Recognition by leukocyte formyl peptide receptors:

Promiscuous binding or pattern recognition?

Johan Bylund

Göteborg University 2002

Recognition by leukocyte formyl peptide receptors:

Promiscuous binding or pattern recognition?

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Göteborgs universitet kommer att offentligt försvaras på Avdelningen för Reumatologi och Inflammationsforskning, föreläsningssalen våning 3 i Mikrobiologihuset, Guldhedsgatan 10A, Göteborg.

Fredagen den 7 juni 2002, kl. 13.00

av

Johan Bylund

Fil. Mag.

Fakultetsopponent: Universitetslektor Maria Fällman

Avhandlingen baseras på följande delarbeten:

I. Problems in identifying microbial-derived neutrophil activators, focusing on *Helicobacter pylori*
Johan Bylund and Claes Dahlgren
Trends in Microbiology 2002: 10(1): 12-14

II. Proinflammatory activity of a cecropin-like antibacterial peptide from *Helicobacter pylori*
Johan Bylund, Thierry Christophe, Francois Boulay, Thomas Nyström, Anna Karlsson and Claes Dahlgren
Antimicrobial Agents and Chemotherapy 2001: 45(6): 1700-1704

III. Lipopolysaccharide-induced granule mobilization and priming of the neutrophil response to *Helicobacter pylori* peptide Hp(2-20), which activates the formyl peptide receptor-like 1
Johan Bylund, Anna Karlsson, Francois Boulay and Claes Dahlgren
Accepted for publication in Infection and Immunity

IV. NADPH-oxidase activation in murine neutrophils via formyl-peptide receptors
Johan Bylund, Marie Samuelsson, L. Vincent Collins and Anna Karlsson
Submitted

Recognition by leukocyte formyl peptide receptors:

Promiscuous binding or pattern recognition?

Johan Bylund

The Phagocyte Research Laboratory
Department of Rheumatology and Inflammation Research
Faculty of Medicine, University of Göteborg,
SE-413 46 Göteborg, Sweden

Abstract

Neutrophil granulocytes play an important role in the early stages of microbial infection. The neutrophils have to leave the blood stream and migrate out into the tissue where they phagocytose microbes and cell debris from damaged host tissues. Their antimicrobial substances might also contribute to the tissue injury commonly associated with inflammation. Migration to the inflammatory site is directed by chemoattractants, which guide and activate neutrophils via specific receptors. One important class of receptors is the group of formyl peptide receptors (FPRs) of which two members are expressed on human neutrophils, FPR and FPR-like 1 (FPRL1). These receptors display large sequence homologies and belong to a larger family of G-protein-coupled receptors. FPR recognizes formylated peptides generated during bacterial growth and can thus be viewed as a "pattern recognition receptor", while FPRL1 was until recently an orphan receptor with unknown functions and agonists.

A cecropin-like antibacterial peptide from the gastric pathogen *Helicobacter pylori*, Hp(2-20), was found to be a complete neutrophil activator that mediates chemotaxis, induces granule mobilization and activates the NADPH-oxidase to release oxygen free radicals. The receptor utilized by Hp(2-20) was identified as FPRL1. This receptor has in the last years been shown to recognize a large number of peptides/proteins, many of which represent cleavage products of full-length proteins in themselves unable to activate the receptor. Thus, also FPRL1 could be considered a "pattern recognition receptor" activated indirectly by the proteolytic cascades accompanying tissue damage. The Hp(2-20)-induced activity was increased when neutrophil storage-organelles were mobilized to the plasma membrane by incubation with bacterial lipopolysaccharide (LPS). A large pool of FPRL1 was found in the easily mobilized gelatinase granules, implying that the enhanced response was due to receptor upregulation by granule mobilization. Also murine neutrophils responded to FPR/FPRL1 agonists, an activation partly subjected to the same regulatory events as human neutrophils. However, important differences between cells from the two species were also found. Neutrophils from mice and men differ not only in relative abundance, but also in receptor arsenals, suggesting that humans and mice have developed distinct sensitivities towards different agonists due to co-evolution with different pathogens.

Key words: neutrophils, chemoattractant, FPRL1, NADPH-oxidase, LPS, priming, subcellular organelles, human, murine

ISBN 91-628-5257-4

Recognition by leukocyte formyl peptide receptors:

Promiscuous binding or pattern recognition?

Johan Bylund

GÖTEBORGS
UNIVERSITET

The Phagocyte Research Laboratory

Department of Rheumatology and Inflammation Research

Faculty of Medicine, University of Göteborg,

SE-413 46 Göteborg, Sweden

Göteborg 2002

Recognition by leukocyte formyl peptide receptors:

Promiscuous binding or pattern recognition?

Johan Bylund

The Phagocyte Research Laboratory
Department of Rheumatology and Inflammation Research
Faculty of Medicine, University of Göteborg,
SE-413 46 Göteborg, Sweden

Abstract

Neutrophil granulocytes play an important role in the early stages of microbial infection. The neutrophils have to leave the blood stream and migrate out into the tissue where they phagocytose microbes and cell debris from damaged host tissues. Their antimicrobial substances might also contribute to the tissue injury commonly associated with inflammation. Migration to the inflammatory site is directed by chemoattractants, which guide and activate neutrophils via specific receptors. One important class of receptors is the group of formyl peptide receptors (FPRs) of which two members are expressed on human neutrophils, FPR and FPR-like 1 (FPRL1). These receptors display large sequence homologies and belong to a larger family of G-protein-coupled receptors. FPR recognizes formylated peptides generated during bacterial growth and can thus be viewed as a "pattern recognition receptor", while FPRL1 was until recently an orphan receptor with unknown functions and agonists.

A cecropin-like antibacterial peptide from the gastric pathogen *Helicobacter pylori*, Hp(2-20), was found to be a complete neutrophil activator that mediates chemotaxis, induces granule mobilization and activates the NADPH-oxidase to release oxygen free radicals. The receptor utilized by Hp(2-20) was identified as FPRL1. This receptor has in the last years been shown to recognize a large number of peptides/proteins, many of which represent cleavage products of full-length proteins in themselves unable to activate the receptor. Thus, also FPRL1 could be considered a "pattern recognition receptor" activated indirectly by the proteolytic cascades accompanying tissue damage. The Hp(2-20)-induced activity was increased when neutrophil storage-organelles were mobilized to the plasma membrane by incubation with bacterial lipopolysaccharide (LPS). A large pool of FPRL1 was found in the easily mobilized gelatinase granules, implying that the enhanced response was due to receptor upregulation by granule mobilization. Also murine neutrophils responded to FPR/FPRL1 agonists, an activation partly subjected to the same regulatory events as human neutrophils. However, important differences between cells from the two species were also found. Neutrophils from mice and men differ not only in relative abundance, but also in receptor arsenals, suggesting that humans and mice have developed distinct sensitivities towards different agonists due to co-evolution with different pathogens.

ISBN 91-628-5257-4

Preface

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Problems in identifying microbial-derived neutrophil activators, focusing on *Helicobacter pylori.*
Johan Bylund and Claes Dahlgren
Trends in Microbiology 2002: 10(1): 12-14

II. Proinflammatory activity of a cecropin-like antibacterial peptide from *Helicobacter pylori.*
Johan Bylund, Thierry Christophe, François Boulay, Thomas Nyström, Anna Karlsson and Claes Dahlgren
Antimicrobial Agents and Chemotherapy 2001: 45(6): 1700-1704

III. Lipopolysaccharide-induced granule mobilization and priming of the neutrophil response to *Helicobacter pylori* peptide Hp(2-20), which activates the formyl peptide receptor-like 1.
Johan Bylund, Anna Karlsson, François Boulay and Claes Dahlgren
Accepted for publication in Infection and Immunity

IV. NADPH-oxidase activation in murine neutrophils via formyl-peptide receptors
Johan Bylund, Marie Samuelsson, L. Vincent Collins and Anna Karlsson
Submitted

Contents

Abbreviations

AD	Alzheimer's disease
DAG	diacylglycerol
fMLF	*N*-formyl-methionyl-leucyl-phenylalanine
FPR	formyl peptide receptor
FPRL1	formyl peptide receptor-like 1
FPRL2	formyl peptide receptor-like 2
GPCR	G-protein-coupled receptor
HBP	heparin binding protein
hCAP18	human cationic antimicrobial protein, 18 kD
Hp-NAP	*Helicobacter pylori* neutrophil activating protein
IP_3	inositol 1,4,5-trisphosphate
LJP	localized juvenile periodontitis
LPS	lipopolysaccharide
LXA_4	lipoxin A_4
MPO	myeloperoxidase
PAF	platelet activating factor
PAMP	pathogen-associated molecular pattern
PI3K	phosphoinositide 3-kinase
PIP_2	phosphatidylinositol 4, 5-bisphosphate
PIP_3	phosphatidylinositol 3, 4, 5-trisphosphate
PKC	protein kinase C
PLC	phospholipase C
ROS	reactive oxygen species
SAA	serum amyloid A
TLR	Toll-like receptor
TNFα	tumor necrosis factor α
uPAR	urokinase-type plasminogen activator receptor

1. Introduction

The neutrophils are the most abundant cells among the human blood leukocytes and play an important role in combating the early stages of infection as well as disposing of cell debris upon tissue damage. For these purposes the neutrophils have to leave the circulation and migrate out into the tissue where they phagocytose microbes and release their impressive arsenal of antimicrobial substances and degradative enzymes. A proper and tightly controlled regulation of the release of these substances are of utmost importance and failure to do so may cause serious tissue damage and result in a variety of inflammatory disease states. As part of the innate immune defense the neutrophils are, in contrast to the cells of the adaptive immune system, not dependent on gradual maturation of specific recognition, but instead rely on preformed, germ-line encoded, receptor structures. These receptors can recognize infectious agents, directly or indirectly through opsonins, as well as a variety of "danger signals" calling for an inflammatory response. An important class of receptors responsible for this recognition in neutrophils is the group of formyl peptide receptors (FPRs). These are serpentine seven transmembrane spanning G-protein coupled structures belonging to the chemoattractant family of receptors and neutrophils express two different FPRs (out of the three human variants), namely FPR and FPR-like 1 (FPRL1). The former receptor is known to recognize a variety of *N*-formylated peptides generated during bacterial growth. The latter functions as a low affinity receptor for formylated peptides but has lately been shown to recognize a number of seemingly unrelated peptides/proteins/lipids and has thus come to be regarded as a promiscuous receptor. This review will mainly focus on FPRL1 in terms of regulation, effector functions affected by its activation, possible involvement in different clinical settings and whether the promiscuous feature of FPRL1 can be regarded as a concept of pattern recognition and a way of reacting to seemingly unspecific danger signals.

2. Leukocyte functions induced by chemoattractants

The recruitment of neutrophils to sites of infection or inflammation is a rapid process dependent on directed cellular migration, a process known as chemotaxis. This migration occurs along a gradient of chemical mediators, chemoattractants, of both exogenous and endogenous origin. Chemoattractants include bacterial products, products of the complement cascade (e.g., C5a), a variety of cytokines known as chemokines and some secreted lipids derived from phospholipid metabolism (i.e., platelet activating factor (PAF) and leukotriene B_4). These substances can induce additional responses in neutrophils apart from guiding their migration to an inflammatory focus, including granule mobilization and activation of the NADPH-oxidase. Chemoattractants are thus involved at several stages in the mission of a neutrophil and gradually alters it from being a resting cell that circulates the blood stream into becoming an actively cytotoxic cell in the inflamed tissue.

2.1 Chemotaxis

The directed migration of a neutrophil is a highly complex process, depending not only on actin dynamics but also to a high degree on integrin-mediated adhesion. When a neutrophil is exposed to a biochemical gradient of a chemoattractant, the cell adopts a polarized morphology with a leading edge pointed towards the highest concentration of chemoattractant. A highly controlled actin equilibrium featuring polymerization in the leading edge and depolymerisation in the trailing edge keeps the cell in motion (20). *In vivo*, the directed migration of neutrophils to an inflammatory focus is an even more complicated process involving a constant interplay between the migrating neutrophil and the surrounding cells and tissues in addition to various endogenous molecules affecting the state of the neutrophil. An example of this intricate interplay is diapedesis, the process where the neutrophils pass the endothelial cell layer of the blood vessels and move into the surrounding tissue. Activation of the endothelial cells is required for the initial communication with circulating neutrophils. Upon stimulation with e.g., cytokines or complement factors the endothelial cells rapidly upregulate their surface expression of P-selectin (12) that recognizes carbohydrates present on the surface of resting neutrophils (79). This is a transient interaction of low affinity and causes the neutrophils to slow down and roll along the endothelium. The next step involves activation of the neutrophils by low concentrations of chemoattractants of exogenous or endogenous origin, e.g., IL-8 or PAF produced by activated endothelial cells (98). This results in activation of the neutrophils' surface-localized integrins that bind to extracellular matrix proteins and mediate a high affinity interaction and thus

a more firm attachment between the neutrophils and the endothelial cells. Ligation of the integrins also induces a signal transduction cascade in the neutrophils leading to their spread along the endothelium and altered sensitivity to other stimuli (10, 87). Neutrophils with occupied integrins can then be stimulated to release heparin-binding protein (HBP) that binds to an as yet unidentified receptor present on the endothelial cells. The binding of HBP induces mobilization of intracellular calcium as well as the formation of actin-stress fibers spanning the endothelial cells. These events lead to an increase in endothelial monolayer permeability indicating that HBP released from activated neutrophils actively induces endothelial cells to contract and permit the passage of the neutrophils (44). When the neutrophils have passed the barrier consisting of the blood vessel endothelium, they begin to migrate along the chemotactic gradient toward the inflammatory focus.

2.2 Granule mobilization

Mature neutrophils show very low levels of *de novo* protein synthesis. Instead they rely on the function of preformed proteins stored in a variety of intracellular storage organelles called granules (16). The granules are membrane enclosed vesicles formed during maturation of the neutrophils in the bone marrow and contain both soluble matrix proteins and membrane-associated molecules. In addition, storage organelles called secretory vesicles are formed by endocytosis of the plasma membrane during the late stages of neutrophil maturation. With respect to orientation, the granule membrane is organized in an "inverted" fashion in order to obtain the correct functional direction of membrane-associated molecules (e.g., receptors) upon fusion of the granule membrane with the plasma membrane. The mobilization of granules, degranulation/secretion/exocytosis, is an important process starting upon neutrophil attachment to the endothelium, continuing during diapedesis and chemotaxis and ending at the inflammatory site with release (extracellular or phagosomal) of microbicidal substances.

To date, four different granule subsets have been identified and classified according to content of matrix and membrane proteins and the propensity to undergo exocytosis and there appears to be a logical correlation between the content of the granules and the order in which they are mobilized. The most easily mobilized organelles are the secretory vesicles that upon exocytosis secrete plasma proteins and supply the plasma membrane with chemotactic- and adhesion- receptors, the latter promoting the interaction with the endothelial cells. The gelatinase granules are the next organelles to become mobilized, supplying the plasma membrane with more chemotactic receptors and releasing matrix-degrading enzymes to facilitate diapedesis and chemotaxis. Even more stimulation is required for the specific granules to be mobilized. These granules contain phagocytic receptors and some

antimicrobial substances. However, the major part of the microbicidal substances is present in the azurophil granules that also contain a variety of lysosomal enzymes. The azurophil granules are delivered to the engulfed prey by phagolysosomal fusion and are not secreted extracellularly (15).

As outlined above, some granules need very little stimulation, such as a very low dose of a chemoattractant, in order to become mobilized to the cell surface. Other granules require higher doses of chemoattractant to undergo exocytosis. Apart from chemoattractants, other proinflammatory substances without chemotactic activity can also mediate degranulation e.g., tumor necrosis factor α (TNF-α) (41) and bacterial lipopolysaccharide (LPS) (Paper III). In line with the fact that a low dose of chemoattractant stimulates exocytosis of fewer granule types than does a higher dose, the difference in propensity to undergo exocytosis between the different granule types has been correlated with levels of cytosolic calcium (69, 89). Whether signal transduction mechanisms could explain also the relative difference in secretory potency between different chemoattractants remains to be established.

It is also worth to mention that the classification of the neutrophil granules is not definite and with all probability there exists granules that do not fit the current scheme and it seems logical to assume that the distinctions between the granule subsets are not absolute.

2.3 Activation of the NADPH-oxidase

The microbicidal arsenal of the neutrophils can be divided in an oxygen-independent branch, including antibacterial peptides/proteins and catalytic enzymes that are stored primarily in the azurophil granules (see above), and an oxygen-dependent branch. The oxygen-dependent substances comprise a variety of reactive oxygen species (ROS) formed as a result of NADPH-oxidase activation. The NADPH-oxidase is a membrane-bound electron transport chain that ferries electrons from cytoplasmic NADPH to molecular oxygen on the opposite side of the membrane, resulting in the formation of highly reactive superoxide anion and hydrogen peroxide. These compounds can then be further processed, either by the product of nitric oxide synthase to form the very reactive peroxynitrite molecule, or by myeloperoxidase (MPO) (26). The MPO is localized in the azurophil granules of the neutrophils and catalyses the reaction of reduced oxygen species with halides forming hypohalous acid and subsequently other toxic halogenated compounds.

The NADPH-oxidase is a complex enzyme system consisting of both membrane-bound subunits ($gp91^{phox}$ and $p22^{phox}$) and cytosolic components ($p40^{phox}$, $p47^{phox}$ and $p67^{phox}$) and the proper assembly of these subunits into

an active enzyme is a highly regulated process (reviewed in (5, 25)). The neutrophils harbor two pools of NADPH-oxidase, one localized in the plasma membrane that upon activation releases ROS extracellularly, and one localized in granule membranes that generates intracellular ROS. Activation of the NADPH-oxidase in response to chemoattractants results in an extracellular release of ROS that does not only have deleterious effects on microbes, but may also inflict serious damage on surrounding tissues. The significance of the intracellularly generated ROS has not been clearly established, although they have been implicated as signaling molecules (47, 117) regulating e.g., apoptotic processes (71). The localization of the ROS generation is determined by the nature of the activator (26) and the signal transduction events leading to an intracellular oxidative burst have been shown to, in part, differ from the events leading to an extracellular release of ROS (60). Interestingly, the ability of neutrophils to generate ROS intracellularly seems not to be a preserved feature between species, since murine neutrophils appear to be devoid of an activatable pool of intracellular NADPH-oxidase (Paper IV).

3. Chemotactic Receptors

Comparing the various chemoattractant receptors that have been identified over the years reveals several important similarities in structure and function. Chemoattractant receptors belong to a group of G-protein coupled receptors (GPCRs) with a serpentine orientation in the plasma membrane, starting with an extracellular amino terminus followed by seven helical transmembrane domains and an intracellular carboxyl terminus. This family of receptors have structures and to varying degrees also signaling components that are common throughout the animal kingdom and implemented in a variety of biological contexts (78). For example, the receptors responsible for detecting odors in sensory neurons in man (33) as well as in lower eukaryotes, e.g., insects, are seven transmembrane spanning GPCRs (19, 112). It is interesting to note that the chemotactic receptors that are used to guide inflammatory cells are similar to those involved in the complex process of odor detection. Thus, it is perhaps not too farfetched to say that the neutrophils "smell" their way to an infected tissue.

The carboxyl terminus of the chemoattractant receptors contains potential phosphorylation sites that function as regulatory elements in receptor internalization and termination of signaling. This part of the receptor has also, together with other cytoplasmic parts and transmembrane domains, been implicated in the interaction with the signal transducing G-protein (92, 107), while the extracellular parts and also certain transmembrane regions are involved in agonist binding (85, 90).

One of the most studied chemoattractant receptors is the formyl peptide receptor (FPR) that enables neutrophils to "sniff" their way towards a bacterial infection. The fact that neutrophils are attracted by bacterial colonization and growth in the infected tissue has been known for a long time (54). The phenomenon gained a molecular explanation by the discovery that *N*-formylated peptides can function as potent chemoattractants (106), while similar peptides lacking the formyl group were devoid of chemotactic activity. Since bacteria but not eukaryotic cells start their protein synthesis with a *N*-formylated methionine residue, *N*-formylated peptides seem like logical molecules for the neutrophil to use as recognition pattern when responding to bacterial invasion. Later research has shown that *N*-formylated peptides are indeed a major contributing factor to the chemotactic potential of different bacterial species (77, 103).

Soon after the initial observation that formylated peptides, in particular the prototypic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLF), function as chemoattractants, the quest began to unravel the mechanism by which neutrophils react to these peptides. Gradually, it became clear that the

interaction between the formylated peptides and the neutrophil is a highly specific receptor-ligand interaction. The initial work to purify and characterize the potential receptor by biochemical means generated data showing that the receptor was glycosylated (32, 75, 88) and tightly associated with the plasma membrane (8). Furthermore, it was shown that treatment of guinea pig neutrophils with pertussis toxin inhibited functional responses mediated via the potential receptor, suggesting that G-proteins are involved in the signaling (14). Due to the tight association of FPR to the plasma membrane, attempts to purify the receptor were at large unsuccessful, but as outlined above, the receptor was already partially characterized when the high affinity receptor for formylated peptides, the human FPR, was first cloned by Boulay in 1990 (17). The results obtained during the cloning of FPR also implicated the existence of related receptor variants and later work has identified two additional FPR-related receptors, the FPRL1 (9, 86, 128) and FPRL2 (9).

In humans, the expression patterns of the formyl peptide family of receptors (FPR, FPRL1 and FPRL2) differ between leukocytes. Neutrophils express FPR and FPRL1, while monocytes have been shown to express the complete set (34). Receptors of the FPR family have also been found in other cell types, e.g., dendritic cells, hepatocytes, microglial cells and astrocytes (66, 94). Expression of FPRL1 has been reported in a variety of epithelial cell lines (48), although the functional consequences of this expression remain to be established. As described in Fig. 1, the degree of similarity between the human FPRs is relatively high, with the transmembrane domains and the cytosolic parts showing the highest conservation. The genes encoding the three receptor variants are all clustered on chromosome 19q13.3 (86) suggesting that their differences have arisen by divergent evolution after relatively late duplication events.

3.1 Exploring the FPRs, *in vitro* and *in vivo*

Most of the information regarding the FPRs, their regulation and specificities has evolved from *in vitro* experiments with varying degrees of complexity. Cloning and transfection of a particular receptor in an unrelated cell type that is not normally expressing the receptor, and stimulation with purified agonists represent the most clear-cut and less complex experimental set-ups. The use of transfected FPRs has enabled detailed analyses of affinities for different agonists and by mutating the receptor sequence, knowledge concerning more sophisticated receptor-associated processes has been gained. For example, the parts of FPR involved in agonist binding and G-protein coupling (85) was defined by this approach, as was the molecular requirements for FPR internalization (95).

Although the use of transfected cell lines has undoubtedly generated large amounts of interesting data, this approach suffers from several important drawbacks when it comes to translating the results to the *in vivo* situation, such as the heterogeneity in expression levels and the fact that the transfected receptor may behave erratic when expressed outside of its normal habitat. To remedy the latter and add a level of complexity to the experimental system, purified agonists and purified cells (e.g., neutrophils) are often used. The variety of receptors expressed to different extents by the neutrophil better represents the proper background on which to test the effects of an agonist. In order to further complicate the set-up, non-purified agonists obtained from their natural habitats (such as bacterial extracts) can be used on a mixture of cells aimed to mimic an inflammatory infiltrate.

The experimental system most employed to ensure a maximal level of complexity and resemblance of human inflammatory processes are animal models. Various animal models have enabled a much-increased understanding of various aspects of inflammation and neutrophil physiology, not least due to the possibility of performing genetic manipulations in a controlled genetic background to investigate the importance of a particular gene. This approach was used by Murphy and co-workers to investigate the role of the murine FPR homologue in an infectious model. Neutrophils from the mutant mice were shown to be defect in chemotaxis against fMLF and mice lacking this receptor were found to be more susceptible to *Listeria monocytogenes* infection and were defect in bacterial clearance, implying an important role for FPR *in vivo* (43). In animal models other than murine, investigators have reported varying degrees of affinity for formylated peptides and varying sequence homologies of the responsible receptor. The rabbit FPR, for example, is 78% identical to its human counterpart and shows very similar binding properties (129). On the other hand, porcine neutrophils have been shown to be totally unresponsive to fMLF (35) and neutrophils from horse have been shown to respond functionally to fMLF by granule mobilization, but are inert with regards to chemotaxis (110). These data imply that the responsiveness to formylated peptides is not a trait of high inter-species conservation.

Even though there are a number of ways to make an *in vitro* system more *in vivo*-like one has to be aware that the events taking place in a test tube always represent much-simplified versions of extremely complex processes taking place in the human body. For instance, a very complex mixture of pro- and anti-inflammatory substances acting in concert influences the inflammatory process, and the combined effect of a mixture of agonists is not always merely the sum of the individual effects induced by the agonists (discussed in Paper I). Furthermore, an agonist that exerts activating effects on one

particular cell type may well have (secondary) inhibiting effects on other cells (11).

Despite all drawbacks in employing over-simplified *in vitro* systems and even various *in vivo* models, studies using highly purified agonists in single receptor systems are often necessary to enable proper interpretation of data generated from more complex settings, and the combined knowledge from experiments obtained with different levels of complexity and validity will greatly increase our understanding of the FPRs and their role in various inflammatory processes.

3.2 The FPR receptors of mice and men

There are three different members of the human FPR family whose expression pattern differs between leukocytes. Human neutrophils have been shown to express FPR and FPRL1, while monocytes in addition express also FPRL2 (34). The most extensively used systems for *in vivo* studies of inflammatory processes are, as mentioned above, murine models that offer the great asset of genetic manipulations in the murine genome. There are six genes with homologies to the human FPRs in the murine genome. Out of these six genes, only three seem to be expressed in leukocytes, namely *fpr1, fpr-rs1* and *fpr-rs2* (42). It is at present not known whether the expression

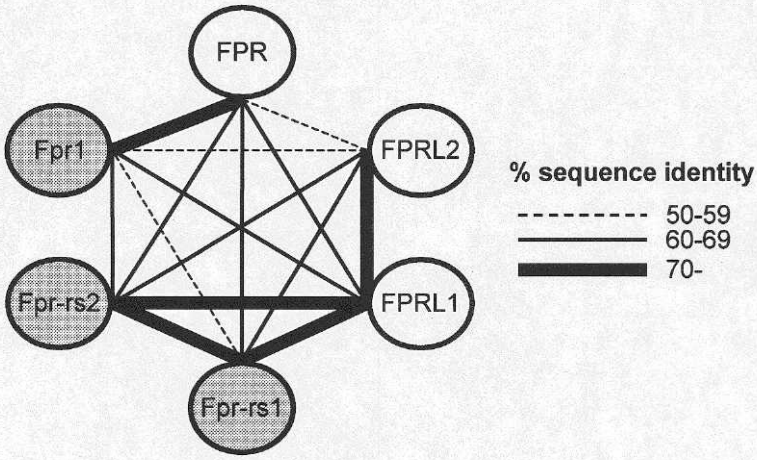

Figure 1. *Structural relationships among the members of the human and murine FPR families. The figure depicts cross-wise comparisons of amino-acid identities between the FPR receptors present on human (open symbols) and murine (filled symbols) leukocytes. Exact amino acid (and nucleotide) identities can be found in (42).*

patterns of these receptors differ between the different murine leukocytes. The human and murine FPRs can be subdivided by sequence relationships into two distinct groups (Fig. 1). The first group consists of FPR and its murine orthologue Fpr1, whose amino acid sequences are 76% identical, while the second group consists of the murine Fpr-rs1 and Fpr-rs2 (with a reciprocal amino acid identity of 81%) and the human FPRL1 and FPRL2 (72% identity between themselves). Both Fpr-rs1 and Fpr-rs2 are more closely related to FPRL1 than to FPRL2 (42).

Despite the extensive use of murine models in inflammation research, few reports describing the activity and potency of different agonists on murine neutrophils have been published. It is, however, clear that the prototypic FPR agonist fMLF is a much less potent stimulus of murine than of human neutrophils (Paper IV, 115). Nonetheless, Fpr1 has been identified as the orthologue of the human FPR and has been shown to be a functional receptor for formylated peptides (43). Moreover, fMLF has been claimed to bind and activate not only Fpr1, but also Fpr-rs2, although with even lower affinity (55).

Our knowledge about the agonist specificities of the murine receptors is insufficient, but activation through these receptors seems to result in processes similar to those in humans, e.g., chemotaxis (43), degranulation (61) and ROS production (Paper IV). Furthermore, murine and human FPR-signaling is at least in some parts governed by the same regulatory events e.g., priming by LPS and homologous desensitization (Paper IV). Despite this similarity in regulation of chemoattractant receptor signaling, the fact that the neutrophils from mice and men differ not only in the relative abundance and receptor arsenal, but also in the ability to produce intracellular ROS, suggests that this particular cell type may perform partially different functions in the two species (Paper IV). That neutrophils are the most abundant white blood cell in humans, making up a total of 60-70% of the peripheral blood leukocytes, while being considerably less abundant in murine blood, contributing only approximately 10-15% (21), also implicates a different and perhaps less critical or different role of neutrophils in murine immunity. However, as opposed to this supposal it has been shown that neutrophil-depleted mice display increased susceptibility to experimental infections with e.g., *Staphylococcus aureus* (125).

Taken together, it is of great importance to expand our understanding of the murine physiology, cell function and receptor repertoire before directly translating results obtained in murine models of inflammation into a human setting.

3.3 FPRL1

FPRL1 was originally cloned by screening a cDNA library from a neutrophil-like cell line, HL-60, using the cDNA for FPR as a probe (86), and found to share 69% sequence identity with FPR. The sequences are particularly similar in the transmembrane domains and intracellular loops, suggesting that FPRL1 transmits the same signals as FPR, but has different agonist preference (130).

3.3.1 Agonists of FPRL1

Initially, FPRL1 was considered an orphan receptor, but was later found to function as a low-affinity receptor for fMLF with approximately 1000-fold lower affinity as compared to the FPR (34). The FPRL1 was also reported to function as a high-affinity receptor for the anti-inflammatory lipid mediator lipoxin A_4 (LXA_4; (39)).

LXA_4 has been found to induce calcium mobilization and chemotaxis in monocytes (72, 101), but neither neutrophils nor FPRL1 transfected cells responded with calcium mobilization upon LXA_4 stimulation (39, 114). LXA_4 was initially discovered as an inhibitor of immune responses (reviewed in (104)) and has been shown to inhibit neutrophil functions (38, 68). These somewhat contradictory data has led to speculations of differential activation of second messengers in monocytes and neutrophils by LXA_4, i.e., that the same receptor induces an inhibitory signal in neutrophils while inducing an activating signal in monocytes. The attempts to define specific LXA_4-induced inhibitory signaling via FPRL1 has failed and the doubts regarding the correctness of the initial findings and speculations that the LXA_4 effects are mediated, at least in part, via a receptor different from FPRL1 is getting stronger (24, 80, 114).

In line with the fact that FPRL1 shares a high degree of sequence identity with FPR, a number of agonists seem to be shared between the two receptors (Table 1). The synthetic hexapeptide WKYMVm was isolated from a random peptide library as a potent stimulant of both monocytes and neutrophils (7, 108). Later it was found to be an agonist for both FPR and FPRL1, with approximately 300 times higher affinity for the latter (27). The WKYMVm contains a D-methionine in its carboxy terminus and by substituting this right-handed amino acid for its natural left-handed counterpart, generating WKYMVM, the receptor specificity is shifted and the resulting peptide has no affinity for FPR (23). Furthermore, both variants of the hexapeptide have affinity for FPRL2, which is the third member of the FPR family.

By the use of transfected HEK 293 cells, the acute phase protein serum amyloid A (SAA) was shown to be a specific agonist for FPRL1 (114),

suggesting that its potent chemotactic activity on monocytes and neutrophils (6) is mediated by this receptor. Apart from SAA, being a protein of 104 amino acids, mainly peptides shorter than 40 amino acids have been identified as activators of FPRL1 and as mentioned above, many of the peptides bind to two or three members of the FPR family, although with different affinities (Table I). Among these peptides, some are derived from the HIV-1 envelope proteins gp120 and gp41, implicating a role for FPRs in HIV infection (66). However, no experimental evidence describing a direct interaction of any FPR receptor with HIV-1 envelope proteins has been published. Furthermore, two amyloidogenic peptide fragments, the 20 amino acid long PrP106-126 derived from the human prion (67) and the 42 amino acid form of amyloid β ($A\beta_{42}$) (65), have been shown to activate FPRL1, indicating a possible involvement of FPRL1 in neurodegenerative diseases.

Two FPRL1 agonists of particular interest are the C-terminal cleavage fragment of the human cathelicidin, LL-37 (28), and the N-terminal part of a ribosomal protein from *Helicobacter pylori*, Hp(2-20) (Paper II), which are both cecropin-like α-helical antimicrobial peptides (49, 96). LL-37 is the 37 amino acid antimicrobial peptide cleaved off from human cationic antimicrobial protein (hCAP18) that is the only identified human cathelicidin. Epithelial cells have been shown to produce hCAP18, but the protein is also found in the specific granules of neutrophils (28). The cleavage of hCAP18 into LL-37 is mediated by proteinase-3 (found mainly in the azurophil granules of neutrophils) and appears to be a strictly extracellular event, as no cleavage could be observed in phagolysosomes of neutrophils (111). This seems to indicate that the main function of LL-37 is not the killing of a phagocytosed prey, but is instead executed extracellularly. Hence, in addition to its bactericidal effect, LL-37 may function as a chemoattractant by ligation of FPRL1 and thereby recruit phagocytes to a site of infection in a positive feedback manner. The peptide thus displays an intriguing functional dualism in that it is both proinflammatory and directly bactericidal.

A similar functional dualism is displayed by the *H. pylori*-derived Hp(2-20). This 19-residue peptide has a potent antibacterial effect against a broad range of microorganisms, although not against *H. pylori* itself and it has therefore been suggested to act on competing bacterial species present in the gut (97). Apart from these intriguing findings, we have shown that Hp(2-20) is also a complete neutrophil activator in that it is chemotactic, induces degranulation and activates the NADPH-oxidase to release ROS (Paper II). These effects are mediated by FPRL1 in neutrophils (Paper II) and with all probability through both FPRL1 and FPRL2 in monocytes (11).

Table I. Agonists for FPRL1

Agonist	Origin	Alternative FPR preference	References
WKYMVm	peptide library	FPR, FPRL2	(23, 27)
WKYMVM	peptide library	FPRL2	(23)
fMLF[1]	bacteria	FPR	(34)
Hp(2-20)	*H. pylori*	FPRL2	(Paper II, 11)
LL-37	neutrophils, endothelial cells		(28)
SAA	acute phase protein		(114)
$A\beta_{42}$	amyloid β	FPR	(65)
PrP106-126	prion protein		(67)
mitochondrial peptide	mitochondrial peptide		(22)
N36	HIV-1, gp 41		(64)
T21/DP107	HIV-1, gp 41	FPR	(113)
F-peptide	HIV-1, gp 120		(30)
V3-peptide	HIV-1, gp 120		(109)
MMK-1	peptide library		(57)
LXA_4	lipid metabolite	?[2]	(39, 80)
uPAR	plasminogen activator receptor		(99)

[1]All agonists in the table have a preference for FPRL1, except for fMLF that exhibits higher affinity for FPR.

[2]Receptor identity unknown, although probably no FPR (see text and (80) for details).

In addition to the examples given above, FPRL1 has been shown to have affinity for a number of other peptides (Table I). Neither of the agonists shows any reciprocal sequence homologies and are thus seemingly unrelated, supporting the notion of FPRL1 as a promiscuous receptor.

3.3.2 Signal transduction induced by FPRL1

Binding of a chemoattractant to its neutrophil receptor induces a number of cellular responses in a hierarchical manner, regulated by the concentration of the chemotactic factor. A directed migration is mediated by low concentrations of agonist and an increase in concentration results in partial degranulation with a concomitant alteration of the plasma membrane constitution. Higher concentrations of chemoattractant have the potential of activating the neutrophil's cytotoxic and antimicrobial responses, including ROS production and further degranulation. The signal transduction events of chemoattractant-induced cellular activation have been quite extensively investigated (121) and seem to involve numerous parallel pathways that with regard to the exact casual connections remain to be elucidated. Certain chemoattractants induce only a subset of cellular effector functions while others trigger the complete set and whether this is due to the fact that the various functional cellular responses are triggered by distinct signal transduction events (differentially induced by different chemoattractants), is also a matter of speculation.

It should be pointed out that the knowledge about FPR signaling far exceeds what is known about the signal transduction pathways employed by FPRL1. Based on the similarities of the two receptors, both regarding biological functions, sequence homologies, and sensitivity to pertussis toxin (Paper II) and other pharmacological modulators, it is reasonable to believe that many of the signaling characteristics of FPR holds true also for FPRL1 (130).

The binding of an agonist to a seven-transmembrane spanning GPCR leads to a dissociation of heterotrimeric G-proteins in the plasma membrane into α and $\beta\gamma$ subunits, resulting in activation of phosphoinositide 3-kinase (PI3K) and phospholipase C (PLC). The FPR has been shown to be functionally coupled to inhibitory G-proteins (45, 62, 124) and as a consequence, FPR mediated responses can be specifically inhibited by pertussis toxin. The membrane phosphatidylinositol 4, 5-bisphosphate (PIP_2) is converted by PLC into the secondary messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). The lipid DAG can activate members of the protein kinase C (PKC) family, a process in which DAG synergizes with cytosolic Ca^{2+}. Cytosolic Ca^{2+} levels are in turn elevated as a consequence of the second messenger IP_3, which promotes a release of Ca^{2+} from intracellular calcium stores (63). Clearly, the activation of PKC and the elevation of

cytosolic Ca^{2+} levels are key events in FPR signaling, although other events are probably also of importance, such as the PIP_2 conversion into phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3) by the PI3K. That these and consecutive signaling steps are related to the cellular processes governed by the FPR has been established (56), although the details about how this is mediated remains to be established.

Despite the limited knowledge about the details of FPRL1-mediated signal transduction it has been shown that also FPRL1 signaling is sensitive to pertussis toxin and is characterized by elevated levels of cytosolic Ca^{2+} concentrations (Paper II, 23, 27).

3.3.3 Cellular responses mediated by FPRL1

Many of the known FPRL1 agonists (Table 1) have been identified by exogenous expression of FPRL1 in various cell lines and using elevation of cytosolic Ca^{2+} concentrations as a means of monitoring signaling activity. In some cases the identified FPRL1 agonists have been studied in the context of endogenously expressed FPRL1 on neutrophils and monocytes and in general, these agonists have been shown to mediate the same leukocyte effector functions as discussed above. In short, different FPRL1 agonists have been shown to mediate chemotaxis of neutrophils and monocytes (Paper II, 11, 23, 27, 28, 114), granule mobilization in neutrophils (Paper II), and activation of the NADPH-oxidase resulting in a release of oxygen free radicals from both neutrophils and monocytes (Papers, II & III, 11, 23, 27).

3.3.4 Pathophysiological roles for FPRL1

Localized juvenile periodontitis (LJP) is a debilitating periodontal disease featuring aggressive bone destruction (126) that is associated with a defect in the patient's host defense against numerous oral bacteria. In a recent study, 29 out of 30 patients diagnosed with LJP were shown to carry mutations in the *FPR* gene (51), resulting in impaired chemotaxis to formylated peptides. Similar findings of patients with defect allelic variants of FPRL1, showing a definite role of this receptor in human pathophysiology, have not yet been described. However, based on the fact that activation of neutrophils *via* FPRL1 results in a massive release of ROS and other potentially tissue destructing substances (Paper II, 23, 27), it seems plausible that this receptor, or modified allelic variants, should be involved in various pathological states involving inflammatory components.

One infectious disease in which FPRL1 potentially is involved is the severe gastric inflammation caused by the bacterium *Helicobacter pylori*. We have shown that the *H. pylori*-derived antibacterial peptide Hp(2-20) activates neutrophils *via* FPRL1 (Paper II). Since *H. pylori* do not normally penetrate

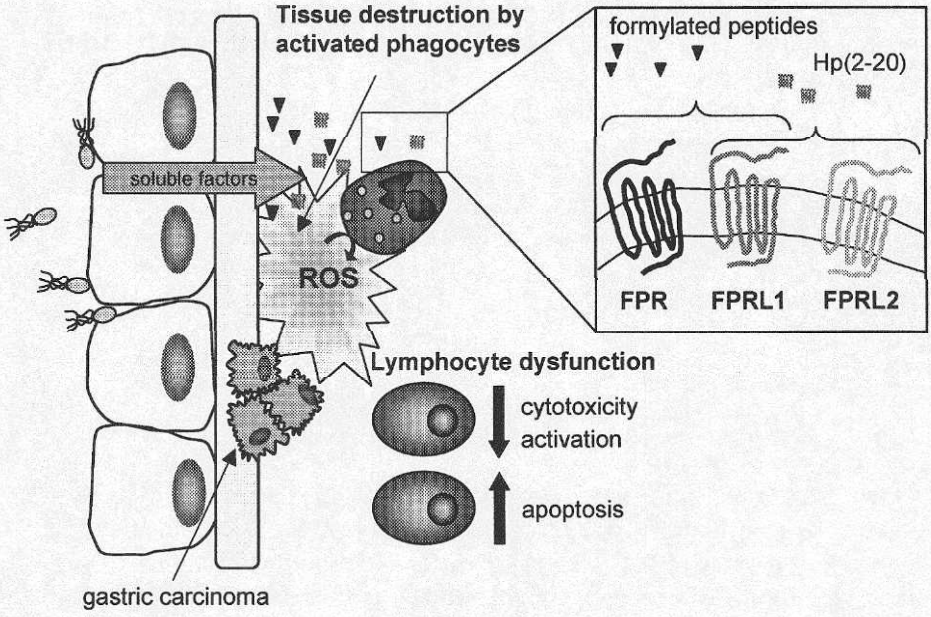

Figure 2. *Model of immune-regulation in the gut mucosa infected with Helicobacter pylori. The inflammatory response is mediated by soluble substances, e.g., formylated peptides and Hp(2-20) that can cross the gut epithelium and attract and activate both neutrophils and monocytes via members of the FPR family. The phagocyte activation results in tissue damage due to the release of ROS and a variety of proteolytic enzymes. The ROS released upon activation may also have secondary effects in that it mediates dysfunction of lymphocytes normally associated with defense against gastric cancer, i.e., NK cells and T cells (see text for details).*

into the sub-epithelial lamina propria, the direct bacteria-induced inflammation is likely to be dependent on soluble factors (3, 74, 105), such as Hp(2-20), that can cross the epithelial layer and form a chemotactic gradient to attract inflammatory cells. It has been speculated that an inflammatory response with concomitant tissue damage would be beneficial for *H. pylori*, by promoting a release of nutrients from the epithelial lining enabling continued bacterial growth and persistence in the mucosal tissue (13). Furthermore, the ROS generated upon FPRL1 activation can also have more specific pathophysiological effects apart from the tissue destruction accompanying the inflammation. We have shown that Hp(2-20) is also a monocyte chemoattractant and activates these cells to produce ROS that have secondary effects on other immune cells (Fig. 2, (11)). More specifically, the Hp(2-20)-induced ROS functionally inhibits lymphocytes that normally are associated with defense against gastric cancer, i.e., NK cells and T cells, and also triggers apoptosis in these cells. Based on these findings we have

suggested that Hp(2-20), and consequently FPRL1 (and possibly also FPRL2), not only contributes to the accumulation and activation of neutrophils and monocytes in chronic gastritis, but also that the ROS released from the activated phagocytes may be of relevance to the increased cancer risk in *H. pylori* infected gastric tissue due to its inhibitory effects on NK and T cells (Fig. 2).

The study discussed above points to the value of increasing the complexity of an *in vitro* system. By using a mixture of cells aimed at mimicking the mononuclear cell infiltrate of a *H. pylori* infected gastric tissue (1), we were able to establish indirect effects of a FPRL1 agonist on cell types that in themselves do not respond to the agonist. It should be noted that this concept of indirect regulatory effects is in no way unique to FPRL1 and its agonists, but is in theory valid for all kinds of receptor/agonist pairs capable of evoking a high enough level of ROS release, e.g., fMLF/FPR (84).

Other pathological states where FPRL1 has been postulated to play a role include the neurodegenerative disorders Alzheimer's disease (AD) and prion diseases (known as Creutzfeldt-Jakob disease, scrapie or bovine spongiform encephalopathy, depending on affected species). Both types of disorders are characterized by the formation of plaques in the brain constituting an inflammatory site with complex cellular reaction (100, 122). Present in the plaques are activated microglial cells, the brain equivalent of monocytes, which are believed to be the direct mediators of the inflammatory state seen in AD. These microglial cells express FPRL1 and amyloidogenic protein fragments such as the 42 amino acid form of amyloid β ($A\beta_{42}$) and a peptide derived from the human prion protein (PrP106-126), have been shown to activate microglial cells *via* FPRL1 (65, 67, 127).

3.4 Regulation of FPRL1

3.4.1. Subcellular localization –exposure through granule mobilization

Neutrophils have very little, if any, *de novo* protein synthesis and instead rely upon storing preformed molecules (including chemoattractant receptors) in intracellular granules for their function. The regulated exocytosis of the different granules ascertains upregulation of the receptors to the cell surface in a controlled manner (15). We have shown that FPRL1 is localized in different mobilizable subcellular compartments in the resting neutrophil (Paper III). It is a well-known fact that the neutrophil responses to chemoattractants can be enhanced by prior exposure to various inflammatory mediators, a process known as priming. This issue has been extensively studied, in particular regarding the production of ROS in response to chemoattractants, where priming agents can make cells hyper-responsive to

chemoattractant stimulation without activating the NADPH-oxidase *per se*. Examples of priming agents are proinflammatory cytokines such as TNF-α and microbial-derived substances such as LPS. There are numerous hypotheses regarding the mechanism underlying the priming phenomenon, e.g., alterations of intracellular signaling pathways (increased protein phosphorylation, phospholipase activity, intracellular Ca^{2+} changes and cross talk between Ca^{2+} increase and tyrosine phosphorylation), altered assembly of the NADPH-oxidase, and proteolytic processing of cell surface proteins (29, 50, 52, 116, 119, 123). One important aspect of priming has been proposed to be granule mobilization with a concomitant increase in cell surface expression of receptors (4, 59). In the case of FPRL1 we have shown that LPS treatment mobilizes the secretory vesicles and gelatinase granules (organelles that harbor FPRL1), leading to increased levels of the receptor in the plasma membrane (Paper III). Furthermore, we showed that cytoplasts, consisting of an organelle-free cytoplasm (thus devoid of granules) surrounded by plasma membrane (102), could not be primed by LPS, indicating that receptor upregulation through granule mobilization is an important feature of LPS-mediated priming.

The primed state accomplished *in vitro* by e.g., incubation with LPS corresponds to the primed state of neutrophils having extravasated *in vivo* with regards to functional modifications such as granule mobilization and L-selectin cleavage (40). Exudated neutrophils have also been shown to display an increased responsiveness to both fMLF and the lactose-binding lectin galectin-3 as compared to peripheral blood cells, implying that priming via granule mobilization is an important process also *in vivo* (59).

The degranulation observed in *in vivo* migrated neutrophils resembles that of cells primed *in vitro* in that a strict hierarchical order of degranulation between the different granule types is employed. As mentioned above, also low doses of chemoattractants promote granule mobilization (Paper II) and therefore these substances may prime neutrophils to subsequent chemoattractant-induced activation. However, mechanisms also exist to avoid a continuous positive feedback-regulated priming/activation of neutrophils.

3.4.2. Desensitization – termination of signaling capacity

When neutrophils have been activated by a chemoattractant they rapidly become refractory to further or subsequent stimulation with the same agonist or another agonist using the same receptor (Papers II & IV). This phenomenon, called homologous desensitization, has been described not only for chemoattractant receptors such as FPR, FPRL1, PAFR, C5aR, IL8R (120), but also for other GPCRs such as the rhodopsin (70) and β-adrenergic

receptors (31). For many GPCRs, homologous desensitization has been implicated as an important physiological feedback mechanism that protects against acute and chronic receptor over stimulation (37) and in all probability this is the case also for the chemoattractant receptors.

Upon ligand stimulation of chemoattractant receptors the signal is terminated by uncoupling the receptor from the G-protein as a result of receptor phosphorylation. This uncoupling is probably the cause for the desensitization, together with internalization of the ligated receptor. A family of GPCR kinases mediates the initial phosphorylation of the carboxyl terminus of the receptor. After phosphorylation, arrestins bind to the receptors and sterically prevents binding and activation of the G-protein. Arrestins are a group of adaptor proteins whose first member was identified in the retina as an inactivator of light-activated rods (91). The binding of arrestins also targets most GPCRs to clathrin-coated vesicles for internalization (37). Prossnitz and co-workers have, however, recently shown that the internalization of chemoattractant receptors, such as FPR and C5a, occurs independently of both arrestin and clathrin (46, 73), indicating that these receptors are internalized through alternative pathways. Nonetheless, the carboxy-terminal phosphorylation is a key event in both the desensitization and agonist-induced uptake of FPR. This was shown in experiments using cells expressing a mutated FPR that lacked the potential carboxy-terminal phosphorylation sites. These cells showed normal migration to fMLF, but were defect in both desensitization and fMLF-induced internalization (55, 93). With regards to FPRL1, the exact mechanisms underlying desensitization and internalization are yet to be established. The receptor, however, exhibits similar homologous desensitization on a functional level as FPR (Paper II, 11, 23).

In addition to homologous desensitization, a number of studies describe a phenomenon called heterologous desensitization or cross-desensitization, where the activation of a certain chemoattractant receptor results in inactivation also of receptors not occupied by agonists (2). This effect is supposedly the result of phosphorylation of un-liganded receptors by various second messenger-activated kinases, but may also be influenced by other signaling events downstream of the G-protein (2, 66, 120). Although more work is needed in order to elucidate the precise mechanisms behind cross-desensitization it seems clear that different chemoattractant receptors can affect each other at several different levels.

As mentioned in Paper I, yet another form of desensitization or downregulation of FPRL1 responses may exist. We have found that stimulation of neutrophils with the *H. pylori* neutrophil activating protein (Hp-NAP, (105)) makes the cells unable to respond to the FPRL1 agonist

Hp(2-20). The fact that the Hp-NAP-stimulated cells still respond normally to other FPRL1 agonists (Bylund, unpublished data) implicates the existence of a novel ligand-specific, as contrasted to the established receptor-specific, desensitization process. Although the mechanism behind this interesting interaction is yet to be determined, this finding suggest that receptor desensitization is not the only way of downregulating signaling through FPRL1.

4. The FPR family – a receptor family for pattern recognition?

The main distinction between the adaptive and the innate immune defenses lies in the repertoire of protective mechanisms and receptors used for immune recognition. The T and B cells of the adaptive immune system rely on receptors that are not encoded in the germ-line or predestined to recognize particular antigens. Instead, a large arsenal of receptors are generated at random and then selected for clonal expansion upon recognition of the antigen for which they, by chance, are specific. This complex system ensures the generation of lymphocytes bearing high affinity receptors for practically any imaginable antigen. Regardless of how useful such receptors may be, it takes time to expand the specific cell clones, and the structural information cannot be passed on to coming generations but each new generation has to go through the same procedure. In contrast, the innate immune system relies on germ-line encoded receptors with predetermined specificities. A drawback in this approach is that the human genome not possibly could harbor specific receptors for the wide range of microorganisms encountered during a life span. Furthermore, the much shorter generation time and higher mutation frequency of the microorganisms would undoubtedly lead to humans being "outsmarted" by the microbes before long. Therefore, the innate immune system has adopted a strategy to recognize a few, highly conserved, antigens present on a large group of microorganisms by so-called pattern recognition receptors (83).

Some of the most studied examples of pathogen-associated molecular patterns (PAMPs) recognized by pattern recognition receptors are LPS, mannans, bacterial flagellin, peptidoglycan, unmethylated bacterial DNA sequences, double stranded RNA and lipoteichoic acids, representing clearly non-self antigens shared by, and essential for, large groups of microbes. In higher organisms, pattern recognition receptors are usually present on a wide array of cell types, not least on antigen presenting cells, and the activation of these receptors serves to mobilize the combined forces of the adaptive and innate immunity in order to combat the infection. Many of the PAMPs are recognized by a class of pattern recognition receptors known as Toll-like receptors (TLRs), present in organisms as diverse as insects and mammals (81). The TLRs are not 7-transmembrane spanning receptors signaling via G-protein activation, instead the signal transduction utilizes a number of different adaptor proteins and kinases (82). Ultimately, TLR activation leads to the release of NF-κB that gains access to the nucleus and functions as a transcriptional activator of a number of inflammatory- and immune response genes (118). Human neutrophils have been shown to express at least three TLRs, namely TLR1, -2 and -4. These receptors have been implicated in

responses to e.g., peptidoglycans/lipoteichoic acids and LPS by other cells (53), although direct evidence of their role in mediating neutrophil responses are currently lacking.

Although not traditionally regarded as a pattern recognition receptor, the chemoattractant receptor FPR can in a way be classified as such. Formylation of newly synthesized proteins is a unique signature of bacterial metabolism and thus, the presence of formylated peptides would serve as a highly relevant PAMP, being both non-self and essential for bacterial metabolism. The importance of proper recognition of formylated peptides have been described in a murine model where mice lacking Fpr1 were clearly more susceptible to bacterial infections than wild type mice (43). Another interesting notion in this context is that also mitochondria produce formylated peptides, a reminder of their bacterial heritage. These peptides are presumably released upon tissue damage and may thus contribute to the proinflammatory signal accompanying such an event (94).

If FPR can be regarded as a pattern recognition receptor, is this also the case for FPRL1? The recruitment of neutrophils is desirable not only in case of infection, but also during the inflammatory event accompanying tissue damage caused by mechanical injury or trauma. In the event of injury cells are damaged, promoting a leakage of endogenous molecules not normally present in the extracellular space. Accompanying the destruction of cells, a plethora of proteases and other hydrolytic enzymes are activated. For example, upon epithelial damage, the constant equilibrium involved in blood coagulation is affected, including very complex proteolytic cascades such as the thrombin-generating pathway and the fibrinolytic system (18). These events may lead to the generation of peptide fragments not normally present extracellularly which could function as a danger signal to alert the innate immune system. It is interesting to note that many of the known activators of FPRL1 represent cleavage products of full-length proteins that in themselves lack affinity for the receptor (e.g., $A\beta_{42}$, PrP106-126, LL-37, mitochondrial peptide). Recently, a direct link between the fibrinolytic machinery and the inflammatory response was demonstrated and a naturally occurring cleaved form of the urokinase-type plasminogen activator receptor (uPAR) was shown to induce chemotaxis in FPRL1-transfected cells (99). Thus, the pattern recognized by FPRL1 may not be pathogen-associated, but rather FPRL1 can be said to be a sensor of (endogenous) danger signals constituted by different proteolytic cascades. This type of recognition bears resemblance with the prototypic pattern recognition receptor in *Drosophila*, Toll, which is not activated directly by any known PAMP, but instead recognizes the proteolytically cleaved form of the endogenous protein Spaetzle. Normally, the full-length Spaetzle is not recognized by Toll but upon cleavage of the protein in response to the proteolytic cascade (which in itself is a host

defense event triggered by e.g., bacterial infection), the resulting carboxy-terminal fragment activates the receptor. In this context Toll is not really a pattern recognition receptor but rather a sensor of the danger signal constituted by the proteolytic cascade (58).

The presence of a neutrophil receptor capable of recognizing a danger signal composed of partially degraded peptides would contribute to the infiltration of neutrophils that can dispose of cellular debris resulting from the initial injury. An exception to the hypothesis that FPRL1 recognizes proteolytically processed peptide fragments is SAA. This acute phase protein does not require proteolytic cleavage to gain affinity for FPRL1 but on the other hand SAA is generated predominately in association with inflammation and/or tissue injury (76). Furthermore, hCAP18 is processed into the FPRL1 ligand LL-37 as a result of neutrophil activation (111) and could thus serve to sustain inflammation in a positive feedback manner in a similar way as SAA. Regarding the microbial-derived agonists of FPRL1 i.e., the HIV-1 derived peptides and Hp(2-20), they could possibly represent microbial strategies to hijack the innate immune system, in the case of *H. pylori* in order to promote the release of nutrients following inflammatory damage of the gut epithelium (13).

Clearly, not just any cleaved peptide can function as an FPRL1 agonist and much more work has to be done in order to elucidate the specific structural requirements of peptide fragments capable of activating FPRL1, i.e., the actual structure of the recognized pattern. The different FPRL1 agonists do not show any similarities on the sequence level, giving rise to the notion of a promiscuous receptor. On the other hand, agonists may well show similarities on higher levels, e.g., in the physico-chemical properties of the correctly folded peptide. In this context it is interesting to note that LL-37 and Hp(2-20) both adopt a positively charged, amphipathic, α-helical structure, which is usually the minimal requirement for cecropin-like peptides to exert antibacterial effects (36). The antibacterial effect of cecropin-like peptides is probably dependent on non-specific interactions with bacterial membranes and is thus quite different from the specific interaction with FPRL1 displayed by Hp(2-20) and LL-37. However, these two peptides clearly have structural features in common. Cecropin-like antibacterial peptides in general do not activate neutrophils and while the ability to form a positively charged α-helix is necessary to exert any bactericidal effect it is not a sufficient criterion to activate FPRL1 (Bylund, unpublished data).

5. Concluding remarks

During the last decade an impressive amount of knowledge has been gained about the FPR family in terms of structures, expression patterns, signaling processes, biological roles and regulation of its signaling. The majority of what is known today would have been impossible to address before the genes encoding the different receptors were cloned and exogenous expression possible. A lot more information can be found on the FPR as compared with its two homologues FPRL1 and FPRL2, which is probably due to the existence of a prototypic agonist (fMLF) for this receptor. It is interesting to note that FPRL1 has switched from being an orphan receptor to being considered a promiscuous receptor, with numerous described ligands. This has been a process of just a few years and simultaneously FPRL1 has switched positions from a FPR homologue to being an important receptor in its own right. While research on FPR continues, the development of selective high-affinity agonists (and antagonists) for FPRL1 will undoubtedly generate large amounts of interesting data in the future and possibly uncover unique features of this receptor. Hopefully, future research will also reveal whether FPRL1 should still be considered a promiscuous receptor with affinity for a number of unrelated agonists, or if the apparently unrelated agonists turn out to share a common pattern on a higher level than the sequence level. This common pattern may well be on a physico-chemical level of the folded peptide/protein or could be of a more subtle nature where FPRL1 could be considered a pattern recognition receptor or sensor of danger signals. Similar to FPRL1, an expansion in knowledge may be expected for the third FPR variant, FPRL2, in coming years. Hitherto, we have shown that FPRL2 is a functional monocyte receptor for various FPRL1 agonists (11, 23), although no specific, high-affinity FPRL2 agonists have yet been described. In all probability, increased understanding of FPRL2 as an important receptor in its own right may help to complete the picture and bring about a more precise understanding of the FPR family and its role in inflammatory processes.

6. Acknowledgements

Thanks...

Claes Dahlgren for excellent supervision and for having a great attitude to science,

Anna Karlsson for collaborations, lunches, travels, discussions and more,

All other members (past and present) of the **Phagocyte Research Laboratory**, for highly appreciated contributions to the daily life, on- and off- lab,

CGWNMFF for giving me a sound (?) perspective on things through skateboarding, partying, skiing, hanging out, slacking, philosophical bull-shitting etc. and for being the best of friends,

My family for encouragement and support,

All other friends and colleagues -you know who you are,

Anna & Mange for always being there.

7. References

1. Agnihotri, N., D. K. Bhasin, H. Vohra, P. Ray, K. Singh, and N. K. Ganguly. 1998. Characterization of lymphocytic subsets and cytokine production in gastric biopsy samples from *Helicobacter pylori* patients. *Scand J Gastroenterol* 33:704-709.
2. Ali, H., R. M. Richardson, B. Haribabu, and R. Snyderman. 1999. Chemoattractant receptor cross-desensitization. *J Biol Chem* 274:6027-6030.
3. Allen, L. A. 2000. Modulating phagocyte activation. The pros and cons of *Helicobacter pylori* virulence factors. *J Exp Med* 191:1451-1454.
4. Almkvist, J., J. Faldt, C. Dahlgren, H. Leffler, and A. Karlsson. 2001. Lipopolysaccharide-induced gelatinase granule mobilization primes neutrophils for activation by galectin-3 and formylmethionyl-Leu-Phe. *Infect Immun* 69:832-837.
5. Babior, B. M. 1999. NADPH oxidase: an update. *Blood* 93:1464-1476.
6. Badolato, R., J. M. Wang, W. J. Murphy, A. R. Lloyd, D. F. Michiel, L. L. Bausserman, D. J. Kelvin, and J. J. Oppenheim. 1994. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J Exp Med* 180:203-209.
7. Bae, Y. S., Y. Kim, J. H. Kim, P. G. Suh, and S. H. Ryu. 1999. Trp-Lys-Tyr-Met-Val-D-Met is a chemoattractant for human phagocytic cells. *J Leukoc Biol* 66:915-922.
8. Baldwin, J. M., J. P. Bennett, and B. D. Gomperts. 1983. Detergent solubilisation of the rabbit neutrophil receptor for chemotactic formyl peptides. *Eur J Biochem* 135:515-518.
9. Bao, L., N. P. Gerard, R. L. Eddy, Jr., T. B. Shows, and C. Gerard. 1992. Mapping of genes for the human C5a receptor (C5AR), human FMLP receptor (FPR), and two FMLP receptor homologue orphan receptors (FPRH1, FPRH2) to chromosome 19. *Genomics* 13:437-440.
10. Berton, G., S. R. Yan, L. Fumagalli, and C. A. Lowell. 1996. Neutrophil activation by adhesion: mechanisms and pathophysiological implications. *Int J Clin Lab Res* 26:160-177.
11. Betten, A., J. Bylund, T. Cristophe, F. Boulay, A. Romero, K. Hellstrand, and C. Dahlgren. 2001. A proinflammatory peptide from *Helicobacter pylori* activates monocytes to induce lymphocyte dysfunction and apoptosis. *J Clin Invest* 108:1221-1228.
12. Bevilacqua, M. P. 1993. Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 11:767-804.
13. Blaser, M. J. 1993. *Helicobacter pylori*: microbiology of a 'slow' bacterial infection. *Trends Microbiol* 1:255-260.
14. Bokoch, G. M., and A. G. Gilman. 1984. Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin. *Cell* 39:301-308.
15. Borregaard, N., and J. B. Cowland. 1997. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 89:3503-3521.
16. Borregaard, N., K. Theilgaard-Monch, O. E. Sorensen, and J. B. Cowland. 2001. Regulation of human neutrophil granule protein expression. *Curr Opin Hematol* 8:23-27.
17. Boulay, F., M. Tardif, L. Brouchon, and P. Vignais. 1990. Synthesis and use of a novel N-formyl peptide derivative to isolate a human N-formyl peptide receptor cDNA. *Biochem Biophys Res Commun* 168:1103-1109.
18. Boyle, E. M., Jr., E. D. Verrier, and B. D. Spiess. 1996. Endothelial cell injury in cardiovascular surgery: the procoagulant response. *Ann Thorac Surg* 62:1549-1557.
19. Carlson, J. R. 2001. Functional expression of a *Drosophila* odor receptor. *Proc Natl Acad Sci* U S A 98:8936-8937.
20. Cassimeris, L., and S. H. Zigmond. 1990. Chemoattractant stimulation of

polymorphonuclear leucocyte locomotion. *Semin Cell Biol* 1:125-134.

21. Chervenick, P. A., D. R. Boggs, J. C. Marsh, G. E. Cartwright, and M. M. Wintrobe. 1968. Quantitative studies of blood and bone marrow neutrophils in normal mice. *Am J Physiol* 215:353-360.

22. Chiang, N., I. M. Fierro, K. Gronert, and C. N. Serhan. 2000. Activation of lipoxin A(4) receptors by aspirin-triggered lipoxins and select peptides evokes ligand-specific responses in inflammation. *J Exp Med* 191:1197-1208.

23. Christophe, T., A. Karlsson, C. Dugave, M. J. Rabiet, F. Boulay, and C. Dahlgren. 2001. The synthetic peptide Trp-Lys-Tyr-Met-Val-Met-NH2 specifically activates neutrophils through FPRL1/lipoxin A4 receptors and is an agonist for the orphan monocyte-expressed chemoattractant receptor FPRL2. *J Biol Chem* 276:21585-21593.

24. Christophe, T., A. Karlsson, M. J. Rabiet, F. Boulay, and C. Dahlgren. 2002. Phagocyte activation by Trp-Lys-Tyr-Met-Val-Met, acting through FPRL1/LXA4R, is not affected by Lipoxin A4. *Submitted.*

25. Clark, R. A. 1999. Activation of the neutrophil respiratory burst oxidase. *J Infect Dis* 179 Suppl 2:S309-317.

26. Dahlgren, C., and A. Karlsson. 1999. Respiratory burst in human neutrophils. *J Immunol Methods* 232:3-14.

27. Dahlgren, C., T. Christophe, F. Boulay, P. N. Madianos, M. J. Rabiet, and A. Karlsson. 2000. The synthetic chemoattractant trp-lys-tyr-met-val-DMet activates neutrophils preferentially through the lipoxin A(4) receptor. *Blood* 95:1810-1818.

28. De, Y., Q. Chen, A. P. Schmidt, G. M. Anderson, J. M. Wang, J. Wooters, J. J. Oppenheim, and O. Chertov. 2000. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* 192:1069-1074.

29. DeLeo, F. R., J. Renee, S. McCormick, M. Nakamura, M. Apicella, J. P. Weiss, and W. M. Nauseef. 1998. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J Clin Invest* 101:455-463.

30. Deng, X., H. Ueda, S. B. Su, W. Gong, N. M. Dunlop, J. L. Gao, P. M. Murphy, and J. M. Wang. 1999. A synthetic peptide derived from human immunodeficiency virus type 1 gp120 downregulates the expression and function of chemokine receptors CCR5 and CXCR4 in monocytes by activating the 7-transmembrane G-protein- coupled receptor FPRL1/LXA4R. *Blood* 94:1165-1173.

31. Dohlman, H. G., J. Thorner, M. G. Caron, and R. J. Lefkowitz. 1991. Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem* 60:653-688.

32. Dolmatch, B., and J. Niedel. 1983. Formyl peptide chemotactic receptor. Evidence for an active proteolytic fragment. *J Biol Chem* 258:7570-7577.

33. Dryer, L., and A. Berghard. 1999. Odorant receptors: a plethora of G-protein-coupled receptors. *Trends Pharmacol Sci* 20:413-417.

34. Durstin, M., J. L. Gao, H. L. Tiffany, D. McDermott, and P. M. Murphy. 1994. Differential expression of members of the N-formylpeptide receptor gene cluster in human phagocytes. *Biochem Biophys Res Commun* 201:174-179.

35. el-Awar, F. Y., D. L. Ochs, R. H. Pyle, and H. P. Misra. 1990. Lack of formyl-methionyl-leucyl-phenylalanine receptors on porcine neutrophils. *Am J Vet Res* 51:1561-1564.

36. Epand, R. M., and H. J. Vogel. 1999. Diversity of antimicrobial peptides and their mechanisms of action. *Biochim Biophys Acta* 1462:11-28.

37. Ferguson, S. S. 2001. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* 53:1-24.

38. Filep, J. G., C. Zouki, N. A. Petasis, M. Hachicha, and C. N. Serhan. 1999. Anti-inflammatory actions of lipoxin A(4) stable analogs are demonstrable in human whole blood: modulation of leukocyte adhesion molecules and inhibition of neutrophil-endothelial interactions. *Blood* 94:4132-4142.

39. Fiore, S., J. F. Maddox, H. D. Perez, and C. N. Serhan. 1994. Identification of a human cDNA encoding a functional high affinity lipoxin A4 receptor. *J Exp Med* 180:253-260.

40. Follin, P. 1999. Skin chamber technique for study of in vivo exudated human neutrophils. *J Immunol Methods* 232:55-65.

41. Forsberg, M., R. Lofgren, L. Zheng, and O. Stendahl. 2001. Tumour necrosis factor-alpha potentiates CR3-induced respiratory burst by activating p38 MAP kinase in human neutrophils. *Immunology* 103:465-472.

42. Gao, J. L., H. Chen, J. D. Filie, C. A. Kozak, and P. M. Murphy. 1998. Differential expansion of the N-formylpeptide receptor gene cluster in human and mouse. *Genomics* 51:270-276.

43. Gao, J. L., E. J. Lee, and P. M. Murphy. 1999. Impaired antibacterial host defense in mice lacking the N-formylpeptide receptor. *J Exp Med* 189:657-662.

44. Gautam, N., A. Maria Olofsson, H. Herwald, L. F. Iversen, E. Lundgren-Akerlund, P. Hedqvist, K. E. Arfors, H. Flodgaard, and L. Lindbom. 2001. Heparin-binding protein (HBP/CAP37): A missing link in neutrophil- evoked alteration of vascular permeability. *Nat Med* 7:1123-1127.

45. Gierschik, P., M. Steisslinger, D. Sidiropoulos, E. Herrmann, and K. H. Jakobs. 1989. Dual Mg2+ control of formyl-peptide-receptor-G-protein interaction in HL 60 cells. Evidence that the low-agonist-affinity receptor interacts with and activates the G-protein. *Eur J Biochem* 183:97-105.

46. Gilbert, T. L., T. A. Bennett, D. C. Maestas, D. F. Cimino, and E. R. Prossnitz. 2001. Internalization of the human N-formyl peptide and C5a chemoattractant receptors occurs via clathrin-independent mechanisms. *Biochemistry* 40:3467-3475.

47. Gopalakrishna, R., and S. Jaken. 2000. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 28:1349-1361.

48. Gronert, K., A. Gewirtz, J. L. Madara, and C. N. Serhan. 1998. Identification of a human enterocyte lipoxin A4 receptor that is regulated by interleukin (IL)-13 and interferon gamma and inhibits tumor necrosis factor alpha-induced IL-8 release. *J Exp Med* 187:1285-1294.

49. Gudmundsson, G. H., B. Agerberth, J. Odeberg, T. Bergman, B. Olsson, and R. Salcedo. 1996. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur J Biochem* 238:325-332.

50. Guthrie, L. A., L. C. McPhail, P. M. Henson, and R. B. Johnston, Jr. 1984. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J Exp Med* 160:1656-1671.

51. Gwinn, M. R., A. Sharma, and E. De Nardin. 1999. Single nucleotide polymorphisms of the N-formyl peptide receptor in localized juvenile periodontitis. *J Periodontol* 70:1194-1201.

52. Hallett, M. B., and D. Lloyds. 1995. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. *Immunol Today* 16:264-268.

53. Hallman, M., M. Ramet, and R. A. Ezekowitz. 2001. Toll-like receptors as sensors of pathogens. *Pediatr Res* 50:315-321.

54. Harris, H. 1954. Role of chemotaxis in inflammation. *Physiol. Rev.* 34:529-562.

55. Hartt, J. K., G. Barish, P. M. Murphy, and J. L. Gao. 1999. N-formylpeptides induce two distinct concentration optima for mouse neutrophil chemotaxis by differential interaction with two N-

formylpeptide receptor (FPR) subtypes. Molecular characterization of FPR2, a second mouse neutrophil FPR. *J Exp Med* 190:741-747.

56. Hirsch, E., V. L. Katanaev, C. Garlanda, O. Azzolino, L. Pirola, L. Silengo, S. Sozzani, A. Mantovani, F. Altruda, and M. P. Wymann. 2000. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 287:1049-1053.

57. Hu, J. Y., Y. Le, W. Gong, N. M. Dunlop, J. L. Gao, P. M. Murphy, and J. M. Wang. 2001. Synthetic peptide MMK-1 is a highly specific chemotactic agonist for leukocyte FPRL1. *J Leukoc Biol* 70:155-161.

58. Imler, J. L., and J. A. Hoffmann. 2000. Signaling mechanisms in the antimicrobial host defense of *Drosophila*. *Curr Opin Microbiol* 3:16-22.

59. Karlsson, A., P. Follin, H. Leffler, and C. Dahlgren. 1998. Galectin-3 activates the NADPH-oxidase in exudated but not peripheral blood neutrophils. *Blood* 91:3430-3438.

60. Karlsson, A., J. B. Nixon, and L. C. McPhail. 2000. Phorbol myristate acetate induces neutrophil NADPH-oxidase activity by two separate signal transduction pathways: dependent or independent of phosphatidylinositol 3-kinase. *J Leukoc Biol* 67:396-404.

61. Kenny, M. T., H. L. Torney, and F. J. Balistreri. 1989. Comparative effect of the naphthalenic ansamycins rifamycin SV, rifampin and cyclopentylrifampicin on murine neutrophil function. *Int J Immunopharmacol* 11:915-920.

62. Klinker, J. F., I. Schwaner, S. Offermanns, A. Hageluken, and R. Seifert. 1994. Differential activation of dibutyryl cAMP-differentiated HL-60 human leukemia cells by chemoattractants. *Biochem Pharmacol* 48:1857-1864.

63. Krause, K. H., D. Pittet, P. Volpe, T. Pozzan, J. Meldolesi, and D. P. Lew. 1989. Calciosome, a sarcoplasmic reticulum-like organelle involved in intracellular Ca2+-handling by non-muscle cells: studies in human neutrophils and HL-60 cells. *Cell Calcium* 10:351-361.

64. Le, Y., S. Jiang, J. Hu, W. Gong, S. Su, N. M. Dunlop, W. Shen, B. Li, and J. Ming Wang. 2000. N36, a synthetic N-terminal heptad repeat domain of the HIV-1 envelope protein gp41, is an activator of human phagocytes. *Clin Immunol* 96:236-242.

65. Le, Y., W. Gong, H. L. Tiffany, A. Tumanov, S. Nedospasov, W. Shen, N. M. Dunlop, J. L. Gao, P. M. Murphy, J. J. Oppenheim, and J. M. Wang. 2001. Amyloid {beta}42 activates a G-protein-coupled chemoattractant receptor, FPR-Like-1. *J Neurosci* 21:RC123.

66. Le, Y., J. J. Oppenheim, and J. M. Wang. 2001. Pleiotropic roles of formyl peptide receptors. *Cytokine Growth Factor Rev* 12:91-105.

67. Le, Y., H. Yazawa, W. Gong, Z. Yu, V. J. Ferrans, P. M. Murphy, and J. M. Wang. 2001. Cutting Edge: The neurotoxic prion peptide fragment PrP(106-126) is a chemotactic agonist for the G protein-coupled receptor formyl peptide receptor-like 1. *J Immunol* 166:1448-1451.

68. Lee, T. H., P. Lympany, A. E. Crea, and B. W. Spur. 1991. Inhibition of leukotriene B4-induced neutrophil migration by lipoxin A4: structure-function relationships. *Biochem Biophys Res Commun* 180:1416-1421.

69. Lew, P. D., A. Monod, F. A. Waldvogel, B. Dewald, M. Baggiolini, and T. Pozzan. 1986. Quantitative analysis of the cytosolic free calcium dependency of exocytosis from three subcellular compartments in intact human neutrophils. *J Cell Biol* 102:2197-2204.

70. Liebman, P. A., K. R. Parker, and E. A. Dratz. 1987. The molecular mechanism of visual excitation and its relation to the structure and composition of the rod outer segment. *Annu Rev Physiol* 49:765-791.

71. Lundqvist-Gustafsson, H., and T. Bengtsson. 1999. Activation of the granule pool of the NADPH oxidase accelerates apoptosis in human neutrophils. *J Leukoc Biol* 65:196-204.

72. Maddox, J. F., M. Hachicha, T. Takano, N. A. Petasis, V. V. Fokin, and C. N. Serhan. 1997. Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor. *J Biol Chem* 272:6972-6978.

73. Maestes, D. C., R. M. Potter, and E. R. Prossnitz. 1999. Differential phosphorylation paradigms dictate desensitization and internalization of the N-formyl peptide receptor. *J Biol Chem* 274:29791-29795.

74. Mai, U. E., G. I. Perez-Perez, J. B. Allen, S. M. Wahl, M. J. Blaser, and P. D. Smith. 1992. Surface proteins from *Helicobacter pylori* exhibit chemotactic activity for human leukocytes and are present in gastric mucosa. *J Exp Med* 175:517-525.

75. Malech, H. L., J. P. Gardner, D. F. Heiman, and S. A. Rosenzweig. 1985. Asparagine-linked oligosaccharides on formyl peptide chemotactic receptors of human phagocytic cells. *J Biol Chem* 260:2509-2514.

76. Malle, E., and F. C. De Beer. 1996. Human serum amyloid A (SAA) protein: a prominent acute-phase reactant for clinical practice. *Eur J Clin Invest* 26:427-435.

77. Marasco, W. A., S. H. Phan, H. Krutzsch, H. J. Showell, D. E. Feltner, R. Nairn, E. L. Becker, and P. A. Ward. 1984. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. *J Biol Chem* 259:5430-5439.

78. Marinissen, M. J., and J. S. Gutkind. 2001. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* 22:368-376.

79. McEver, R. P., K. L. Moore, and R. D. Cummings. 1995. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J Biol Chem* 270:11025-11028.

80. McMahon, B., C. Stenson, F. McPhillips, A. Fanning, H. R. Brady, and C. Godson. 2000. Lipoxin A4 antagonizes the mitogenic effects of leukotriene D4 in human renal mesangial cells. Differential activation of MAP kinases through distinct receptors. *J Biol Chem* 275:27566-27575.

81. Medzhitov, R., and C. Janeway, Jr. 2000. The toll receptor family and microbial recognition. *Trends Microbiol* 8:452-456.

82. Medzhitov, R., and C. Janeway, Jr. 2000. Innate immunity. *N Engl J Med* 343:338-344.

83. Medzhitov, R., and C. Janeway, Jr. 2000. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 173:89-97.

84. Mellqvist, U. H., M. Hansson, M. Brune, C. Dahlgren, S. Hermodsson, and K. Hellstrand. 2000. Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. *Blood* 96:1961-1968.

85. Miettinen, H. M., J. S. Mills, J. M. Gripentrog, E. A. Dratz, B. L. Granger, and A. J. Jesaitis. 1997. The ligand binding site of the formyl peptide receptor maps in the transmembrane region. *J Immunol* 159:4045-4054.

86. Murphy, P. M., T. Ozcelik, R. T. Kenney, H. L. Tiffany, D. McDermott, and U. Francke. 1992. A structural homologue of the N-formyl peptide receptor. Characterization and chromosome mapping of a peptide chemoattractant receptor family. *J Biol Chem* 267:7637-7643.

87. Nathan, C., S. Srimal, C. Farber, E. Sanchez, L. Kabbash, A. Asch, J. Gailit, and S. D. Wright. 1989. Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. *J Cell Biol* 109:1341-1349.

88. Painter, R. G., M. Schmitt, A. J. Jesaitis, L. A. Sklar, K. Preissner, and C. G. Cochrane. 1982. Photoaffinity labeling of the N-formyl peptide receptor of human polymorphonuclear leukocytes. *J Cell Biochem* 20:203-214.

89. Perez, H. D., S. Marder, F. Elfman, and H. E. Ives. 1987. Human neutrophils contain subpopulations of specific

granules exhibiting different sensitivities to changes in cytosolic free calcium. *Biochem Biophys Res Commun* 145:976-981.

90. Perez, H. D., R. Holmes, L. R. Vilander, R. R. Adams, W. Manzana, D. Jolley, and W. H. Andrews. 1993. Formyl peptide receptor chimeras define domains involved in ligand binding. *J Biol Chem* 268:2292-2295.

91. Pfister, C., M. Chabre, J. Plouet, V. V. Tuyen, Y. De Kozak, J. P. Faure, and H. Kuhn. 1985. Retinal S antigen identified as the 48K protein regulating light-dependent phosphodiesterase in rods. *Science* 228:891-893.

92. Prossnitz, E. R., O. Quehenberger, C. G. Cochrane, and R. D. Ye. 1993. The role of the third intracellular loop of the neutrophil N-formyl peptide receptor in G protein coupling. *Biochem J* 294:581-587.

93. Prossnitz, E. R. 1997. Desensitization of N-formylpeptide receptor-mediated activation is dependent upon receptor phosphorylation. *J Biol Chem* 272:15213-15219.

94. Prossnitz, E. R., and R. D. Ye. 1997. The N-formyl peptide receptor: a model for the study of chemoattractant receptor structure and function. *Pharmacol Ther* 74:73-102.

95. Prossnitz, E. R., T. L. Gilbert, S. Chiang, J. J. Campbell, S. Qin, W. Newman, L. A. Sklar, and R. D. Ye. 1999. Multiple activation steps of the N-formyl peptide receptor. *Biochemistry* 38:2240-2247.

96. Putsep, K., C. I. Branden, H. G. Boman, and S. Normark. 1999. Antibacterial peptide from *H. pylori*. *Nature* 398:671-672.

97. Putsep, K., S. Normark, and H. G. Boman. 1999. The origin of cecropins; implications from synthetic peptides derived from ribosomal protein L1. *FEBS Lett* 451:249-252.

98. Rainger, G. E., A. C. Fisher, and G. B. Nash. 1997. Endothelial-borne platelet-activating factor and interleukin-8 rapidly immobilize rolling neutrophils. *Am J Physiol* 272:H114-122.

99. Resnati, M., I. Pallavicini, J. M. Wang, J. Oppenheim, C. N. Serhan, M. Romano, and F. Blasi. 2002. The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. *Proc Natl Acad Sci U S A* 99:1359-1364.

100. Rogers, J., and Y. Shen. 2000. A perspective on inflammation in Alzheimer's disease. *Ann N Y Acad Sci* 924:132-135.

101. Romano, M., J. F. Maddox, and C. N. Serhan. 1996. Activation of human monocytes and the acute monocytic leukemia cell line (THP-1) by lipoxins involves unique signaling pathways for lipoxin A4 versus lipoxin B4: evidence for differential Ca2+ mobilization. *J Immunol* 157:2149-2154.

102. Roos, D., A. A. Voetman, and L. J. Meerhof. 1983. Functional activity of enucleated human polymorphonuclear leukocytes. *J Cell Biol* 97:368-377.

103. Rot, A., L. E. Henderson, T. D. Copeland, and E. J. Leonard. 1987. A series of six ligands for the human formyl peptide receptor: tetrapeptides with high chemotactic potency and efficacy. *Proc Natl Acad Sci U S A* 84:7967-7971.

104. Samuelsson, B., S. E. Dahlen, J. A. Lindgren, C. A. Rouzer, and C. N. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237:1171-1176.

105. Satin, B., G. Del Giudice, V. Della Bianca, S. Dusi, C. Laudanna, F. Tonello, D. Kelleher, R. Rappuoli, C. Montecucco, and F. Rossi. 2000. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. *J Exp Med* 191:1467-1476.

106. Schiffmann, E., B. A. Corcoran, and S. M. Wahl. 1975. N-formylmethionyl peptides as chemoattractants for leucocytes. *Proc Natl Acad Sci U S A* 72:1059-1062.

107. Schreiber, R. E., E. R. Prossnitz, R. D. Ye, C. G. Cochrane, and G. M. Bokoch. 1994. Domains of the human neutrophil N-formyl peptide receptor involved in G

protein coupling. Mapping with receptor-derived peptides. *J Biol Chem* 269:326-331.

108. Seo, J. K., S. Y. Choi, Y. Kim, S. H. Baek, K. T. Kim, C. B. Chae, J. D. Lambeth, P. G. Suh, and S. H. Ryu. 1997. A peptide with unique receptor specificity: stimulation of phosphoinositide hydrolysis and induction of superoxide generation in human neutrophils. *J Immunol* 158:1895-1901.

109. Shen, W., P. Proost, B. Li, W. Gong, Y. Le, R. Sargeant, P. M. Murphy, J. Van Damme, and J. M. Wang. 2000. Activation of the chemotactic peptide receptor FPRL1 in monocytes phosphorylates the chemokine receptor CCR5 and attenuates cell responses to selected chemokines. *Biochem Biophys Res Commun* 272:276-283.

110. Snyderman, R., and M. C. Pike. 1980. N-Formylmethionyl peptide receptors on equine leukocytes initiate secretion but not chemotaxis. *Science* 209:493-495.

111. Sorensen, O. E., P. Follin, A. H. Johnsen, J. Calafat, G. S. Tjabringa, P. S. Hiemstra, and N. Borregaard. 2001. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 97:3951-3959.

112. Stensmyr, M. C., M. C. Larsson, S. Bice, and B. S. Hansson. 2001. Detection of fruit- and flower-emitted volatiles by olfactory receptor neurons in the polyphagous fruit chafer *Pachnoda marginata* (Coleoptera: Cetoniinae). *J Comp Physiol* 187:509-519.

113. Su, S. B., J. Gao, W. Gong, N. M. Dunlop, P. M. Murphy, J. J. Oppenheim, and J. M. Wang. 1999. T21/DP107, A synthetic leucine zipper-like domain of the HIV-1 envelope gp41, attracts and activates human phagocytes by using G-protein-coupled formyl peptide receptors. *J Immunol* 162:5924-5930.

114. Su, S. B., W. Gong, J. L. Gao, W. Shen, P. M. Murphy, J. J. Oppenheim, and J. M. Wang. 1999. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J Exp Med* 189:395-402.

115. Sugawara, T., M. Miyamoto, S. Takayama, and M. Kato. 1995. Separation of neutrophils from blood in human and laboratory animals and comparison of the chemotaxis. *J Pharmacol Toxicol Methods* 33:91-100.

116. Surette, M. E., N. Dallaire, N. Jean, S. Picard, and P. Borgeat. 1998. Mechanisms of the priming effect of lipopolysaccharides on the biosynthesis of leukotriene B4 in chemotactic peptide-stimulated human neutrophils. *Faseb J* 12:1521-1531.

117. Suzuki, Y. J., H. J. Forman, and A. Sevanian. 1997. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 22:269-285.

118. Takeuchi, O., and S. Akira. 2001. Toll-like receptors; their physiological role and signal transduction system. *Int Immunopharmacol* 1:625-635.

119. Tauber, A. I., A. B. Karnad, K. L. Hartshorn, J. B. Myers, and J. H. Schwartz. 1989. Parameters of neutrophil activation: models of priming and deactivation. *Prog Clin Biol Res* 297:297-309.

120. Tomhave, E. D., R. M. Richardson, J. R. Didsbury, L. Menard, R. Snyderman, and H. Ali. 1994. Cross-desensitization of receptors for peptide chemoattractants. Characterization of a new form of leukocyte regulation. *J Immunol* 153:3267-3275.

121. Uhing, R. J. S., R. 1999. Chemoattractant stimulus-response coupling. *In* Inflammation. Basic Principles and Clinical Correlates. J. I. S. Gallin, R., eds. Raven Press, New York, p. 607-626.

122. Van Everbroeck, B., E. Dewulf, P. Pals, U. Lubke, J. J. Martin, and P. Cras. 2002. The role of cytokines, astrocytes, microglia and apoptosis in Creutzfeldt-Jakob disease. *Neurobiol Aging* 23:59-64.

123. Watson, F., and S. W. Edwards. 1998. Stimulation of primed neutrophils by soluble immune complexes: priming leads to enhanced intracellular Ca2+ elevations, activation of phospholipase D, and

activation of the NADPH oxidase. *Biochem Biophys Res Commun* 247:819-826.

124. Wenzel-Seifert, K., J. M. Arthur, H. Y. Liu, and R. Seifert. 1999. Quantitative analysis of formyl peptide receptor coupling to g(i)alpha(1), g(i)alpha(2), and g(i)alpha(3). *J Biol Chem* 274:33259-33266.

125. Verdrengh, M., and A. Tarkowski. 1997. Role of neutrophils in experimental septicemia and septic arthritis induced by *Staphylococcus aureus*. *Infect Immun* 65:2517-2521.

126. Wisner-Lynch, L. A., and W. V. Giannobile. 1993. Current concepts in juvenile periodontitis. *Curr Opin Periodontol*:28-42.

127. Yazawa, H., Z. X. Yu, Takeda, Y. Le, W. Gong, V. J. Ferrans, J. J. Oppenheim, C. C. Li, and J. M. Wang. 2001. Beta amyloid peptide (Abeta42) is internalized via the G-protein-coupled receptor FPRL1 and forms fibrillar aggregates in macrophages. *Faseb J* 15:2454-2462.

128. Ye, R. D., S. L. Cavanagh, O. Quehenberger, E. R. Prossnitz, and C. G. Cochrane. 1992. Isolation of a cDNA that encodes a novel granulocyte N-formyl peptide receptor. *Biochem Biophys Res Commun* 184:582-589.

129. Ye, R. D., O. Quehenberger, K. M. Thomas, J. Navarro, S. L. Cavanagh, E. R. Prossnitz, and C. G. Cochrane. 1993. The rabbit neutrophil N-formyl peptide receptor. cDNA cloning, expression, and structure/function implications. *J Immunol* 150:1383-1394.

130. Ye, R. D., and F. Boulay. 1997. Structure and function of leukocyte chemoattractant receptors. *Adv Pharmacol* 39:221-289.

Populärvetenskaplig sammanfattning på svenska i form av E-mailkorrespondens mellan författaren och hans humanistiskt skolade faster

----- Original Message -----
From: Barbro Bylund <BARBRO@nola.ornskoldsvik.se>
To: <johan.bylund@microbio.gu.se>
Sent: Tuesday, February 05, 2002 10:55 AM

Hej för åttonde gången!

Nu krånglar min dator eller jag alldeles förskräckligt.

Jag skulle bara maila och fråga efter dels den exakta titeln på din blivande avhandling dels några meningar på vanlig svenska, som talar om vad du sysslar med om det nu går att förklara. Kanske på den nivån att en natuvetargymnasist skulle få ett hum.
Hälsningar från Fastern

From: "Johan Bylund" <johan.bylund@microbio.gu.se>
To: "Barbro Bylund" <BARBRO@nola.ornskoldsvik.se>
Subject: Re:
Date sent: Tue, 5 Feb 2002 11:25:31 +0100

Hoppla, det blir inte helt lätt. Ok, arbetsnamnet är "Pattern recognition by formyl peptide receptors in neutrophils" Den handlar om HUR vita blodkroppar (neutrofiler) känner att de behövs och "luktar" sig fram till det område i kroppen som behöver dem. Detta är mycket bildligt talat, ett konkret exempel -man skär sig i fingret (vilket innebär att det kommer in en massa bakterier) och väldigt fort blir det rött, svullet, ömt osv. dvs. det blir inflammation. Inflammation är helt enkelt en ansamling och aktivering av neutrofiler (en typ av vita blodkroppar) som på nåt sätt känner av att det finns bakterier i såret och skyndar sig dit. Är du med?
De molekyler som känner av att bakterier finns i såret sitter på ytan av de vita blodkropparna och kallas receptorer (ännu ett ord i titeln -du ser vi betar av dem!). Dessa receptorer liknar väldigt mycket den typ av receptorer som vi t.ex luktar med och är grymma på att särskilja olika molekyler (tex. luktämnen ifall det rör sig om dessa, eller olika molekyler som kommer ifrån bakterier i mitt fall), och min avhandling rör en speciell klass av receptorer som alltså sitter på neutrofilerna och medför igenkänning av t.ex bakterier. Vad jag har pysslat med är att kolla på hur dessa receptorer fungerar, regleras, signalerar och vilka cell-processer de styr, och jag lanserar en liten hypotes om att en specifik receptor (FPRL1 är det poetiskt högtravande namnet) som tidigare trots vara promiskuös (japp, det är uttrycket som används för att indikera att receptorn kan känna igen en massa olika molekyler utan några likheter sinsemellan), i själva verket kan sägas reagera på en sorts "varningssignaler" i form av sönderbrutna molekyler som bildas i samband med vävnadsskada. Igenkänning av varningssignaler = pattern recognition.
Har jag tappat dig fullständigt?? Hursom, dessutom har jag gjort ett litet knäck av att kolla hur vissa cell-funktioner (som styrs av dessa receptorer) fungerar i möss, och eftersom det är en hel del skillnader mot hur det står till i människa (på vårt labb jobbar vi normalt med mänskliga blodceller som vi tar ifrån blodgivarblod) och då möss används så rackarns

mycket i olika typer av inflammationsforskning och resultaten översätts ofta rakt av till människan, försöker vi tala om att det inte är så enkelt utan att neutrofiler skiljer sig rätt mycket åt i Möss och Människor (japp, jag funderar på att referera till Steinbeck). Sen hänger jag in en direkt jämförelse av hur den klass av receptorer jag jobbar med skiljer sig mellan de olika arterna. För att popularisera tillställningen så diskuterar jag oxå direkta sjukdomstillstånd då "mina" receptorer med största sannolikhet spelar en viktigt roll t.ex, magsår, reumatism, alzheimers mfl.
Huga, det är riktigt svårt att förklara populärvetenskapligt vad tusan man pysslar med, är det nåt som verkar helt obegripligt så får du säga till.
Hoppas allt är lysande! /johan

From: Barbro Bylund <BARBRO@nola.ornskoldsvik.se>
To: <johan.bylund@microbio.gu.se>
Sent: Wednesday, February 06, 2002 10:25 AM

Johan, vad intressant och skojigt du berättar om din forskning. Jag fattade det mesta efter ett par långsamma läsningar. Den där sammanfattningen tycker jag du ska skicka till flera. Det jag undrar över är om dina laborationer stöder tesen att FPRL1 reagerar på de där varningssignalerna. Är det kanske lika viktigt ur forskningssynpunkt att upptäcka att så inte är fallet?
Du behöver inte snällt svara på stubben men någon gång när du har tid. / Babbi

---

From: "Johan Bylund" <johan.bylund@microbio.gu.se>
To: "Barbro Bylund" <BARBRO@nola.ornskoldsvik.se>
Subject: Re:
Date sent: Wed, 6 Feb 2002 20:25:31

Njae både och. Mina arbeten som jag baserar avhandlingen på handlar om
1) Interferens mellan olika substanser som aktiverar cellerna genom bla. FPRL1
2) Identifiering av en helt ny aktivator av FPRL1 (substansen kommer ifrån bakterien som orsakar magsår och kan därför hjälpa till att förklara den elakartade inflammationen som finns vid magsår)
3) Reglering av FPRL1 -det är känt sen förr att om de vita blodkropparna först stimuleras med vissa faktorer så kan aktiviteten som medieras via FPRL1-liknande receptorer öka rätt dramatiskt. Vi har visat att denna ökning beror på att FPRL1 lagras i små bubblor inuti cellen och dessa bubblor släpps lösa vid stimulering av cellen --> fler FPRL1 molekyler på cellen = högre effekt vid stimulering
4) Karaktärisering av FPRL1 liknande receptorer hos möss, och specifikt fokuserat på en av de cellulära effekter som dessa receptorer styr (bildningen av fria syreradikaler).

För att sy ihop detta så skriver jag avhandlingen som en översiktsartikel (tänkt att publiceras i sig) över denna typ av receptorer. Vinklingen mot varningssignaler bygger jag dels på andra arbeten jag gjort (som undersöker specificiteten hos FPRL1 map. hur aktivatorn ser ut) samt en massa andra publicerade artiklar. Jag försöker sammanfatta fältet så att säga. Det finns såklart alltid undantag som bekräftar regeln, så visst finns det fall där min hypotes inte riktigt går i hamn, men fokuseringen på "pattern recognition" är rätt spekulativ och inte alls bevisad.
Har förresten fått mkt snygga resultat idag (toppen) men oxå fått en artikel refuserad av folk som vägrar förstå sig på geniala experiment ;) (botten). Upp och ner hela tiden alltså!
Ha det fint! /j

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK

Digitaltryckt & Bunden
Vasastadens Bokbinderi AB
Göteborg 2002