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Review

An emerging role for Serine Protease Inhibitors in T lymphocyte immunity and beyond

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ABSTRACT

Serine proteases control a wide variety of physiological and pathological processes in multi-cellular organisms, including blood clotting, cancer, cell death, osmo-regulation, tissue re-modeling and immunity to infection. T lymphocytes are required for adaptive cell mediated immunity and serine proteases are not only important for effector function but also homeostatic regulation of cell numbers. *Ser*ine *P*rotease *In*hibitors (Serpins) are the physiological regulators of serine proteases activity. In this review, I will discuss the role of serpins in controlling the recognition of antigen, effector function and homeostatic control of T lymphocytes through the inhibition of physiological serine protease targets. An emerging view of serpins is that they are important promoters of cellular viability through their inhibition of executioner proteases. This will be discussed in the context of the T lymphocyte survival during effector responses and the development and persistence of long-lived memory T cells. The potent anti-apoptotic properties of serpins can also work against adaptive cell immunity by protecting viruses and tumors from eradication by cytotoxic T cells (CTL). Recent insights from knock-out mouse models demonstrate that these serpins also are required for hematological progenitor cells and so are critical for the development of lineages other than T lymphocytes. Given the emerging role of serpins in multiple aspects of lymphocyte immunity and blood development I will review the progress to date in developing new immunotherapeutic approaches based directly on serpins or knowledge gained from identifying their physiologically relevant protease targets.

1. Serine Protease Inhibitors

Homeostatic regulation of serine proteases is mainly achieved through interaction with inhibitors belonging to the *Ser*ine *P*rotease *In*hibitor (serpin) superfamily [1]. Inhibitory serpins have a common mode of action: each contains a variable C-terminal reactive center loop (RCL) resembling the substrate of its cognate protease. Serpins regulate proteolytic pathways by acting as "suicide substrates" and inactivating serine proteases through the formation of a 1:1 complex [1] (Fig. 1). On protease binding, the RCL is cleaved between the two residues designated P_1 and P'_1 and it undergoes a conformational change that distorts the protease and irreversibly locks the serpin-protease complex. Most serpins are secreted and constitute about 10% of plasma proteins. In vertebrates, clade B serpins belong to a large intracellular family [2]. The intracellular serpins ($serpin_{IC}$) lack cleavable N-terminal signal peptides and dwell within the nucleo-cytoplasmic compartment. $Serpin_{IC}$ inhibit several key proteases that trigger cell death and so there is an emerging view that they are key regulators of cell survival.

In addition to inhibiting serine proteases, some $serpins_{IC}$ are cross-class specific and inhibit cysteine proteases. The $serpin_{IC}$ Cytokine response modifier A (CrmA) from the cowpox virus and SERPINB9 (PI9) from humans can inhibit caspases [3–6]. Cross-class specific $serpin_{IC}$ also inhibit papain-like, lysosomal cysteine cathepsins, such as cathepsin B, V, L, K and H. For example, Serine Protease Inhibitor 2A (Spi2A) (Serpina3g) from mice inhibits the serine protease cathepsin G and also papain-like, lysosomal cysteine cathepsins such as cathepsin B, V, L, K and H [7]. This cross-class specificity of Spi2A for cysteine cathepsins is also a property another mouse serpin SQN-5 (Serpinb3a) and the human serpin SCCA1 (SERPINB3) [8,9]. The general evolutionary relevance of cross-class specific $serpin_{IC}$ is illustrated by SRP-6, which inhibits lysosomal cysteine cathepsins in *Caenorhabditis elagans* [10]. The mechanism by which cysteine proteases are inhibited involves the cleavage of the $serpin_{IC}$, in some cases involving as stable covalent complex [3,4,8,10] and in other cases not [3,7]. Cellular inhibitors of apoptosis (c-IAPs) [11] are physiological inhibitors of caspases

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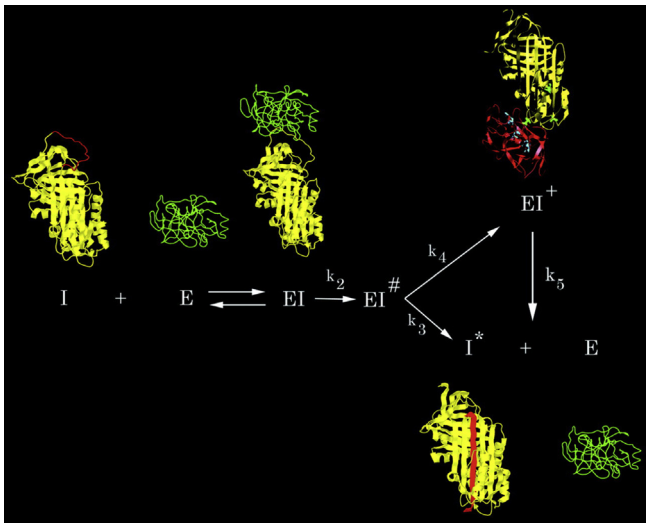

Fig. 1. Serpin mechanism of action. Fate of the serpin and proteinase complex *via* the branched pathway. The serpin (I) inhibition of proteinase (*E*) proceeds *via* an initial noncovalent, Michaelis-like complex (*EI*) that involves no conformational change within the proteinase or the body of the serpin. Subsequent peptide bond hydrolysis results in an acyl-enzyme intermediate (*EI*#) that progresses to either a kinetically trapped loop-inserted covalent complex (*EI*+, inhibitory pathway) or a cleaved serpin (I*) and free proteinase (noninhibitory or substrate pathway). The serpin body is in *yellow*. Free serine proteinase is in *green* and covalently bound proteinase is in *red*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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and cystatins inhibit papain-like cysteine cathepsins [12]. However, these inhibitors do not act like suicide substrates, as do serpins, but rather inactivate by through high affinity non-covalent binding. Therefore, cross-class specific inhibition by $serpin_{IC}$ is a mechanism that therefore has evolved along together with other molecular inhibitors to control cysteine protease activity.

1.1. Programmed cell death

Programmed cell death (PCD) is defined as an active process that is dependent on signaling processes within the dying cell [13]. Morphological criteria can be used to divide PCD into three subclasses: classic apoptosis, apoptosis-like PCD and necrosis-like PCD [13]. All three forms of death involve the activity of executioner serine proteases, caspases and cysteine proteases. In apoptosis, chromatin condenses to compact and apparently discrete geometric figures; in contrast apoptosis-like PCD is characterized by less-compact, lumpy chromatin masses [13]. Both forms of apoptosis usually require mitochondrial outer membrane permeabilization, which is controlled by the Bcl-2 family of proteins and the release of AIF, cytochrome c and Smac-a/Diablo [14]. Necrosis-like PCD occurs either in the complete absence of chromatin condensation or at best with chromatin clustering to form loose speckles [13]. Autophagy, which is characterized by the formation of large lysosome-derived cytosolic vacuoles, and death-receptor-induced necrotic death are examples of active death programs that lack chromatin condensation and are classified as necrosis-like PCD [13]. Although the morphology of necrosis-like PCD is indistinguishable from that of cells dying from accidental necrosis, the former is the result of active cellular processes that rely on Reactive Oxygen Species (ROS) [15–17], poly (ADP) ribose polymerase [18], autophagolysome formation [19], and cathepsins [20].

There is a large overlap between the biochemical pathways leading to different forms of PCD, which is reflected in the executioner proteases involved (Fig. 2). Serine proteases, such as granzyme B (GrB) and granzyme A can trigger all three morphological forms of PCD [21]. Caspases on the other hand are usually associated with either classical apoptosis or apoptosis-like PCD [22] and are activated after stimulation of death receptors (*e.g.* caspase 8) or by the release of proteins from damaged mitochondria (*e.g.* caspase 9) [14]. Lysosomal cathepsins, when released into the cytoplasm, trigger a spectrum of PCD morphologies, ranging from necrosis-like autophagy to classical apoptosis [23]. Cathepsins, especially the cysteine cathepsins B and L and the aspartyl cathepsin D participate in both caspase-dependent and caspase-independent PCD induced by several stimuli, including death receptors of the Tumor Necrosis Factor Receptor (TNFR) family [24], B cell receptors [25], T cell receptor (TCR) [26], the p53 tumor suppressor protein [27] and ROS [28]. Depending on the cell type and the stimuli, cathepsins may function upstream [24,29], or down stream of caspases [30,31], or even in the absence of caspase activity [30]. The relative amount of cathepsin released into the cytoplasm after lysosomal membrane permeabilization (LMP) is thought to determine whether PCD is necrotic or apoptotic (Fig. 2). Low level leakage of cathepsins trigger mitochondrial permeabilization and apoptosis through Bid activation, whereas larger scale lysosomal rupture triggers necrotic PCD [23]. Since $serpins_{IC}$ can inhibit all three classes of executioner proteases the potential exists for them to protect from not only classical apoptosis, but also apoptosis-like PCD or necrosis-like PCD.

2. Serpins and programmed cell death

All forms of cell death require the activity of proteases and $serpin_{IC}$ have evolved to protect cells from cell death by not only inhibiting serine proteases but also other executioner proteases. The first description of an anti-apoptotic function of $serpin_{IC}$ comes from work on CrmA from the cowpox virus. CrmA inhibits the serine protease GrB, which is used by T lymphocytes to kill infected cells [3–6]. Mammalian $serpin_{IC}$, which inhibit GrB, have also been identified in humans – proteinase inhibitor 9 (PI9 – SERPINB9) [32] and mice – Serine Protease Inhibitor 6 (Spi6) [33]. In addition to inhibiting executioner serine proteases, some are cross-class specific $serpin_{IC}$ that inhibit cysteine cathepsins. CrmA can inhibit both caspases 1 and 8 at physiological rate constants, whereas only caspase 1 is inhibited efficiently by PI9 [3–6]. Cross-class specific $serpins_{IC}$ can also inhibit executioner cathepsins. Spi2A from mice inhibits the serine protease cathepsin G and also cysteine cathepsins such as cathepsin B, V, L, K and H [7]. Spi2A protects from apoptotic PCD by inhibiting cathepsin B-mediated cleavage of Bid after stimulation by TNF-R) [7] (Fig. 2). Spi2A can also protect from necrotic PCD induced by ROS or TNF-α in the absence of caspase activity, presumably because cathepsin B can induce both apoptotic and necrotic PCD [34]. Spi2A is a physiological target of NF-κB [7,35] and can substitute for the transcription factor in protecting cell from the lysosomal pathways of PCD [7]. The evolutionary relevance of cytoprotection by cathepsin-specific $serpins_{IC}$ is underlined by SRP-6, which protects in *C. elagans* from necrotic PCD during development [10]. SQN-5 in the mouse and the human serpin SCCA1 (SERPINB3) [8,9] are other examples of cross-class specific $serpin_{IC}$ that inhibit cysteine cathepsin, but whether they are physiologically relevant inhibitors of PCD remains to be determined.

2.1. Programmed cell death and T lymphocytes

Programmed cell death plays a critical role in both the antigen-independent and antigen-dependent development of T lymphocytes. Two types of cellular selection take place in the thymus to shape the repertoire of antigen specificities displayed by T cells. Auto-reactive T cells are eliminated by a process referred to as negative selection through the direct induction of apoptosis