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Anterior Uveitis Accompanies Joint Disease in a Murine Model Resembling Ankylosing Spondylitis

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Ankylosing spondylitis · Uveitis · Murine

Abstract
Background: Uveitis is often associated with a systemic inflammatory disease such as ankylosing spondylitis. Our understanding of the eye's susceptibility to immune-mediated uveitis as in the apparent absence of infection has been limited by a relative lack of experimental models. Here we sought to assess whether ocular inflammation occurs in a previously described murine model of proteoglycan-induced spondylitis, wherein mice develop progressive spondylitis, sacroiliitis and peripheral arthritis – features common to the clinical presentations of ankylosing spondylitis. ***Methods:*** Using intravital microscopy we examined the ocular inflammatory response after the onset of arthritis in mice that overexpressed the T cell receptor (TCR) specific for a dominant arthritogenic epitope of cartilage proteoglycan [TCR-Tg (transgenic) mice] or BALB/c controls. ***Results:*** Immunized TCR-Tg mice showed a significant increase in the number of rolling and adhering cells within the iris vasculature compared to adjuvant control mice. Cellular infiltration within the iris tissue, as assessed by intravital microscopy and histology, was also increased. Our initial temporal analysis has revealed that immunized TCR-Tg mice show a significant increase in intravascular inflammation by 2 weeks after immunization, but it diminishes at 4 weeks after immunization. ***Conclusions:*** Although these data are preliminary, this model has the potential to clarify the mechanisms accounting for the coexistence of eye and sacroiliac inflammation as occurs in patients with ankylosing spondylitis.

Introduction

Uveitis is often associated with systemic, rheumatic inflammatory diseases such as ankylosing spondylitis (AS), sarcoidosis, juvenile idiopathic arthritis or Behcet's disease. AS is considered one of the most prevalent of the rheumatic diseases [1, 2] and intriguingly patients with AS demonstrate an increased incidence of anterior uveitis. Approximately 40% of the patients with AS develop anterior uveitis, indicating uveitis is a frequent extra-articular manifestation of this disease [3]. Moreover, anterior uveitis represents the most commonly encountered type of uveitis [4]. The uveitis associated with AS is typically unilateral anterior with a sudden onset and subsequent complete resolution, but it can be followed by recurrent attacks of ocular inflammation [5]. Relatively young patients are afflicted and it is a potentially vision-threatening disease. Thus, this particular clinical entity is of importance due to its prevalence, impending visual complications and strong association with the systemic manifestations of AS.

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The immunological mechanisms of uveitis associated with AS are poorly understood. There is a strong genetic predisposition associated with the development of AS and the expression of the human leukocyte antigen, HLA-B27 [6, 7], and ~90% of the AS patients with uveitis express HLA-B27 [8, 9]. However, most people within the general population that express HLA-B27 do not develop the disease, indicating that its pathogenesis and the onset of uveitis involve other genetic or environmental factors. Thus, investigation of uveitis as appears specifically in patients with AS is crucial to our comprehension of this ocular disease.

Our understanding of the eye's susceptibility to immune-mediated uveitis as occurs in the apparent absence of infection in AS has been limited by a relative lack of experimental models. While several rodent models have been developed to study AS, including the *ank/ank* mice and the HLA-B27 transgenic (Tg) rodents, in certain respects they are lacking an accurate replication of the disease as is observed clinically in AS patients. Progressive AS occurs in *ank/ank* mice that bear a homozygous autosomal recessive gene defect [10]. Clinically, the *ank/ank* mice show some similarities to humans with AS, as the disorder involves the peripheral and axial joints, including the sacroiliac joint [11–14]. However, unlike the human form of the disease, the progressive ankylosis is largely noninflammatory and results from metabolic dysregulation involving excessive calcium deposition. Indeed, the disease severity is controlled by the calcium phosphate inhibitor phosphocitrate [15].

The effects of overexpression of HLA-B27 on the disease have been investigated in mice and rats bearing the transgene for human HLA-B27 (as reviewed by Taurog et al. [16]). In HLA-B27 Tg rats which express both HLA-B27 and human β2-microglobulin, a spontaneous disease develops that resembles spondylitis in humans with AS [17, 18]. The development of disease within this model is mediated by T cells and B27 but is also influenced by other factors such as the gut micoflora [19]. The disease is ameliorated by raising the rats in a germfree state [20]. Mice transgenic for HLA-B27 with and without intact β2-microglobulin [21–24] also develop peripheral arthritis, but there is an apparent lack of spine involvement – a crucial feature of AS in humans. There has been some question as to the relevance of the HLA-B27 Tg mice as an appropriate model for investigation of the clinical form of AS. Notably, the spontaneous onset of uveitis in all of these rodent models of AS has not been observed.

While a specific 'autoantigen' has yet to be identified in AS, there is increasing evidence to support a role for immunity to the proteoglycan aggrecan in the pathology of this disease. A main constituent of the extracellular matrix of articular cartilage, aggrecan is a large proteoglycan comprised of a core protein (~220 kDa) that binds hyaluronan (hyaluronic acid) at its N-terminally located G1 globular domain [25–28]. Most of the arthritogenic epitopes of aggrecan appear to be localized to the N-terminal G1 domain in a 'cryptic' position, which is typically masked by the presence of glycosaminoglycan side chains. Immunity to cartilage proteoglycan was reported in patients with AS several years ago [29, 30]. More recently, cellular immune responses to aggrecan in AS patients have been examined and isolated T cell clones have been demonstrated to recognize the G1-immunodominant epitopes of aggrecan [31, 32]. The majority of these T cells are CD3+, CD4+ and CD8– in their phenotype with an apparent Th1 cytokine profile.

Experimental immunity to the proteoglycan aggrecan induces progressive spondylitits and peripheral arthritis in mice that appears to involve events very similar to those in the human disease [33–35]. Immunization of BALB/c mice with the proteoglycan aggrecan isolated from human cartilage initiates inflammation within the sacroiliac joints, intervertebral discs and articular cartilage, the endplates and entheses. It ultimately progresses to total destruction of the nucleus and endplates, as observed in the human end-stage form of AS [33, 34]. Immunization selectively with recombinant human G1 domain [36] also induces spondylitis in mice. Studies have demonstrated that T cell clones of diseased mice recognize immunodominant epitopes of G1 as reported in humans [37]. Thus, the chronology of pathological events in this model involving the sacroiliac joint, the spine and peripheral arthritis all resemble human AS.

Fundamental features of the pathology of AS such as inflammation of entheses and the axial skeleton may be due to the molecular composition of ligament entheses and nucleus of the spinal cord that are enriched with the hyaluronic-acid-binding proteoglycan aggrecan. In addition to these principle sites where inflammation initially develops, aggrecan is localized within other sites of inflammation observed in AS such as the peripheral joints as well as extra-articular tissue sites including the arterial media of the aorta and the anterior uveal tract, sclera and endoneurium of the optic nerve within the mammalian eye [38–40]. Thus, it is intriguing to speculate that the pathology of extra-articular tissues such as the eye as observed in patients with AS may result from cross-reactive immunity to the immunologically related aggrecan that could be localized within this tissue.

Materials and Methods

We sought to investigate whether ocular inflammation occurs in the previously established mouse model of spondylitis as induced by immunization with the proteoglycan aggrecan. We immunized age-matched female mice (20–24 weeks) that overexpress the T cell receptor (TCR) for the G1 epitope of aggrecan or their wild-type BALB/c controls. These mice have been previously described [41, 42]. The Tg animals allowed us to test how an increased percentage of a CD4+ T cell population reactive to G1 aggrecan might alter the incidence and/or severity of uveitis in mice. The mice were immunized with deglycosylated proteoglycan that was purified from human cartilage in the presence of the adjuvant dimethyldioctadecylammonium bromide, which lacks known Toll-like receptor agonists, as previously described [43]. Ocular inflammation was assessed by intravital microscopy [44] at 12 weeks following immunization. We chose this particular time point as both groups of immunized mice have developed maximal arthritis with 100% incidence; albeit the TCR-Tg mice exhibit an exacerbated form of the disease compared to immunized BALB/c controls [41, 42].

Results

In response to immunization with proteoglycan we found that the TCR-Tg mice showed a significant increase in the intravascular inflammatory response as the number of rolling and adhering leukocytes within the iris vasculature was increased compared to adjuvant-treated control mice. However, we observed only a minimal increase in the intravascular inflammatory response in immunized wild-type BALB/c mice at this same time point. In the process of imaging, we noted that some mice did not exhibit ocular inflammation, while the mice that did develop ocular inflammation typically had inflammation within only 1 eye. Comparison of the onset of ocular inflammation between the TCR-Tg and BALB/c controls revealed that the TCR-Tg mice showed a greater percent incidence as well as a severer form of the disease compared to the immunized BALB/c mice. We have not examined later time points in immunized BALB/c mice to rule out the possibility of delayed onset of uveitis in the BALB/c mice compared to the TCR-Tg mice. Our initial histological assessment of eyes collected from TCR-Tg mice at 12 weeks after immunization is suggestive of an increased presence of leukocytes within the anterior chamber of the eye as well as a mixed infiltrate of leukocytes within the limbal region of the eye.

We have begun to assess the kinetics of the ocular inflammatory response to immunization with proteoglycan in the TCR-Tg mice. Our initial studies have revealed that within 2 weeks following immunization a significant intravascular inflammatory response occurs. We found that the number of rolling and adhering leukocytes within the iris vasculature was significantly increased at this time, but by 4 weeks following immunization the ocular inflammatory response had diminished. Intriguingly, at 12 weeks following immunization TCR-Tg once again showed increased ocular inflammation. These data suggest that recurrent or episodic patterns of ocular inflammation might appear similar to what is observed clinically with uveitis in patients with AS.

Conclusions

In conclusion, these findings are the first to indicate that ocular inflammation occurs spontaneously in a mouse model of spondylitis. Our data suggest that the presence of an increased CD4+ T cell population reactive to G1 aggrecan (TCR-Tg mice) leads to a greater incidence and severity of intravascular inflammation due to immunization with proteoglycan. Ocular inflammation may manifest itself as episodic bouts of inflammation as in patients with AS, as immunized TCR-Tg mice showed an initial increase in the leukocytic-vascular inflammatory response that subsequently subsided and then increased at a later stage of disease. This model may prove to be a valuable tool for investigation of the mechanisms involved in uveitis as observed clinically in patients with AS.

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