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SnSAG5 is an alternative surface antigen of *Sarcocystis neurona* strains that is mutually exclusive to SnSAG1☆

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Abstract

Sarcocystis neurona is an obligate intracellular parasite that causes equine protozoal myeloencephalitis (EPM). Previous work has identified a gene family of paralogous surface antigens in *S. neurona* called SnSAGs. These surface proteins are immunogenic in their host animals, and are therefore candidate molecules for development of diagnostics and vaccines. However, SnSAG diversity exists in strains of *S. neurona*, including the absence of the major surface antigen gene *SnSAG1*. Instead, sequence for an alternative SnSAG has been revealed in two of the SnSAG1-deficient strains. Herein, we present data characterizing this new surface protein, which we have designated SnSAG5. The results indicated that the protein encoded by the *SnSAG5* sequence is indeed a surface-associated molecule that has characteristics consistent with the other SAGs identified in *S. neurona* and related parasites. Importantly, Western blot analyses of a collection of *S. neurona* strains demonstrated that 6 of 13 parasite isolates express SnSAG5 as a dominant surface protein instead of SnSAG1. Conversely, SnSAG5 was not detected in SnSAG1-positive strains. One strain, which was isolated from the brain of a sea otter, did not express either SnSAG1 or SnSAG5. Genetic analysis with *SnSAG5*-specific primers confirmed the presence of the *SnSAG5* gene in Western blot-positive strains, while also suggesting the presence of a novel *SnSAG* sequence in the SnSAG1-deficient, SnSAG5-deficient otter isolate. The findings provide further indication of *S. neurona* strain diversity, which has implications for diagnostic testing and development of vaccines against EPM as well as the population biology of *Sarcocystis* cycling in the opossum definitive host.

Keywords: Equine protozoal myeloencephalitis; Sarcocystis; Diagnosis; Vaccine; Apicomplexa; Coccidia; Strain diversity

1. Introduction

Sarcocystis neurona is an apicomplexan parasite that is known to be the primary cause of equine protozoal myeloencephalitis (EPM), a prominent neurologic disease in horses of North, Central, and South America. The natural life cycle of *S. neurona* includes the

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opossum definitive host (Fenger et al., 1995) and a variety of small mammal intermediate hosts. In addition, viable *S. neurona* have been isolated from sea otters with neurologic disease (Lindsay et al., 2001; Miller et al., 2001; Thomas et al., 2007). Numerous serologic surveys have shown that the exposure rate of North American horses to *S. neurona* is quite high, but EPM has been estimated to occur in less than 1% of the equine population (MacKay et al., 2000). While it is apparent that infection of horses does not always result in neurological disease, it remains unclear what factors influence the relationship between infection with *S. neurona* and the occurrence of clinical disease.

A gene family of paralogous surface antigens (SnSAGs) has been described in *S. neurona* (Howe et al., 2005). These antigens, which were initially identified from a database of *S. neurona* expressed sequence tags (ESTs) (Howe, 2001; Li et al., 2003), are localized to the surface of the merozoite stage and are also present throughout intracellular development of the *S. neurona* schizont. Much like the TgSAG and TgSRS surface antigen orthologues that were characterized in *Toxoplasma gondii* (Lekutis et al., 2001), these *S. neurona* surface proteins produce strong immune responses in infected animals (Ellison et al., 2002; Hoane et al., 2005; Howe et al., 2005; Liang et al., 1998) and are therefore candidate molecules for development of serologic assays and vaccines for EPM. Interestingly, strain-specific variation in the SnSAGs has been observed among 14 *S. neurona* strains that included 8 EPM isolates from a variety of geographic locations (Howe et al., 2008). Of particular significance, the *SnSAG1* locus, which is highly transcribed in the UCD1 and SN3 strains of *S. neurona* (Ellison et al., 2002; Howe et al., 2005), was shown by immunologic and genetic analyses to be absent from seven of the 14 *S. neurona* strains. Importantly, the extensive EST data for other genetic loci (*SnSAG2* and *SnSAG3*, for example) revealed few sequence differences between the SnSAG1-positive strain SN3 and the SnSAG1-deficient strain SN4, thus implying that these are the same species of *Sarcocystis*. These findings have obvious ramifications for development of EPM diagnostics and protective immunization. As well, the genetic variations observed in the strains that were tested may have interesting implications regarding genetic and phenotypic heterogeneity in the *S. neurona* population circulating in nature.

Examination of the *Sarcocystis* EST database suggests the presence of several additional SnSAG paralogues in the SnSAG1-minus SN4 strain of *S. neurona*. In particular, dbEST sequence accession CO748328 from strain SN4 exhibited 60% and 34% sequence similarity to SnSAG1 and SnSAG4, respectively (Howe et al., 2008), and was nearly 100% identical to a putative SnSAG sequence that had been amplified from the SnSAG1-deficient SN-MU1 strain of *S. neurona* (Accession No. AAP72018) (Hyun et al., 2003). The study presented herein characterized the protein encoded by the putative SnSAG sequence, which we have designated *SnSAG5*. Notably, both protein and genetic analyses of a collection of parasite isolates demonstrated that the *SnSAG5* and *SnSAG1* loci are mutually exclusive of one another in individual strains of *S. neurona*.

2. Materials and methods

2.1. Parasite cultures

Parasite cultures were maintained by successive passage in bovine turbinate cells, and extracellular merozoites were harvested for analyses, as described previously (Howe et al., 2005). The 13 strains of *S. neurona* examined in this study are listed in Table 1 and were described previously in detail (Howe et al., 2008).

2.2. SnSAG gene cloning and sequence analysis

A tentative consensus (TC) sequence of the putative SnSAG from strain SN4 was obtained from the Dana Farber Cancer Institute's *S. neurona* Gene Index (SnGI) (Quackenbush et al., 2001), which is a resource for analyses of sequence assemblies comprised of the *S. neurona* EST sequence data and cDNAs identified by traditional approaches. Based on the sequence assembly

Table 1
Strains of *Sarcocystis neurona* analyzed in this study

Strain	Animal
SN2	EPM Horse
SN3	EPM Horse
SN4	EPM Horse
SN5	EPM Horse
SN-UK3	EPM Horse
SN6	EPM Horse
SN-VT1[a]	EPM Horse
SN7	EPM Horse
SN-MU1	EPM Horse
SN-OT1	Myeloencephalitic Otter
SN138[b]	Raccoon
SN744[b]	Raccoon
SN-CAT2	Cat

[a] Single-cell clone of strain SN6.
[b] Independent cultures of strain SN37R (Sofaly et al., 2002).