Cell. Mol. Life Sci. 62 (2005) 2947–2955
1420-682X/05/242947-9
DOI 10.1007/s00018-005-5483-7

Cellular and Molecular Life Sciences

Trefoil factor family 2 deficiency and immune response

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Online First 5 December 2005

Abstract. The protective effect of Trefoil Factor Family (TFF) proteins in the gastrointestinal tract by promoting the healing of injured mucosa is well known. An increasing body of evidence connects TFFs, especially, TFF2 and TFF3, with a possible role in immune regulation. TFF2 is able to inhibit lipopolysaccharide-induced nitric oxide production in monocytes and can potently limit leukocyte recruitment at the site of injury. An analysis of gene expression in gastrointestinal tissue of TFF2-deficient mice reveals some new aspects of TFF2's role in the immune response.

Key words. TFF2; gastrointestinal defense; TFF2 deficiency; gene expression; immune response.

Gastrointestinal defense

The epithelial surface of the gastrointestinal (GI) tract is perpetually exposed to a mixture of potentially harmful agents such as acid, microbes and toxins, as well as useful nutrients. This enormous surface area (on the order of 400 m^2), is a place of an exciting but precarious functional balance between defensive and absorptive functions. The gastrointestinal mucosa protects the host against invading pathogens and allergens, and at the same time absorbs nutrients and regulates the uptake of antigens through tightly-packed microvilli.

The intestinal epithelial cells, which are protected by a thick layer of mucus and a glycocalyx [1], constitute a barrier due to the expression of a variety of junctional complexes, including tight junctions, adhesion junctions and desmosomes. While this anatomy helps to prevent penetration by foreign agents, intestinal epithelial cells also express major histocompatibility complex (MHC) class II receptors (MHC II) that facilitate antigen presentation to immune cells as needed. The gastrointestinal epithelial cells can also recognize certain highly conserved microbial structures through surface Toll-like receptors (TLRs) [2], and thus initiate a cellular signaling response on their own [3]. The first line of defense against microbial pathogens is the innate immune response along with other nonspecific defense systems [4]. The latter includes gastric acid, mucus, intact epithelial layer forming tight junctions, digestive enzymes, peristaltic movement, alternative complement pathways, phagocytes, trefoil factor peptides [5] and more recently recognized antimicrobial peptides such as defensins and cathelicidins [2, 6]. Together, they act to enhance the blocking of pathogen contact with epithelial cells and clearance of the pathogens.

The intestinal mucosa contains a highly specialized immune system which differs in many aspects from those in other compartments. The primary inductive sites are organized lymphoid aggregates, so-called 'Peyer's patches', located in the mucosa of the small and large intestine. Peyer's patches are unique among lymphoid tissues in that they contain no afferent lymphoid vessels. All lymphoid cells traffic into Peyer's patches via migration from the bloodstream across the high endothelial venules, mediated by mucosa-specific adhesion molecules. Isolated

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lymphoid follicles are also present in high numbers in the mucosa of the colon and the appendix. Following ingestion, antigens and microorganisms are transported from the gut lumen via specialized M cells, which are scattered among conventional epithelial cells overlying the dome of Peyer's patch follicles. Subepithelial region dendritic cells are the main antigen-presenting cells that bind bacterial products with their TLRs. Signals initiated by the interaction of TLRs with specific microbial patterns direct the subsequent inflammatory response [7].

Dendritic cells in the subepithelial dome process antigen as relative immature cells and then migrate to the T cell, region where they present antigens to naive T cells. Such T cells in the follicles provide help for the B cells switch to immunoglobulin (Ig)A production. IgA is the major mucosal immunoglobulin and can help dispose of antigens without provoking the complement cascade. Following IgA switch and affinity maturation, B cells migrate, via efferent lymphatic vessels, from Peyer's patches to the mesenteric lymph node and finally to the lamina propria where they undergo terminal differentiation into plasma cells. The lamina propria is the main effector site of the intestinal immune response and contains a large and heterogeneous group of lymphoid and myeloid cells, such as lymphocytes, macrophages, dendritic cells, neutrophils and mast cells. This highly integrated cell network in the mucosal immune system expresses specific co-stimulatory and adhesion molecules and produces effector molecules such as cytokines [8].

Minor disruption of the GI surface layer of the cells occurs frequently, such that maintaining epithelial integrity is of crucial importance. Normal epithelial repair requires restitution and regeneration. During restitution, within minutes after injury, epithelial cells spread and migrate across the basement membrane to re-establish surface-cell continuity [9]. Trefoil factor family (TFF) proteins play an important role in restitution by influencing the migration of cells without promotion of proliferation or tumorigenesis. Regeneration occurs later on and involves proliferation and differentiation of epithelial cells and restoration of specialized elements [10].

Trefoil peptides

TFFs are members of a unique family of proteins characterized by one or more three-looped structural motifs (trefoil or P-motif) held together by disulphide bonds. The mammalian members of this family are TFF1 (formerly pS2), TFF2 (formerly spasmolytic polypeptide, SP) and TFF3 (formerly intestinal trefoil factor, ITF) [11]. TFF peptides are major secretory products of normal mucous epithelia, where they exhibit an important role in mucosal defense through both protective and reparative mechanisms. TFF peptides are predominantly expressed in the GI tract but also in some other, predominantly epithelial tissues, such as the respiratory tract, salivary glands, uterus, conjuctiva and even brain [12]. Comparison of the high-resolution structure of human trefoils by using H nuclear magnetic resonance (NMR) spectroscopy [13] demonstrated that trefoils have significant structural and electrostatic differences in the loop2/loop3 regions, which suggests that each trefoil peptide has a specific target or group of target molecules. This finding, together with the recent data proving the existence of TFF heteromers with their interacting proteins [14], points to different functions for the structurally similar TFF peptides.

Trefoils are strongly induced after epithelial damage [15] and facilitate both short-term (restitution) and long-term (glandular re-epithelialization) repair processes by stimulating cell migration [16, 17], inhibiting apoptosis [18] and reducing antigen access to the healing epithelium by augmenting the barrier function of mucus [19]. In addition, they are regulated by both pro-inflammatory [20] and anti-inflammatory cytokine expression [21–23], and have been postulated to participate in the mucosal immune response by stimulating immunocyte migration [24].

There are numerous in vivo studies clearly documenting positive effects of all three TFF peptides on repair of ulcers in the GT tract [25, 26]. All three trefoil genes have been 'knocked out' in separate mouse models, with only *TFF1* knockout showing a drastic effect [27], while TFF2- and TFF3-deficient mice displayed mild phenotypes unless chemically challenged [28, 29]. TFF1-deficient mice developed antropyloric adenoma and occasionally carcinomas, suggesting a tumor suppressor function for TFF1. Accordingly, the altered expression or deletion of the *TFF1* gene is frequently observed in human gastric carcinomas [30]. TFF3 knockout animals showed increased sensitivity to intestinal damage with increased intestinal apoptosis [31]. The latest TFF deficiency model, *TFF2* knockout mice, has provided further new insights into the role of TFFs [28, 32].

Mouse models of TFF2 deficiency

At the morphologic level, TFF2-deficient mice exhibited a significant reduction in numerous parameters, including gastric mucosal thickness, total cell number and mucus neck cell number per gland, gastric gland height and gastric mucosal proliferation rate (reduced by 33,6%). Interestingly, they did show an increase in endocrine cell number per horizontal length of mucosa. TFF2 deficiency was associated with a twofold increase in gastric acid secretion and a slightly greater susceptibility to gastric ulceration by indomethacin treatment [28]. The number of indomethacin-induced gastric ulcerations

was not significantly different between wild-type and TFF2-deficient mice, but there was a notable increase in the damage score (depth of ulcer injury and degree of associated inflammation) in the mutant mice. This finding supports previous reports showing that recombinant TFF2 given to the rats resulted in significant protection against nonsteroidal anti-inflammatoryy drug (NSAID)- and ethanol-induced gastric ulceration [33, 34]. Elevated basal acid and stimulated acid secretion, along with a noted increase in activated parietal cells and decreased gastrin levels, suggest a role of TFF2 in acid suppression. This effect is consistent with previous reports demonstrating suppression of acid secretion after administration of TFF2 to rats [35, 36]. At a first glance, the phenotype of the TFF2-deficient mice does not appear to be a striking one but in fact a more dramatic phenotype (perhaps revealing the true function of TFF2) is hidden below at the molecular level.

Modulation of gene expression due to TFF2 deficiency

Our investigation of alterations in gene expression (mouse expression array MGU74, Affymetrix) due to TFF2 deficiency in the major TFF2-expressing tissue (gastric antrum and Brunner's glands) has provided some new insights into the function of TFF2 [32]. Out of 12,000 analyzed genes examined in our microarray analysis, 128 genes had significantly modulated expression, and the majority are implicated in immune regulation (fig. 1). The change in expression of some of the more interesting genes was confirmed by quantitative real-time polymerase chain reaction (PCR) (Table 1).

The most prominent upregulated genes belong to the cryptdin family (mouse orthologues of human alpha-defensins), which have been shown to play a crucial role in innate immunity. An important mechanism of innate defense is linked to the production of endogenous antimicrobial peptides, many of which are secreted into the lumen of the intestine. The best characterized of these peptides are the alpha-defensins (called cryptdins in mice), which are produced by the Paneth cells of the small intestine [37]. Defensins are vital contributors to innate host antimicrobial defense (direct microbicidal effect), and recent evidence points to their role as enhancers of adaptive immunity [38]. The alpha-defensins have a wide-acting spectrum of activity, and their antimicrobial activity is effective against both Gram-positive and Gram-negative bacteria. Some of the cryptdins (cryptdin 2 and cryptdin 3) show paracrine action [39]. They are able to form pores in neighboring epithelial cells and stimulate secretion of some interleukins [40].

Table 1. Relative changes of mRNA expression between wild type and *TFF2*$^{-/-}$ animals obtained by microarray analysis (n = 3 each) and quantitative real time PCR (qPCR) (n = 12 each) in different gastrointestinal regions.

Gene	Pyloric antrum with Brunner's glands		qPCR	
	Microarray	qPCR	Duodenum	Jejunum/ileum
PSMB5	–4.0	–2.2 ± 0.05*	–2.1 ± 0.92*	–1.9 ± 0.85*
LMP2	2.0	1.5 ± 0.16*	1.5 ± 0.31*	1.3 ± 0.28*
LMP7	2.0	1.0 ± 0.46	1.2 ± 0.34	1.5 ± 0.26*
TAP1	2.4	2.0 ± 0.25*	1.1 ± 0.67	1.1 ± 0.75
BAG2	–2.0	–1.6 ± 0.67*	–1.5 ± 0.55*	–1.8 ± 1.20*
CRBP2	2.6	3.3 ± 0.22*	2.0 ± 0.37*	1.7 ± 0.22*
APO AIV	3.7	3.0 ± 0.34*	1.7 ± 0.17*	1.5 ± 0.30*
CATHEPSINC	–4.8	–1.2 ± 1.41	2.0 ± 0.21*	1.5 ± 0.19*
CRIP	2.3	2.2 ± 0.27*	2.3 ± 0.10*	1.0 ± 0.47
MMP7	n.a.	–1.0 ± 1.2	–1.1 ± 0.65	–1.4 ± 0.59
TIMP3	–1.8	1.5 ± 0.45	1.3 ± 0.46	1.5 ± 0.27
TFF3	5.6	41.0 ± 0.04*	1.9 ± 0.26*	–1.4 ± 0.68
MUC3	4.9	1.2 ± 1.08	2.1 ± 0.83*	2.0 ± 0.26*

Data are expressed as mean fold changes ±SD. Significantly changed expression compared with wild-type mice is marked with (*)($p<0.05$) (n.a. not available). Proteasome subunit 5 (PSMB5); proteosome subunit, beta type 9 (LMP2); proteasome subunit Lmp7 (PSMB8); Mus musculus transporter 1(TAP1); Bcl2-associated athanogene 2 (BAG2); cellular retinol binding protein 2 (CRBP2); apolipoprotein A-IV (APO AIV); Cysteine-rich intestinal peptide (CRIP); matrix metalloproteinase 7 (MMP7); tissue inhibitor of metalloproteinase 3 (TIMP3); mucin 3 (MUC3); trefoil factors (TFF); glyceraldehyde-3-phosphate dehydrogenase (mGAPDH); β-glucuronidase (GUS); Mouse housekeeping protein (mHKG) [32].

Figure 1. Data set of signal log ratios of nine different comparisons between wild-type and *TFF2*$^{-/-}$ mice for relevant significantly changed genes. The presented genes have a signal intensity >100 and are significantly changed according to one class analysis of the Significance Analysis of Microarrays (SAM) program, with a false discovery rate of 0,004%. Changes in expression ≥1.6 were considered. The expression pattern of the genes is represented in the horizontal strips, where the tones of red and green represent respectively the degrees of up- and downregulation. More intense color indicates a higher degree of change: red for up- and green for downregulation. The difference in expression of some genes (see K3xWT1; lane 7) is due to individual variability [32].

Considerable numbers of Paneth cells are found in the small intestine, where the number of bacteria is low in comparison to the colon. Paneth cells originate from intestinal stem cells located at the interface of the villus and the crypt; these stem cells then migrate downward to the bottom of the crypt to eventually form Paneth cells. It is thought that secretion of antimicrobial peptides into the upper crypt by Paneth cells contributes to the protection of stem cells. Defensins are stored with other antimicrobial peptides in apical cytoplasmic granules, and their secretion can be stimulated by bacteria and their products [6]. In addition to the finding of increased expression of alpha-defensins messenger RNA (mRNA) in TFF2-deficient mice, we found upregulation of another abundant Paneth cell product, the so-called cystein-rich intestinal protein mRNA (CRIP) [32]. CRIP has a significant role in the regulation of cytokine balance and the immune response. A 3–7-fold overexpression of CRIP achieved in a transgenic mice model caused a change in the population of leukocytes [41] and altered the pattern of cytokine expression and consequently the immune response [42]. Transgenic CRIP overexpressing mice had only 50% of the white blood cell count found in non-transgenic animals. Differential leukocyte counts showed that transgenic animals had proportionately fewer lymphocytes and more monocytes, eosinophils and neutrophils. Flow cytometry data suggested that these transgenic mice overexpressing CRIP have more CD4+/CD8+ thymic lymphocytes. Taken together, these data suggest that CRIP plays a significant role in the differentiation or maturation of cells with rapid turnover such as those found in the intestine and immune system.

The alteration in the expression of genes expressed in Paneth cells, that is associated with TFF2 deficiency, is not surprising given earlier studies that monitored the uptake of intravenously applied radioactively labeled TFFs [43, 44]. These experiments showed that intravenously applied radioactive ^{125}I-TFF2 was mainly taken up by the kidney (40%) and the gastrointestinal tract (14%), where it was mainly located in the gastric body, pylorus and duodenum. ^{125}I -pTFF2 from the circulatory system was taken up by mucous neck cells, parietal cells, the mucus layer and the pyloric glands of the stomach. In the intestine, it was concentrated in Brunner's glands and the Paneth cells, while in the colon it appeared in cells in the lower part of the crypts. Thus, labeled TFF2 was found in cells that normally produce only TFF2 and not the other TFFs. Intravenously applied radioactively labeled TFF1 and TFF3 were also collected by these TFF2-producing cells (the same that collected ^{125}I-TFF2) and not by TFF1- or TFF3-producing cells [44]. Autoradiographic studies showed that grains representing ^{125}I-TFF2 that were primarily outside the cells initially were found intaracellularly 15 min later. The endocrine effects of TFF2 implicate

the presence of a basolateral localized cellular receptor, which allows uptake of peptide secreted via endocrine or paracrine pathways. The TFF2-binding cells in the gastrointestinal tract seem to have basolateral, receptor-like activity to all three TFF peptides. Recently, a new TFF1 interacting protein was described [14] as a member of the BRICHOS domain protein family. The same domain is present in a new TFF2 interacting protein [W. Otto, personal communication], and it is possibly connected with dementia, respiratory stress and cancer [45].

TFF2 deficiency and MHC class I presentation relevant genes

The other group of immune response-relevant genes whose expression was altered in the setting of TFF2 deficiency includes genes involved in MHC class I presentation. The immune system provides continual surveillance against bacterial and viral infections and cancer by monitoring whether cells are synthesizing foreign or mutant proteins. In this process, MHC class I molecules bind oligopeptide fragments and display them on a surface. The appearance of a mutant or foreign protein on the cell surface stimulates cytotoxic T lymphocytes to kill the affected cell. MHC class I molecules are often downregulated on the surface of tumor cells, probably helping tumor cells to evade killing by cytotoxic T cells [46].

The majority of these antigenic oligopeptides are generated in the cytoplasm as side products of the ubiquitin-proteasome protein degradation pathway. The dominant protease is the multi-subunit ATP-dependent protease called the proteasome. Most of the oligopeptides generated by the proteasome are further degraded into amino acids, while a fraction of them escape complete destruction. The oligopeptides generated are transported into the endoplasmatic reticulum (ER) by an integral ER protein known as the transporter associated with antigen processing (TAP), which is actually a heterodimer consisting of TAP1 and TAP2 subunits. MHC class I molecules are also present in the ER, where they are retained by interactions with various molecular chaperones (calnexin, calreticulin, tapasin etc.). The binding in the ER of an 8–10-residue oligopeptide stabilizes MHC class I heterodimers, which are then transported to the cell surface. Oligopeptides are bound by MHC class I molecules in the ER and delivered to the cell surface, allowing surface presentation of the bound oligopeptide. The immune system appears to modify the proteasome function by displacing the housekeeping subunits (PSMB5 and PSMB6) with LMP2 and LMP7 subunits, whose expression may be induced by increased levels of interferon (IFN)-γ [47] or by tumor necrosis factor (TNF)-α [48]. These immunoproteasomes facilitate antigen presentation presumably by altering the cleavage specificities of proteasomes [48, 49]. Degraded short peptides are moved from the cytosol into the lumen of the ER by the TAP transporter composed of TAP1 and TAP 2 subunits and are finally transported through the Golgi complex to the plasma membrane.

Microarray analysis of the TFF2-deficient mice revealed significant change in expression of several genes involved in MHC class I antigen presentation [32], such as different proteosomal subunits (PSMB5, LMP2 and LMP7), TAP1 and BAG2 (BLC2-associated anthanogen 2), a member of the BAG protein family that can regulate chaperone activity. The modulation of proteasomal subunit expression suggests a possible difference in the proteasomal composition and formation of immunoproteasomes [50] in TFF2-deficent mice. Immunoproteasomes are usually formed as a part of immune adaptation to INF-γ stimulation [51]. It has been shown that deficiency of the immunosubunits LMP2 or LMP7 reduces the cytotoxic T lymphocyte repertoire and thus the efficiency of the immune response [52, 53]. Impairment of immunoproteasome formation has been observed as a consequence of oncogenesis [54, 55] and virus-induced immune evasion strategies [56].

TFF2-deficient mice showed an upregulation of the TAP1 subunit in the pyloric antrum and Brunner's glands [32]. It is interesting to notice that some viruses, such as the human papilloma virus [57] and the Epstein Bar virus [58], may evade immune recognition by downregulating MHC class I cell surface expression via downregulation of the TAP1 gene, thereby affecting the transport of peptides into the ER. Downregulation of TAP1 was observed in a variety of tumor tissues [49], and it was observed that the induction of TAP1 through vaccinia virus expression vectors inhibited tumor development in mice [59, 60].

The gastrointestinal tissue of TFF2-deficient mice showed significant downregulation of BAG2, a member of BAG family that interacts with chaperones [61]. BAG-family proteins regulate chaperone activities through their interaction with Hsc70/Hsp70. BAG2 has been recently recognized as a member of a possibly novel signaling pathway in the response to cellular stress [62]. Overexpression of BAG-family proteins is found in several cancers, and their overexpression enhances cell survival and proliferation [63]. BAG-family proteins participate in a wide variety of cellular processes, including cell survival (stress response), proliferation, migration and apoptosis. Emerging knowledge about BAG-family proteins indicates that there may be a mechanism for influencing signal transduction through non-covalent post-translational modifications [64].

The alteration of genes involved in immune responses in the non-inflamed gut of TFF2-deficient mice points toward a potential role for TFF2 in immune response regulation. It is of interest that macrophages from TFF2-deficient animals show increased basal nuclear factor

NFκB (NF-κB) activation and increased response to pro-inflammatory cytokines [T. Wang, personal communication] that additionally connects TFF2 with regulation of immune response. At this point it is unclear whether this is a direct effect of TFF2 deficiency or a consequence of indirect mechanisms leading to altered homeostasis; nevertheless, taken together, these observations point to TFF2's involvement in immune response.

An interesting consequence of TFF2 deficiency in gastric pylorus and Brunner's glands is a strong (almost 40-fold) upregulation of *TFF3* gene expression. It is not clear whether the change in TFF3 expression is a consequence of chromosomal rearrangements in the *TFF2* gene or a consequence of physiological adaptation to TFF2 deficiency in order to maintain the homeostasis. In the case of TFF1-deficient mice, TFF3 expression in the stomach was unchanged, while the expression of the *TFF2* gene was completely abolished [27], such that with respect to the stomach, TFF1-deficient mice appeared almost as 'double knockouts' (of *TFF1* and *TFF2*). In light of new data regarding the potential role of TFFs in immune regulation, the marked phenotype of TFF1-deficient mice might be related to alterations in both TFF1 and TFF2 expression in these animals.

TFFs and modulation of immune response

In previous studies, TFF2 and TFF3 were detected in lymphoid tissues [24]. We have recently confirmed these findings, and also demonstrated the absence of TFF2 in the spleen and thymus of TFF2-deficient mice (fig. 2). In addition, published studies showing strong upregulation of TFF2 in the lung by diverse allergens and by the interleukin (IL)-4 and IL-13 [65] raises the possibility that TFF2 may have an important role in the pathogenesis of asthma. TFF2 could potentially contribute to pathogenic processes common to allergic lung response and gastrointestinal injury. The significant presence of TFF2 and TFF3 in spleen, thymus, lymph nodes and bone marrow, together with recent data implicating TFFs influence in modulation of the inflammatory response, raises many new possibilities with respect to the primary function of TFFs. The role of TFF2, and TFFs in general, seems to be evolving from that of simple secretory proteins that interact with mucins towards more complex proteins involved in various aspects of inflammatory reactions, such as the ability to regulate nitric oxide (NO) production and modulate expression of adhesion molecules.

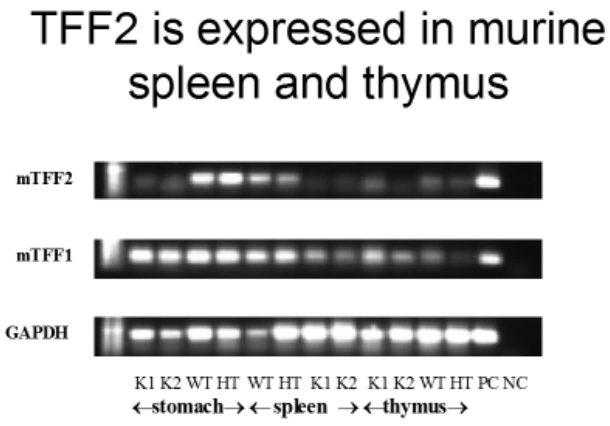

Figure 2. TFF2 mRNA is expressed in murine spleen and thymus. Expression of TFF2 mRNA detected by RT-PCR is completely abolished in stomach, spleen and thymus of $TFF2^{-/-}$ mice. Expression of TFF1 is maintained in stomach and slightly downregulated in spleen of TFF2-deficient animals. K1,K2: $TFF2^{-/-}$ animals; WT, $TFF2^{+/+}$ animals; HT, $TFF2^{-/+}$ animals; PC, positive control; NC, negative control.

NO is an important regulator of gut inflammatory responses, and in acute injury NO is produced by constitutively active nitric oxide synthase (cNOS) and has beneficial effects on the mucosa by increasing blood flow and releasing various local repair mediators [66]. Conversely, in chronic *inflammatory* states, NO is generated by the inducible nitric oxide synthase (iNOS) within infiltrating macrophages and neutrophils [67], leading to production of reactive nitrogen species, including peroxynitrite and NO_2 [68]. These free-radical species are highly damaging and induce further inflammation, leading to ongoing tissue injury. Both NO and nitrotyrosine production are mainly localized to macrophages and neutrophils in the submucosa [69]. The primary function of neutrophils is in the acute inflammatory response, where they migrate towards damaged tissue and invade microorganisms, which they ingest and destroy. The level of NO in enterocytes is normally low. Recent data indicate that iNOS-derived NO in enterocytes is a key mediator of early villous re-epithelialization following acute mucosal injury. The increase in iNOS-derived NO in enterocytes mediates epithelial healing prior to the onset of inflammation by stimulating restitution, mucus production and cytoprotection [70–73]. At first glance, TFFs appear to exhibit opposite effects on iNOS-derived NO production. TFF3 stimulates NO production through iNOS in the non-transformed rodent intestinal epithelial cell line, IEC-18 [74]. On the other hand, TFF2 is able to inhibit LPS-induced (but not basal) NO production in a monocyte cell line [75]. This effect is in agreement with the finding that exogenous TFF2 administered to a rat colitis model inhibits myeloperoxidase (MPO) activity and reduces the inflammatory infiltration [76]. TFF2 potently limits leukocyte recruitment in experimental intestinal inflammation by reducing the expression of vascular adhesion component-1 (VCAM-1) and by reducing leukocyte adhesion to intestinal venules [77]. These data suggest that TFF2 can play an anti-inflammatory role by inhibiting local mobilization of immune cells in colitis.

TFFs can also modulate the expression of the plasma membrane protein known as Decay-Accelerating Factor (DAF) or CD55, which is an important mucosal defense protein that was recently recognized as a key molecule that regulates the interplay between complement and T cell immunity in vivo [78]. DAF is widely expressed on peripheral tissues as well as on T and B lymphocytes, macrophages and dendritic cells [79]. The TFF3 peptide may potentially serve a protective role against complement activation through the induction of DAF in intestinal epithelial cells. DAF prevents the assembly, and accelerates the dissociation, of autologous C3/C5 convertases, and thereby prevents complement activation on the cell surface [80], protecting host tissues from autologous complement injury. DAF is a negative modulator of T cell (adaptive) immunity and suppresses T cells through mechanisms involving complement regulation [81]. In the human intestinal tract, DAF is expressed sporadically on the luminal surface of the epithelial cells, and its expression is upregulated during inflammation [82, 83]. Complement activation initiates numerous defense mechanisms intended to protect tissue from invading organisms [84]. Uncontrolled complement activation in the gut can lead to tissue damage and promote intestinal inflammation, so it is crucial that complement activation is appropriately modulated by proteins such as DAF. The TFF3 peptide induces DAF expression and thus may enhance protection against complement activation in intestinal epithelial cells. TFF3 likely acts to stimulate DAF expression through actions on the basolateral surface of epithelial cells, which are typically exposed in the setting of mucosal injury, allowing access to TFF3 and other factors involved in mucosal protection and restitution.

Conclusion

Recent studies of TFFs implicating these secreted proteins in numerous aspects of immune regulation have begun to clarify findings published earlier indicating a high level of TFF2 expression in lymphoid organs. Altered expression levels (mainly upregulation) in gastric mucosa of TFF2-deficient animals of genes linked to immunity, such as defensins and MHC class I-associated genes, implicate TFF2 in immune regulation. It is likely that future studies that explore TFF's role in the immune response will provide further clarification of the primary function of trefoil factor family members.

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