Project number: 6082(B)
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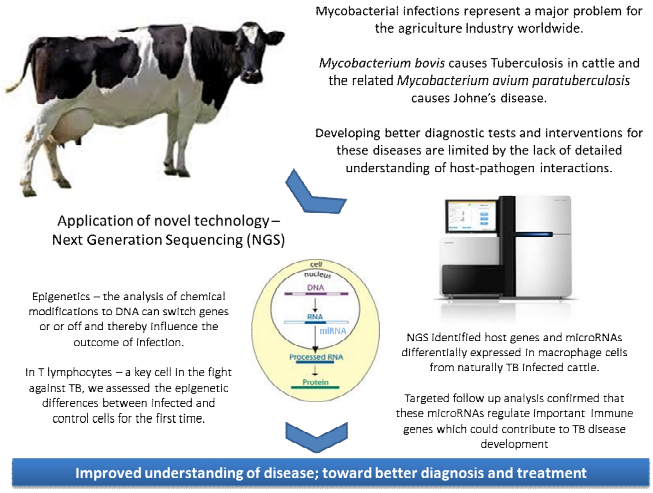
Investigating the genetic and molecular mechanisms contributing to bovine infectious disease



Key external stakeholders:

DAFM, Teagasc commercial partners, dairy industry

Practical implications for stakeholders:

- The complex interplay between mycobacteria and their bovine host is complex and multi-layered.
- This work has identified specific molecules (genes and microRNAs) that are changed in response to TB and Johne's disease in cattle.
- Certain changes may be useful as biomarkers of infection thereby enabling the development of improved diagnostics.

Main results:

- Epigenetics is an emerging field and has much to offer in terms of understanding phenotypes of agricultural interest – including disease. This project has uncovered differences in epigenetic profile in key immune cells between TB infected and healthy control cattle. This is completely novel and adds a whole new layer to our understanding of TB infection.
- Advances in next-generation sequencing technology allow us to take a global picture of the changes which occurs in cells as a result of TB infection. Using NGS, this project identified changes in the abundance of immune genes and microRNAs in macrophages challenged with *M. bovis* and *M. paratuberculosis*. These biomarkers could represent future targets for improved diagnosis.

Opportunity / Benefit:

This project has identified targets that warrant investigation in additional animals and in more detail, to ascertain their value as novel biomarkers of mycobacterial infection.

Collaborating Institutions:

TCD
UCD

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Teagasc project team: Dr. Kieran Meade (Project Leader)
Dr. David Lynn
External collaborators: Prof. Cliona O'Farrelly,TCD
Prof. David MacHugh, UCD

1. Project background:

Bovine tuberculosis is one of the most economically significant infectious diseases of cattle in Ireland and globally, resulting in economic losses of approximately €2 billion annually. The causative agent is *Mycobacterium bovis*, a rod-shaped, Gram positive, acid-fast bacterium that is a member of the Mycobacterium tuberculosis complex (MTBC), a group of highly related pathogens that are spread via an airborne route. A related mycobacterium from the same complex causes Johne's disease in cattle. Costs associated with this disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* cannot be accurately estimated as reliable diagnostics do not exist. Developments in diagnostics and therapeutics for these diseases require detailed understanding of the interactions that occur at a molecular level between these sophisticated pathogens and their bovine host.

2. Questions addressed by the project:

1. Does infection by *M. bovis* lead to changes in the epigenetic profile of T cells?
2. What are the consequences of these epigenetic changes for T cell function in diseased cattle?
3. What are the consequences of MAP infection on macrophage gene expression?
4. What are the consequences of MAP infection on macrophage miRNA expression?

3. The experimental studies:

- *M. bovis* infection (causative agent of TB in cattle):
- Sub-project 1: Cattle with natural TB infection were sourced from the DAFM high biosecurity unit in Backweston, Co. Dublin. Age and sex matched controls were sourced from a farm with a clean infection history for TB. T cells were separated from whole blood using fluorescence-activated cell sorting in Trinity College Dublin. ELISA analysis to confirm infection status was performed in UCD. Libraries were generated from extracted RNA and DNA and sent for commercial sequencing. Bioinformatic data analysis identified differentially expressed genes and methylated regions.
- Sub-project 2: RNA from TB-stimulated alveolar macrophages were obtained from collaborators in UCD. Libraries were generated from extracted RNA and miRNA and sent for commercial sequencing. Bioinformatic data analysis identified differentially expressed genes and miRNA. Transfection of a synthetic mimic of one miRNA (miR-146) into a bovine macrophage cell-line (Bomac) was performed to validate results.

- *M. avium* subsp. *paratuberculosis* (MAP) infection (causative agent of Johne's disease in cattle): Sub-project 3: RNA-seq transcriptomics study was performed in bovine monocyte-derived macrophage (MDM) samples obtained from seven age- and sex-matched Holstein-Friesian cattle that were infected with MAP across a six-hour infection time course with parallel non-infected control MDM samples. Libraries were generated from extracted RNA and sent for commercial sequencing. Bioinformatic data analysis identified differentially expressed genes and differentially regulated pathways.

4. Main results:

- *M. bovis* infection (causative agent of TB in cattle):
- Sub-project 1: Results indicate that DNA methylation is important for T lymphocyte differentiation during TB infection, leaving cells poised to respond upon encountering antigen. It was also found that CD4+ T lymphocytes have a reduced proliferative capacity in *M. bovis* infected cattle, which is likely due to immunoregulation by TGF-β. Findings also suggest that DNA methylation regulates a small number of genes that are concurrently differentially expressed during *M. bovis* infection.
- Sub-project 2: This work has for the first time identified the differential expression of more than 40 miRNAs in aveolar macrophages (AMs) at several time-points following infection with *M. bovis* and revealed that these miRNAs play important roles in targeting genes that are functionally relevant for mycobacterial pathogenesis, suggesting that miRNAs play a key role in tuning the complex interplay between *M. bovis* survival strategies and the host immune response.

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- *M. avium* subsp. *paratuberculosis* infection (causative agent of Johne's disease in cattle):
- Sub-project 3: Results suggest that genes encoding subunits of the nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) transcription factor complex and genes encoding regulatory or signal transduction proteins that affect NF-κB activity were found to be overrepresented as at 2 hpi and 6 hpi post-challenge, thereby shedding light on the regulatory pathways stimulated in these cells in response to MAP.

5. Opportunity/Benefit:

This project developed a strong foundation for our research into mycobacterial infection and has identified targets that may have potential for the development of improved diagnostic tests.

6. Dissemination:

Main publications:

1. Casey, M.E., Meade, K.G., Nalpas, N.C., Taraktsoglou, M., Browne, J.A., Killick, K.E., Park, S.D., Gormley, E., Hokamp, K., Magee, D.A. and MacHugh, D.E. (2015) 'Analysis of the Bovine Monocyte-Derived Macrophage Response to Mycobacterium avium Subspecies Paratuberculosis Infection Using RNA-seq' *Front Immunology* 4 (6):23. doi: 10.3389/fimmu.2015.00023. eCollection 2015. PMID: 25699042.
2. Doherty, R., O' Farrelly, C. and Meade, K.G. (2014) 'Comparative epigenetics: relevance to the regulation of production and health traits in cattle' *Animal Genetics* 45 (1):3-14. doi: 10.1111/age.12140. Epub 2014 Jul 1. Review. PMID: 24984755.
3. Doherty, R., O'Farrelly, C. and Meade, K.G. (2013) 'Epigenetic regulation of the innate immune response to LPS in bovine peripheral blood mononuclear cells (PBMC)' *Vet Immunology Immunopathology* 15:154(3-4):102-10. doi: 10.1016/j.vetimm.2013.05.004. Epub 2013 May 10. PMID: 23764468.

Popular publications:

Teagasc TResearch - 'Epigenetics – Linking Nurture to Nature', Autumn 2011

7. Compiled by: Dr Kieran Meade

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