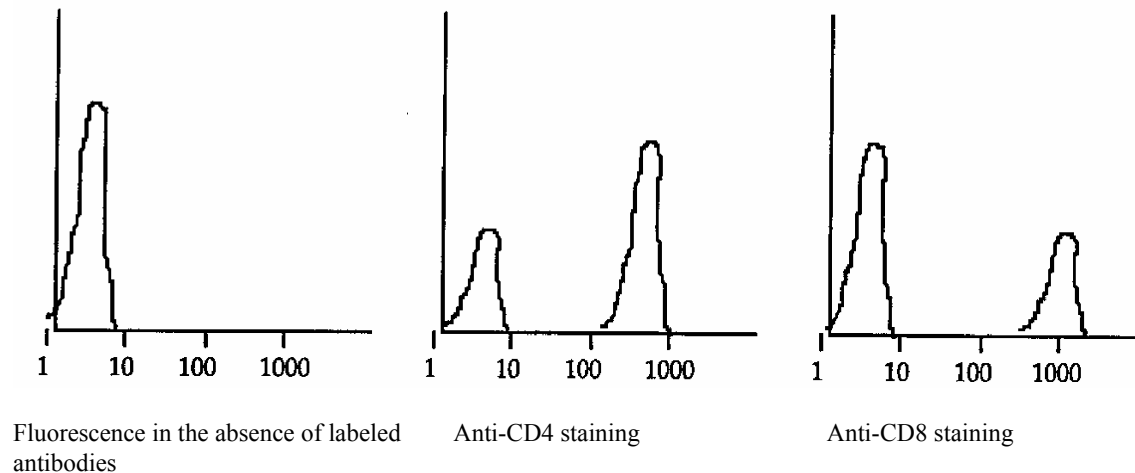
Anti-V β 7 staining

Question 1. *How can you explain these results?*

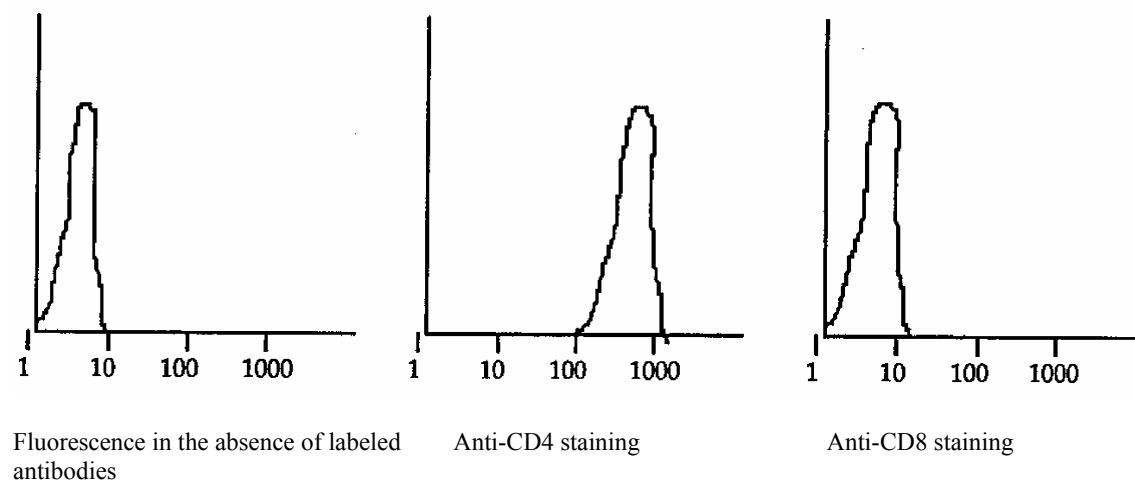
Another staining experiment is performed on the same cells with anti-CD4 and anti-CD8 antibodies coupled to fluorochromes. Results are shown on Figure 2.

Figure 2:

Labeling of non-transgenic female T lymphocytes



Labeling of transgenic female T lymphocytes



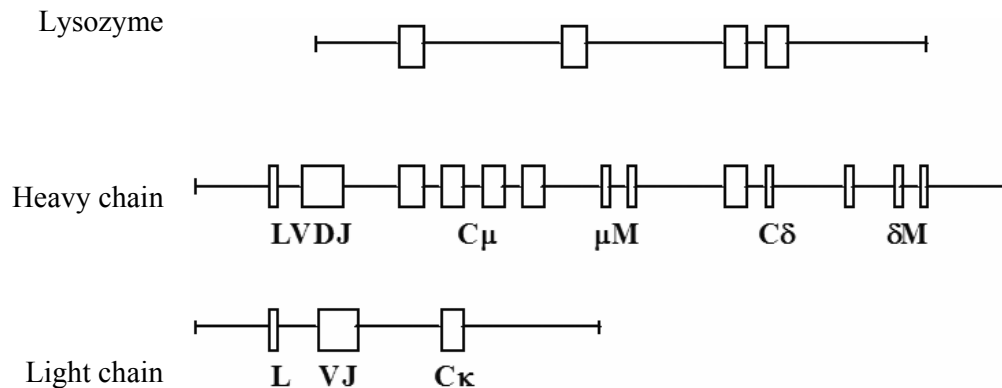
Question 2. *Explain the origin of these observations?*

N.B.: The experiments above were performed with female mice.

Question 3. *Could you predict what would be observed with male mice?*

II.

Three types of transgenic mice were obtained with the transgene shown on the Figure below:



- Lys-Tg mice bear the lysozyme transgene ubiquitously expressed at the embryonic stage.
- Ig-Tg mice bear immunoglobulin (Ig) heavy and Ig light chain transgenes derived from an anti-lysozyme antibody.
- Double-transgenic mice (Dbl-Tg) bear all transgenes (lysozyme and IgH/IgL chains).

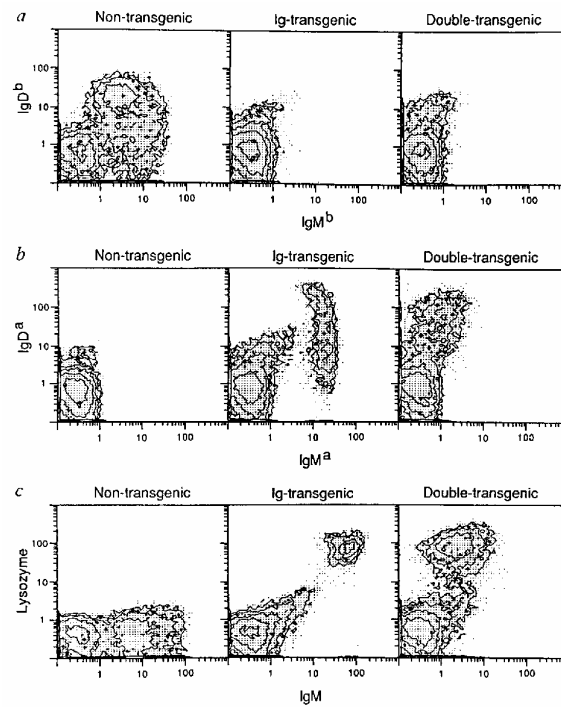
The anti-lysozyme response is studied in the Lys-Tg mice, with a C57BL/6 (B6) or C57BL/6 x CBA genetic background. Mice are immunized with lysozyme alone or lysozyme coupled to horse red blood cells (GRC). After immunization, the anti-lysozyme antibody titer is determined together with the capacity of lymph node lymphocytes to proliferate in the presence of antigen-presenting cells and lysozyme.

Immunized mice	Immunization antigen	Antibody titer (μg/ml)	Thymidine uptake (cpm)
B6	Lysozyme	<1	2 000
	Lysozyme-GRC	1 000	4 000
B6 Lys-Tg	Lysozyme	<1	2 200
	Lysozyme-GRC	<1	1 800
B6 x CBA	Lysozyme	1 200	40 000
	Lysozyme-GRC	1 500	48 000
B6 x CBA Lys-Tg	Lysozyme	<1	1 800
	Lysozyme-GRC	<1	1 900

Question 1. Explain the difference of behavior between B6 and B6 x CBA mice as well as the effect of the transgene in these mice.

The expression of transgenic immunoglobulin is studied in Ig-Tg or Dbl-Tg mice by immunofluorescence. It must be noted that the transgenes originate from a BALB/c (Igh^a haplotype) hybridoma when B6 mice are of the Igh^b haplotype.

Question 2. Analyze the results presented in the figure below which shows the fluorescence analysis of spleen cells from transgenic or non-transgenic B6 mice.



Spontaneous IgM^a secretion is measured together with the number of anti-lysozyme plaque forming cells in Ig-Tg and Dbl-Tg mice.

Mouse strain	IgM ^a (μg/ml)	Number anti-lysozyme plaque forming cells per spleen
B6 Ig-Tg	40	9 450
B6 Dbl-Tg	2	<100

Question 3. Are these results in accordance with the immunofluorescence analysis presented in Question 2? Explain.

Transfer experiments are then performed into irradiated B6 recipient mice. 10^5 spleen cells from non-immunized transgenic or non-transgenic mice are transferred together with $5 \cdot 10^6$ spleen cell from a GCR-immunized B6 mouse. After immunization with lysozyme-GRC, the anti-lysozyme antibody serum titer is determined in recipient mice. Results are presented in the Table below.

Transferred cells		Lysozyme-GRC antigen	Anti-lysozyme antibody titer
10^5 cells	$5 \cdot 10^6$ cells "GRC sensitized "		
B6	B6	-	<1
B6 Ig-Tg	B6	-	<1
B6 Dbl-Tg	B6	-	<1
B6	B6	+	<1
B6 Ig-Tg	B6	+	40
B6 Dbl-Tg	B6	+	<1

Question 4. What is the nature of the lymphocytes involved in the unresponsiveness of double-transgenic mice to lysozyme?