Establishment of a bovine C-type lectin map reveals both conserved and species-specific recognition patterns that impact on mycobacterial recognition

A. Holder[1], S.A.F. Jegouzo[2], M. Salavati[1], J. Hammond[3], J.C. Schwartz[3], B. Rosen[4], T. Smith[4], J.F. Medrano[5], M.E. Taylor[2], K. Drickamer[2], D. Werling[1]

[1]Royal Veterinary College, Department of Pathobiology and Population Sciences; [2]Faculty of Natural Sciences, Department of Life Sciences, Imperial College; [3]The Pirbright Institute; [4]Animal Genomics and Improvement Laboratory, US Department of Agriculture; [5]Department of Animal Science, University of California.

Introduction

Recognition of glycans by pattern recognition receptors (PRRs) contributes to anti-pathogen immune responses. C-type lectin receptors (CLRs) have been well defined in human and mice (http://www.imperial.ac.uk/research/animallectins/) and are capable of sensing glycans present in pathogens to activate mainly innate immune responses, such as phagocytosis, antigen processing and presentation, and subsequent T cell activation. The ability of CLRs to elicit and shape adaptive immunity plays a critical role in the inhibition of pathogen spread within the host. However, certain pathogens exploit CLRs for their entry into host cells to avoid immune recognition. To understand these mechanisms, a detailed analysis of the CLRs present within different mammalian genomes has been undertaken, while specific CLRs known to be involved in mycobacterial recognition have then been investigated further.

Comparative organisation of C-type lectin receptor genes

Bovine orthologues to human and mouse CLR genes were identified in a newly published bovine genome (ARS-UCDv1.2) using the Basic Local Alignment Search Tool (BLAST). The bovine genome was found to contain orthologues of most human and mouse CLR genes. As in humans and mice, bovine CLR genes were found to cluster in regions on a small number of chromosomes (an example is shown in Figure 1), however the distribution of the genes on the chromosomes varies between the different species. The majority of bovine genes appear to occur as a single copy, unlike some mouse genes (e.g. CLEC4A) where multiple paralogues exist.

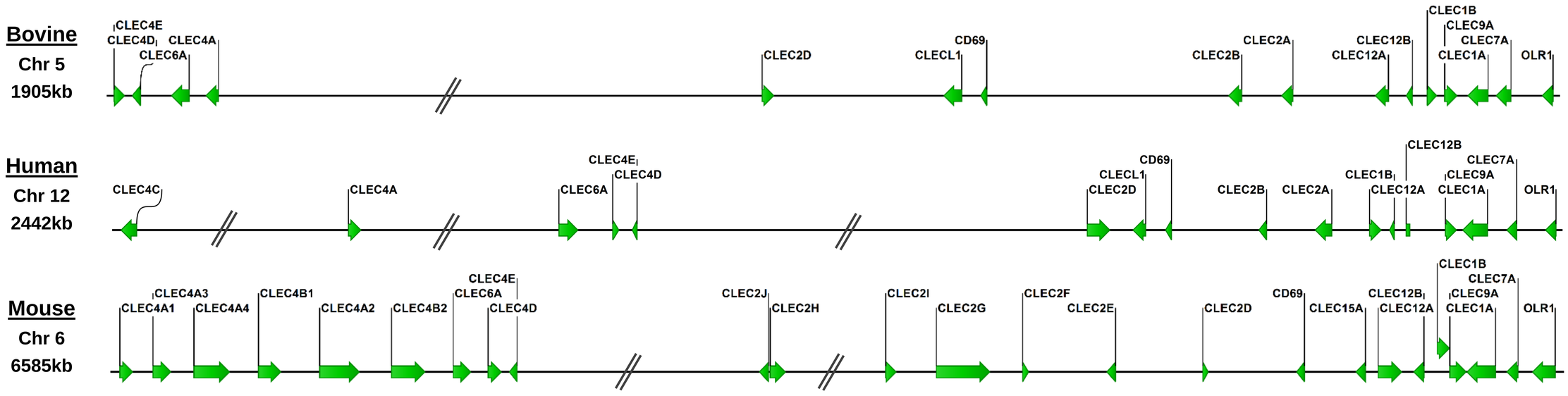

Figure 1. Comparative organisation of genomic regions containing C-type lectin genes in selected species. Genomic regions shown are not to scale

Characterisation of bovine DC-SIGN

Bovine DC-SIGN (CD209), identified on chromosome 7 of the new bovine genome, appears to exist as a single copy gene. In contrast, the human and mouse genes for DC-SIGN exist as multiple paralogues, 2 and 9 copies respectively (Figure 2). Despite variation between orthologues, the ligand binding properties of bovine DC-SIGN appear to be conserved, as demonstrated by similar binding of FITC labelled gp120 to both human and bovine MoDCs, which was blocked by a polyclonal anti-DC-SIGN antibody (Figure 3).

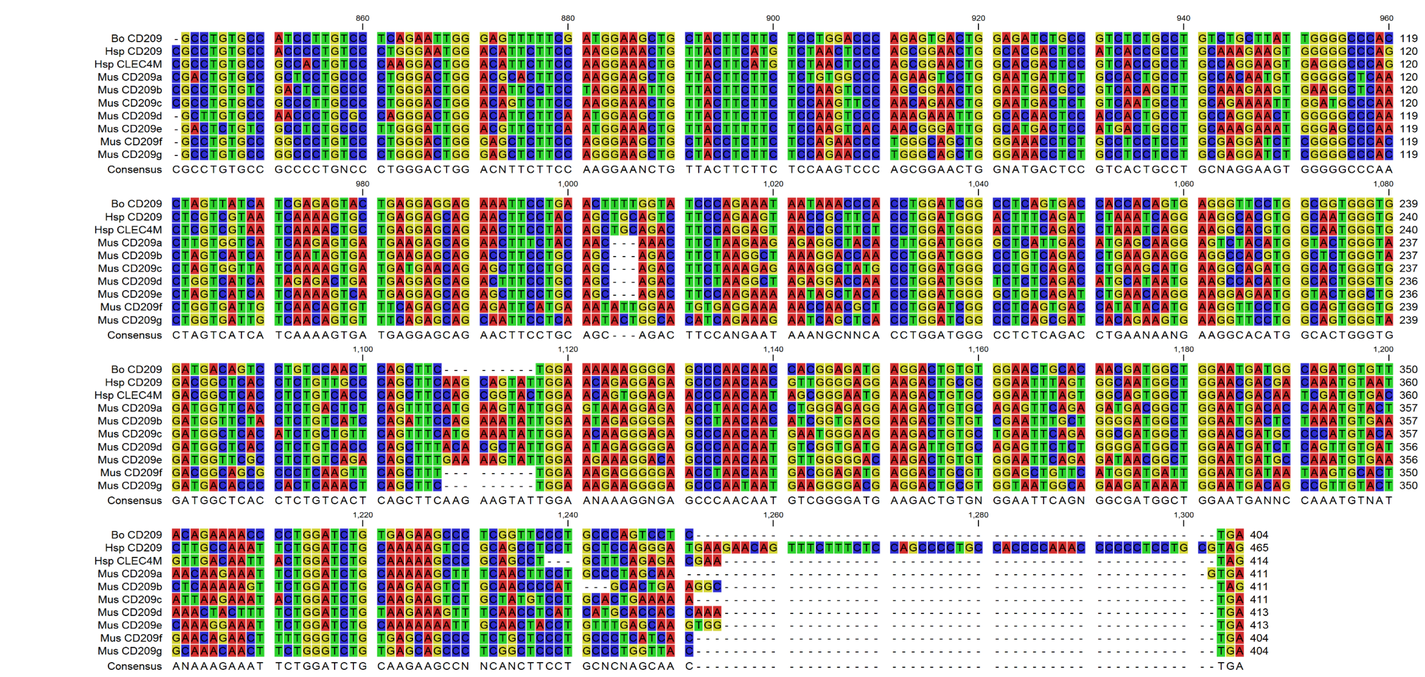

Figure 2. Multiple sequence alignment of partial sequences for bovine, human and mouse DC-SIGN genes. The region shown encodes the carbohydrate recognition domain.

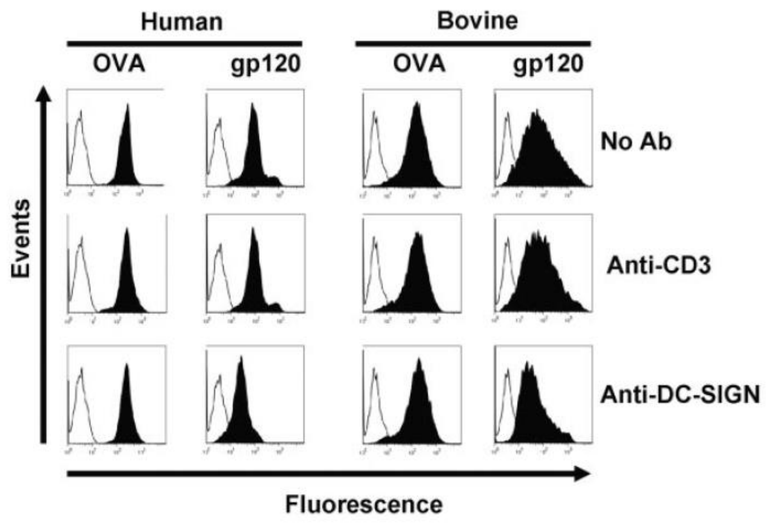

Figure 3. Human and bovine MoDCs were incubated with FITC-OVA (left columns) or FITC-gp120 (right columns) for 30 min at 37°C in the absence or presence of anti-CD3 antibody or anti-DC-SIGN pAb. Open histograms represent untreated cells.

Characterisation of bovine Mincle

Bovine Mincle (CLEC4E), also located on chromosome 7, shares 84% sequence identity with human Mincle (Figure 4). However, crystallographic analysis, site-directed mutagenesis and binding studies have identified subtle differences in a secondary binding site and adjacent hydrophobic surfaces that provide docking sites for acyl side chains of trehalose dimycolate, a key glycolipid on the surface of *M. bovis and M. tuberculosis* (Figure 5). Antibodies generated to the carbohydrate recognition domain of bovine mincle, which have been shown to bind to macrophages in bovine PBMCs cultured for 24hrs (Figure 6), might represent a potential method for disrupting the interaction between Mincle and the mycobacterium.

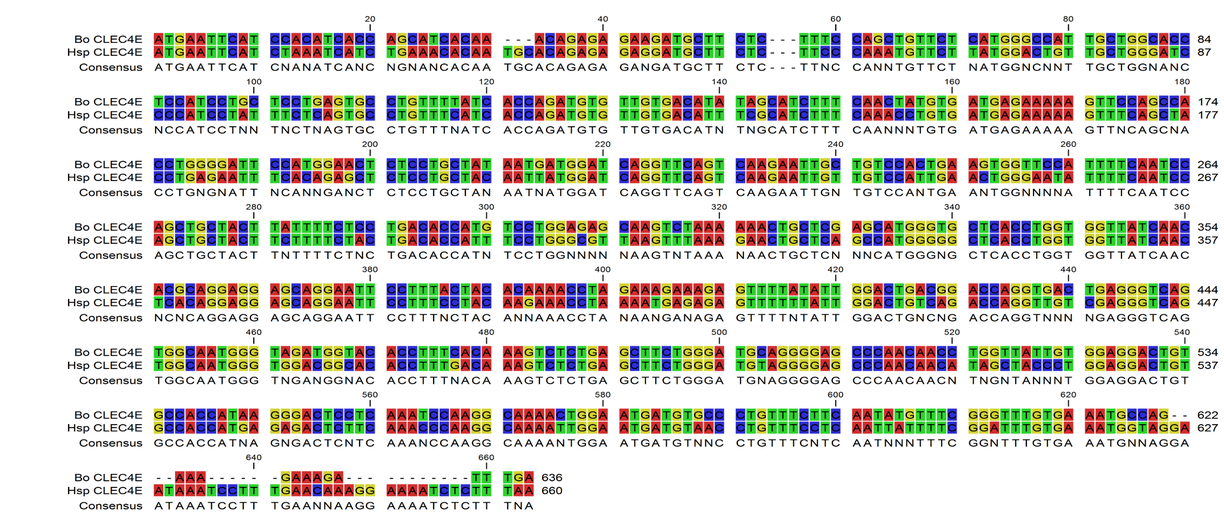

Figure 4. Sequence alignment of the genes for bovine and human mincle (CLEC4E).

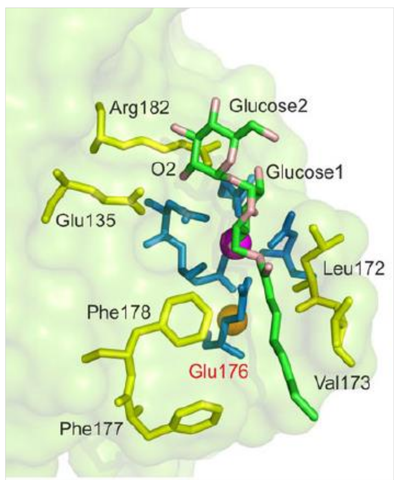

Figure 5. Structure of bovine mincle with trehalose bound. Side chains highlighted in dark green form the primary sugar-binding site by ligating to the conserved Ca^{2+}, shown in magenta, and by making hydrogen bonds with OH groups on the first glucose residue in trehalose. Side chains that form hydrogen bonds with the second glucose residue are highlighted in yellow, as are the side chains proposed to form the binding site for the acyl side chain attached to trehalose.

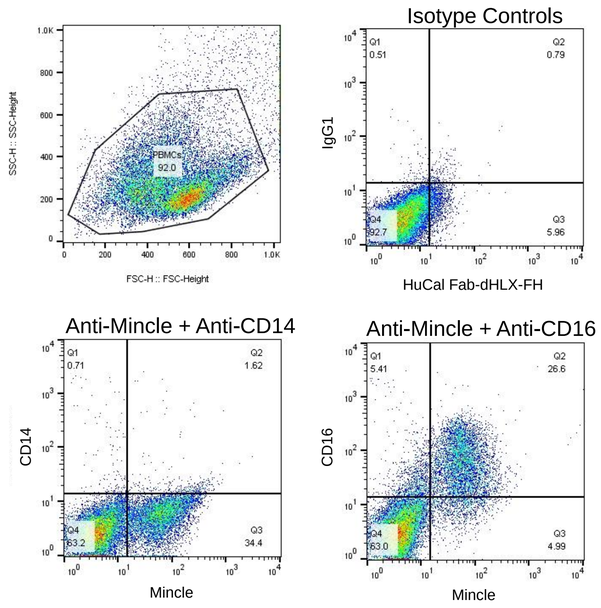

Figure 6. Bovine PBMCs cultured for 24hrs were stained with the antibodies indicated and analysed by flow cytometry. Data presented are dot plots of 20,000 cells.

Conclusions

Identification and comparative analysis of CLRs in the bovine genome, and an understanding of their function might provide new avenues for understanding host pathogen interactions. This in turn might provide a basis for designing new intervention strategies by either interrupting this interaction or by developing adjuvants that mimic the ability of pathogens to stimulate immune responses.

References:

1. Yamakawa Y, Pennelegion C, Willcocks S, Stalker A, Machugh N, Burt D, Coffey TJ, Werling D. J Leukoc Biol. 2008 Jun;83(6):1396-403. Identification and functional characterization of a bovine orthologue to DC-SIGN.
2. Feinberg H, Jégouzo SA, Rowntree TJ, Guan Y, Brash MA, Taylor ME, Weis WI, Drickamer K.J Biol Chem. 2013 Oct 4;288(40):28457-65. Mechanism for recognition of an unusual mycobacterial glycolipid by the macrophage receptor mincle.
3. Jégouzo SA, Harding EC, Acton O, Rex MJ, Fadden AJ, Taylor ME, Drickamer K. Glycobiology. 2014 Dec;24(12):1291-300. Defining the conformation of human mincle that interacts with mycobacterial trehalose dimycolate.