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Short title: TRYPANOSOME RESISTANCE BIOLOGY

Genomics Approaches to Study the Biology Underlying Resistance to Trypanosomiasis - Some Unexpected Lessons

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Abstract: An international multidisciplinary consortium is conducting a programme of research on the host response to trypanosome infection. This builds upon quantitative trait loci (QTL) mapping which identified genome regions influencing susceptibility to pathology following *T. congolense* infection in both cattle and mice. The approach uses expression analysis to examine the response of both susceptible and resistant strains and a series of novel informatics tools to identify pathways which are activated as a result of challenge, and those which are differentially used by resistant and susceptible strains. Of particular interest are those pathways which simultaneously satisfy both criteria, i.e. are significantly differentially activated and contain genes within QTL regions. However, it is important to stress that it is not required that the genes within the QTL region are differentially expressed themselves.

Tsetse fly transmitted trypanosmiasis is of enormous economic importance; it is responsible for the exclusion of livestock from vast tracts of West and Central Africa. Closely related species of trypanosomes cause sleeping sickness in humans, which is of great, and increasing, public health significance. Resistance to infection has been described in several breeds of cattle, especially the N'Dama, and in mouse models. Following infection with *Trypanosoma congolense,* both resistant and susceptible cattle breeds become infected and show initial anaemia, but the resistant genotype quickly recovers and continues to grow while the susceptible animals show increasingly severe anaemia, cachexia and eventually death. The total cost to Africa has been estimated to exceed $1 billion per annum [1].

Quantitative trait loci (QTL) affecting resistance in both the mouse and cow have been mapped [2-4] and we are following this with an expression analysis. In the study of host resistance and susceptibility, one very important challenge in the analysis of microarray data (or indeed, any phenotype data) is distinguishing relevant from irrelevant differences, and differences that are consequences of pathology from those which are causes of pathology. There are many clear differences in the biology of different animal strains, and there are many differences between healthy and sick animals. The challenge is to use objective unbiased methods to analyse expression data in order to extract the causative changes. We have developed some novel methodologies for this [5].

The comparison of murine and bovine systems has proven to be highly informative. In particular, the use of congenic mouse lines in which fragments of resistant genome surrounding the QTL have been placed on a susceptible background has proven to be a powerful means of studying the action of QTL, and several trypanotolerance QTL co-localize to QTL involved in a range of other traits. There are a number of cases in which genes of susceptible origin show an altered pattern of expression as a result of the presence of a distant gene of resistant origin. For example, a structural polymorphism within Daxx within the QTL was correlated with altered expression of p53, which is regulated by Daxx and is on a different chromosome.

We have identified the genes that are most significantly differentially expressed over an infection time course and examined the pathways in which they are involved. This has proven to be a highly informative approach and there are strong indications that survival or death is a result of differential use of few very generic pathways. In all cases, there are candidate genes within QTL involved in these pathways, many of which are not differentially expressed themselves. There is strong crosstalk between pathways which are normally considered as separate entities. This is mediated by a small number of key molecules which are highly connected and represent critical points through which a wide range of responses may be modulated.

The power such systems-based approaches to array data is that they can generate findings of relevance well beyond the system being investigated. The results of our trypanosomiasis studies illustrate this, having relevance for human survival following sepsis and many other forms of stress, and suggesting common mechanisms of adaptation to disease resistance across mammalian species.

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