ANALYSIS OF T CELL RECEPTOR β VARIABLE (TCR Vβ) REGION GENE EXPRESSION IN SHEEP WITH MAEDI-VISNA VIRUS (MVV) INFECTION

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Maedi-Visna virus (MVV) is a member of the subfamily lentivirinae, which causes a persistent, chronic active inflammatory disease in sheep potentially affecting many body systems. MVV is a macrophage-tropic lentivirus which infects accessory cells of the immune system, leading to lymphocyte proliferation. In order to study the role of $CD8^+$ T lymphocytes in immunity to MVV it would be helpful to be able to identify the presence of virus specific precursor cytotoxic T lymphocytes (pCTL) in cell preparations or tissues.

Sheep chronically, experimentally infected with MVV were used as a source of lymphocytes which were analysed for Vβ RNA expression before and after *in vitro* stimulation with autologous MVV infected skin cell monolayers to produce active anti-MVV CTL. In this study the method for analysis of the Vβ gene usage in response to MVV was evaluated. RNA was extracted from $CD8^+$ and CTL cell populations and cDNA was amplified by PCR using specific primers for TCR Vβ gene families. The comparative analysis of each Vβ product between the different samples was achieved by densitometric analysis of the signals on the Southern blot membrane after hybridization with specific digoxigenin (DIG) oligonucleotide probes.

This study does show that the PCR method used in this study to analyse TCR Vβ gene has the potential to use for screening of large numbers of sheep. The development of this method for analysis of the TCR Vβ repertoire in populations of lymphocytes in sheep is much faster than the three-week CTL culture and assay. In addition the method practised in this study includes a semi-quantitative analysis, which indicates the proportional increase in the level of TCR Vβ mRNA in the lymphocytes.

We are interested in finding out whether we would be able to find an immunodominant TCR Vβ response to a persistent infection of monocytes, macrophages and dendritic cells in the circulation. Sufficient numbers of sheep were analysed to determine any definite trends in TCR Vβ expression. Using the method practised in this study we were able to suggest four candidates out of 16 genes identified so far namely Vβ 1.2, 2.1, 7.1 and 24.1 for future analysis of TCR Vβ region usage in sheep against MVV infection. At the same time we were able to exclude the involvement of several genes namely Vβ 4.1, 6.1, 10.1, 13.2, 17.1 and 28.1.2 in the $CD8^+$ T cell response to MVV.

In conclusion it is necessary to look for other more sensitive methods in future to definitely find which Vβ gene is used by $CD8^+$ T lymphocytes.