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DIVERSE CLINICAL FUNCTIONS OF SERINE PROTEASE AND ITS INHIBITORS

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ABSTRACT

Serine protease performs normal biological functions in cell. Serine protease are involves in coagulation, inflammation and immunity. Wide variety organisms produce serine proteases including virus, bacteria, animals and plants. Serine proteases are mono-meric enzymes. Serine protease are produced as inactive enzymes called zymogen and transported to other organs where they needed, which are then be cleaved and activated in those organs. They have a key role in malignancy as different carcinoma cells produced serine protease to promote Tumor growth. Malignant cells also produce serine protease inhibitor to protect the tumor cells from proteolysis. Malignant cells with serine protease expression have a very poor prognosis. Serine proteases are involved in inflammation. They serve as chemo-attractant for different pro-inflammatory cells and also involves in cleavage and activation of different pro-inflammatory cytokines. Serine proteases are also produced by different pathogens and helps pathogens in adherence and biofilm formation inside the host and increase pathogenicity. Serine protease inhibitors are very important in order to stop the abnormal proteolysis generate by serine protease. Serine protease inhibitors are also produced by a wide variety of organisms. Serine protease inhibitors involve in the enhancement of immunity by protecting the memory T cells from program cell death.

KEYWORDS: Zymogen, malignancy, pro-inflammatory cytokines, biofilms, memory T cells.

INTRODUCTION

Proteins are the functional units of cell. It produced to perform functions which are critical for the cell growth and development. Protein synthesis and proteolysis are the most important function for cell metabolism. Proteolysis is used in different cellular function including coagulation, inflammation, immunity, digestion, development etc. these enzymes have catalytic mechanism for the selective cleavage specific substrate. Serine protease is group of protease family which is very important in the proteolysis and has very close relationship with cell differentiation andcell growth. They often produced as zymogens which are inactive form activated after specific but limited cleavage of the polypeptide chain. Example of this activation is activating the trypsin by cleaving the trypsinogen using enteropeptidase (Huber & Bode, 1978).

Serine protease has different types including trypsin, chymotrypsin, elastase etc. Trypsin is the most important serine protease because it activates the chymotrypsinogen into chymotrypsin by cleaving Arg 15 - Ile 16 bond of the chymotrypsinogen. Trypsin is also activating the elastase by specific cleavage of its polypeptide chain. Serine protease also found in many organism including bacteria and helminthes and it plays a very crucial role in host-parasite relationship. The balance between the protease and there inhibitor is very much important. The misbalance will leads to different diseases. Inhibitors are also very helpful in inhibiting the pathogenic serine protease which can cause disease. In this review we focus on the clinical functions of serine protease and its inhibitors.

Role of Proteases in Cancer

Different research studies are published from several years that shown relationship between protease and tumor growth at metastasis and primary site (Yang *et al*., 2009). Expression of proteolytic enzymes is induced bytumor cell into neighboring non-cancerous cells to favor the tumor expansion by manipulating their activity (Zucker *et al*., 2000). One kind of serine protease urokinase type plasminogen activator is well investigated to establish its relationship with tumor growth and metastasis (henneke *et al*., 2010). Different studies has shown that their enzyme activity regulation and expression are closely related to malignant phenotype of tumors. Type II transmembrane serine protease (matriptase) is involved in progression, angiogenesis and

degradation of extracellular matrix of some epithelial cancers (*Nakamura et al*., 2009). The hepatocyte growth factor activator inhibitor (HAI-1) in normal cells inhibits the activity of the serine protease. Expression of metriptase is still there while on the other hand there is a loss of HAI-1 in progression of human prostate cancer. Ratio of these two products of gene is serves as a biomarker for human prostate cancer and may establish the efficacy of chemo-preventive and therapeutic interventions.

One of the best characterized serine protease is trypsin. This protease plays a very critical role in cellular physiology as well as pathology e.g. inflammation, atherosclerosis, cancer (Borg, 2004). The trypsin is produced as inactive trypsinogen in pancreas and transported to the duodenum where trypsinogen is activated by conversion of the trypsinogen to trypsin using an enzyme enteropeptidase. Other trypsin expressing cells as well as carcinoma cells also have the trypsin activating enteropeptidase (Miyata *et al*., 1999). An imbalance of protease and its inhibitor may lead to apathophysiological condition, pancreatitis. Pancreatic secretory trypsin inhibitor (PSTI) which is produced to protects pancrease from premature activity of trypsin (O'Keefe *et al*., 2005). PSTI is produced and secreted by mucosa of the normal gastrointestinal tract to protect it from proteolytic breakdown. On the other hand tumor cells alsoproduced same peptide known as tumor associated trypsin inhibitor to protect tumor cells from proteolysis (Stenman *et al*., 1991).

Trypsin is involved in colorectal cancer and it promotes proliferation, metastasis and invasion (soreide *et al*., 2006). Colorectal cancer with trypsin expression has very short disease free survival as well as very poor prognosis. How trypsin involved in cancer progression, its understanding is still emerging. It seems like trypsin involved in both "protease anti-protease system" and also by activating other protease cascades. Trypsin co expressed and activates the matrix metalloproteases (MMP's) which are known to involve in facilitation of metastasis and invasion of Tumor (Nyberg *et al*., 2002). Trypsin is co-expressed with other MMP's including MMP-2, MMP-7 and MMP-9 that seems to be very important in progression proliferation and invasion of tumor. MMP's are involves in initiation of metastasis and invasion as well as conversion into carcinoma from adenoma. Co-expression of MMP's and trypsin together in tumor environment has a prime importance in activation of MMP's and it also explains the damaging effect of trypsin on prognosis of colorectal cancer. Protease-activated receptors 2 (PAR-2) and trypsin act together in an autocrine loop that promotes proliferation, metastasis, invasion by various mechanisms of which the synthesis of prostaglandin is very important (Jahan *et al*., 2008). (figure.1) Presentation of ligand sequence on extracellular domains of receptor and after the site specific proteolysis of N-terminus by trypsin and activation of PAR-2, explains its participation in tissue differentiation and growth, repair and regeneration, malignant transformation and inflammatory response regulation (Adams *et al*., 2011).

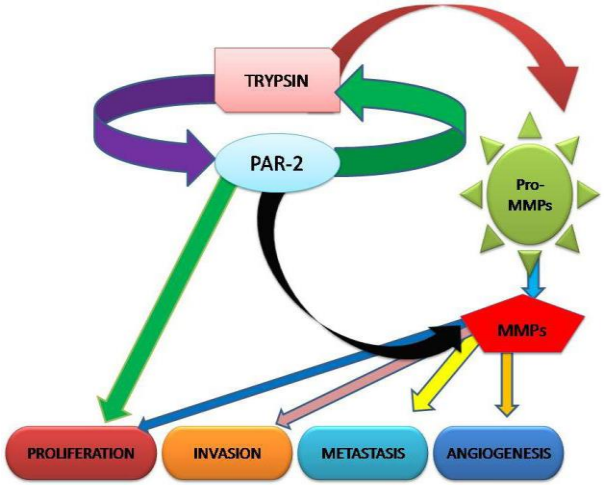

Figure 1: Interaction of trypsin with PAR-2 and the matrix metalloproteases (MMPs) and its role in carcinogenesis.

Role of Serine Protease in Inflammation

Inflammation is natural phenomena which used by the body against injury or damage to the tissues. It delivers the defensive tools to the site of injury. Tissue damage implies disruption of normal histological features with recruitment of leukocytes very rapidly and changes to the blood vessels while inflammatory mediators coordinate the response in a way that preserves both circulation and vascular activity while allows the extravasations of leukocytes (Sharony *et al*., 2010). Serine protease family has an active and important role in pro-inflammatory response. There are different members of serine protease family which are involved in pro inflammatory response. These proteases send their signals by interacting with specific cell-membrane receptors. Protease activated receptors are the class of trans-membrane G proteins play a crucial role in inflammation, cardio vascular biology and thrombosis (Leger *et al*., 2006). All protease activated receptors describe till date is expressed in different types of cells and modulate the responses to the coagulation proteases during inflammatory states and thrombosis (Hollenberg *et al*., 2002).

Elastase: is a major serine protease has a pro-inflammatory effect. It degrades the inter epithelial E-cadherin and inter-endothelial VE-cadherin promotes the permeability through these cell layers (Carden *et al*., 1998). Elastase is also stimulates the secretion of the granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8 and IL6 from epithelial cells (Nakamura *et al*., 1992) on the other hand in same time it can degrades the IL-8,IL-1, IL-1band IL-2. This effect can further enhance the migration of leukocytes and can propagate the inflammation. The effect of the proteolysis is depends totally on the balance between the anti-

inflammatory and pro-inflammatory states. In many diseases imbalance of proteases and their inhibitors play an important role in the progression of pathological conditions.

Cathepsin-G: Cathepsin-G is another member of serine protease family that hydrolysis many proteins. Cathepsin-G also plays an important role in pro-inflammatory response (Owen *et al*., 1999). It play an important role in chemoattractant for leukocytes including T-cells and in degradation of cytokines as well as extracellular components. It plays a key role in tissue modeling by it elastolytic activity (Boudier *et al*., 1991). Cathepsin-G Modulate coagulation and remodeling of tissues at the site of inflammation and injury By using the mechanism of cleaving and activating the G protein-coupled receptors (GPCR's).

PR-3: has an indirect role in pro-inflammatory response. IL-18 has role in inflammatory states which is cleaved and activated by the Caspase1. But the PR-3 activates the IL-18 independent of caspase-1 activity confirmed by using a caspase-1 deficient mice model (Tsutsui *et al*., 1999).

Thrombin: It is another serine protease which plays a critical role in regulation of homeostasis and vascular integrity. It is the key enzyme of Inflammatory mediator, angiogenesis factor, coagulation cascade,regulator of vascular cell function and platelet agonist (Mann *et al*., 2003).In malignancy generation of thrombin through the activation of tissue factor-dependent coagulation cascade is also well described (Rickles *et al*., 2003). Tissue injury and inflammation activates the thrombin which modulates the activity of MOS of the vascular growth factors which explains the angiogenic properties of thrombin.

Table 1: Serine protease which plays active role in inflammatory events.

Protease	Targets	Function	References
Elastase	E-cadherin, GM-CSF, IL-1, IL-2,IL-6, IL8, p38MAPK, TNFa, VE-cadherin	Degrades ECM components Regulates inflammatory response Activates pro-apoptotic signaling	(Sharony *et al.*, 2010)
Cathepsin G	EGF, ENA-78, IL-8, MCP-1, MMP-2, MT1-MMP, PAI-1, RANTES, TGFb, TNFa	Degrades ECM components Chemo-attractant of leukocytes Regulates inflammatory response Promotes apoptosis	(Owen and Campbell,1999)
PR-3	IL-18, IL-8, JNK, p38MAPK,ENA-78, TNFa	-Activates pro-apoptotic signaling -Promotes inflammatory response	(Robache-Gallea *et al.*, 1995)
Thrombin	FGF-2HB-EGF, Osteo-pontin, PDGF, VEGF	Strengthens VEGF-induced proliferation Inflammatory mediator Induces cell migration Regulates haemostasis	(Leger *et al.*, 2006)

Bacterial Serine Proteases Involves in Pathogenicity

Different bacteria which cause pathogenecity secrete highly specialized proteins. Gram-negative bacteria use a most common mechanism for delivering their virulence factors is the type V secretion pathway (Pallen *et al*., 2003). It includes a C-terminal translocator and an N-terminal passenger domain; these are known as classical autotransporters. Different autotransporter proteins provide the bacteria in adherence, cytotoxicity, and pathogenecity it also protects the bacteria against the different antimicrobial activities.

EspC: It is involved in the cleavage of hemoglobin, pepsin and coagulation factor V like substrates. It is also hypothesized that *EspC* also help the bacteria to utilize the heme as an iron source for its growth as it is a hemin-binding protein (Drago-Serrano *et al*., 2006), by the inactivation of coagulation-factor V this virulence trait could be enhanced. It involved in promoting the cytotoxic effect and intestinal colonization of bacteria and perhaps enhanced diarrhea which is some time bloody and most of the time profuse watery diarrhea.

Sig-A: is an enterotoxin which may contribute to cell destruction, watery diarrhea and also for the inflammatory events. Different types of virulence determinants actively act in shigellosis and pathogenesis. A high level of anti-Sig-A serum titer is observed in a pet sera of a patient infected with *Shigella flexneri* explains that the Sig A is produced in vivo (Al-Hasani *et al*., 2009).

Sat: Before triggering the cell detachment and cytoskeleton disruption sat induce autophagy which causes vacuolation of cell cytoplasm (Lievin-Le*et al*., 2011). Using lysosomal machinery it triggers degradation of the cell's own components. It shows that autophagy is tightly regulated process (Yang *et al*.,

2010). It is a normal process which plays an important part in cell homeostasis, development and growth but if it is intentionally trigger it can harm the host cell and might be served as the virulence strategies of bacteria. Cell death triggered by Sat is actually the cytotoxic effect which is induced by the class-1 SPATE's.

Pet: It is predominantly distributed in many other clinical *Enteroaggregative E.coli* isolates was found originally encoded in the pAA2 which is a virulence plasmid of the prototype *Etnteroaggregaive E.coli* strain (Eslava *et al*., 1998). Pet is one of the most conclusively studied class-1 SPATE till date. The purified Pet protein is found to trigger the cytopathy in HT29 C1 and HEp-2 cells, phenotype dependence of catalytic serine protease motif found in the pet passenger domain which mediates the activity of proteolysis (Navarro-Garcia *et al*., 1999).

Table 2: Bacterial different serine proteases which are involves in virulence and cause different diseases.

Protein	Species	Disease	Function/ Effect	References
Sat	-*Uropathogenic E. coli* -*Shigella* -*Enteroaggregative E. coli*, -*Diffuse- Adhering E. coli*	-Cystitis, pyelonephritis -Watery Diarrhea-Bloody, mucoid diarrhea	- Enterotoxin, Cytotoxin, -autophagy, impairment of tight junctions	(Boisen*et al*., 2009), (Fernando and James, 2014)
Pet	-*Enteroaggregative E. coli*	-Watery Diarrhea	-Cytotoxin -Enterotoxin	(Henderson *et al*., 1999)
SigA	-*Shigella*, -*Enteroaggregative E. coli*	Watery diarrhea -Bloody, mucoid diarrhea	-Enterotoxin -Cytotoxin	(Al-Hasani*et al*., 2009)
EspC	-*Enteropathogenic E. coli*	Watery diarrhea	-Cytotoxin, -Enterotoxin	(Dutta*et al*., 2002)

Serine Protease from Parasitic Helminthes

Humans are constantly threatened by the infectious parasites which are the most important pathogens worldwide. The parasitic helminthes could be divided into three classes, trematode, nematodes and cestodes (Yang *et al*., 2015). Protease releases from the parasite play a key role in infection and the life cycle of the parasite. Serine protease mostly is the digestive protease involves in the host tissue penetration and the metabolic processes for the pathogen. Serine protease additionally performs the key role in development, reproduction and host immune system evasion (Dzik JM 2006).

Serine protease of parasitic nematodes

Trichinella: cause infection in a wide variety of animals it is an intracellular parasite. The life cycle is completed in a single host it starts with the invasion in skeletal muscle cells and epithelial. A recent study shows a substantial serine protease activity against the different structural proteins at crude extracts and excretion-secretion of muscle stage larva while an adult and newborn larva degrades the hematic protein. During the parasitic infection host humoral and mechanical barriers are breakdown using this stage-specific proteolysis. In a sensitize host antibodies attach with these serine protease and inhibit them to impair the parasite (Ros-Moreno *et al.*, 2000). At different stages of T. spiralis multiple serine proteases identified which suggest that serine protease super-family is present in T. spiralis. Stage specific location, expression, and presence of a regulation domain may have a different function in parasite infection (Yang Y *et al.*, 2015).

Anisakis Simplex: is another example of infectious nematode which causes the gastrointestinal tract infection, which might be caused by eating of undercooked raw sea food that contains the larva of A. simplex. A. simplex larva after entering the body penetrates the submucosa, mucosa and muscularis of host intestine and stomach might be able to migrate to the liver, omentum, gall bladder or pancrease. A study reveals that trypsin-like serine protease found in the secretion of larvathat degrades connective tissues and extra cellular matrix (Sakanari *et al.*, 1990). In another study 4 serine protease genes were isolated from A. simplex using a consensus region of mammalian serine protease degenerate oligo-nucleotide primer. One of these nematode genes is 67% identical with trypsin II gene of rat. It is observed that the intron-exon junctions are conserved in between both rat and nematode while align the 2 genes, confirming the functional and structural similarity between 2 genes. So, it explains that the infective larval serine protease might be involved in digestion and degradation of host tissues (Sakanari *et al.*, 1989).

Serine protease of parasitic termatode

***Fasciola gigantic/hepatica*:** are the parasites which are involved in causing liver fluke disease. It effects sheep and cattle, it also very important human disease. A serine protease Dipeptidylpeptidase from *Fasciola hepatica* is purified using ion exchange chromatography. This serine protease in expressed by immature and mature flukes as well as expressed by newly excysted juvenile fluke. A study shows that dipeptidylpeptidase converts the host macromolecules into peptides. These peptides are

ingested by the parasite which helps in its growth and development (Carmona *et al.*, 1994). In another study the *Fasciola gigantic* serine protease is separated it is also finds out that the stability and activity of serine protease is depends on temperature and divalent cat-ions. This study reveals that the development of *fasciola gigantic* is increased when proteolytic activity is increased which suggest that serine protease has a key role in the physiology of the parasite but the precise function of protease is still unknown (Mohamed *et al.*, 2005).

***Schistosoma mansoni*:** is the causative agent of *schistosomiasis* which is a serious human disease of tropical areas; millions of peoples affected by this disease every year. It begins with the invasion of intact skin by *cercariae*. Several studies have shown that a trypsin-like serine protease "cercarial elastase" released by the parasite, which is one of the major histolytic protease which involved in skin invasion. It also indicates that it is only expressed in *cercariae* and it is stage-specific. It is only produced in the acetabular gland cells of larvae which further explain that carcariae elastase is regulated in a specialized cell within a limited development (Newport *et al.*, 1998). A protease activity essay reveals that secretion of *cercariae* which contains a serine protease with trypsin-like activity can be involved in host invasion. Another study demonstrates that trypsin-like serine protease is the contaminant of intermediate host snail (Salter *et al.*, 2002).

Serine protease of parasitic Cestode

***Spirometra mensoni*:** It is a cestode it *plerocercoid* larvae is the causative agent of spargsnosis which is usually result from ingesting contaminated water or food. It is usually resides in skin where it form nodule but it can migrate to any part of the body. In the extract of plerocercoids, 3 neutral serine protease have been found (Kong *et al.*, 1994). Study performed by kong *et al.* identified 1 chymotrypsin-like serine protease involves in the cleavage of bovine myelin basic protein and human recombinant interferon-g and 2 trypsin-like serine protease shows collagenolytic activities. In infected patients a high anti body response have seen which suggest that in serologic diagnosis, they could be the potential antigens in human sparganosis (Kong *et al.*, 1994).

Serine Protease Inhibitors from Different Sources

Animals and plants have developed a specialized defense system against pathogenic microorganism including bacteria, virus and fungi. This defense system has ability to inhibit the proteins secreted by these microorganisms. Protease inhibitors are the proteins which form a high affinity stoichiometric complex with protease in order to inhibit the hydrolytic activity of proteases (Jin *et al.*, 2009). Most protease inhibitors bind to the active site of protease make the enzyme incapable of activity by just forming a protease-inhibitor complex. Protease regulates different functions including synthesis, activation and turnover of all proteins. Proteolysis is a very controlled mechanism if it gets uncontrollable it is dangerous for the cellular functions. Different checkpoints are developed by the nature to control this mechanism known as protease inhibitors. Uncontrolled proteolysis is involved in different diseases such as systemic inflammatory response syndrome, hepatitis, arthritis, emphysema, pancreatitis etc (Catherine 2009). Protease inhibitors play a key role in defense system against the microorganisms and insets and act as antibody against some viruses and fungi. These inhibitor targets the hydrolytic enzymes of the microorganism to inhibit them which ultimately leads to the inhibition of growth and development of microorganism. Protease inhibitors from extracts of plant play a key role in synthesis of antimicrobial agents as they developed a potential inhibition against different pathogenic microorganisms (Jin *et al.*, 2009).

Largest inhibitors family distributed throughout nature is serine protease inhibitors. Serine protease inhibitors that inhibit chymotrypsin or trypsin are of low molecular mass molecules (from 3-25 kD). Serine protease inhibitors can be classified into three groups based on their substrate specificity such as elastase like, trypsin-like chymotrypsin-like (Barrett *et al.*, 1998). Serine protease inhibitors based on the source can be classified structurally into 13 different families. Six families of Serine protease inhibitors are of microbial or mammalian origin they are Bovine pancreatic trypsin inhibitors family, *Hirudin* family, the *Chelonianin* family, the *Kazal* family, *Serpins* family and *Streptomyces subtilisin* inhibitors family. These families are distinguished from each other by cystein content, mass and number of reactive sites. (Richardson 1977).

Bovine pancreatic trypsin inhibitor: Bovine pancreatic trypsin inhibitors are isolated from the egg yolk there was no cross reactivity with inter-alpha-trypsin inhibitor, alpha1-protinase inhibitor and show activity against pancreas and lungs (Melrose *et al.*, 2000).Serine protease inhibitors *Hirudin*which is isolated from the leech's saliva and it is thrombin specific inhibitor which is a serine protease involved in the coagulation of blood (Rydel *et al.*, 1990). Kazal et al was identified *Kazal*family. They are the inhibitors of double heads which inhibits chymotrypsin and trypsin simultaneously (Mistry *et al.*, 1997). *R-Elfin* and *secretory leukocytes* protease inhibitors are very familiar member to the *Chelonianin* protease inhibitors. It is stabilized by 7 disulfide bridges it is double headed and inhibits elastase and trypsin (Stergios Doumas *et al.*, 2005).

Serpins is the largest super family of serine protease inhibitors found in wide variety of organisms such as bacteria, virus, animals and plants. They irreversibly inhibit Serine protease. Barley is a potent inhibitor of chymotrypsin and trypsin with reactive sites which overlapped and active against *spodoptura litura* and *Agrotis ipsilon* (Carbonero *et al.*, 1993).

Role of Serine Protease Inhibitors in Immunity

Secretory leukocyte protease inhibitors (*SLPI*): It is first isolated from the secretions of human parotide gland. It shields within the lungs by providing an important component of human anti-protease. Against the protease it provides a significant protection, through its C-terminus domain, such as Cathepsin G. SLPI is released by different inflammatory cells including mast cells, neutrophils and macrophages. It is estimated that the concentration of SLPI in nasal secretion is 2.5 mg/ml (Lee *et al*., 1993) and 0.1-2mg/ml in BAL fluid. It is also suggested that SLPI down regulates the macrophage responses against bacterial lipopolysaccharides thus shields the tissues from inflammatory products. SLPI level and lipopolysaccharides is the most important mediator in bacterial endotoxic shock in patients with sepsis (Sallenave *et al*., 1999). It seems that SLPI production is induced by lipopolysaccharides from macrophages directly or TNF-α, IL-1b IL-10 and IL-6 (Gipson *et al*., 1999).

Systemic antiprotease a-1-protease inhibitors: it is the member of serpins superfamily of proteins which is the prototypic member of serine protease inhibitors, it perform an important role in inactivation of Neutrophile Elastase and some other proteases including proteinase 3 and cathepsin G. Systemic antiprotease a 1-protease inhibitor is produced in small quantities by some immune system cells as well as by some epithelial surfaces (Paakko *et al*., 1996), Primarily these inhibitors are produced by hepatocytes. The major inducer of a-1-protease inhibitors is the cytokin on costatin-M in bronchial epithelial cells (Sallenave *et al*., 1997).

a-1-Protease inhibitor deficiency is a genetic disorder of lungs. In the early-onset of emphysema is result from the low level of a-1-protease inhibitor secretion. It was early believed that in the respiratory tract the decrease secretion of a-1-protease inhibitor can leads to prolong and unopposed activity of neutrophil elastase, recent studies reveals that when polymerize the mutated variant Z it may act as chemo-attractant for neutrophils when secreted, may be pro-inflammatory in the a-1-protease inhibitor deficient lung and adding to the excessive neutrophil elastase burden and neutrophill (Parmar *et al*., 2002). Its role as an anti-protease like SLPI and elafin, a-1-protease inhibitors possess important pleitropic pro inflammatory or anti-inflammatory properties, which are totally condition dependent. These effects including the regulation of expression of pro-inflammatory cytokines including IL-8, IL-1 b, IL-6, TNF-α and monocyte chemotactic protein by monocytes and blocking of pro-inflammatory effect of human (Spencer *et al*., 2004).

Role of serpins in the development of memory T-cells

Immunological memory is defined as the persistence of the reactive state by antigenic challenge and it is a very important for vertebrate's immune system. Memory lymphocytes are the important entity on which this persistence of immunological memory is dependent, which are functionally prior to naïve lymphocytes and increase the frequency of pathogenic-specific cells. Different studies support a model for memory T lymphocytes developemnent known as differential model (Kaech *et al*., 2002). The effector T cell are very susceptible to programmed cell death, small stochastic changes in small proportion of memory T cell precurse may facilitates the escape of effectors from programmed cell death which is the differentiation, memory cells required. It might be involved in the encoding of antiapoptotic factors in memory cells precursors which are upregulated by the protective genes. Different studies have shown that serpins which are produced in cytotoxic T cell controls the memory T cells in many ways. Serine protease inhibitor 2A is the inhibitor of cathepsins and cysteine which protect the cells from programmed cell death activated by lysosomal pathway, which upregulate the precursors of memory T cell as well as memory T cells them self. Over expression of serine protease 2A can increase the amount of memory T cells (Liu *et al*., 2004).

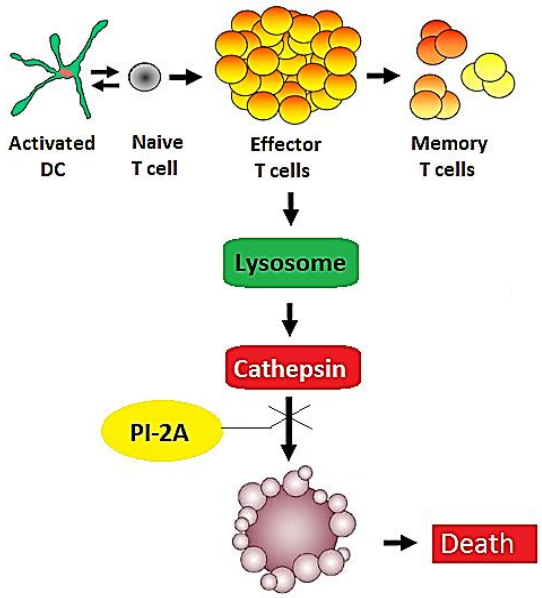

Figure 2: Protease inhibitor 2A inhibits the cathepsins which protects Memory T-cell from programmed cell death.

CONCLUSION

Serine protease and its inhibitor have a wide range of functions including normal functions such as coagulation immunity and cell homeostasis. Serine protease also has pathological functions like pancreatitis etc. It plays an active role in cancer proliferation and metastasis. Serine protease is also produced by other pathogenic organisms and serves as pathogenic agent. Its inhibitors play an important role in immunity boost. The serine protease inhibitors produced by cancer cells help to protect tumor cells from proteolysis. Serine protease and it inhibitors might play a critical role in the cure of cancer in future. Inhibitors of other sources used as drug to cure different

disease caused by the abnormal behavior of serine protease. It also helps to study the pathogenic relations of serine protease to eradicate different pathogens.

REFERENCES

1. Adams M.N., R. Ramachandran, M.K. Yau, J.Y. Suen, D.Y. Fairlie, M.D. Hollenberg and J.D. Hooper. Structure function and pathophysiology of protease activated receptors. *Pharmacol Ther*, 2011; 130: 248-282.
2. Al-Hasani K et al. The immunogenic siga enterotoxin of shigella flexneri 2a binds to hep-2 cells and induces fodrin redistribution in intoxicated epithelial cells. *PLoS One*, 2009; 12: 2-23.
3. Barrett A.J., N.D. Rawlings and J.F. Woessner. *Handbook of proteolytic enzymes*. Academic Press: New York, 1998.
4. Boisen N et al. Short report: High prevalence of serine protease autotransporter cytotoxins among strains of enteroaggregative escherichia coli. *Am J Trop Med Hyg*, 2009; 80: 294-301.
5. Borge T.K. It's the matrix! Ecm proteases, and cancer. *Am. J. Pathol*. 2004; 1141-1142.
6. Boudier C., G. Godeau, W. Hornebeck, L. Robert and J.G. Bieth. The elastolytic activity of cathepsin g: An ex vivo study with dermal elastin. *Am J Respir Cell Mol Biol*, 1991; 4: 497-503.
7. Carbonero P., J. Royo, I. Díaz, F. García-maroto, E. Gonzalez-hidalgo, C. Gutierrez and P. Casanera, Cereal inhibitors of insect hydrolases (a-amylases and trypsin): Genetic control, transgenic expression and insect pests. Spain, 1993.
8. Carden D., F. Xiao, C. Moak, B.H. Willis, S. Robinson-Jackson and S. Alexander. Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. *Am J Physiol*, 1998; 275: 385-392.
9. Carmona C., S. McGonigle, A.J. Dowd, A.M. Smith, S. Coughlan, E. McGowran and J.P. Dalton. A dipeptidylpeptidase secreted by fas¬ciola hepatica. *J Parasitology*, 1994; 109: 113-118.
10. Catherine M.G. and G.M. Noel. Protease and anti proteases in chronic neutrophilic lung disease-relevance to drug discovery. *Br J Pharmacol*, 2009; 158: 1048-1058.
11. Drago-Serrano M.E., S.G. Parra and H.A. Manjarrez-Hernandez. Espc, an autotransporter protein secreted by enteropathogenic escherichia coli (epec), displays protease activity on human hemoglobin. *FEMS Microbiol Lett*, 2006; 265: 35-40.
12. Dutta PR. et al. Functional comparison of serine protease autotransporters of enterobacteriaceae. *J Infect Immun*, 2002; 70: 7105-7113.
13. Dzik J.M. 2006. Molecules released by helminth parasites involved in host colonization. *Acta Biochim Pol*, 53: 33-64.
14. Eslava C. et al. Petan autotransporter enterotoxin from enteroaggregative escherichia coli. *J Infect Immun*, 1998; 66: 3155-3163.
15. Fernando R.P. and P.N. James. Bacterial serine proteases secreted by the autotransporter pathway: Classification, specificity and role in virulence. *Cell Mol Life Sci*, 2014; 71: 745-770.
16. Gipson T.S., N.M. Bless, T.P. Shanley, L.D. Crouch, M.R. Bleavins, E.M. Younkin, V. Sarma, W. Gibbs D.F.Tefera, et al. x. Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. *J Immunology*, 2014; 162: 3653-3662.
17. Henderson I.R. et al. Involvement of the enteroaggregative escherichia coli plasmid-encoded toxin in causing human intestinal damage. *J Infect Immun*, 1999; 67: 5338-5344.
18. Henneke I., S. Greschus, R. Savai, M. Korfei, P. Markart, P. Mahavadi, T. Ralph, R.T. Schermuly, et al. Inhibition of urokinase activity reduces primary tumor growth and metastasis formation in a murine lung carcinoma model. *Am. J. Respir. Crit. Care Med*, 2010; 181: 611-619.
19. Hollenberg M.D. and S.J. Compton. Proteinase-activated receptors. *Pharmacol Rev*, 2002; 54: 203-217.
20. Huber R. and W. Bode. Structural basis of the activation and action of trypsin. *J Acc. Chem Res*, 1978; 11: 114-122.
21. Jahan I., J. Fujimoto, S. Mahfuzul, E. Sato and T. Tamaya. Role of protease activated receptor-2 in lymph node metasstasis of uterine cervical cancers. *BMC Cancer*, 2008; 8: 301.
22. Jin-Young K., P. Seong-Cheol, H. Indeok, C. Hyeonsook, N. Jae-Woon, H. Kyung-Soo and P. Yoonkyung. Protease inhibitors from plants with antimicrobial activity. *Int. J. Mol. Sci*, 2009; 10: 2860-2872.
23. Kaech S.M., S. Hemby, E. Kersh and R. Ahmed. Molecular and functional profiling of memory cd8 t cell differentiation. *Cell*, 2002; 111: 837-851.
24. Kazal L.A., D.S. Spicer and R.A. Brahinsky. Isolation of a crystalline trypsin inhibitor-anticoagulant protein from pancreas. *J. Am. Chem. Soc*, 1948; 70: 304-340.
25. Kong Y., Y.B. Chung, S.Y. Cho, S.H. Choi and S.Y. Kang. Characterization of three neutral proteases of spirometra mansoni plerocercoid. *J Parasitology*, 1994; 108: 359-368.
26. Lee C.H., Y. Igarashi, R.J. Hohman, H. Kaulbach, M.V. White and M.A. Kaliner.. Distribution of secretory leukoprotease inhibitor in the human nasal airway. *Am Rev Respir Dis*, 1993, 147: 710-716.
27. Leger A.J., L. Covic and A. Kuliopulos. Protease-activated receptors in cardiovascular diseases. *J Circulation*, 2006; 114: 1070-1077.
28. Lievin-Le M.V. et al. Secreted autotransporter toxin (sat) triggers autophagy in epithelial cells that relies on cell detachment. *J Cell Microbiol*, 2011; 13: 992-1013.
29. Liu N., T. Phillips and M. Zhang. Serine protease inhibitor 2a is a protective factor for memory t cell

development. *Nature Immunology*, 2004; 5: 919-926.
30. Mann K.G., K. Brummel and S. Butenas. What is all that thrombin for? *J Thromb Haemost*, 2003; 1: 1504-1514.
31. McDonnell J., J.M. Lobner, W.B. Knight, M.W. Lark, B. Green, M. Poe and V.L. Moore. Comparison of the proteoglycanolytic activities of human leukocyte elastase and human cathepsin g in vitro and in vivo. *Connect Tissue Res*, 1993; 30: 1-9.
32. Melrose J., S. Smith, K. Rodgers, C. Little, D. Burkhardt and P. Ghosh. Immunolocalisation of bpti-like serine proteinase inhibitory proteins in mast cells, chondrocytes and intervertebral disc fibrochondrocytes of ovine and bovine connective tissues. An immunohistochemical and biochemical study. *J Histochem Cell Biol*, 2000, 114: 137-146.
33. Mistry R., P.D. Snashall, N. Totty, S. Briskin, A. Guz and T.D. Tetley. Purification and characterization of a novel - type serine proteinase inhibitor of neutrophil elastase from sheep lung. *Biochim. Biophys. Acta*, 1997; 1342: 51-61.
34. Miyata S., N. Koshikawa, S. Higashi, Y. Miyagi, Y. Nagashima and S. Yanoma. Expression of trypsin in human cancer cell lines and cancer tissues and its tight binding to soluble form of alzheimer amyloid precursor protein in culture. *J. Biochem (Tokyo)*, 1999; 125:1067-1076.
35. Mohamed S.A., A.S. Fahmy, T.M. Mohamed and S.M. Hamdy. Proteases in egg, miracidium and adult of fasciola gigantica. Characterization of serine and cysteine proteases from adult. *Comp Biochem Physiol B Biochem Mol Biol*, 2005; 142: 192-200.
36. Nakamura H., K. Yoshimura, N.G. McElvaney and R.G. Crystal. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest*, 1992; 89: 1478-1484.
37. Nakamura K., A. Hongo, J. Kodama, F. Abarzua, Y. Nasu, H. Kumon and H.Y. YUJI. Expression of matriptase and clinical outcome of human endometrial cancer. *Antic. Res*, 2009; 29: 1685-1690.
38. Navarro-Garcia F. etal. Cytoskeletal effects induced by pet, the serine protease enterotoxin of enteroaggregative escherichia coli. *Infect Immun*, 1999; 67: 2184-2192.
39. Newport G.R., J.H. McKerrow, R. Hedstrom, M. Petitt, L. McGarrigle, P.J. Barr and N. Agabian. Cloning of the proteinase that facilitates infection by schistosome parasites. *J Biol Chem*, 1988; 263: 13179-13184.
40. Nyberg P., M. Moilanen, A. Paju, A. Sarin, U.H. Stenman, T. Sorsa and T. Salo. Mmp-9 activation by tumor trypsin-2 enhances in vivo invasion of human tongue carcinoma cells. *J. Dent. Res*, 2002; 81: 831-835.
41. O'Keefe S.J., R.B. Lee, J. Li, S. Stevens, S. Abou-Assi and W. Zhou. Trypsin secretion and turnover in patients with acute pancreatitis. *Am. J. Physiol. Gastrointest. Liver. Physiol*, 2005, 289: 181-187.
42. Owen C.A. and E.J. Campbell. The cell biology of leukocyte-mediated proteolysis. *J Leukoc Biol*, 1999; 65: 137-150.
43. Paakko P., M. Kirby, R.M. du Bois, A. Gillissen, V.J. Ferrans and R.G. Crystal. Activated neutrophils secrete stored alpha 1-antitrypsin. *Am J Respir Crit Care Med*, 1996, 154: 1829-1833.
44. Pallen M.J., R.R. Chaudhuri and I.R. Henderson. Genomic analysis of secretion systems. *Curr Opin Microbiol*, 2003; 6: 519-527.
45. Parmar J.S., R. Mahadeva, B.J. Reed, N. Farahi, K.A. Cadwallader, M.T. Keogan, D. Bilton, E.R. Chilvers, et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: A new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol*, 2002; 26: 723-730.
46. Richardson M. The protease inhibitors of plants and microorganisms. *J Phytochemistry*, 1977; 16: 159-169.
47. Rickles F.R, S. Patierno and P.M. Fernandez. Tissue factor, thrombin, and cancer. *Chest*, 2003; 124: 58-68.
48. Robache-Gallea S., V. Morand, J.M. Bruneau, B. Schoot, E. Tagat, E. Realo, S. Chouaib and S. Roman-Roman. In vitro processing of human tumor necrosis factor-alpha. *J Biol Chem*, 1995; 270: 23688-23692.
49. Ros-Moreno R.M., C. Vázquez-López, C. Giménez-Pardo, C. de Armas-Serra and F. Rodríguez-Caabeiro. A study of proteases throughout the life cycle of trichinella spiralis. *J Folia Parasitol (Praha)*, 2000; 47: 49-54.
50. Rydel T.J., K.G. Ravichandran, A. Tulinsky, W. Bode, R. Huber, C. Roitsch and J.W. Fenton. 2nd the structure of a complex of recombinant hirudin and human alpha-thrombin. *Science*, 1990; 249: 277-280.
51. Sakanari J.A. and J.H. McKerrow. Identification of the secreted neutral proteases from anisakis simplex. *J Parasitol*, 1990; 76: 625-630.
52. Sakanari J.A., C.E. Staunton, A.E. Eakin, C.S. Craik and J.H. McKerrow. Serine proteases from nematode and protozoan parasites: Isolation of sequence homologs using generic molecular probes. *Proc Natl Acad Sci USA*, 1989; 86: 4863-4867.
53. Sallenave J.M., G.M. Tremblay, J. Gauldie and C.D. Richards. Oncostatin m, but not interleukin-6 or leukemia inhibitory factor, stimulates expression of alpha1-proteinase inhibitor in a549 human alveolar epithelial cells. *J Interferon Cytokine Res*, 1997; 17: 337-346.
54. Sallenave J.M., S.C. Donnelly, I.S. Grant, C. Robertson, J. Gauldie and C. Haslett. Secretory leukocyte proteinase inhibitor is preferentially increased in patients with acute respiratory distress syndrome. *Eur Respir J*, 1999; 13: 1029-1036.
55. Salter J.P., Y. Choe, H. Albrecht, C. Franklin, K.C. Lim, C.S. Craik and J.H. McKerrow. Cercarial

elastase is encoded by a functionally conserved gene family across multiple species of schistosomes. *J Biol Chem*, 2002; 277.
56. Sharony R., Y. Pey-Jen, P. Joy, C.G. Aubrey, M. Paolo and P. Giuseppe. Protein targets of inflammatory serine proteases and cardiovascular disease. *Journal of Inflammation*, 2010; 7(45): 1-17.
57. Soreide K., EA Janssen, H Korner and JPA Baak. Trypsin in colorectal cancer: Molecular biological mechanisms of proliferation, invasion and metastasis. *J. Pathol*, 2006; 209: 145-156.
58. Sotiropoulou G., G. Pampalakis and E.P. Diamandis. Functional roles of human kallikrein-related peptidases. *J Biol Chem*, 2009; 284: 32989-32994.
59. Spencer L.T., G. Paone, P.M. Krein, F.N. Rouhani, J. Rivera-Nieves and M.L. Brantly. Role of human neutrophil peptides in lung inflammation associated with alpha1-antitrypsin deficiency. *Am J Physiol Lung Cell Mol Physiol*, 2004; 286: 514-520.
60. Stenman U.H., E. Koivunen and O. Itkonen. Biology and function of tumor-associated trypsin inhibitor. *Scand J. Clin. Lab. Invest. Suppl*, 1991; 207: 5- 9.
61. Stergios D., K. Alexandros and S. Panagiotis. Anti-inflammatory and antimicrobial roles of secretory leukocyte protease inhibitor. *Infect Immun*, 2005; 73: 1271-1274.
62. Tsutsui H., N. Kayagaki, K. Kuida, H. Nakano, N. Hayashi, K. Takeda, K. Matsui, S. Kashiwamura, et al. Caspase-1-independent, fas/fas ligand-mediated il-18 secretion from macrophages causes acute liver injury in mice. *Immunity*, 1999; 11: 359-367.
63. Yang Y., H.H. Hao, Z.Y. Yin and C.W. Weibo. Molecular imaging of proteases in cancer. *J Canc Gro and Metast*, 2009; 2: 13-27.
64. Yang Y., Y.J. Wen, Y.N. Cai, I. Vallée, P. Boireau, M.Y. Liu and S.P. Cheng. Serine proteases of parasitic helminths. *Korean j parasitol*, 2015; 53: 1-11.
65. Yang Z. and D.J. Klionsky. Mammalian autophagy: Core molecular machinery and signaling regulation. *Curr Opin Cell Biol*, 2010; 22: 124-131.
66. Zucker S., J. Cao and W.T. Chen. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene*, 2000; 19: 6642-6650.