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New target against inflammatory diseases: transglutaminase 2

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Summary

Transglutaminase (TGase) 2 is an enzyme that is widely used in many biological systems for generic tissue stabilization or immediate defense for wounds. Many reports showed that TGase 2 is aberrantly activated in tissues and cells and contributes to a variety of diseases, including neurodegenerative diseases and autoimmune diseases. In most cases, TGase 2 appears to be a factor in the formation of inappropriate proteinaceous aggregates that may be cytotoxic. However, in other cases, such as celiac disease, arthritis, lupus, and amyotrophic lateral sclerosis, TGase 2 is involved in the generation of autoantibodies. This suggests the possibility that inappropriate expression and/or presentation of TGase 2 to T cells might contribute to these diseases in genetically predisposed individuals. We and others have found that TGase 2 expression is also increased in the inflammation process. Furthermore, we also demonstrated a reversal of inflammation by TGase inhibition. This review will examine a possibility of TGase inhibitors as therapeutic agents in a variety of inflammatory diseases.

Key words: transglutaminase • transglutaminase inhibitor • inflammation • autoimmune disease

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INTRODUCTION

Transglutaminase 2 (TGases, EC 2.3.2.13) is a particularly interesting enzyme to consider in the context of pathology because its many functions generally involve both protection and prevention of bodily injury, or tissue remodeling and repair²⁷. Their most common function is to catalyze the formation of isopeptide linkages between the carboxamide group of protein-bound glutamine residues and the ϵ -amino group of protein-bound lysine residues. Aberrantly activated TGase 2 contributes to a variety of diseases, including neurodegenerative diseases, atherosclerosis, inflammatory diseases, autoimmune diseases, and fibrosis³⁸. Although TGase 2 appears to be responsible for the formation of insoluble deposits in neurodegenerative diseases, it is not clear whether cross-linked inclusion itself is pathogenic. In other diseases, protein deposit is not a common factor for TGase 2 induction. Increased TGase activity is easily found both in diseased tissues with inflammation and in cells with inflammatory stress. The detail mechanism of TGase 2 contribution to the inflammatory process remains to be answered. Recently, however, we opened a possibility that TGase inhibitors can reverse the inflammatory process in the conjunctivitis⁶² and uveitis¹⁴ models.

INFLAMMATION

Recent studies by myself and others indicate that inhibition of TGases will be a profitable new approach to the treatment of at least some types of inflammation. Inflammation processes are complex biochemical phenomena that are manifested physiologically in tissues by edema, pain, and leukocyte infiltration. Currently, the most effective drugs for inflammation are glucocorticoids. Glucocorticoids induce many proteins, such as lipocortins, as inhibitory proteins of phospholipase A₂ (PLA₂, EC 3.1.1.4)²⁶. PLA₂ plays a key role in the pathogenesis of allergic conjunctivitis. Miele et al.⁴⁴ identified a region of sequence similarity between uteroglobin and lipocortin. Furthermore, they designed several synthetic peptides (nona-peptides) corresponding to the region of highest similarity between uteroglobin and lipocortin-1. They named these peptides anti-flamins, corresponding to uteroglobin residues 39-47 and lipocortin-1 residues 246-254. Both peptides were shown to be PLA₂ inhibitors *in vitro* and were effective in a classic model of acute inflammation in carrageenan-induced rat footpad edema.

Interestingly, treatment of purified PLA₂ with TGase 2 strikingly increased PLA₂ activity *in vitro*. TGase 2-catalyzed conformation of PLA₂ can be brought

about by formation of an intramolecular ϵ -(γ -glutamyl)-lysine cross-link¹⁹ or by incorporation of polyamines¹⁸. These observations suggest that TGase 2-mediated modification may activate PLA₂ *in vivo*, following an influx of calcium. Increased TGase 2 expression has been reported for many inflammatory diseases, such as celiac's disease²⁵, Crohn's disease²⁰, and sporadic inclusion body myositis¹⁷. The pathological roles of TGase 2 in those diseases might be associated with activation of PLA₂. Therefore we hypothesized that blocking both TGase 2 and PLA₂ activities may ameliorate PLA₂-mediated inflammation. To test this hypothesis, a series of new recombinant peptides using sequences from AFs and pro-elafin (cementin) were constructed as competitive inhibitors of PLA₂ and TGase 2, respectively. We showed that the recombinant peptides abolish the TGase 2-catalyzed activation of PLA₂ *in vitro* and have a pronounced anti-inflammatory effect on allergic conjunctivitis *in vivo*⁶². In this study, we have shown that rationally designed peptide inhibitors for TGase 2 are potent anti-inflammatory agents in allergic conjunctivitis⁶². This concept can be applied to PLA₂-mediated (arachidonic acid-mediated) inflammatory diseases. Interestingly, Taggart et al.⁶⁴ demonstrated that over-expression of elafin containing pro-elafin domain (TGase-inhibitory domain) prevents lipopolysaccharide-induced nuclear factor κ B activation without proteasome inhibition. This suggests that TGase 2 may be involved in the regulation of inflammatory signaling beyond activation of PLA₂.

AUTOIMMUNE DISEASES

Celiac disease

TGase inhibitors may have a role in the treatment of celiac disease. This disease is characterized by damage to the upper small intestine, causing effacement of the villi and producing a characteristically flat mucosa with markedly hypertrophic crypts (inflammatory bowel disease). Consequently, patients with celiac disease usually have difficulty with absorbing nutrition. This disease is found in genetically predisposed individuals (mostly HLA-DQ2⁺ and -DQ8⁺) who mount heightened T cell-mediated and humoral immune responses to gluten. The immunological reaction to gluten in the upper small bowel, where ingested dietary components are concentrated, leads to inflammation and the characteristic features of disease⁴³.

Progress in understanding the mechanism of the disease has been enhanced by the discovery that TGase 2 is an autoimmune antigen of celiac disease²².

Immunoprecipitation of proteins from fibrosarcoma cells with IgA from celiac disease patients led to the identification of TGase 2 as the dominant endomysial autoantigen. Based on a series of *in vitro* experiments, TGase 2 is thought to be responsible for generating neopeptides of gliadin through deamidation of glutamine residues. In this hypothesis, auto-antibody generation against TGase 2 cannot be explained at all. TGase 2, however, also cross-links itself onto gliadin *in vitro*. More likely, the cross-linked TGase may act as a hapten for the formation of antibodies against gliadin *in vivo*. This is a plausible hypothesis because serum anti-TGase antibody titer falls dramatically when wheat products are removed from the diet. Thus, the presentation of fragments of gliadin cross-linked to TGase 2 results in antibodies against gliadin, TGase 2, and the cross-linked proteins. The greater preponderance of anti-TGase antibodies presumably reflects the greater antigenicity of this antigen.

TGase 2 expression in jejunal biopsies of celiac disease patients is elevated 3.2-fold¹¹ and it is theoretically possible that this contributes to celiac disease by increasing the frequency of gliadin-TGase 2 cross-linking and/or deamidation. Increase of TGase 2 expression by proinflammatory cytokines is a possible novel mechanism. A plausible hypothesis for the increase in TGase activity in celiac disease duodenal mucosa is induction of the enzyme by cytokines present in this inflamed tissue. The inflammatory infiltrates of the jejunal tissues of celiac disease patients are rich in T cells. T cells are not the only source of cytokines, as duodenal biopsies from celiac disease patients show increases in interferon (IFN)- γ of greater than a 1,000-fold relative to samples from normal individuals. Elevations in interleukin (IL)-2, IL-4, IL-6, and tumor necrosis factor (TNF)- α have also been reported in celiac disease patients⁵¹. We have shown that IFN- γ can induce expression of TGase 2 in the rat IEC-6 small intestinal cells and, interestingly, transforming growth factor (TGF)- β suppresses TGase 2 expression in that system³⁹. Therefore it was suggested that the increased expression of TGase 2 leading to pathogenesis of celiac disease in genetically predisposed individuals (HLA-DQ2⁺) could be due to increased IFN- γ signaling, loss of TGF- β signaling, or both, in the small intestine.

Taken together, the above observations suggest that TGase 2 is involved in the pathogenesis of celiac disease. More work is necessary to ascertain whether its role is direct or indirect. In either case, TGase 2 inhibitors could be useful in the treatment of this fairly common chronic disease.

Sporadic inclusion body myositis and inflammatory myopathies

Sporadic inclusion body myositis (SIBM) is the most common progressive muscle disorder that affects older individuals. This disease is characterized by a progressively worsening weakness in the proximal and distal limbs that does not respond to steroid therapy. There is as yet no useful therapy for this disease. Askanas et al.⁵ hypothesized that the overexpression of the β -amyloid precursor protein and its abnormal deposition may precipitate the muscle-fiber destruction characteristic of inclusion body myositis. This hypothesis further suggested a role for TGases in inclusion body myositis based on the putative involvement of these enzymes in the formation of the β -amyloid aggregates in Alzheimer's disease. Total TGase enzyme activity is elevated 16-fold in SIBM tissue¹¹. Interestingly, pharmacological agents designed to attenuate the progression of symptoms of Alzheimer's disease also have an inhibitory effect on TGase-induced β -amyloid cross-linking *in vitro*, for instance indomethacin, phenelzine, tacrine, and deferoxamine⁶⁷. This suggests that appropriate TGase inhibitors may be already available for treating this relatively common disease.

To test whether an increase in TGase 2 expression in SIBM is a common factor in muscle inflammation, idiopathic inflammatory myopathies, including dermatomyositis (DM), polymyositis (PM), and sporadic inclusion body myositis were analyzed. Using immunocytochemistry and quantitative RT-PCR, the level of TGase 2 expression was found to be significantly increased in DM and PM¹⁶. DM and PM do not present any deposition of inclusion bodies containing highly cross-linked amyloid and other proteins. Therefore a plausible role of TGase 2 in the cascade of debilitating muscle diseases may be a contribution to the inflammatory process.

Other autoimmune diseases

Autoantibodies against TGase 2 are found in the blood of patients with dermatitis herpetiformis⁵⁵. Dermatitis herpetiformis is characterized by blistering of the extensor surfaces and sensitivity to gluten. Thus the presence of anti-TGase 2 antibodies in celiac disease and dermatitis herpetiformis may reflect a common etiology. In addition, the antibodies in dermatitis herpetiformis may arise in a similar manner to that described above for celiac disease. Consistent with this view is the observation that the anti-TGase 2 antibodies found in dermatitis herpetiformis are mainly of the IgG class. The IgA immunoglobulins are predominantly associated with

Table 1. Autoantibodies against TGase substrates in autoimmune disease

TGase substrate /autoantigen	Autoimmune disease	References for autoimmunity	TGase substrate
Actin	hepatitis	52	50
	necrobiosis lipidica	33	
	lupus (human)	1	
Keratins	hepatitis		12
	necrobiosis lipidica		
Histone H2B	lupus (human and murine)		6
Lipocortin I	lupus (murine)	36	4
Myosin	lupus (human)		24
	myasthenia gravis	13	
Troponin	necrobiosis lipidica		8
Tubulin	hemolytic anemia	34	53
	lupus (human)		
Acetylcholine receptor	myasthenia		35
Gliadin	celiac disease	22	60
Collagen	autoimmune disease with subepidermal blisters	28	10
	bullous pemphigoid	15	
	goodpasture disease	9	
	rheumatoid arthritis	21	
Myelin basic protein	multiple sclerosis	65	61

external secretions, including those of the digestive tract. Antibodies against TGase 2 also occur in the blood of patients with type I diabetes⁷. Type I diabetes is a common disease, although not as common as late-onset type II diabetes. Given the above observations, I would like to suggest that TGase 2 generates autoantibodies in a variety of autoimmune disorders by cross-linking potential autoantigens and acting as a hapten. This hypothesis is supported by numerous reports of the participation of TGase substrates in autoimmune diseases (Table 1). They occur in the blood in systemic lupus erythematosus⁵⁸ and in the synovial fluid in rheumatoid arthritis⁶⁶. TGase 2 involvement in the pathogenesis of common autoimmune diseases requires further investigation. However, accumulating data suggest that specific TGase inhibitors might be very useful in treating chronic autoimmune diseases, some of which are very common chronic diseases.

FIBROSIS, INCLUDING LIVER FIBROSIS (CIRRHOSIS)

TGase 2 is involved in the pathogenesis of fibrosis, since TGase 2 plays a key role in the process of wound healing and scar formation³. Fibrosis, like inflammation, is a physiological process that becomes pathological when it goes too far. TGase inhibitors have been proposed as agents that may prevent hypertrophic scar tissue in human skin, for instance non-

-toxic amine compounds such as aminoacetonitrile, cadaverine, putrescine, and spermidine²³. TGase activity is increased in pathological (paraquat-induced) pulmonary fibrosis³⁰. In 1997, Johnson et al.³⁷ showed that characteristic TGase cross-linking product is highly increased in the renal fibrosis model induced by rat subtotal nephrectomy. This model disorder may be associated with loss of renal tubule integrity³⁷.

TGases are involved in the stabilization of the extracellular matrix by cross-linking matrix proteins. TGase 2 is associated with the extracellular surfaces, although the mechanism by which this protein traverses plasmalemma membranes is not known⁴⁸. A number of the major matrix proteins have been identified as TGase substrates, including fibronectin⁴⁷, fibrinogen³¹, osteonectin², osteopontin⁶³, collagens^{40, 46, 54}, vitronectin⁵⁹, collagen-tailed acetylcholinesterase³², elafin⁴⁹, and plasminogen activator inhibitor 2⁵⁶. Another TGase substrate of interest, which is inserted into the extracellular matrix, is latent TGF (LTGF)- β ^{41, 57}. LTGF- β cannot be activated without binding to matrix by TGase 2. Active TGF- β is released from the insoluble LTGF- β . TGase 2 is important in the development of the extracellular matrix since TGase 2 regulates the availability of this cytokine in the matrix. Perturbation of the formation of the extracellular matrix has been implicated in a number of pathological conditions, including cancer metastasis⁴⁵, as well as several of the already mentioned conditions, including pathological fibrosis, atherosclerosis, and celiac disease.

Liver fibrosis represents the response of liver to damage by toxic, infectious, or metabolic agents. The process leading to liver fibrosis resembles the process of wound healing, including the three phases following tissue injury: inflammation, synthesis of collagenous and noncollagenous extracellular matrix components, and tissue remodeling (scar formation). In 1997, Mirza et al.⁴⁵ showed that TGase activity is increased at the early stage of hepatic fibrogenesis induced by CCl₄ in the rat. That finding suggests a possible role for this enzyme in stabilizing the fibrotic bands during liver fibrogenesis⁶⁸. Rapamycin (an immunosuppressive agent) inhibited extracellular matrix deposition in the rat model of liver fibrogenesis as determined by mRNA levels of procollagen and TGF- β 1, and inhibited TGase 2 activity as well³. Recently, Grenard et al.²⁹ found that TGase-mediated cross-linking occurs in liver extracellular matrix during the early, inflammatory stage of liver fibrosis, whereas cross-linking by pyridinoline occurs mostly later in the fibrotic process. TNF- α expression is upregulated in early hepatic inflammation, and Kuncio et al.⁴² showed that liver cells treated with TNF- α increase TGase 2 expression.

TGase 2 appears to be involved in the early pathogenesis of fibrogenesis, indicating that it is an excellent target for the design of novel inhibitors to treat this very common condition.

CONCLUSIONS

We suggest that TGase 2 is strongly associated with the pathogenesis of many inflammatory diseases.

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