P.O.R. ABRUZZO – OBBIETTIVO 3 PER IL 2000/2006
PROTOCOLLO DI INTESA TRA REGIONE ABRUZZO,
COMITATO DI COORDINAMENTO REGIONALE DELLE UNIVERSITA' ABRUZZESI
E
UFFICIO SCOLASTICO REGIONALE
PER L'ATTUAZIONE DEL MACROPROGETTO
INNOVAZIONE, COMPETITIVITÀ, GOVERNANCE
(PROGETTO REGIONALE FORMAZIONE TECNICO SCIENTIFICA
E
PROGETTO IN_CO: AZIONI INTEGRATE PER LO SVILUPPO DI
"INTERMEDIARI DELLA CONOSCENZA TECNOLOGICA, ORGANIZZATIVA E GESTIONALE")
"ASSEGNI REGIONALI PER ATTIVITÀ DI RICERCA E ALTA FORMAZIONE " IN MATERIE TECNICO SCIENTIFICHE, INTERVENTO IC4E – SOTTO - UNIVERSITÀ DEGLI STUDI DI TERAMO-

ASSEGNISTA DI RICERCA:
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Nome istituzione a cui afferisce laboratorio ospitante:

Nome e qualifica del responsabile del laboratorio ospitante:

Durata soggiorno laboratorio ospitante:
N. 1 trimestre

Work Plan:

The Major Outer Membrane Protein (MOMP) of *Chlamydia/Chlamydophila* is a 40 kDa protein that comprises approximately 60% of the total outer membrane protein content (Caldwell *et al*., 1981). It is strongly immunogenic is regarded as a candidate antigen for protective vaccines (Brunham and Rey Ladino, 2005). Immunity to *Chalmydophila abortus* infection has been shown to be conferred by a 110 kDa oligomeric (probably trimeric) form of MOMP (De Sa *et al*., 1995). Bacterial expression of recombinant MOMP has been problematic. The full length protein appears to be toxic for *E. coli* (Koehler *et al*., 1992; Manning and Stewart, 1993). Therefore, alternative expression systems need to be explored. The baculovirus expression vector system (BEVS) is one such system. BEVS is a versatile and powerful eukaryotic expression system for recombinant proteins (Smith *et al*., 1983). The advantage of a eukaryotic system such as BEVS is that it permits the folding, post-transcriptional modification and assembly of the recombinant expressed protein, preserving conformational epitopes that may be important for immune recognition.

Both humoral and cell-mediated immunity (CMI) play a role in host protection to *C. abortus* infection in sheep (Entrican and Wheelhouse, 2006). The intracellular habitat of the bacterium suggests that CMI is of primary importance, which has been confirmed by numerous studies. Experimental chlamydial infection of mice has revealed that protection is conferred by MHC class II-restricted $CD4^{+ve}$ T cells, and that this mechanism predominates over $CD8^{+ve}$ T cells and antibody (Sue and Caldwell, 1995). However, protection correlates with the production of IFN-γ by either $CD4^{+ve}$ or $CD8^{+ve}$ T cells (Igietseme, 1996). IFN-γ mediates protection by limiting the availability of essential nutrients such as tryptophan and iron, or by the generation of toxic compounds such as nitric oxide that restrict chlamydial growth (Rottenberg *et al*., 2002).

The aim of this project is to determine if sheep that are immune to *C. abortus* can respond to baculovirus-expressed recombinant *C. abortus* MOMP (Bac-rMOMP). The Bac-rMOMP system

was developed at the University of Teramo and has been described previously (Meridiani *et al*. 2006). Cell-mediated immune responses in sheep are studied routinely at the Moredun Research Institute and a variety of techniques are available (Wattegedera *et al*., 2004). For the purposes of this study, three assays will be performed:

1. The lymphocyte transformation test (LTT) will be performed to evaluate lymphocyte proliferation in response to Bac-rMOMP stimulation. Immune cells will be derived from Enzovax-vaccinated sheep, cells from non-vaccinated naïve sheep will serve as negative controls. Cells will be cultured with with medium only (baseline control), Bac-rMOMP (test antigen), an irrelevant recombinant protein expressed in BEVS (negative control antigen), concanavalin A (positive control mitogen) and with heat- or UV-inactivated *C. abortus* elementary bodies (positive control antigen). Cells will be cultured for 96 h, ^{3}H thymidine will then be added and the plates incubated for a further 18 h before being harvested and proliferation measured as counts per minute as determined in a beta scintillation counter.

2. Culture supernatants will be harvested at 96 h from LTT plates as described above and tested for the presence of IFN-γ by ELISA.

3. The phenotype of the responding cells in the LTT will be evaluated by CFSE labelling and surface staining for T cell markers.

Outputs

This project will unite the expertise of two laboratories to advance the development of strategies to control chlamydial abortion in sheep.

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