INFECTION AND IMMUNITY, Mar. 2006, p. 1941–1943 Vol. 74, No. 3
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.3.1941–1943.2006

Synthesis of Syndecan-1 by Skeletal Muscle Cells Is an Early Response to Infection with *Trichinella spiralis* but Is Not Essential for Nurse Cell Development

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Received 29 July 2005/Returned for modification 23 August 2005/Accepted 23 November 2005

Trichinella spiralis **creates a unique intracellular habitat in striated muscle. We report that a proteoglycan, syndecan-1, is induced early in infection yet is not essential for habitat development and exerts a modest influence on the immune response. This report is the first to address the requirement for a specific muscle protein in trichinellosis by using mice deficient in the relevant gene.**

Muscle infection by *Trichinella spiralis* is marked by a series of dramatic morphological and biochemical changes in the host cell, producing a structure referred to as a nurse cell (14). The molecular mechanisms that regulate these complex cellular changes are largely unknown, and protein markers for induction of the infected-cell phenotype are limited (9). We have found that syndecan-1, a heparan sulfate (HS)-bearing proteoglycan (HSPG), is produced early in infection and is retained in the cytoplasm rather than being transported to the surface of the nurse cell. Syndecan-1 binds to fibroblast growth factors (7), inhibits myoblast differentiation (11), and anchors cells to the extracellular matrix (17). In addition, syndecan-1 modulates immune responses, as demonstrated in a model of lung allergy induced by an *Aspergillus* antigen (18). Similarly, HSPGs have been implicated in the control of myeloid cell proliferation in granulomas induced by *Schistosoma mansoni* (1, 8).

Our observation that infected cells produce syndecan-1, together with the knowledge that syndecan-1 binds to collagen types IV and VI (16) (components of the nurse cell capsule [13]), prompted us to hypothesize that syndecan-1 would be influential in parasite development or host cell transformation. To test this hypothesis, 8-week-old C57BL/6J (wild-type [WT]) mice (Jackson Laboratory, Bar Harbor, ME) and syndecan-1-deficient ($sdc1^{-/-}$) mice (Baylor College of Medicine, Houston, TX) were infected intravenously with 25,000 newborn larvae. Parasite preparation and synchronous infections were carried out as described previously (3). At 5, 10, 22, and 51 days postinfection (dpi), mice were killed, and histological sections of tongues were prepared and stained with a rat monoclonal antibody to syndecan-1 (clone 281-2; BD Pharmingen, San Diego, CA) using the ABC Vectastain Elite kit (Vector Labs, Burlingame, CA) (3). Syndecan-1 was evident as early as 5 dpi (Fig. 1A), remained detectable into the chronic stage of infection (Fig. 1D), and localized to the cytoplasm of infected cells. Infected tongues from $sdc1^{-/-}$ mice did not stain with an antibody to syndecan-1 (Fig. 1E), nor did uninfected WT muscle cells. Despite prolonged production of syndecan-1 by infected cells, we found that *T. spiralis* infection progressed normally in mice lacking the proteoglycan. Larvae invaded cells and developed normally in $sdc1^{-/-}$ mice, as ev-

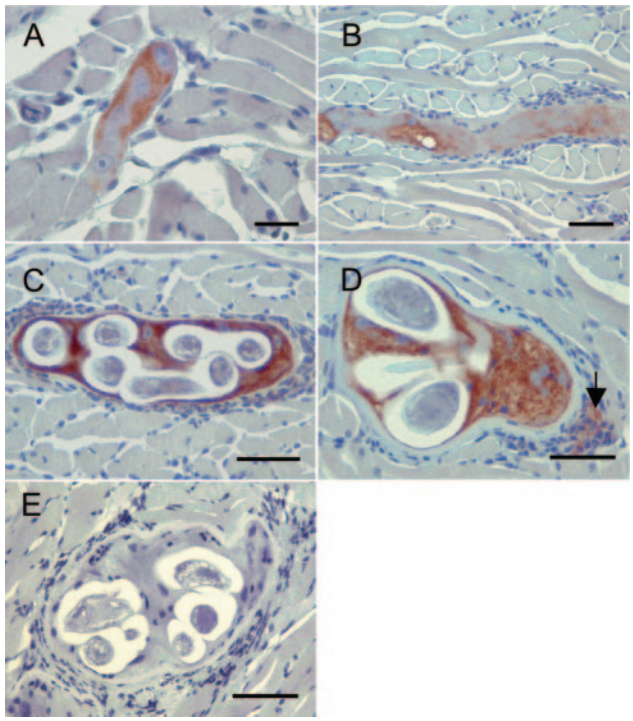

FIG. 1. Syndecan-1 is produced by muscle cells infected with *T. spiralis* but is not required for nurse cell development. Sections of infected tongues from WT (A to D) or $sdc1^{-/-}$ (E) animals were stained with an antibody to syndecan-1. All sections were counterstained with hematoxylin. (A) At 5 dpi, syndecan-1 is in the cytoplasm of the infected cell. (B) At 10 dpi, syndecan-1 is diffusely distributed within the infected cell. (C and D) At 20 dpi (C) and 50 dpi (D), the cytoplasm of a mature nurse cell stains intensely with anti-syndecan-1. Diffuse, extracellular syndecan-1 is detected in the infiltrate surrounding nurse cells (arrow in panel D). (E) At 20 dpi, nurse cells of $sdc1^{-/-}$ mice do not contain syndecan-1. Bars, 25 μm (A) and 50 μm (B to E).

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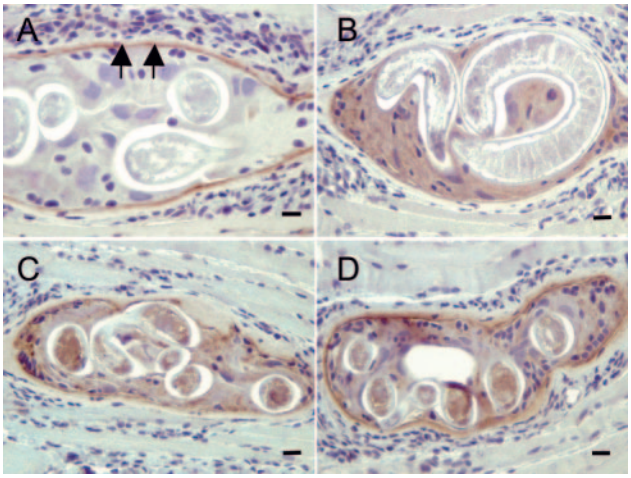

FIG. 2. Investigation of nurse cell syndecan-1 structure. Infected diaphragms from WT (A to C) or sdc1$^{-/-}$ (D) mice at 22 dpi were stained with an antibody to perlecan (A), a polyclonal antibody to the carboxy terminus of syndecan-1 (B), or an antibody to heparan sulfate (C and D). Bars, 10 μm (A) and 20 μm (B to D). Arrows indicate perlecan in the nurse cell basement membrane (A).

idenced by larval morphology and collagen capsule formation (Fig. 1E). Similar numbers of mature first-stage larvae were recovered from WT and sdc1$^{-/-}$ mice and were comparable in their ability to infect, mature, and reproduce when passaged in C57BL/6 mice (data not shown). Although we observed no requirement for syndecan-1 for as long as 50 dpi in our experiments, *T. spiralis* survives in the muscle for months to years, and our data cannot rule out a role for syndecan-1 in the longer term.

The cytoplasmic localization of syndecan-1 in nurse cells contrasts with the cell surface location of the proteoglycan in epithelial cells (6) and plasma cells (12), where the molecule facilitates adhesion and proliferation (4). Modification of syndecan-1 by HS is critical for biological activity (10), localization (19), and immunoregulatory capacity (18). We explored potential causes for retention of syndecan-1 in the nurse cell cytoplasm. In order to detect a general defect in proteoglycan synthesis or transport, we stained infected muscles with antibodies specific for HS (clone F58-10E4; Seikagaku Corporation, Tokyo, Japan) or for perlecan (clone A7L6; NeoMarkers, Fremont, CA), a large (>500-kDa) multidomain HSPG that is a common constituent of basement membrane. We detected HS in the cytoplasm and cell margins of both WT (Fig. 2C) and sdc1$^{-/-}$ (Fig. 2D) mice, indicating that infected muscle cells synthesize HS irrespective of syndecan-1 production. The HS content of nurse cells from sdc1$^{-/-}$ mice may be associated with other HSPGs. Perlecan was detected at the margins of uninfected and infected myotubes, indicating that infected cells can synthesize large, complex HSPGs and transport them appropriately (Fig. 2A). If syndecan-1 mRNA is alternatively spliced in muscle cells, as has been reported to occur in mouse embryos (15), a truncated core protein may exhibit altered activity or localization. We did not detect alternatively spliced transcripts by PCR (data not shown). Staining with antibodies specific for the amino terminus (Fig. 1; clone 281-2) or the carboxy terminus (18) of the protein yielded similar patterns

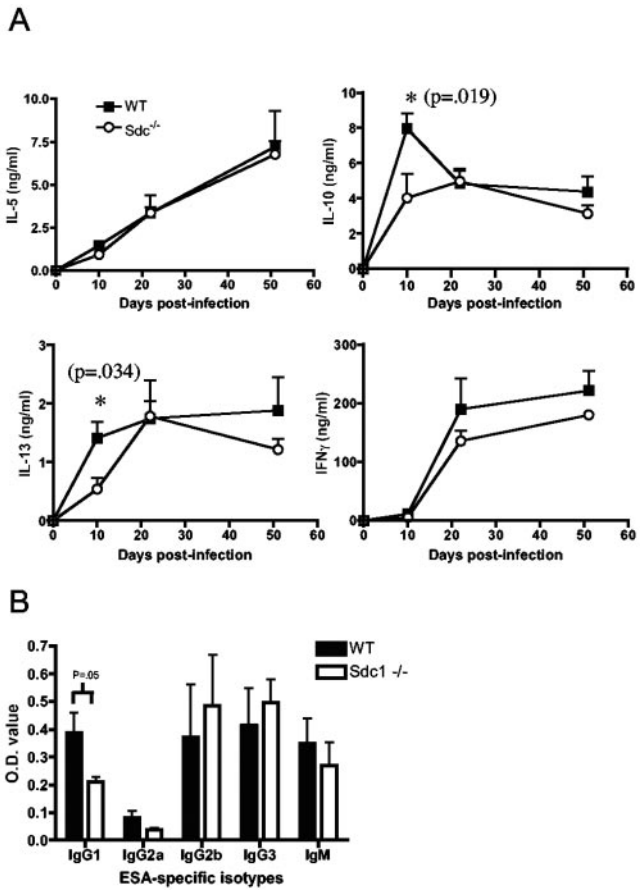

FIG. 3. sdc1$^{-/-}$ mice produce less IL-10, IL-13, and parasite-specific IgG1 than WT mice. (A) Cytokine responses of cervical lymph node cells from WT and sdc1$^{-/-}$ mice infected with *T. spiralis*. Leukocytes were collected at the indicated times postinfection, cultured, and restimulated with anti-CD3. IL-5, IL-10, IL-13, and gamma interferon (IFN-γ) were measured in supernatants collected after 72 h. (B) Sera collected at 50 dpi were diluted 5,000-fold (IgG1) or 100-fold (IgG2a, IgG2b, IgG3, and IgM) and evaluated by ELISA for ESA-specific antibodies. Means and standard deviations are shown for groups of four to five mice. Asterisks indicate statistical significance as determined using Student's *t* test.

(Fig. 2B), suggesting that the protein was intact in the infected cell. It is possible, however, that nurse cell syndecan-1 is heterogeneous, including intact as well as truncated molecules.

Based on recent findings that syndecan-1 limits TH2-driven inflammation by an HS-dependent mechanism (18), we hypothesized that sdc1$^{-/-}$ mice would generate larger areas of inflammatory reaction around nurse cells. To test this hypothesis, we measured cytokine production by cervical lymph node cells restimulated in vitro with plate-bound anti-CD3 (5). Cells cultured from sdc1$^{-/-}$ mice at 10 dpi yielded significantly reduced concentrations of IL-10 and IL-13 compared with WT cells (Fig. 3A). IL-4 is rarely detected in our cultures (WT or sdc1$^{-/-}$) (data not shown), for reasons that have not been elucidated. To test whether the reduced levels of IL-13 early in infection influenced the B-cell response, we measured serum antibodies to parasite excretory-secretory antigens (ESA; prepared as described in reference 2) by an enzyme-linked immunosorbent assay (ELISA) (3). A deficiency in IL-13 production

correlated with a reduced TH2-driven, parasite-specific immunoglobulin G1 (IgG1) response in sdc1$^{-/-}$ mice (Fig. 3B). Despite these differences, the volume (Fig. 1C and D) and composition (CD4-, CD8-, B220-, and major histocompatibility complex class II-positive cells) (data not shown) of local cellular infiltrates were similar in the two groups of mice. We did not detect syndecan-2 or -4 in infected cells from either sdc1$^{-/-}$ or WT mice (data not shown). Thus, these members of the syndecan family did not compensate for the absence of syndecan-1 in nurse cells of sdc1$^{-/-}$ mice.

In summary, we have shown that syndecan-1 is produced by muscle cells following infection by *T. spiralis*. Mice deficient in syndecan-1 support normal larval development but display modestly reduced TH2 responses during infection. The lack of a more dramatic phenotype in these mice during infection might be explained by compensatory activities of HSPGs in the *T. spiralis* nurse cell.

This research was supported by grant NIH-AI14490 from the National Institutes of Health. D.P.B. was supported by training grant T32-AI07643.

We thank Gerard Pineiro for helpful comments and Anita Hesser for assistance with manuscript preparation.

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Editor: J. F. Urban, Jr.