Marie Curie International Reintegration Grants (IRG)

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THE CONTRIBUTION OF THE EXOSPORIUM OF *BACILLUS ANTHRACIS* TO SURVIVAL

The exosporium is not unique to *Bacillus anthracis* and is present in all members of the *B.cereus* group and some clostridia. The function of this complex structure plays in the survival of these spores, some of which are pathogens, is as yet unknown.

The research objectives of the proposal are as follows;

1. Determine the role the exosporium plays in the environmental survival of *B.anthracis*
2. Determine the contribution of the exosporium to biocide resistance
3. In collaboration with colleagues in the US we will determine the contribution of the exosporium to virulence and *in vivo* germination

Progress at 18th months

With support from the Marie Curie fellowship a fully functional research laboratory compliant with all UK national safety and security regulation has been established at Cardiff university to determine the contribution of the spore exosporium to the biology of *Bacillus anthracis*. To our knowledge this facility represents the only non-government laboratory approved in the UK to work with this organism. In preliminary studies we have characterized the properties of wildtype spores and have determined their ability to germinate in response to previously described germination triggers both *in vitro* and in soil microcosms. These results will be compared to those obtained with spores which lack an exosporium either as a consequence of physical disruption (sonication) or mutation. While sonicated spores have been generated, efforts to isolate enzyme specific mutants have been hampered by regulatory and technical problems which are in the process of being resolved. As a risk reduction strategy we have pursued a parallel non-specific mutation approach based on mitomycin C and phenotypic screening assays. A notable success to date has been the development of a fully defined minimal media capable of supporting the growth of *B.anthracis*.

To determine the contribution of the exosporium to biocide resistance we have assayed the sensitivity of wildtype *B.anthracis* spores to a range of commercially available biocides. In preliminary studies sonicated spores were found to be considerably more sensitivity to Chlorox (a chlorine releasing biocide) than their wildtype counterparts suggesting that exosporium contributes to resistance by acting as a physical barrier and a non-specific, passive, target which reacts with the biocide before it has the chance to reach essential molecules located in the spore core. The sensitivity of exosporium deficient spores to other biocides is currently being determined.

While the majority of the effort to date has focused on characterising survival in the environment and resistance to biocides, studies have been undertaken to

characterise the intracellular survival of of *B.anthracis* spores following uptake by macrophages. This work, performed in collaboration with US colleagues, has highlighted the key role that bacterial arginase plays in modulating the innate immune response of infected cells. In addition to strengthening links forged with US colleagues this work has yielded a manuscript which has been accepted by the journal Current Microbiology.

Impact

The exosporium is not unique to *B.anthracis* and is present in all members of the *B.cereus* group and some clostridia. The function this complex structure plays in the survival of these spores, some of which are pathogens, is as yet unknown. By determining the contribution of the exosporium to environmental and *in vivo* survival we will have a better understand of the role of this neglected structure. This work will also provide data to facilitate the design of disinfection technologies better able to deal with the treat posed by exosporium expressing spore formers such as *B.anthracis* and *Clostridium difficile*. Finally by characterizing the contribution that the exosporium makes to the intracellular survival of *B.anthracis* we will be able to design therapeutic modalities with which to treat individuals exposed to spore former organisms.

The support of the Marie Curie fellowship has played a key role in enabling the researcher to establish a UK based research effort and has been instrumental in securing research contracts from UK Government Departments to support additional *B.anthracis* related research projects. In addition it has enabled Dr Baillie to expand his research efforts to include work on *C.difficile*, an organism responsible for considerable morbidity and mortality amongst hospital patients in the UK, which forms a spore structurally similar to that of *B.anthracis*. A PhD studentship supervised by Dr Baillie has been established in collaboration the universities medical school which will draw on the parallels between the two organisms and will identify ways in which biocides could be used more effectively to prevent the patient to patient spread of this pathogen