Clinical Immunology (2014) 153, 254–263

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Suppression of collagen-induced arthritis with a serine proteinase inhibitor (serpin) derived from myxoma virus☆

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Received 8 April 2014; accepted with revision 3 May 2014
Available online 17 May 2014

KEYWORDS
Collagen arthritis;
Serpin;
Rheumatoid;
Myxoma;
Immunotherapy

Abstract Many viruses encode virulence factors to facilitate their own survival by modulating a host's inflammatory response. One of these factors, secreted from cells infected with myxoma virus, is the serine proteinase inhibitor (serpin) Serp-1. Because Serp-1 had demonstrated anti-inflammatory properties in arterial injury models and viral infections, it was cloned and evaluated for therapeutic efficacy in collagen-induced arthritis (CIA). Clinical severity was significantly lower in the Serp-1 protocols ($p < 0.0001$) and blinded radiographs indicated that the Serp-1 group had significantly less erosions than the controls ($p < 0.01$). Delayed-type hypersensitivity was lower in the Serp-1 group but antibody titers to type II collagen were not significantly altered. Recipients had minimal histopathologic synovial changes and did not develop neutralizing antibodies to Serp-1. These results indicate that Serp-1 impedes the pathogenesis of CIA and suggests that the therapeutic potential of serine proteinase inhibitors in inflammatory joint diseases, such as rheumatoid arthritis, should be investigated further.

Abbreviations: Serp-1, serine proteinase inhibitor; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; DTH, delayed-type hypersensitivity; MMP, matrix metalloproteinases; RCL, reactive center loop; PAI, plasminogen activator inhibitors (type-1 and -2); uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor.

☆ This work was supported in part by NIH Grant AR-42200 and Viron Therapeutics, Inc., London, Ontario, Canada.

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http://dx.doi.org/10.1016/j.clim.2014.05.003
1521-6616/

1. Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory joint disease, affecting 1 to 2% of the adult population. The synovial membrane is the main site of inflammation and a primary source of inflammatory cytokines, proteinases, and up-regulated cells [1,2]. Although the precise etiology of RA remains elusive, it is broadly characterized as an autoimmune disease in which mononuclear cells, lymphocytes (B and T cells), dendritic cells and natural killer cells accumulate in the synovium. Autoantibodies activate the complement cascade, while activated T-cells, macrophages and fibroblasts produce pro-inflammatory cytokines. These cytokines lead to inflammatory cell migration, osteoclast and macrophage activation, as well as angiogenesis that support the expanding pannus. Ultimately, cytokine-activated inflammatory cells release enzymes such as matrix metalloproteinases, serine proteinases and cathepsins that are destructive to the cartilage and bone [3].

Although the inflammatory pathways of RA are complex and not fully understood, translational research efforts have led to current targeted therapies. Inflammatory disease has been controlled by countering specific factors, such as inhibiting a single cytokine through the actions of TNFα antagonists or by inhibiting mechanisms such as angiogenesis [4]. The goal of these new therapies is to attenuate inflammation and thereby delay or halt disease progression. The rodent collagen-induced arthritis (CIA) model is a well established antigen-induced autoimmune based disease. It shares many features with RA including the infiltration of inflammatory cells into the joints, the proliferation of synovial cells, pannus formation, and ultimately cartilage and bone destruction [5]. The CIA model has been used to show the importance of monocytes and macrophages in the development of arthritis. Macrophages not only migrate and accumulate in areas of tissue damage, release proinflammatory cytokines, but also possess Fc-receptors which enable disease progression from the development of anti-collagen antibodies to tissue destruction [6].

The CIA model has also been used to illustrate how enzymes of the fibrinolytic system such as plasminogen and urokinase are also important components of inflammatory cascades that contribute to the pathology of arthritis [7,8]. Targeting enzymes of the fibrinolytic pathway and reducing monocyte infiltration to sites of injury offer a potentially novel way to treat autoimmune and inflammatory based diseases.

Serp-1 (Fig. 1) is a 55 kDa secreted viral glycoprotein that has been demonstrated to reduce monocyte infiltration into sites of injury in a number of animal models of disease [9]. Originally identified as a viral virulence factor from myxoma virus that reduced the influx of monocytes to sites of infection [10–12], Serp-1 belongs to the ancient *serpin* superfamily of *ser*ine *p*roteinase *in*hibitors. These are homologous proteins that share a common tertiary structure and are found widely dispersed through evolution, from primitive organisms like bacteria and protozoa, invertebrates such as the horseshoe crab, as well as mammals living today [9,13]. The inhibitory serpins have evolved to regulate proteinase cascades and control processes such as thrombosis, fibrinolysis, complement activation, tissue remodeling, inflammation, and apoptosis [14,15]. Viruses have evolved to incorporate genes encoding serpins for viral defense to control their host's serpin-regulated inflammatory pathways [16]. Such virulence factors could represent a new source of virus-derived immunotherapeutics [17].

The anti-inflammatory potential of purified Serp-1 protein has been examined in a number of acute injury models. In balloon-injury and stent implant models where the aorta is injured by angioplasty, Serp-1 administration reduced monocyte infiltration into the site of vascular injury and reduced the extent of restenosis in numerous species [18]. In models of organ (renal, cardiac) allograft transplant, the administration of a combination of Serp-1 and an immunosuppressant (cyclosporine) reduced monocyte infiltration into the transplanted organ, which improved the histopathological evidence of chronic rejection, including transplant vasculopathy [19–21]. In aortic transplant and mouse herpes virus 68 (MHV68) infectious vasculitis models, Serp-1 treatment alone, without additional immunosuppressant, also reduced inflammation and arterial plaque growth. The inhibition of monocyte migration into the transplant organ was an effective therapeutic strategy that was independent of the presence of the immunosuppressant [20]. Another model evaluated ovalbumin induced inflammation and found that intra-articular injection of purified Serp-1 significantly decreased chronic inflammatory infiltration and tissue destruction [22]. In the work presented here, we characterize the anti-inflammatory properties and therapeutic potential of purified Serp-1 protein in the rat CIA model of RA.

2. Materials and methods

2.1. Animals

The animal experiments were performed in accordance with the Institutional Animal Care and Use Committee at UCLA. Pathogen free syngeneic LOU rats were maintained and bred in an in-house colony. Female rats, weighing 125–150 g (8–10 weeks old), were used in all experiments.

2.2. Serp-1

A stable CHO cell-line that secretes the glycosylated Serp-1 protein into the media was created using a standard mammalian expression vector containing a gene encoding the Serp-1 protein (accession #P12393). The Serp-1 protein was purified from the CHO cell-line conditioned serum-free media using standard chromatography methods and dialyzed into PBS before storage at −80 °C as previously described [18].

2.3. Induction of CIA

To induce arthritis, anesthetized rats were injected intradermally on day 0 with 0.5 mg of native chick type II collagen (Elastin Products Co., Owensville, MO), solubilized in 0.1 M acetic acid, and emulsified in equal volume of IFA (Difco Laboratories, Detroit, MI) [23,24]. Disease onset of one or both hind limbs typically developed in 90–100% of control rats on days 10–12.