Cell surface expression of PrP^C on subsets of peripheral blood cells from sheep of different genotypes

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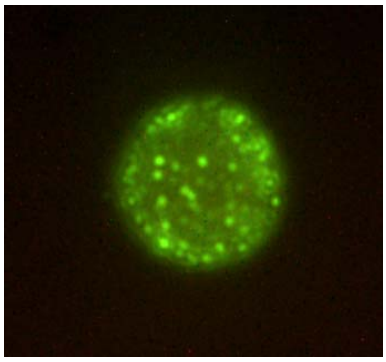
Background and Aims

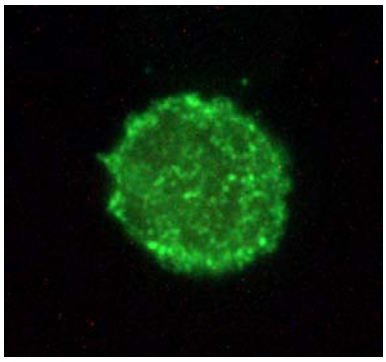
It is well established that different genotypes of sheep differ widely in their susceptibility to development of scrapie, a sheep disease of the Transmissible Spongiform Encephalopathy (TSE) family caused by aggregation of misfolded proteins in the brain. The reasons for the differences in susceptibility are not known but are obviously important with regards to both the understanding of the pathogenesis of scrapie and for the development of means to control scrapie and other prion related diseases. The pathogenic prion protein (PrP^{Sc}) is furthermore thought to be in close contact with the normal prion protein (PrP^C) during the conformational change of PrP^C to PrP^{Sc}. With a firm establishment of scrapie infectivity present in blood from sheep terminally ill with scrapie, blood PrP^C became an interesting aspect. We therefore undertook a study to elucidate the connection, if any, between the level of PrP^C expression and expression pattern on the surface of blood cells and the susceptibility/resistance towards scrapie.

Methods

Blood cells from 30 healthy Norwegian Rygja sheep, grouped after genotype/scrapie susceptibility were investigated by flow cytometry for level of PrP^C expression on the following types of peripheral blood mononuclear cells: CD4+ T-cells, CD8+ T-cells, WC1+ gamma-delta T-cells, CD14+ monocytes and CD21+ B-cells, using commercially available monoclonal antibodies. For detection of PrP^C the 6H4 monoclonal antibody from Prionics was used. Quantitation of cell surface expressed PrP^C molecules, was performed with flow cytometry using Low Level Quantum Simply Cellular calibration beads from Bang's Laboratories. CD positive cell populations were sorted out on a BD FACS Vantage to allow fluorescence microscopy of pure single cell type populations through cytospin preparation. In addition to the above-mentioned non-treated sheep a group of 6 Norwegian Rygja sheep experimentally inoculated with scrapie was analysed in the same way.



Figur 1: Left -Typical patchy PrP^C expression pattern. Here a gamma-delta T-cell. **Right –** CD marker (WC1) on a gamma-delta T-cell.



Results

All PrP^C expressing cell types were found to have a very patchy PrP^C expression pattern (Fig. 1). This was evident in all genotypes investigated. A significant difference was found between levels of PrP^C expression in scrapie susceptible sheep genotypes (V(A/V) RR QQ) compared to moderately susceptible and resistant genotypes (AV RR QR and AA RR R(Q/R). In the susceptible genotype, CD14+ monocytes, WC1+ gamma delta T-cells and CD21+ B-cells expressed approximately twice as many PrP^C molecules as the other genotypes with CD14+ monocytes expressing the highest number of PrP^C molecules (approximately 7700 PrP^C molecules pr cell) (Fig. 2). We found no difference between the blood cell surface expression of PrP^C in infected and non-infected sheep.

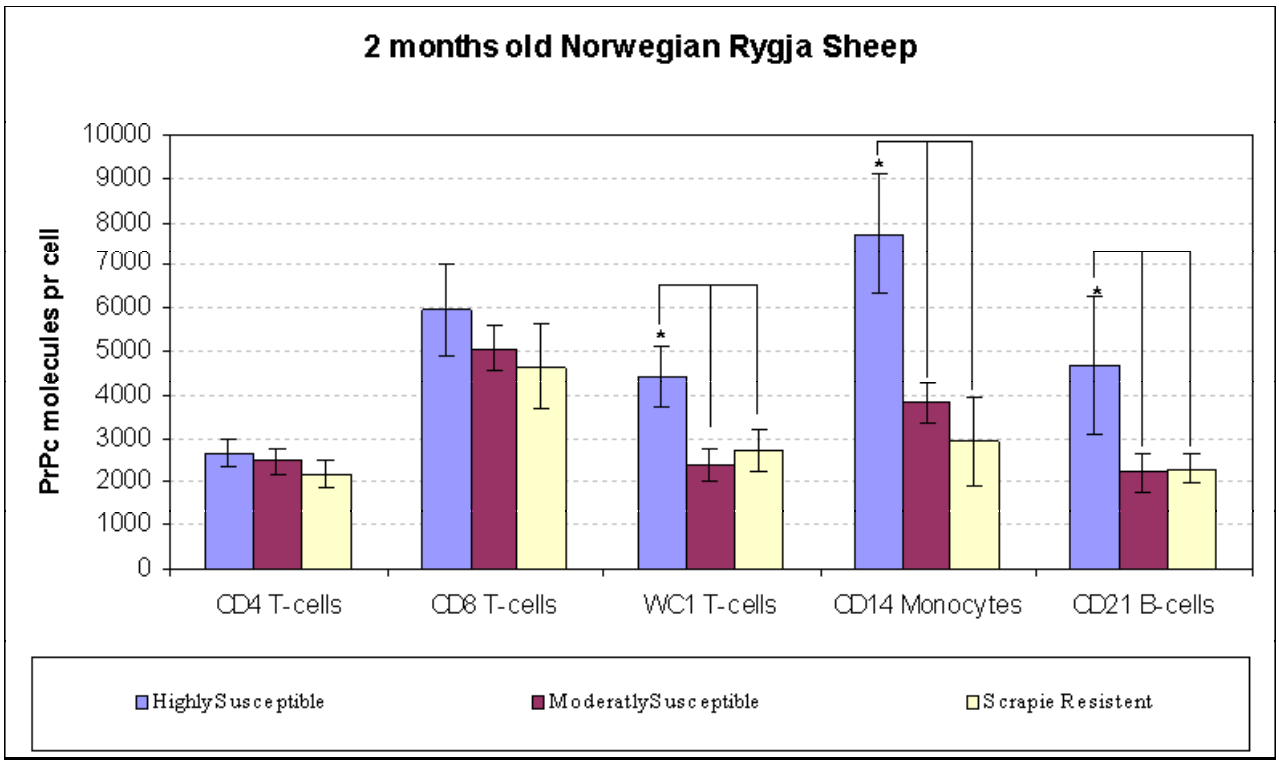


Figure 2: Cell surface expression of PrP^C molecules (± standard deviation) on subsets of ovine blood cells from healthy sheep. * = p<0,05.

Conclusion

In conclusion, this study shows a correlation between scrapie susceptibility and cell surface expression of PrP^C on sheep blood monocytes, gamma delta T-cells and B-cells. The most susceptible sheep genotype showed almost twice the expression of PrP^C on these cells compared with less susceptible and resistant genotypes. This could point to the level of cell surface PrP^C expression as an important factor in susceptibility towards scrapie infection. Furthermore, the high level of PrP^C expression on monocytes suggests that this cell type could play a role in propagation of the infection. The PrP^C expression pattern supports the evidence of patchy PrP^C expression in coated pits/caveolae on neurons, and very likely connected to the function of PrP^C. This could point toward that the function of PrP^C is not just neuron specific.

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