Journal of Medicinal Chemistry
Miniperspective

J. Med. Chem. **2009,** *52,* 6505–6510 **6505**
DOI: 10.1021/jm9008136

Trefoil Factor Family (TFF) Peptides and Chemokine Receptors: A Promising Relationship

Werner Hoffmann[†]

Institut für Molekularbiologie und Medizinische Chemie, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, D-39120 Magdeburg, Germany

Received June 5, 2009

Introduction to TFF Peptides and Their Multiple Roles

The TFF[a] (trefoil factor family) domain is an ancient cysteine-rich shuffled module forming the basic unit for the family of secretory TFF peptides (in short, TFFs).[1−7] Three mammalian TFFs are known consisting of either one (TFF1 and TFF3) or two TFF domains (TFF2). The corresponding human genes form a cluster on chromosome 21. TFFs are characteristic secretory products of the mucous epithelia. Here, they play a key role in the maintenance of the surface integrity for these delicate epithelia in health and disease. Furthermore, TFFs have also been detected in much lower concentrations in the blood, the immune system, and the central nervous system (CNS). However, the physiological relevance of TFFs in these systems has so far been underemphasized.

Many of the multiple biological functions of TFFs are expected to be triggered by receptor activation. However, most attempts to isolate TFF binding proteins with characteristics of typical receptors have failed.[8,9] Only recently, the chemokine receptor CXCR4 has been described as a low affinity receptor for TFF2 by Wang and co-workers.[10] This review discusses implications of that milestone paper.

TFFs in Mucous Epithelia. Systematic studies on the biosynthesis and localization of TFFs particularly in the human body demonstrated their predominant expression in various mucous epithelia, such as the alimentary, the respiratory, and the urogenitary tracts, the conjunctiva, and the inner ear.[11] Here, they are often cosecreted with various mucins.[12] TFF3 seems to represent the standard TFF peptide found in all of these organs, whereas TFF1 and TFF2, as the predominant gastric TFF peptides, have probably more specialized functions. There are numerous in vivo studies in rodents clearly indicating potent protective and healing effects for all three TFFs after various mucosal damages.[6,11] However, luminal application is clearly superior to systemic delivery.[13] Furthermore, all three lines of TFF-deficient mice show mucosal abnormalities. For example, TFF1-deficient mice develop obligatory antropyloric adenomas, 30% of which progressed to carcinomas.[14] TFF2-deficient mice have an increased number of parietal cells and show increased susceptibility to gastric injury,[15] whereas TFF3-deficient mice show a decreased resistance to colonic injury.[16] Generally, TFFs support various complementary mucosal defense and repair mechanisms by their multiple molecular functions including (1) formation of mucous barriers, (2) enhancement of rapid mucosal repair by cell migration (a process termed "restitution"), (3) modulation of mucosal differentiation processes, and (4) modulation of the mucosal immune response.[6]

TFFs are characteristic constituents of mucous gels and thus protect these delicate epithelia.[12] However, only TFF2 changed the viscosity of gastric mucin preparations.[17,18] This is consistent with the fact that TFF2 is noncovalently bound to gastric mucins.[19]

There are many in vitro studies clearly demonstrating a motogenic effect for all three TFFs; i.e., these peptides enhance cell migration.[11] This has been shown for gastric, intestinal, bronchial, and oral epithelial cells, as well as the cornea. Generally, restitution is an important component in the early stages of mucosal repair preceding inflammation, the latter being critical for cancer progression. Restitution begins within minutes after injury and is achieved by the migration of neighboring cells into the wounded area.[20,21] This complex multistep process has been observed in the gastric and intestinal mucosae, the respiratory tract, the renal epithelium and the urothelium, the gall bladder epithelium, the oral epithelium, and the cornea. Restitution requires continuous blood flow and includes at least (1) a reduction of cell−cell contacts, (2) a shift of the cell polarity from polarized epithelial cells (with apical−basal polarity) to polarized migrating cells (with planar polarity), (3) migration of cells, (4) repolarization of cells (morphological restitution), and (5) restoration of barrier function (transmucosal epithelial resistance, functional restitution).[21] Thus far, besides being motogenic, TFFs have been reported to change the growth and migration patterns of cells, they induced scattering of transformed cells, and they modulate adherens and tight junctions.[21−23] Furthermore, TFFs show also an antiapoptotic effect.[24] This ensures that rapid repair by cell migration is not disturbed by cell death due to an intimate relationship between cell migration and cell survival.[21] TFFs also exhibit a proangiogenic activity that would support restitution.[25]

TFFs clearly promote chemotactic migration, but they do not show a chemokinetic effect.[26] Studies concerning

[†]Contact information. E-mail: werner.hoffmann@med.ovgu.de.

[a] Abbreviations: CNS, central nervous system; EGF, epidermal growth factor; ERK, extracellular-signal related kinase; FAK, focal adhesion kinase; GPCR, G-protein-coupled receptor; IL, interleukin; JAK/STAT, Janus kinase signal transducers and activation of transcription; JNK, c-jun-N-terminal kinase; MIF, macrophage migration inhibitory factor; NFκ-B, nuclear factor κ-B; PI3-K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC-γ, phospholipase C-γ; PTX, pertussis toxin; Pyk-2, proline-rich tyrosine kinase 2; SDF-1, stromal cell-derived factor 1 (CXCL12); TNF-α, tumor necrosis factor-α; TFF, trefoil factor family.

Published on Web 09/01/2009

TFF-triggered signaling cascades are rare and include extracellular-signal related kinases 1 and 2 (ERK1/2), c-Jun-N-terminal kinase (JNK), phosphatidylinositol 3-kinase (PI3-K), signal transducers and activation of transcription 3 (STAT3).[12,27−29] Migration has been reported to depend on protein kinase C (PKC), ERK1/2, and Src family of tyrosine kinases but not on PI3-K, p38, or cAMP-dependent kinase.[27,28] In contrast, the antiapoptotic effect of TFF3 requires PI3-K.[27] Of major interest, TFF1 and TFF3 differ in their ability to induce the activation of STAT3.[29]

There are also increasing indications that TFFs modulate various mucosal differentiation processes, e.g., in the respiratory tract and the stomach.[30,31] These processes are crucial for the continuous regeneration of mucous epithelia from stem cells.[31] For example, TFF1 is required for the commitment program of mouse oxyntic epithelial progenitors.[32]

Pathologically, TFFs are ectopically expressed during various inflammatory diseases, particularly in the gastrointestinal tract.[2,6,7,11,12,33] Here, a prominent glandular structure known as the ulcer-associated cell lineage (UACL) is a prominent site of TFF synthesis.[2]

TFFs in the Immune System. There are also clear indications that TFFs participate in immune responses and inflammatory processes. Expression of TFF2 and TFF3 has been observed also in lymphoid tissues, such as spleen, thymus, lymph nodes, and bone marrow, and they stimulate migration of monocytes.[34] Here, particularly TFF2 is present in macrophages and lymphocytes.[35] Interestingly, the inflammatory responses of these immune cells were dysregulated in TFF2-deficent mice, suggesting that TFF2 not only controls gastric repair but also is a negative regulator of both gastrointestinal inflammation and systemic immune responses.[35,36]

TFFs in the CNS. TFF3 is a typical neuropeptide of oxytocinergic neurons of the supraoptic and paraventricular nuclei.[11] However, its major expression site in the CNS is the cerebellum, where it is postnatally regulated with a maximum between P15 and P20.[37] There are multiple indications for TFF3 modulating neuronal activity. For example, injected TFF3 showed fear-modulating activities.[11]

TFF2 Is a Low Affinity Ligand for CXCR4. Only recently, the chemokine receptor CXCR4 has been convincingly demonstrated to be activated by TFF2 at a concentration of about 5×10^{-7} M.[10] This concentration is quite high compared with that of the major physiological CXCR4 ligand stromal cell-derived factor (SDF-1/CXCL12) which is already active below 10^{-9} M.[38,39] However, the low affinity binding observed is in perfect agreement with a previous study showing a maximum motogenic effect of TFF2 precisely at that concentration.[40] TFF2 was able to attenuate CXCL12−CXCR4 mediated chemotaxis, and the TFF2-triggered signaling was abrogated with the specific CXCR4 antagonist AMD3100.[10] Furthermore, TFF2 responsiveness of CXCR4 negative AGS cells could be induced upon expression of the CXCR4 receptor. However, responses triggered by TFF2 were not congruent with those obtained after CXCL12 treatment, e.g., the kinetics of the Ca^{2+} flux and the phosphorylation of ERK1/2 were different.[10]

CXCR4: A Unique Chemokine Receptor with Multiple Roles

The CXCR4 receptor belongs to the family of chemokine receptors, which comprises more than 20 seven transmembrane domain G-protein-coupled receptors (GPCR) sharing

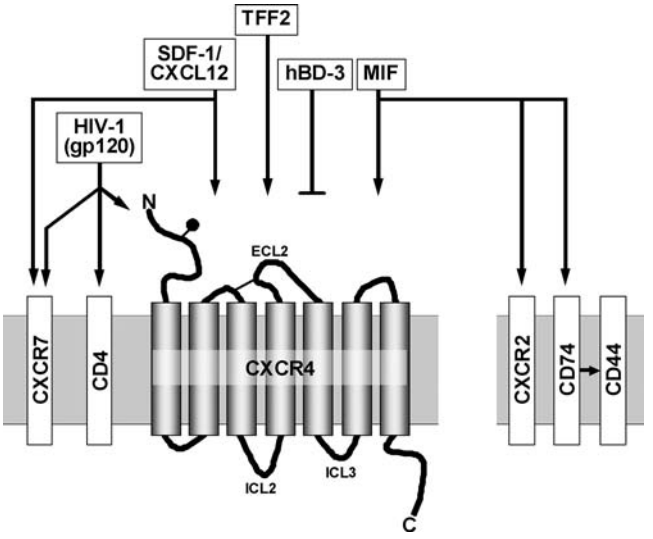

Figure 1. CXCR4 receptor ligands. Schematic illustration of CXCR4 activation by stromal cell-derived factor (SDF-1/CXCL12), TFF2, and macrophage migration inhibitory factor (MIF) as well as inhibition by human β-defensin-3 (hBD-3) and binding of HIV-1 by gp120. Furthermore, ligation of these peptides with other receptors, such as CXCR7, CD4, CXCR2, CD74, and CD44, is shown. The N-glycosylation site used in human CXCR4 is indicated by a knob. The functionally particularly important extracellular and intracellular loops of CXCR4 are also depicted (ECL2, ICL2, ICL3) as well as the disulfide bridge between ECL1 and ECL2. Furthermore, the N- and the C-terminal regions of CXCR4 are indicated.

25−85% identity on the amino acid level.[39,41,42] Usually, chemokine receptors bind more than one chemokine and the more than 50 different chemokines are known bind to multiple receptors. However, the receptor CXCR4 (previously: LESTR, fusin) is outstanding in many respects; e.g., it represents a primordial member of this family most distantly related to all other members, and it is the only one critical for life. Furthermore, CXCR4 exists in different isoforms as the result of differential splicing, which affects the length of the N-terminal portion, as well as various post-translational modifications, such as N-glycosylation, tyrosine sulfation, and modification of serine-18 by chondroitin sulfate.[39,41] Interestingly, all these modifications accumulate in the N-terminal extracellular region of CXCR4 (see Figure 1) and at least sulfation at tyrosine-21 affects binding of the physiological ligand stromal cell-derived factor (SDF-1/CXCL12).[39] CXCR4 is also capable of forming homo- and heterodimers which are present in lipid rafts.[39] Thus, the presence of a huge variety of different forms may underlie the multiple functions and cellular responses by CXCR4.

Multiple Roles of CXCR4. Generally, CXCR4 together with its predominant physiological ligand CXCL12 plays a key role for a variety of chemotactic processes regulating the precise migration of cells.[39] Of special note, stem cell trafficking is also regulated by CXCL12−CXCR4 during development as well as in the adult.[43] This explains why the CXCL12−CXCR4 axis has pleiotropic effects on organogenesis during development, for regeneration and repair in adulthood and also for tumorigenesis and metastasis.[44]

The importance of CXCR4 for development is documented by the fact that disruption of CXCR4 is embryonically lethal resulting from failure of hematopoiesis with impaired B-lymphopoiesis and bone marrow myelopoiesis, defective

gastric vasculogenesis, a cardiac septum defect, and defects in cerebellar cell migration. Of note, CXCR4 and CXCL12 deficient mice show similar developmental defects, suggesting a monogamous and nonredundant relationship between this receptor and its major ligand.

In the adult, CXCR4 is expressed in a wide variety of cell types including most hematopoietic cell types, endothelial cells, neurons, microglia, and astrocytes as well as epithelial cells.[41,42] This explains why the CXCL12−CXCR4 axis plays a role for the recruitment of immune cells to inflamed tissues, e.g., during rheumatoid arthritis,[45] and why it is a pivotal player in angiogenesis.[46] In the brain, the distrubution of CXCL12 and CXCR4 overlaps in a number of regions and the CXCL12−CXCR4 pair is capable of directly modulating neuronal activity.[38] Furthermore, the CXCL12−CXCR4 axis is a master regulator of trafficking of both normal and cancer stem cells because both express CXCR4.[44] On the one side, this is very important for the homing of stem cells to injured tissue, for example, in the heart.[47] On the other side, CXCR4 is the most widely expressed chemokine receptor in malignancy and it clearly contributes to tumor metastatic capacity.[48] Finally, CXCR4 is an HIV-1 coreceptor required for entry of T-tropic but not M-tropic strains.[42]

CXCR4 in Epithelia. Both CXCR4 and its ligand CXCL12 are also typically expressed by various mucous epithelia, e.g., in the intestine.[49] Here, this pair contributes to mucosal wound healing by enhancing restitution.[50] Of special note and somewhat surprising, CXCR4 has been reported to have a predominant apical and, to a lesser extent, basolateral distribution on human enterocytes.[51] Furthermore, CXCR4 but not CXCL12 expression is up-regulated by proinflammatory mediators such as IL-1β or tumor necrosis factor-α (TNF-α).[52,53] Additionally, the CXCL12−CXCR4 axis can also trigger secretion of other chemokines from epithelia, such as IL-8, and thus may affect the effects of chronic inflammation.[52]

Activation of CXCR4. For a long time, only a single natural ligand has been known for CXCR4, i.e., CXCL12,[39,41] which later has been shown to bind also to the receptor RDC1/CXCR7.[54] CXCR7 is capable of forming functional heterodimers with CXCR4, and it effectively reduces the level of CXCL12 in the environment by binding and internalizing this chemokine.[55,56] Only recently, macrophage migration inhibitory factor (MIF) was described as a further noncognate ligand for CXCR4.[57] Furthermore, CXCR4 was the first chemokine receptor shown to serve as a coreceptor for entry of T cell tropic HIV-1 strains in a CD4 dependent or independent manner.[41] Interestingly, there are also peptides that compete with the natural ligand CXCL12, such as human β-defensin-3, and act as antagonists of CXCR4.[58] This increasingly complex situation is outlined in Figure 1.

CXCL12-triggered activation of CXCR4 has been studied in detail and is mostly coupled to heterotrimeric G_i proteins, i.e., activation is sensitive to pertussis toxin (PTX).[39] After the release of free $G_{\beta\gamma}$ a major outcome is a chemotactic effect on cell migration (chemotaxis). CXCL12 requires the CXCR4 amino terminus for binding and the second extracellular loop (ECL2) for activation of downstream signaling pathways.[59] In contrast, the third intracellular loop (ICL3) is important for G_i-dependent signaling, and ICL2 and ICL3 as well as the C-terminal part of CXCR4 are needed for chemotaxis.[60] Activation of multiple signal transduction pathways has been reported, such as phospholipase C-γ (PLC-γ), Ca^{2+} mobilization, PKC, ERK1/2, PI3-K, as well as the Janus kinase signal transducers and activation of transcription (JAK/STAT) pathway (which is independent of G_i)[61] and nuclear factor κ-B (NFκ-B).[60−62] Particularly PKC and PI3-K, but not ERK1/2, are required for CXCL12-triggered migration of hematopoetic cells.[62] Furthermore, increased phosphorylation of focal adhesion components and adaptor molecules has been observed, such as proline-rich tyrosine kinase 2 (Pyk-2), p130Cas, focal adhesion kinase (FAK), paxillin, Crk, and Crk-L.[62]

CXCL12 can also induce CXCR4 dimerization, which is a critical step in triggering biological responses.[39,61] Furthermore, CXCL12 causes CXCR4 internalization accompanied by receptor desensitization and uncoupling from G-proteins.[39] Internalization, which is a G_i-independent event, involves phosphorylation of the CXCR4 carboxyterminal cytoplasmatic domain by PKC and GPCR kinases followed by interaction with β-arrestin.[63] Interestingly, there is a disease, the WHIM syndrome, characterized by truncations in the C-terminal domain of CXCR4, which lead to enhanced responsiveness to CXCL12 due to impaired CXCR4 internalization and altered β-arrestin-dependent signaling.[39,63] The latter would be in line with reports that β-arrestin also plays a role in G_i-dependent processes in CXCR4.[60,64]

It is certainly one of the most important challenges in the field to define the molecular mechanisms that link the CXCR4 signaling to the reorganization of the actin cytoskeleton that drives chemotaxis.[39,65] For example, gene expression profiling has recently been reported after activation of the CXCL12−CXCR4 axis identifying 30 differentially expressed genes, such as IL-8 and IL-6.[66]

Possible Functional Implications and Future Perspectives

Comparison of TFFs and CXCL12. On the one side, TFFs and CXCL12 have major common functional elements, such as the chemotactic effect, the regulation of apoptosis, and probably modulation of neuronal activity. They also share some common expression sites, such as mucous epithelia, the immune system, and the CNS. On the other side, there are major differences; e.g., TFFs behave like inflammatory cytokines, whereas CXCL12 is a homeostatic cytokine. Further discrepancies affect the expression levels particularly in mucous epithelia, the phenotype of TFF- and CXCL12-deficient mice, as well as the signaling cascades triggered by these peptides. For example, the TFF2-triggered motogenic effect is independent of PI3-K, whereas CXCL12-induced chemotaxis requires PI3-K.[27,28,62] Furthermore, there are considerable differences between TFF2 and CXCL12 concerning ERK1/2 activation, Ca^{2+} mobilization kinetics, etc., at least in the cells investigated by Dubeykovskaya et al.[10] These data suggest that there may be additional and probably also cell type specific factors affecting the signaling pathways triggered by TFF2 and CXCL12, respectively. Furthermore, TFF2 might have the potential to also activate additional receptors other than CXCR4. One candidate might be CXCR7 which can form functional heteromers with CXCR4. On the basis of many analogous effects of all TFFs, TFF1 and TFF3 also have to be classified as potential CXCR4 ligands.

Functional Implications of a Possible TFF−Chemokine Receptor Axis. Generally, the overlapping expression profiles of TFFs and CXCR4 (and other chemokine receptors as well) at least in mucous epithelia, the immune system, and the CNS allow physiologically relevant activation of CXCR4 by TFFs in these tissues. The following examples illustrate this.

The major expression sites for TFF2 are gastric mucous neck and antral gland cells which are thought to release this peptide apically into the gastric lumen. Here, it is noncovalently bound to the mucus layer and it is also a constituent of the gastric juice.[19] Furthermore, TFF2 is an early response gene induced after gastric injury.[33] During gastric ulcer healing, epithelial CXCR4 expression is up-regulated whereas the CXCL12 level is down-regulated.[10] Thus, TFF2 would be well designed to act as a physiological CXCR4 ligand enhancing restitution of the gastric epithelium. In the past, luminal TFFs have been expected to reach their basolateral receptors only when needed, i.e., after injury and exposure of the basolateral membrane.[21] Such a mechanism has been demonstrated in airway epithelial repair by the epidermal growth factor (EGF) ligand heregulin, enabling selective activation of those cells that directly neighbor the wounded area.[21] However, CXCR4 has been reported to have a predominant apical distribution, at least on enterocytes.[51] Thus, the question emerges of how permanent activation of CXCR4 by TFF2 is avoided. Hypothetically, this could be achieved by inactivation of TFF2 due to binding to the mucus layer and selective release of TFF2 upon injury by a yet unknown mechanism. Such a "reverse" mechanism would be complementary to activation of the basolateral HER receptors by the various EGF receptor ligands in the gastrointestinal tract.

TFF2 and TFF3 have been shown to augment TNF-α-induced IL-6 and IL-8 secretion in bronchial epithelial cells, whereas noninduced cells did not respond to TFF2 and TFF3.[28] This could easily be explained now by up-regulation of CXCR4 in these cells by TNF-α.[52,53] CXCR4 activation then triggers IL-6 and IL-8 expression.[66] Thus, the dramatically altered response of epithelial cells to TFFs during inflammation could well be due to altered CXCR4 levels.

TFF2 and EGF synergistically enhance their motogenic effects but trigger chemotaxis via different signaling cascades.[26,40] This might be due to interaction of CXCR4 with receptor tyrosine kinases which are also able to colocalize in lipid rafts.[48] However, TFF2 alone does not trigger phosphorylation of the EGF receptor HER1.[40] Maybe activation of HER1 occurs only during costimulation with EGF. This would explain the synergism observed (which is more than the sum of both effects).

CXCR4 is well-known as a coreceptor for entry of HIV-1 strains. Thus, it would be very challenging to test whether TFFs are capable of inhibiting this entry route as has already been proven for other CXCR4 ligands.[64] This could be of major importance for mucous epithelia and also for the immune system.

CXCR4 is prominently expressed in the cerebellum (together with CXCL12), and CXCR4- and CXCL12-deficient mice show typical defects in cerebellar migration.[38] Interestingly, TFF3 is predominantly expressed in the cerebellum with a peak between P15 and P20.[37] Furthermore, TFF3-deficient mice exhibit certain motoric abnormalities (Blaschke, Schwegler, and Hoffmann, unpublished data). Thus, ligation of CXCR4 or a related receptor by TFF3 could play an important role for the postnatal development of the cerebellum as well as its function in coordinating motoric activities.

TFF2 is also expressed by macrophages and lymphocytes, and the inflammatory response of these immune cells is dysregulated in TFF2-deficient mice.[35] This is a clear indication that a TFF−CXCR4 axis could be an important player in a variety of immunological processes including T-cell survival.[10]

Future Perspectives. From the analysis of TFF-triggered signaling cascades (e. g., comparing TFF1 and TFF3)[29] as well as from the different phenotypes of the various TFF-deficient animals (e.g., TFF1- and TFF2-deficent mice both lacking a major exocrine peptide of the gastric mucosa)[14,15] one would expect that the three TFFs exert their functions by ligating more than one receptor. Thus, TFFs are expected to play an important role in the orchestration of chemokines and their multiple receptors by increasing the already existing plethora of effects significantly.[41,42] Additionally, this complexity will be further potentiated by the fact that TFFs as well as chemokine receptors are capable of forming a variety of heteromers. Particularly, heterophilic chemokine−chemokine interactions can affect the biological activities profoundly.[67] In this respect it will be very interesting to reinvestigate the biological function of gastric TFF1 which naturally forms a disulfide-linked heterodimer with gastrokine-2.[5,19]

In the future, the detailed knowledge on the molecular mechanisms of TFFs is expected to have impact on a variety of different fields including mucosal protection and repair, tissue regeneration, as well as cancer therapy, immunology, and neurology. Particularly interesting would be the precise knowledge concerning the active domain(s) of TFFs when interacting with CXCR4. However, CXCL12, TFFs, MIF, and β-defensin-3 represent cysteine-containing peptides (CXCL12, TFFs, and MIF also contain tryptophan residues), which do not show significant similarities in their primary structures. The understanding of the active structures could open new and important therapeutic strategies for pharmacological intervention of the extremely versatile chemokine receptor system. A major impact will certainly come from the development of new drugs, such as CXCR4 antagonists.[68]

Acknowledgment. The author thanks Dr. J. Lindquist for critical reading of the manuscript and E. Voss for valuable secretarial assistance and apologizes for not citing the important work of many colleagues because of space limitations.

Biography

Werner Hoffmann earned his academic degrees in 1978 (Mag. rer. nat.) and 1982 (Dr. rer. nat) at the University of Innsbruck, Austria (major, chemistry; doctoral thesis at the Institute of Molecular Biology of the Austrian Academy of Sciences in Salzburg, Austria). Following this he was Postdoctoral Fellow at ZymoGenetics Inc. (Seattle, WA) and later on Junior Group Leader at the Max Planck Institute for Psychiatry (Martinsried, Germany). In 1993 he accepted a call as a Professor of Medicinal Chemistry and Head of the Institute of Molecular Biology and Medicinal Chemistry at the Otto-von-Guericke University Magdeburg. Prof. Hoffmann is the coauthor of 80 peer-reviewed publications. His interests focus on mucosal protection, regeneration, and repair mechanisms with emphasis on the role of TFF peptides.

References

(1) Wright, N. A.; Hoffmann, W.; Otto, W. R.; Rio, M.-C.; Thim, L. Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. *FEBS Lett.* **1997**, *408*, 121–123.
(2) Wright, N. A. Aspects of the biology of regeneration and repair in the human gastrointestinal tract. *Philos. Trans. R. Soc. London, Ser. B* **1998**, *353*, 925–933.

(3) Ribieras, S.; Tomasetto, C.; Rio, M.-C. The pS2/TFF1 trefoil factor, from basic research to clinical applications. *Biochim. Biophys. Acta* **1998**, *1378*, F61–F77.
(4) Taupin, D.; Podolsky, D. K. Trefoil factors: initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 721–732.
(5) Thim, L.; May, F. E. B. Structure of mammalian trefoil factors and functional insights. *Cell. Mol. Life Sci.* **2005**, *62*, 2956–2973.
(6) Hoffmann, W. TFF (Trefoil Factor Family) Peptides. In *Handbook of Biologically Active Peptides*, 1st ed.; Kastin, A. J., Ed.; Elsevier: San Diego, CA, 2006; pp 1147−1154.
(7) Kjellev, S. The trefoil factor family−small peptides with multiple functionalities. *Cell. Mol. Life Sci.* **2009**, *66*, 1350–1369.
(8) Thim, L; Mortz, E. Isolation and characterization of putative trefoil peptide receptors. *Regul. Pept.* **2000**, *90*, 61–68.
(9) Otto, W. R.; Thim, L. Trefoil factor family-interacting proteins. *Cell. Mol. Life Sci.* **2005**, *62*, 2939–2946.
(10) Dubeykovskaya, Z.; Dubeykovskiy, A.; Solal-Cohen, J.; Wang, T. C. Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. *J. Biol. Chem.* **2009**, *284*, 3650–3662.
(11) Hoffmann, W.; Jagla, W. Cell type specific expression of secretory TFF peptides: colocalization with mucins and synthesis in the brain. *Int. Rev. Cytol.* **2002**, *213*, 147–181.
(12) Hoffmann, W.; Jagla, W.; Wiede, A. molecular medicine of TFF-peptides: from gut to brain. *Histol. Histopathol.* **2001**, *16*, 319–334.
(13) Poulsen, S. S.; Kissow, H.; Hare, K.; Hartmann, B.; Thim, L. Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat. *Regul. Pept.* **2005**, *126*, 163–173.
(14) Lefebre, O.; Chenard, M.-P.; Masson, R.; Linares, J.; Dierich, A.; LeMeur, M.; Wendling, C.; Tomasetto, C.; Chambon, P.; Rio, M.-C. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. *Science* **1996**, *274*, 259–262.
(15) Farrell, J. J.; Taupin, D.; Koh, T. J.; Chen, D.; Zhao, C.-M.; Podolsky, D. K.; Wang, T. C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J. Clin. Invest.* **2002**, *109*, 193–204.
(16) Mashimo, H.; Wu, D.-C.; Podolsky, D. K.; Fishman, M. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science* **1996**, *274*, 262–265.
(17) Thim, L.; Madsen, F.; Poulsen, S. S. Effect of trefoil factors on the viscoelastic properties of mucus gels. *Eur. J. Clin. Invest.* **2002**, *32*, 519–527.
(18) Kjellev, S.; Nexo, E.; Thim, L.; Poulsen, S. S. Systemically administered trefoil factors are secreted into the gastric lumen and increase the viscosity of gastric contents. *Br. J. Pharmacol.* **2006**, *149*, 92–99.
(19) Kouznetsova, I.; Laubinger, W.; Kalbacher, H.; Kalinski, T.; Meyer, F.; Roessner, A.; Hoffmann, W. Biosynthesis of gastrokine-2 in the human gastric mucosa: restricted spatial expression along the antral gland axis and differential interaction with TFF1, TFF2 and mucins. *Cell. Physiol. Biochem.* **2007**, *20*, 899–908.
(20) Silen, W.; Ito, S. Mechanisms for rapid re-epithelialization of the gastric mucosal surface. *Annu. Rev. Physiol.* **1985**, *47*, 217–229.
(21) Hoffmann, W. TFF (trefoil factor family) peptide-triggered signals promoting mucosal restituition. *Cell. Mol. Life Sci.* **2005**, *62*, 2932–2938.
(22) Meyer zum Büschenfelde, D.; Tauber, R.; Huber, O. TFF3-peptide increases transepithelial resistance in epithelial cells by modulating claudin-1 and -2 expression. *Peptides* **2006**, *27*, 3383–3390.
(23) Dürer, U.; Hartig, R.; Bang, S.; Thim, L.; Hoffmann, W. TFF3 and EGF induce different migration patterns of intestinal epithelial cells in vitro and trigger increased internalization of E-cadherin. *Cell. Physiol. Biochem.* **2007**, *20*, 329–346.
(24) Taupin, D. R.; Kinoshita, K.; Podolsky, D. K. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 799–804.
(25) Rodrigues, S.; van Aken, E.; van Bocxlaer, S.; Attoub, S.; Ngyen, Q.-D.; Bruyneel, E.; Westley, B. R.; May, F. E. B.; Thim, L.; Mareel, M.; Gespach, C.; Emami, S. Trefoil peptides as proangiogenic factors in vivo and in vitro: implication of cyclooxygenase-2 and EGF receptor signalling. *FASEB J.* **2003**, *17*, 7–16.
(26) Chwieralski, C. E.; Schnurra, I.; Thim, L.; Hoffmann, W. Epidermal growth factor and trefoil factor family 2 synergistically trigger chemotaxis on BEAS-2B cells via different signaling cascades. *Am. J. Respir. Cell Mol. Biol.* **2004**, *31*, 528–537.
(27) Kinoshita, K.; Taupin, D. R.; Itoh, H.; Podolsky, D. K. Distinct pathways of cell migration and antiapoptotic response to epithelial injury: structure−function analysis of human intestinal trefoil factor. *Mol. Cell. Biol.* **2000**, *20*, 4680–4690.
(28) Graness, A.; Chwieralski, C. E.; Reinhold, D.; Thim, L.; Hoffmann, W. Protein kinase C and ERK activation are required for TFF-peptide-stimulated bronchial epithelial cell migration and tumor necrosis factor-α-induced interleukin-6 (IL-6) and IL-8 secretion. *J. Biol. Chem.* **2002**, *277*, 18440–18446.
(29) Rivat, C.; Rodrigues, S.; Bruyneel, E.; Piétu, G.; Robert, A.; Redeuilh, G.; Bracke, M.; Gespach, C.; Attoub, S. Implication of STAT3 signaling in human colonic cancer cells during intestinal trefoil factor 3 (TFF3)−and vascular endothelial growth factor-mediated cellular invasion and tumor growth. *Cancer Res.* **2005**, *65*, 195–202.
(30) Hoffmann, W. TFF (trefoil factor family) peptides and their potential roles for differentiation processes during airway remodeling. *Curr. Med. Chem.* **2007**, *14*, 2716–2719.
(31) Hoffmann, W. Regeneration of the gastric mucosa and its glands from stem cells. *Curr. Med. Chem.* **2008**, *15*, 3133–3144.
(32) Karam, S. M.; Tomasetto, C.; Rio, M.-C. Trefoil factor 1 is required for the commitment progamme of mouse oxyntic epithelial progenitors. *Gut* **2004**, *53*, 1408–1415.
(33) Wong, W.-M.; Playford, R. J.; Wright, N. A. Peptide gene expression in gastrointestinal mucosal ulceration: ordered sequence or redundancy? *Gut* **2000**, *46*, 286–292.
(34) Cook, G. A.; Familari, M.; Thim, L.; Giraud, A. S. The trefoil peptides TFF2 and TFF3 are expressed in rat lymphoid tissues and participate in the immune response. *FEBS Lett.* **1999**, *456*, 155–159.
(35) Kurt-Jones, E. A.; Cao, L. C.; Sandor, F.; Rogers, A. B.; Whary, M. T.; Nambiar, P. R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; Chi, A. L.; Reed, G.; Houghton, J. M.; Fox, J. G.; Wang, T. C. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. *Infect. Immun.* **2007**, *75*, 471–480.
(36) Baus-Loncar, M.; Kayademir, T.; Takaishi, S.; Wang, T. Trefoil factor family 2 deficiency and immune response. *Cell. Mol. Life Sci.* **2005**, *62*, 2947–2955.
(37) Hinz, M.; Schwegler, H.; Chwieralski, C. E.; Laube, G.; Linke, R.; Pohle, W.; Hoffmann, W. Trefoil factor family (TFF) expression in the mouse brain and pituitary: changes in the developing cerebellum. *Peptides* **2004**, *25*, 827–832.
(38) Kitabgi, P.; Mélik-Parsadaniantz, S.; Rostène, W. Chemokines: A New Peptide Family of Neuromodulators. In *Handbook of Biologically Active Peptides*, 1st ed.; Kastin, A. J., Ed.; Elsevier: San Diego, CA, 2006; pp 559−565.
(39) Busillo, J. M.; Benovic, J. L. Regulation of CXCR4 signaling. *Biochim. Biophys. Acta* **2007**, *1768*, 952–963.
(40) Oertel, M.; Graness, A.; Thim, L.; Bühling, F.; Kalbacher, H.; Hoffmann, W. Trefoil factor family-peptides promote migration of human bronchial epithelial cells: synergistic effect with epidermal growth factor. *Am. J. Respir. Cell Mol. Biol.* **2001**, *25*, 418–424.
(41) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* **2000**, *52*, 145–176.
(42) Horuk, R. Chemokine receptors. *Cytokine Growth Factor Rev.* **2001**, *12*, 313–335.
(43) Laird, D. J.; von Andrian, U. H.; Wagers, A. J. Stem cell trafficking in tissue development, growth, and disease. *Cell* **2008**, *132*, 612–630.
(44) Ratajczak, M. Z.; Zuba-Surma, E.; Kucia, M.; Reca, R.; Wojakowski, W.; Ratajczak, J. The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. *Leukemia* **2006**, *20*, 1915–1924.
(45) Nanki, T.; Hayashida, K.; El-Gabalawy, H. S.; Suson, S.; Shi, K.; Girschick, H. J.; Yavuz, S.; Lipsky, P. E. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J. Immunol.* **2000**, *165*, 6590–6598.
(46) Pi, X.; Wu, Y.; Ferguson, J. E., III; Portbury, A. L.; Patterson, C. SDF-1a stimulates JNK3 activity via eNOS-dependent nitrosylation of MKP7 to enhance endothelial migration. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 5675–5680.
(47) Askari, A. T.; Unzek, S.; Popovics, Z. B.; Goldman, C. K.; Forudi, F.; Kiedrowski, M.; Rovner, A.; Ellis, S. G.; Thomas, J. D.; DiCorleto, P. E.; Topol, E. J.; Penn, M. S. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet* **2003**, *362*, 697–703.
(48) Fulton, A. M. The chemokine receptors CXCR4 and CXCR3 in cancer. *Curr. Oncol. Rep.* **2009**, *11*, 125–131.
(49) Zimmermann, N. P.; Vongsa, R. A.; Wendt, M. K.; Dwinell, M. B. Chemokines and chemokine receptors in mucosal homeostasis at the intestinal epithelial barrier in inflammatory bowel disease. *Inflammatory Bowel Dis.* **2008**, *14*, 1000–14011.

(50) Smith, J. M.; Johanesen, P. A.; Wendt, M. K.; Binion, D. G.; Dwinell, M. B. CXCL12 activation of CXCR4 regulates mucosal host defense through stimulation of epithelial cell migration and promotion of intestinal barrier integrity. *Am. J. Physiol.* **2005**, *288*, G316–G326.
(51) Dwinell, M. B.; Eckmann, L.; Leopard, J. D.; Varki, N. M. Chemokine receptor expression by human intestinal epithelial cells. *Gastroenterology* **1999**, *117*, 359–367.
(52) Crane, I. J.; Wallace, C. A.; McKillop-Smith, S.; Forrester, J. CXCR4 receptor expression on human retinal pigment epithelial cells from the blood−retina barrier leads to chemokine secretion and migration in response to stromal cell-derived factor 1α. *J. Immunol.* **2000**, *165* (4372), 4372–4378.
(53) Eddleston, J.; Christiansen, S. C.; Zuraw, B. L. Functional expression of the C-X-C chemokine receptor CXCR4 by human bronchial epithelial cells: regulation by proinflammatory mediators. *J. Immunol.* **2002**, *169*, 6445–6451.
(54) Balabanian, K.; Lagane, B.; Infantino, S.; Chow, K. Y. C.; Harriague, J.; Moepps, B.; Arenzana-Seisdedos, F.; Thelen, M.; Bachelerie, F. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T-lymphocytes. *J. Biol. Chem.* **2005**, *280*, 35760–35766.
(55) Sierro, F.; Biben, C.; Martinez-Munoz, L.; Mellado, M.; Ransohoff, R. M.; Li, M.; Woehl, B.; Leung, H.; Groom, J.; Batten, M.; Harvey, R. P.; Martinez-A, C.; Mackay, C. R.; Mackay, F. Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14759–14764.
(56) Boldajipour, B.; Mahabaleshwar, H.; Kardash, E.; Reichman-Fried, M.; Blaser, H.; Minina, S.; Wilson, D.; Xu, Q.; Raz, E. Control of chemokine-guided cell migration by ligand sequestration. *Cell* **2008**, *132*, 463–473.
(57) Bernhagen, J.; Krohn, R.; Lue, H.; Gregory, J. L.; Zernecke, A.; Koenen, R. R.; Dewor, M.; Georgiev, I.; Schober, A.; Leng, L.; Kooistra, T.; Fingerle-Rowson, G.; Ghezzi, P.; Kleemann, R.; McColl, S. R.; Bucala, R.; Hickey, M. J.; Weber, C. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* **2007**, *13*, 587–596.
(58) Feng, Z.; Dubyak, G. R.; Lederman, M. M.; Weinberg, A. Cutting edge: human β defension 3−a novel antagonist of the HIV-1 coreceptor CXCR4. *J. Immunol.* **2006**, *177*, 782–786.
(59) Doranz, B. J.; Orsini, M. J.; Turner, J. D.; Hoffmann, T. L.; Berson, J. F.; Hoxie, J. A.; Peiper, S. C.; Brass, L. F.; Doms, R. W. Identification of CRCX4 domains that support coreceptor and chemokine receptor functions. *J. Virol.* **1999**, *73*, 2752–2761.
(60) Roland, J.; Murphy, B. J.; Ahr, B.; Robert-Hebmann, V.; Delauzun, V.; Nye, K. E.; Devaux, C.; Biard-Piechaczyk, M. Role of the intracellular domains of CXCR4 in SDF-1-mediated signaling. *Blood* **2003**, *101*, 399–406.
(61) Vila-Coro, A. J.; Rodriguez-Frade, J. M.; de Ana, A. M.; Moreno-Ortiz, M. C.; Martinez-A, C.; Mellado, M. The chemokine SDF-1α triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. *FASEB J.* **1999**, *13*, 1699–1710.
(62) Wang, J.-F.; Park, I.-W.; Groopman, J. Stromal cell-derived factor-1a stimulates tyrosine phosphorylation of multiple focal adhesion proteins and induces migration of hematopoietic progenitor cells: roles of phosphoinositide-3 kinase and protein kinase C. *Blood* **2000**, *95*, 2505–2513.
(63) Lagane, B.; Chow, K. Y. C.; Balabanian, K.; Levoye, A.; Harriague, J.; Planchenault, T.; Baleux, F.; Gunera-Saad, N.; Arenzana-Seisdedos, F.; Bachelerie, F. CXCR4 dimerization and β-arrestin-mediated signaling account for the enhanced chemotaxis to CXCL12 in WHIM syndrome. *Blood* **2008**, *112*, 34–44.
(64) Cheng, J.-Z.; Zhao, J.; Sun, Y.; Hu, W.; Wu, Y.-L.; Cen, B.; Wu, G.-X.; Pei, G. β-Arrestin differentially regulates the chemokine receptor CXCR4-mediated signalling and receptor internalization, and this implicates multiple interaction sites between β-arrestin and CXCR4. *J. Biol. Chem.* **2000**, *275*, 2479–2485.
(65) Jin, T.; Xu, X.; Hereld, D. Chemotaxis receptors and human disease. *Cytokine* **2008**, *44*, 1–8.
(66) Stich, S.; Haag, M.; Häupl, T.; Sezer, O.; Notter, M.; Kaps, C.; Sittinger, M.; Ringe, J. Gene expression profiling of human mesenchymal stem cells chemotactically induced with CXCL12. *Cell Tissue Res.* **2009**, *336*, 225–236.
(67) Weber, C.; Koenen, R. R. Fine-tuning leukocyte responses: towards a chemokine "interactome". *Trends Immunol.* **2006**, *27*, 268–273.
(68) Burger, J. A.; Peled, A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. *Leukemia* **2009**, *23*, 43–52.