Section 4 : Executive summary

The program OD1606 was to develop an understanding of bovine cellular immunology that could be applied to infections of cattle and used to establish strategies appropriate for the control of disease. The initial objective was to apply the techniques, reagents and tools developed to bovine respiratory disease but the findings would be equally applicable to other diseases such as tuberculosis. Dendritic cells are the only accessory cell accepted as being able to induce immune responses in naïve animals. The characterisation of these cells was therefore a major objective. FcR (receptors for antibody) target effector cells and link cellular and humoral immunity and their expression on antigen presenting cells can lead to up and down-regulation of immune responses.

The program has characterised the dendritic cells in the afferent lymph of cattle and established how different dendritic cells in afferent lymph handle different antigens. These studies have shown a new route of uptake of antigen used by dendritic cells. The function of different T cell populations in vivo and in vitro has been investigated in particular how the WC1+ γδTCR+ T cells are activated. A number of FcR for IgG on cattle cells have been cloned for the first time and the FcR binding cattle IgG2 shown to represent a new class of mammalian IgG binding FcR.

The program OD1609 followed on from OD1606 and is in line with MAFF policy aimed at the control of *Mycobacterium bovis* infection in cattle. In particular it was to establish the methodology to determine how the bacterium affects antigen presenting cell (APC) function and to provide data relevant to the design of effective vaccination strategies. The project has focussed on the biological role of the antigen presenting cell in cattle and their role in the regulation of cell mediated immunity to intracellular pathogens, the interaction of *M. bovis* with monocytes and the pathogenesis of disease, markers of the type of T cell response.

An investigation of the association of cattle IgG isotype and a type 1 or type 2 cytokine bias established that although the adjuvant influences the cytokine bias of responding immune T cells this did not correlate with IL-4 or IFNγ synthesis. Thus there is no justification for analysing IgG isotypes in cattle – a difference from the mouse model.

The quantitative real time PCR using the TaqMan has been established for a range of cattle cytokines. RNA production was related to IL-4 and IFNγ protein assayed using ELISA or bioassay. This technology will enable us to investigate the cytokines synthesised by APC and T cells following exposure to *M. bovis* or its antigens and relate the type of immune response to resistance to infection.

Techniques for culturing dendritic cells from cattle monocytes were used to show that the cells became infected with *M. bovis* and that infected cells were effective in inducing T cell responses. Techniques for the culture of dendritic cells from bone marrow have also been developed. These will be exploited to aid the generation of T cell lines and clones and to examine how mycobacteria affect APC and consequently affect the type of T cell response induced. This will involve an analysis determining how surface antigen expression is affected by infection with mycobacteria and what cytokines are synthesised by the APC. Infection with environmental mycobacteria has been show to be the norm in conventionally reared cattle. This will affect the immune response to vaccination and investigations are proposed to determine ways in which vaccination strategies can be modified to induce effective resistance to infection.

Thus, the objective listed at the beginning of this program have been met and techniques and base line data established that we can build on in future proposed studies of M. bovis infection of cattle. These are the subject of proposal XF0301

This focuses on understanding of the key events in the pathogenesis of the disease in cattle and the nature of the protective immune responses that would allow targeted approaches to be applied to the identification of bacterial molecules involved in virulence and immunity and hence enhance the likelihood of developing an effective vaccine. The main components are

(ii) Identification of the T cell responses that are required for immunity: The findings will facilitate selective screening of antigens for use in sub-unit vaccines and indicate the type of vaccine formulation that will be required. Immunological correlates of immunity identified in this work will also be of value for monitoring responses in vaccine trials.

(iii) Clarification of the role of environmental mycobacteria in interference with vaccine responses to *M. bovis*: This issue needs to be resolved to determine whether or not vaccines need to be designed to circumvent such interference.

(iv) Investigation of cytokine expression in DNA vaccines as a means of enhancing vaccine efficacy: Selected cytokines have the potential to bias the immune responses induced by DNA vaccines towards those responses that are required for immunity.

The proposed experiments are consistent with the requirements defined in the MAFF Animal Health and Welfare Research Requirements Document