

The Serpin Saga; Development of a New Class of Virus Derived Anti-Inflammatory Protein Immunotherapeutics

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*Wisdom in infinite thought,
Expanding time, endlessly sought,
An ancient star journeying here
So far, so near, bright seer
This unseen dance
A brilliant chance
—Anonymous*

Abstract

Serine proteinase inhibitors, also called serpins, are an ancient grouping of proteins found in primitive organisms from bacteria, protozoa and horseshoe crabs and thus likely present at the time of the dinosaurs, up to all mammals living today. The innate or inflammatory immune system is also an ancient metazoan regulatory system, providing the first line of defense against infection or injury. The innate inflammatory defense response evolved long before acquired, antibody dependent immunity. Viruses have developed highly effective stratagems that undermine and block a wide variety of host inflammatory and immune responses. Some of the most potent of these immune modifying strategies utilize serpins that have also been developed over millions of years, including the hijacking by some viruses for defense against host immune attacks. Serpins represent up to 2-10 percent of circulating plasma proteins, regulating actions as wide ranging as thrombosis, inflammation, blood pressure control and even hormone transport. Targeting serpin-regulated immune or inflammatory pathways makes evolutionary sense for viral defense and many of these virus-derived inhibitory proteins have proven to be highly effective,

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working at very low concentrations—even down to the femtomolar to picomolar range. We are studying these viral anti-inflammatory proteins as a new class of immunomodulatory therapeutic agents derived from their native viral source. One such viral serpin, Serp-1 is now in clinical trial (conducted by VIRON Therapeutics, Inc.) for acute unstable coronary syndromes (unstable angina and small heart attacks), representing a ‘first in class’ therapeutic study. Several other viral serpins are also currently under investigation as anti-inflammatory or anti-immune therapeutics. This chapter describes these original studies and the ongoing analysis of viral serpins as a new class of virus-derived immunotherapeutic.

Innate Immunity

Many investigators have studied the antigen-dependent, antibody-mediated immune response, which is only found in vertebrates. Over a century ago, however, Ilya Ilyich Mechnikov described a more ancient and yet extraordinarily powerful immune response, known as the innate immune system. Mechnikov studied the response of a transparent starfish (bipinaria) to wood splinters and recorded the early massing of cells around these splinters inside this organism. Mechnikov thus provided the first description of the cell-based innate immune response that forms the first defense response to injury or infection.¹ This ‘inflammatory’, cell-based immune system recognizes and then eradicates or blocks pathogen and parasite infection, invasion and dissemination long before antibodies are formed and the acquired, antibody dependent immune response is activated.²⁻⁶ The innate immune response also orchestrates the first stages of tissue repair after other forms of injury produced by physical or chemical insults.

The vascular endothelium, together with the circulating inflammatory blood cells, monocytes/macrophages, T-lymphocytes and polymorphonuclear leukocytes (also called neutrophils), recognize patterns of microbial molecular expression through pattern recognition receptors (PRR) forming the prelude to this innate response. The PRRs now recognized include toll-like receptors (TLRs), nucleotide binding and oligomerization domain-like receptors (NLRs), C-type lectin-like receptors (CLRs), cytoplasmic double stranded RNA (dsRNA) helicase-like receptors and cytoplasmic dsDNA receptors.^{7,8} These receptors comprise an alarm system that alerts inflammatory cells to danger or infection, signaling through MyD88, NFκB and MAPK signal-transduction pathways. The endothelial cell layer is the innermost layer of cells in the arterial tree and is composed of miles of interconnected cells, a living carpet of cells that encompasses the vasculature, the cardiac valves and the inner chambers of the heart. This endothelial cell layer is in constant contact with the circulating blood. Injury or infection of the endothelium causes loss and/or activation of endothelial cells with increasing expression of selectins on the activated cells. These selectins, when expressed, slow down circulating leukocytes that pass by in the blood stream. Once slowed, mononuclear cells (leukocytes composed of neutrophils, monocytes and lymphocytes) can then recognize cell adhesion molecules and adhere to the endothelium and in turn become activated.⁹ Circulating and activated inflammatory cells also can recognize connective tissue and lipids exposed under areas of damaged endothelium. The activated endothelium expresses increased amounts of selectins and adhesion molecules that further stimulate cell adherence and activation. Once activated, inflammatory mononuclear cells, together with endothelial cells, begin to release chemoattractant proteins, particularly chemokines, which bind to surface glycosaminoglycans (GAGs). Also induced are pro-inflammatory immune signaling molecules, cytokines and growth factors that signal cells to migrate through the vessel wall and into the surrounding tissue, become further activated, proliferate and then release more inflammatory cytokines. Damaged cells also can become apoptotic and act as small cytokine release factories, further stimulating this inflammatory response.

Platelets, small clotting cell fragments derived from megakaryocytes, are also activated at sites of damaged or apoptotic endothelium. Platelets carry reserves of proteins in storage granules that are released upon platelet activation. Initially, platelets adhere to areas of arterial or tissue damage, secreting pro-inflammatory proteins from storage granules into this mix of cells and proteins. This activated and inflamed milieu then further stimulates cell invasion and activation. The clot forming