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Resistance and susceptibility to a protozoan parasite of cattle—Gene expression differences in macrophages from different breeds of cattle

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Abstract

Cattle infected with the tick-borne protozoan, *Theileria annulata*, usually undergo severe morbidity, and mortality ensues in a high proportion of animals. However, we have shown that a *Bos indicus* breed, the Sahiwal, which originates in a *T. annulata* endemic area, is more resistant to the parasite. Although Sahiwals become infected, the breed exhibits fewer clinical signs and recovers from a dose of parasite which is fatal in the Holstein *B. taurus* breed. The Sahiwals have a significantly lower fever response, and lower levels of parasite than the Holsteins. One unusual feature of this disease is the production of acute phase proteins (APP), indicating that the parasite induces high systemic levels of pro-inflammatory cytokines. In the Holsteins there is prolonged production of the APP, α_1-glycoprotein, which, in contrast, is only slightly elevated in the Sahiwals. As the parasite infects macrophages (mϕ), our hypothesis is that the Sahiwals can control the excessive production of pro-inflammatory cytokines in response to infection, and that this control is expressed at the level of the mϕ.

We thus reasoned that the genes underlying the observed difference in resistance to tropical theileriosis, might be identified by investigating gene expression differences in mϕ from both breeds. It is possible that relevant polymorphisms might in themselves result in gene expression differences, so initially we targeted likely candidates. However, we detected no differences in expression of the pro-inflammatory cytokines, tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β) or IL-6, in infected mϕ. As it is more likely that polymorphisms in candidate genes influence the expression of other genes involved in interrelated pathways, we undertook a more global approach. We designed a bovine mϕ specific cDNA microarray, which contains representatives of 5000 different genes expressed in mϕ, and investigated the transcriptional responses of mϕ from both breeds in response to a variety of stimuli, including infection with *T. annulata*. Our results indicate that there are fundamental differences in gene expression in mϕ from both breeds in the way they respond to infection, and even in their pre-infection resting state.

Keywords: *Theileria annulata*; Microarray; Macrophage; Host–pathogen interactions

1. Introduction

The cattle disease, tropical theileriosis, imposes a huge burden on the countries where the causative agent, *Theileria annulata*, is endemic. This protozoan parasite is carried by ticks of the species *Hyalomma*, most commonly *H. anatolicum*, and prevalence of the disease reflects the geographical distribution of these ticks, which extends over a wide region of the World from countries bordering the Mediterranean Sea eastwards to China. It is estimated that over 250 million cattle are at risk in these areas from infection with *T. annulata*, which causes an extremely debilitating and often fatal

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disease, especially in non-indigenous cattle imported to improve productivity (reviewed by Preston et al., 1999; Glass, 2001). Even in sub-clinical cases, tropical theileriosis significantly reduces performance (Gharbi et al., 2006) and this together with the high mortality rate inflicts a huge financial cost, e.g. $384.3 million per annum in India alone (Minjauw and McLeod, 2003).

Cell based vaccines and chemical controls exist (Pipano and Shkap, 2000; Jongejan and Uilenberg, 2004), but have not resulted in eradication of this threat to livestock farming. Furthermore, climate change and increases in global trade may lead to expansion of the habitat of the ticks responsible for spreading this infection. In addition, eradication is unlikely to be possible as there are wild-life reservoirs for the parasite and tick host. Thus, new approaches are needed and these could include breeding for disease resistance. Although non-indigenous cattle (e.g. *Bos taurus*, Holstein) often succumb to *T. annulata* infection, we have shown that a *Bos indicus* breed, the Sahiwal, which originates in a *T. annulata* endemic area, is more resistant to the parasite (Glass et al., 2005). In India, breeding for disease resistance has been adopted as national policy (Minjauw and McLeod, 2003) and anecdotal evidence has suggested that reducing the genetic contribution of imported breeds such as the Holstein in Indian dairy cattle is resulting in reduced incidence of tropical theileriosis.

Identifying markers or genes underlying resistance to this economically important disease would provide a means of selecting resistant animals in a more targeted and efficient way, and could provide an environmentally sound and sustainable approach to reducing the disease. The rapidly increasing genomic resources and information for both the host (Williams, 2004; Womack, 2006) and parasite (Pain et al., 2005; Shiels et al., 2006), can now be harnessed to identify genes and pathways involved in host–pathogen interactions and in disease resistance in an unprecedented manner (Glass and Coussens, 2005). The bovine genome has been sequenced to an eight times coverage (Womack, 2006; see http://hgsc.bcm.tmc.edu/projects/bovine/). In addition, there is now a comprehensive tool kit of bovine genomic resources including genetic and radiation hybrid maps, a whole genome physical map comprising a BAC minimum tiling path, sequence information with nearly one million expressed sequence tags (ESTs), and 2.4 million putative single nucleotide polymorphism (SNP) markers (Williams, 2004) and several SNP genotyping arrays were made available for cattle during the latter part of 2005. A variety of expression microarrays have now been constructed that are suitable for investigating the transcriptome of the bovine immune response (reviewed by McGuire and Glass, 2005). These range from small focussed microarrays such as the bovine immune-endocrine cDNA microarray which represents 167 genes (Tao et al., 2004), to larger microarrays such as our bovine macrophage (mϕ) specific cDNA microarray which represents over 5000 genes (Jensen et al., 2006b), to the more global non-immune specific microarrays, for example the bovine genome array from Affymetrix. For the parasite side of the equation, the *T. annulata* genome has been sequenced (Pain et al., 2005) and the sequences are being mined for genes that potentially could manipulate the host genome (Shiels et al., 2006).

In this paper, we review our current understanding of the immunopathology of the host following *T. annulata* infection, contrasting the responses by susceptible and tolerant breeds. In particular, we concentrate on the consequences of parasite infection of host mϕ and ask what role these cells and the cytokines they produce may play in the genetic resistance to *T. annulata*. Our overarching hypothesis is that gene expression differences in mϕ may be the key to breed specific resistance.

2. Host-parasite interactions

T. annulata has a complex life cycle, involving both the *Hyalomma* tick host as well as the bovine host, but here we will concentrate on the stages that induce pathology in cattle i.e. sporozoites and schizonts (reviewed by Preston et al., 1999; Glass, 2001). Following a tick bite, *T. annulata* sporozoites travel to the draining lymph node, where they predominantly infect *in situ* mϕ in the medulla of the draining lymph node (Glass et al., 1989; Campbell et al., 1995; Campbell and Spooner, 1999). The parasite ‘‘hides’’ from the immune system within these mϕ and modulates its host gene expression programme to facilitate multiplication of the parasite. *T. annulata* sporozoites differentiate through a trophozoite stage to the intracellular form, the schizont which exists apparently ‘‘free’’ in the cytoplasm (Fig. 1a). Following differentiation to the schizont stage, the parasite induces transformation and proliferation of infected cells as evinced by the staining for Ki-67 in infected cells in the lymph node draining the tick attachment site of a cow infected with *T. annulata* (Fig. 1b). The transformation of the host cell is reversible and depends on the presence of live parasite within the cell (Sager et al., 1997). Infection by *T. annulata* also alters the phenotype and function of bovine myeloid cells (Glass and Spooner, 1990; Sager et al., 1997), although direct comparisons