

FINAL REPORT CCFC GRANT

The most prevalent theory of a microbial etiology of Crohn's disease is infection with *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), also the cause of Johne's disease in ruminants. The objective of this one year establishment award granted by CCFC, entitled 'Proteomic identification of human and animal *Mycobacterium avium* subsp. *paratuberculosis* (MAP) strains by mass spectrometry' was to compare protein profiles between *Map* strains isolated from animals with Johne's disease and humans with Crohn's disease using mass spectrometric (MS) techniques, with specific focus on surface and cell wall associated proteins.

Significant progress was made in understanding the surface exposed proteome of MAP and how to use it for the purpose of strain discrimination. The proteomic studies have provided a wealth of information on which the lab has built and will continue building future projects. A complete profile is being constructed of the cell envelope of MAP after hundreds of proteins have been discovered in the cell wall, cell membrane and on the cell surface (several manuscripts in preparation). The studies have identified members of the polymorphic PPE protein family of MAP on the surface of MAP strains. The corresponding gene of one of these proteins was the subject of a detailed study to use sequence differences in this gene to discriminate MAP strains from different sources. We succeeded in discriminating the three subtypes of MAP, type I, II and III. By adopting the denaturing gradient gel electrophoresis technique on these samples we succeeded for the first time in developing a rapid PCR based technique to visibly discriminate all known subtypes at once. The human isolates were shown to belong to the bovine subtype, type II (Griffiths et al., 2008). Two PPE proteins were also selected to be studied in more detail to confirm their surface localization and their immunogenicity (Newton et al., 2008). We have also engaged in a genomic comparison study of the PE and PPE genes in 4 members of the *M. avium* complex (Mackenzie et al., 2009) as preparation for an experimental study to use MAP unique proteins as targets in immune-diagnostic test for CD.

We have learned that the mass spectrometry data obtained from surface exposed proteins can indeed be used as a source of information to develop strain discrimination techniques. However, we have also observed that the differences in amino acid sequences in peptides obtained from different strains are too infrequent to allow efficient screening for strain differences by proteomic mass spectrometry approaches. Basically, the differences that were observed likely originated from random mutations in a few isolates and did not allow discriminatory strain typing. A considerable limitation in this study was the number of available and reliable human isolates. Our preliminary conclusion is that the genomic differences are too infrequent to be easily observable by the proposed techniques and will not lead to discrimination of human and animal isolates.

The lack of available human MAP isolates has urged us to start isolating MAP strains from human biopsies ourselves through our access to the Intestinal Inflammation Tissue Bank, an initiative funded by the chair of the CCFC, Dr. Keith Sharkey of the Faculty of Medicine. Currently biopsies from 45 CD patients, 15 UC and 15 healthy controls have been sampled. This has resulted in the detection of MAP in CD patients and the isolation of a small number of slow growing isolates. A paper describing these findings is in process. In the mean time, we have obtained a grant from the Calgary Laboratory Services to continue the study of CD biopsies and the development of diagnostic tools to identify MAP infection in CD patients.

In conclusion, objectives 1, 2 and 4 of the (originally 3 year) grant proposal were met to a great degree. And this project has provided us with extensive experience in proteomic analyses of the cell envelope of MAP.

Publications

1. Griffiths TA, Rioux K, **De Buck J** (2008) Sequence Polymorphisms in a surface PPE protein distinguish types I, II, and III of *Mycobacterium avium* subsp *paratuberculosis*. *Journal of Clinical Microbiology* 46: 1207-1212.
2. Mackenzie N, Alexander D, Turenne C, Behr M, **De Buck J** (2009) Genomic comparison of the PE and PPE genes of the *M. avium* complex. *Journal of Clinical Microbiology*. In press.
3. Newton, V., Mckenna, S. and **De Buck, J.** (2008) Immunogenicity of two surface exposed PPE proteins of *Mycobacterium avium* subsp. *paratuberculosis*. *Veterinary Microbiology*. In press
4. **De Buck, J.** (2008). Expression, surface localization and immunogenicity of two PPE proteins, 4th Annual JDIP Conference, Lansing, Michigan, US.
5. Griffiths, T., Rioux, K. & **De Buck, J.** (2007). Sequence polymorphisms in a surface exposed PPE protein of *Mycobacterium paratuberculosis*. 9th International Colloquium on paratuberculosis, Tsukuba, Japan.
6. **De Buck, J.** (2008) *Mycobacterium avium* subsp. *paratuberculosis* in biopsies from Calgary IBD patients, 1st Canadian Map researchers Meeting, Banff, Alberta, Canada.