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Short communication

Clinical and histopathological characterization of a large animal (ovine) model of collagen-induced arthritis

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ABSTRACT

Collagen induced arthritis (CIA) is the most studied and used rheumatoid arthritis (RA) model in animals, as it shares many pathological and immunological features of the human disease. The aim of this study was to characterize clinical and immunological aspects of the ovine CIA model, and develop lameness and histopathological scoring systems, in order to validate this model for use in therapeutic trials. Sheep were sensitized to bovine type II collagen (BCII), arthritis was induced by injection of bovine collagen type II into the hock joint and the response was followed for two weeks. Clinical signs of lameness and swelling were evident in all sheep and gross thickening of the synovium surrounding the tibiotarsal joint and erosion on the cartilage surface in the arthritic joints. Leucocyte cell counts were increased in synovial fluid and there was synovial hyperplasia, thickening of the intimal layer, inflammation and marked angiogenesis in the synovial tissue. There was a large influx of monocytes and lymphocytes into the synovial tissue, and increased expression of TNF-α and IL-1β in arthritic intima, angiogenesis and upregulation of VCAM-1. CIA in sheep appears to be an excellent large animal model of RA and has the potential for testing biological therapeutics for the treatment of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder causing synovitis and aggressive synovial hyperplasia. Collagen induced arthritis (CIA), using bovine collagen type II (BCII) as the antigen, is the most studied model of RA in animals as it shares many pathological features of the human disease (Cho et al., 2007). The immune response in CIA results in cartilage degradation, marked synovial hyperplasia with peri-articular inflammation, mononuclear cell infiltration and bone resorption (Brand et al., 2007).

Rodent models of have been used extensively to study mechanisms of action and efficacy of treatments, however, they have limitations with respect to their body mass, strain variability and animal welfare issues associated with polyarthritis. Large animal joints more closely resemble the anatomical size of human joints and their body weight means the magnitude of the mechanical loads acting across the joints during weight-bearing activities is closer to that of humans (Ghosh, 1999).

We previously described an ovine model of CIA using s.c. immunizations with BCII followed by an intra-articular (i.a.) injection (Thorp et al., 1992). In this earlier publication

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we demonstrated that there was a rise in Ab to both ovine and bovine collagen in serum, SF and afferent lymph, and that there were obvious histopathological changes in the joint synovial tissues. The aim of the present study was to further characterize clinical and immunological aspects of the ovine CIA model, and develop lameness and histopathological scoring systems, in order to validate this model for use in therapeutic trials.

2. Materials and methods

Two year-old female merino ewes were obtained locally and housed on site. Sheep were sensitized to collagen by s.c. injection of 1 ml BCII (5 mg/ml) emulsified in Freund's Complete Adjuvant (Sigma–Aldrich, St. Louis, MO, USA) at day 0, followed by s.c. injection of 1 ml BCII (5 mg/ml) in Freund's incomplete adjuvant (Sigma–Aldrich) at day 14. Two weeks later arthritis was induced by i.a. injection of 100 μg BCII in 0.5 ml saline into the left hock (tibio-tarsal) joint. The contralateral joint was not injected, as previous work indicated no detectable changes in the joint 2 weeks after a sham PBS injection (Thorp et al., 1992). Six normal (untreated) sheep were used as controls. They did not undergo the immunization procedure, because this alone has been shown to cause mild synovitis (Thorp et al., 1992). Furthermore, they could not receive Freund's adjuvant because this alone may cause systemic inflammation; therefore the process of immunization was considered a part of the inflammation induction, rather than specifically focussing on the response to i.a. collagen.

Lameness, joint swelling and pain on flexion were assessed weekly until day 28 (BCII i.a.) and then every day until euthanasia with i.v. barbiturate anaesthetic (Lethabarb, Arnolds, Australia) on day 42. A semi-quantitative evaluation system (0–5 scale) to assess lameness was adapted from a previous scoring system developed for dairy cows (Thomsen et al., 2008) and included the parameters of behaviour, standing posture and gait. Similar 4-point scales (0–3) were developed to assess joint swelling and pain elicited on flexion of the hock. The scoring categories for lameness were: 0 (behaviour unaffected, sheep standing squarely on all 4 legs, moving with even strides and able to change direction rapidly), 1 (behaviour unaffected, sheep standing squarely on all 4 legs, but with abnormal stride length. Movement no longer fluent, but still able to change direction easily), 2 (behaviour unaffected, uneven posture and shortened stride. Lameness detected in a straight line, with awkwardness changing direction), 3 (behaviour unaffected, uneven posture and stride. Moving with minimum weight bearing on affected limb), 4 (behaviour altered with individual trying to remain separate from others in the group, posture altered with individual elevating or not bearing weight on affected limb and gait severely affected with affected limb not used) and 5 (behaviour altered with individual trying to remain separate from others in the group, failing to stand unaided and failure to move). The clinical signs for joint swelling were assessed as 0 (none detectable), 1 (barely detectable, but present), 2 (clearly discernible swelling on palpitation) and 3 (very marked joint swelling). Pain on flexion was assessed as 0 (none elicited), 1 (slight discomfort on strong flexion), 2 (clear discomfort with strong flexion) and 3 (severe discomfort even with slight flexion and sheep very reluctant to flex the joint).

Synovial fluid (SF) was collected from the arthritic and contralateral hock joints, and those from normal, control animals at necropsy. The total leucocytes were counted using a Coulter Counter (Model Z1; Beckman Coulter) and differential cell counts of a minimum of 200 cells were made on cytospots stained with Giemsa (ProSciTech, QLD, Australia).

Gross observations were recorded for each tissue and synovial membranes (SM) from the dorsal region of the joints, cartilage from the articular surface of the talus bone, and synovial fluid (SF) were collected. The cartilage on the surface of the talus was assessed using a 5-point scoring system based on the OARSI recommendations for macroscopic scoring of cartilage pathology (Little et al., 2010), but simplified because the primary cartilage injuries were confined to the central trochlear groove of the talus, rather than the whole joint surface. The cartilage erosion scoring system and results are included in supplementary data.

The cytokines TNF-α, IFN-γ IL-6, IL-17A, activin A and IL-10 were measured using ELISA assays. The antibody combinations and kits used are provided as online supplementary data.

Scoring systems developed for microscopic grading of synovial inflammatory changes in RA are specific to human patients (Henderson et al., 1975; Rooney et al., 1988; Krenn et al., 2002; Yamanaka et al., 2010), so it was necessary to develop a modified scoring system for this ovine model of CIA. The scoring system was a semi-quantitative composite of those used for humans with modifications for ruminants (Rooney et al., 1988; Highton et al., 1997; Krenn et al., 2002; Smith et al., 2008; Little et al., 2010) and assessed three parameters of the synovium; namely hyperplasia of the intimal lining cells (intima), stromal activation and inflammatory infiltrate, with each scored from 0–3 with the aggregate score of 0–9 points. For consistency, intimal hyperplasia was specifically evaluated at the predominant cell depth (Rooney et al., 1988). Stromal activation and inflammatory infiltrate were based upon those areas with the greatest alterations (Krenn et al., 2006). Each parameter was observed at low power, before evaluation at high power (40× objective). The pathological changes were scored by two blinded observers and if their scores differed by 2 or more points, the sections were examined by a third blinded observer.

Hyperplasia was based on the thickness of the intima and whether thickening was diffuse or focal: 0 = normal (1–3 cell thickness), 1 = mild (4–5 cells thick in focal areas), 2 = mild diffuse (4–5 cells thick in diffuse areas or moderate 6+ cells in focal areas) and 3 = moderate diffuse (6+ cells in focal areas). Stromal activation was defined as: 0 = normal, absent, 1 = mild (slight cellularity with chronic oedema, slight fibrosis with low cellularity of fibroblasts, endothelial cells and histiocytes), 2 = moderate (chronic oedema, moderate fibrosis with moderate cellularity of fibroblasts, endothelial cells and histiocytes) and 3 = marked (chronic oedema, marked fibrosis with marked cellularity of fibroblasts, endothelial cells and histiocytes). Inflammatory infiltration of lymphocytes, plasma cells and