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Localised mitogenic activity in horses following infection with *Streptococcus equi*

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ABSTRACT

Streptococcus equi subspecies *equi* (*S. equi*) is the causative agent of strangles, a highly contagious upper respiratory disease of equids. *Streptococcus equi* produces superantigens (sAgs), which are thought to contribute to strangles pathogenicity through non-specific T-cell activation and pro-inflammatory response. *Streptococcus equi* infection induces abscesses in the lymph nodes of the head and neck. In some individuals, some abscess material remains into the guttural pouch and inspissates over time to form chondroids which can harbour live *S. equi*. The aim of this study was to determine the sites of sAg production during infection and therefore improve our understanding of their role. Abscess material, chondroids and serum collected from Equidae with signs of strangles were tested in mitogenic assays. Mitogenic sAg activity was only detected in abscess material and chondroids. Our data support the localised *in vivo* activity of sAg during both acute and carrier phases of *S. equi* infection.

Strangles is a highly contagious upper respiratory tract infection of equids caused by *Streptococcus equi* subspecies *equi* (*S. equi*) (Slater, 2007). Clinical signs of disease include fever, mucopurulent nasal discharge, and swollen/enlarged lymph nodes in the neck (Waller et al., 2011). *Streptococcus equi* produces four bacterial superantigens (sAgs): SeeH, SeeI, SeeL and SeeM (Anzai et al., 1999; Artiushin et al., 2002; Paillot et al., 2010b; Proft et al., 2003b). Superantigens are bacterial toxins that disrupt immune responses through non-specific T-cell proliferation and overzealous cytokine production (Fraser and Proft, 2008; Sriskandan et al., 2007). Superantigens are thought to play an important role in the pathogenesis of strangles (Holden et al., 2009; Paillot et al., 2010b; Timoney, 2004). This study aimed to quantify the mitogenic activity of abscess material, chondroid and serum recovered from horses infected with *S. equi* to increase understanding of the role played by these pathogenic factors and their localisation during the acute and persistent stages of infection.

After infection, *S. equi* rapidly translocates to the submandibular and retropharyngeal lymph nodes (RPLN) (Timoney, 2004; Timoney and Kumar, 2008; Waller et al., 2011). Mitogenic activity was measured in lymph node abscess materials from equids that were naturally (N-01 to N-07) or experimentally infected with *S. equi* (E-01 to E-08) (Supplementary technical data), as previously described (Paillot et al., 2010a, 2010b). Experimentally infected ponies were challenged for reasons unrelated to the current study in a controlled strangles challenge model with the *S. equi* strain 4047 (*Se*4047) encoding the sAgs SeeH, SeeI, SeeL and SeeM. Abscess materials from both experimentally and naturally infected sample groups elicited a significant dose dependent increase in PBMC proliferation, with the exception of N07 (Fig. 1A and B). The sAg-dependent mitogenic activity of *Se*4047 has previously been described (Paillot et al., 2010b). All mitogenic samples purified from suspected natural cases of strangles were associated with *S. equi* strains (Supplementary Table S1), which possessed all four *S. equi* sAgs, but none of the *S. zooepidemicus* sAgs (*szeN*, *szeF* and *szeP*) as confirmed by multi-locus sequence typing (MLST) and PCR, respectively (Paillot et al., 2010a, 2010b; Webb et al., 2008). Therefore, the presence of mitogenic factors in abscess material is likely to be associated with the production of *S. equi* sAgs *in vivo* during infection. The level of proliferation was variable between the samples tested and the PBMC donors. PBMC proliferation remained low, which could be explained by limited production of sAgs, their degradation in the abscess material, and/or the cytotoxicity of some of the samples tested.

Xu et al., (2014) have recently suggested that *Staphylococcus aureus* (*S. aureus*) may use sAgs to subvert the neutrophil response into a protective niche. *In vivo* production of the sAg SEA increased *S. aureus* persistence in the liver by promoting hepatic abscess formation in the mouse (Xu et al., 2014). This mechanism involved the synthesis of pro-inflammatory cytokines and neutrophil recruitment. The formation of abscesses (*i.e.* within a fibrin pseudocapsule) is an essential immune process to limit dissemination of the pathogen in the host. However, it can also provide a physical barrier to protect the bacteria from immune cells (Cheng et al., 2011), especially when

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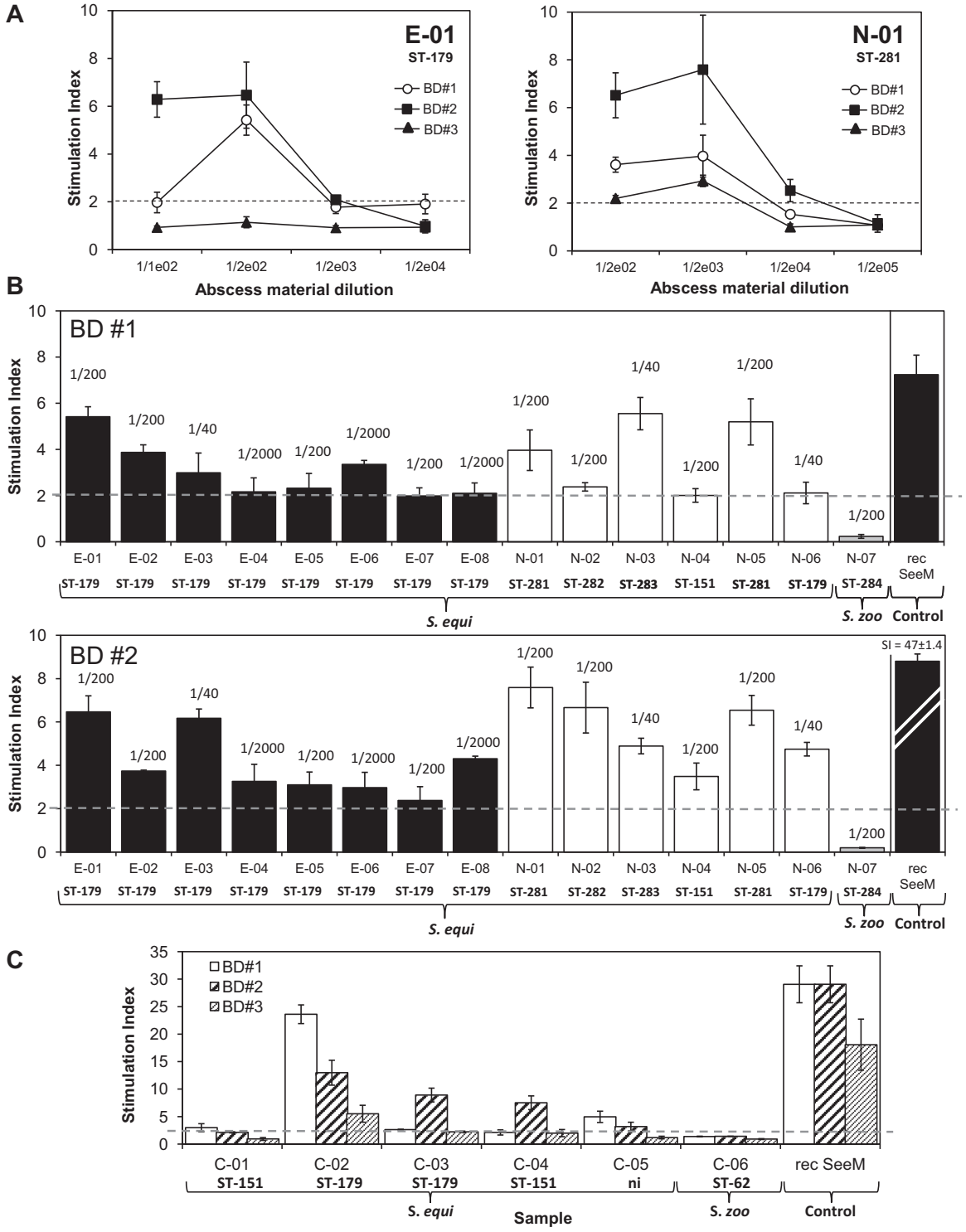

Fig. 1. Abscess material and chondroid elicits mitogenic activity *in vitro*. Example of dose dependent proliferative response obtained with E-01 and N-01 (A), overall abscess extracts (B) and chondroid extracts (C). Results are presented as stimulation index (SI), with the negative control (PBMC culture in medium alone) SI = 1 (not shown) and non-mitogenic response <2. Samples beginning with 'E' are cases which were experimentally infected with *S. equi*. 'N' denotes diagnostic samples from naturally infected horses. The recombinant sAg SeeM (0.125 μg/ml) was used as a positive control (SeeM stimulation index measured during the evaluation of E-01 and C-01 is presented in B and C as an example, respectively). ST indicates the sequence type as determined by MLST. The dashed line represents a threshold at which a proliferation result is considered significant (SI ≥ 2). (B) Abscess material mitogenicity was dose dependent with high concentrations regularly associated with cell cytotoxicity. The lowest dilution (indicated above the relevant bar) without signs of cytotoxicity is shown. Chondroid samples were diluted 1:40 in PBS. Results are representative of results obtained from up to six blood donors (BD#).

combined with pathogen-induced immuno-modulatory mechanisms that impair neutrophil functions, promoting bacteria survival. This novel role for sAgs is compatible with the characteristic abscessation associated with *S. equi* infection and the presence of mitogenic factors in the *S. equi* abscess materials tested here. This is also further supported by the significant association between cases of non-strangles lymph node abscess induced by *S. zooepidemicus* and the presence of sAg genes (Rash et al., 2014).