THE ROLE OF SERUM AMYLOID A IN INFLAMMATORY DISEASE

- PROINFLAMMATORY MEDIATOR OR INERT BIOMARKER?

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Cover illustration photo (Mikael Alsterholm): Neutrophil from the author obtained by skin blister technique after a cross-country skiing tour in Delsjön.

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Abstract: Neutrophils are phagocytes of the innate immune system with prominent roles in host defense and are also believed to be important in autoimmune diseases such as rheumatoid arthritis (RA) by accumulating in inflamed joints and contribute to tissues destruction. Neutrophils differentiate in the bone-marrow and are mature cells when entering circulation with a cytoplasm packed with granules that contain toxic substances in addition to receptors that can be up-regulated to the cell surface during activation. Migration into the affected tissue is directed by mediators from the inflamed site which guides neutrophils along a chemotactic gradient and transfers the cell from resting- into a primed state. The inflammatory process triggers a systemic acute phase response characterized by the production of acute phase proteins (APP). The most prominent APP is serum amyloid A (SAA), the concentration of which can increase thousand fold in response to infection, aseptic inflammation or trauma. Patients with RA often have chronically elevated SAA in blood and joints and SAA has been implicated in the pathogenesis of RA. Recombinant SAA (rSAA) has been suggested to possess proinflammatory activities and act as a chemoattractant for neutrophils via a receptor called FPR2. A peptide (PBP10) with intracellular inhibitory activity was shown to allow discrimination between neutrophil signals mediated by FPR2 and the closely related FPR1. Next, the receptor specificity for rSAA was studied by use of PBP10 and another FPR2 specific inhibitor WRW4. rSAA affinity for FPR2 in transfected cell lines was corroborated and rSAA indeed activated primary human neutrophils. However, FPR2 was not responsible for mediating activation of primary human neutrophils by rSAA. Next, proinflammatory activity of rSAA was compared to that of endogenous SAA in circulation of RA patients. Using a sensitive marker for neutrophil activation in peripheral blood, endogenous SAA in circulation lacked proinflammatory activity and thus differed functionally from rSAA. Synovial neutrophils from patients with inflammatory arthritis and elevated SAA in joint fluid were next studied with respect to activation status. Synovial neutrophils displayed a surprisingly resting phenotype despite having transmigrated from peripheral blood to a compartment with endogenous SAA. Endogenous SAA, both in circulation and in joints, lack the proinflammatory properties present in the recombinant molecule.

Key-words: neutrophils, serum amyloid A, rheumatoid arthritis
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PREFACE

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

I. Fu H, Björkman L, Janmey P, Karlsson A, Karlsson J, Movitz C, Dahlgren C. The two neutrophil members of the formylpeptide receptor family activate the NADPH-oxidase through signals that differ in sensitivity to a gelsolin derived phosphoinositide-binding peptide. BMC Cell Biol. 2004 Dec 29;5(1):50.

II. Björkman L, Karlsson J, Karlsson A, Rabiet MJ, Boulay F, Fu H, Bylund J, Dahlgren C. Serum amyloid A mediates human neutrophil production of reactive oxygen species through a receptor independent of formyl peptide receptor like-1. *J Leukoc Biol. 2008 Feb;83(2):245-53.*

III. Björkman L, Raynes JG, Shah C, Karlsson A, Dahlgren C, Bylund J. The proinflammatory activity of recombinant serum amyloid A is not shared by the endogenous protein in circulation. *Arthritis Rheum.* 2010. *Accepted for publication.*

IV. Björkman L, Christenson K, Ekwall-Hultgård A-K, Dalhgren C, Bylund J. Activation status of neutrophils in the joints of patients with rheumatic disease. *In manuscript.*

To my children Erik and Johan

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ABBREVIATIONS

Anti-CCP	Autoantibodies directed toward citrullinated peptides
APP	Acute phase protein
APR	Acute phase response
C3	Complement factor 3
C5	Complement factor 5
C5a	Complement factor 5a (split product from C5)
CIA	Collagen induced arthritis
CR3	Complement receptor 3
CRP	C-reactive protein
CGD	Chronic granulomatous disease
DMARD	Disease modifying anti-rheumatic drug
fMLF	N-formylmethionyl-leucyl-phenylalanine
FPR	Formyl peptide receptor
G-CSF	Granulocyte colony stimulating factor
GPCR	G-protein coupled receptor
HDL	High-density lipoprotein
IL	Interleukin
LFA-1	Lymphocyte function-associated antigen
LPS	Lipopolysaccharide
MPO	Myeloperoxidase
RA	Rheumatoid arthritis
RF	Rheumatoid factors
ROS	Reactive oxygen species
SAA	Serum amyloid A
SRs	Scavenger receptors
TLR	Toll-like receptor
TNF	Tumour necrosis factor-α

INTRODUCTION

Our body is constantly exposed to microbial threats that need to be combated. One arm of our immune system, called the innate immune system, is rapidly activated in an attempt to eradicate the invading danger by eliciting an inflammatory response with release of potent antimicrobial substances from inflammatory cells. Inflammation can also be activated aseptically in response to *e.g.*, autoantigens, allergens or tissue damage. Inflammation is governed by a plethora of soluble signals from numerous sources in the affected tissues. Neutrophils, phagocytes of the innate immune system, arrive in vast numbers to an inflamed site after leaving the bloodstream and migrating out in the inflamed tissue guided by local gradients of chemoattractants. Neutrophils are armed with several antimicrobial substances that may also cause collateral damage to surrounding tissues. It is therefore vital that the activity of these cells is properly balanced. During the journey from blood to tissue, neutrophils will typically rearrange their surface flora of receptors and acquire an activated phenotype making cells primed for completing their antimicrobial mission. When the threat has been extinguished, neutrophils will gradually disappear from the site as inflammation is terminating. Usually, these processes run smoothly and homeostasis is rapidly recovered, but if inflammatory signals are altered or misprocessed, inflammation fails to terminate and may instead become chronic. Chronic inflammation characterizes many autoimmune diseases, *e.g.* rheumatoid arthritis (RA), and can be coupled to tissue damage.

The inflammatory process, whether triggered by infection, tissue injury or autoimmune disease, also involves a systemic acute phase response where the liver synthesizes a number of distinct acute phase proteins that are released into circulation. Serum amyloid A (SAA) is one of the most prominent of these plasma proteins and its concentration can be chronically elevated in blood and joints of patients with RA. Since the protein has been described as a highly proinflammatory neutrophil chemoattractant, it has been implicated in the pathogenesis of RA in addition to serving as a reliable biomarker for monitoring disease activity.

This thesis deals with SAA in inflammation and focuses on how recombinant SAA activate neutrophils and also addresses whether endogenous forms of this acute phase protein truly possess proinflammatory properties. Furthermore, whether SAA could be important in the pathology of RA or if it simply serves as a biomarker for ongoing inflammation is discussed.

INFLAMMATION

In mammals, the immune system compromises innate and adaptive immunity; both parts are built of large numbers of cellular and humoral components involved in a complex interplay. The innate immune system is present from birth and considered more primitive and less specific than the adaptive immune system and in contrast also lacks immunological memory (1). Innate immunity forms the first line of defense against invaders; it is rapid and aims at recognizing microbes and at eliminate and/or delay their spreading until the slower-acting, but more fine-tuned adaptive immune system is mobilized. Aseptic inflammation triggered by non-infectious stimuli, *e.g.* autoantigens our allergens, will also activate the innate immune system. Infectious or non-infectious agents can induce a fulminant immune response with production of proinflammatory mediators and activation of several cell types leading to the five cardinal signs of inflammation; rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), and functio laesa (loss of function) (2-3). For example, the local cardinal sign of RA is a synovitis which is a joint effusion with massive infiltration of neutrophils and thickening of the synovia leading to restricted joint motion and pain (described below). Inflammation can also lead to systemic responses with induction of an acute phase response (APR) coupled to a release of acute phase proteins (APP).

Given the power of inflammation, the process can be regarded as a double-edged sword. It is of vital importance to effectively protect us against potentially lethal infections, but can also cause severe harm in itself if too forceful or too long-lasting. Whenever acute inflammation fails to terminate, inflammation can become chronic with involvement of the adaptive immunity and inflammatory diseases are often characterized by persistence of the inflammatory infiltrate, tissue hyperplasia and tissue destruction and scarring (4). Key cells of the innate immune response are monocytes, macrophages, dendritic cells and neutrophils. In particular neutrophils migrate in large quantities towards the site of tissue damage or infection and are the first cell to arrive at inflamed foci. Since neutrophils are particularly potent inflammatory cells, it is of crucial importance that the presence and activity of neutrophils is properly balanced, both temporarily and spatially.

NEUTROPHILS

The principal task for neutrophils in our body is to combat invading microbes by phagocytosis and intraphagosomal killing. In humans, neutrophils are the most abundant leukocyte in circulation at concentrations around 1.8 – 7.5 x 10^9 cells/L, constituting about 60- 70% of circulating leukocytes. In contrast, neutrophils in mice are a minority population, making up only 20% of the total circulating white blood cells (5). During acute inflammation, neutrophils can be considered a hit and run type of cell, rapidly accumulating at the inflamed site in vast numbers, but also quick to disappear from the site once the immediate threat is cleared. The clearance is in part due to the fact that neutrophils are short lived cells that spontaneously undergo apoptosis after which they are rapidly cleared in visceral tissues like the liver and spleen (6). Neutrophils mature to fully differentiated cells in the bone marrow and their cytoplasm will be packed with storage organelles; *i.e.* granules, containing different matrix proteins and various membrane bound receptors (7). The granules constitute an arsenal of potent microbicidal compounds (proteolytic enzymes and antimicrobial peptides) that together with the reactive oxygen species (ROS) generated by the so called NADPH-oxidase, make up the weaponry against intruders (8).

Being professional phagocytes, neutrophils are equipped with receptors that recognize infectious agents directly, but also a variety of "danger signals" released from damaged tissue in response to *e.g.*, infection, trauma or autoimmune disease (9-11). Chemotactic gradients of certain inflammatory mediators guide neutrophils into an inflamed site through the binding of specialized chemoattractant receptors. An acutely inflamed tissue is morphologically characterized by a dense infiltration of phagocytes the majority being neutrophils and since they are packed with a multitude of highly toxic compounds, they could contribute to tissue destruction in inflammatory diseases (12). Also, neutrophils can interact with other immune cells by cell–cell contact and secreted products, thereby suggesting neutrophils as immunological decision makers (13). In addition, neutrophils have been shown to possess regulatory roles during acute and chronic microbial infections *e.g.*, by the production of the proinflammatory cytokine interleukin 8 (IL-8) (14) as well as the antiinflammatory cytokine interleukin-10 (15-16).

Thus, neutrophils are central in acute inflammation, but can also be present in vast numbers in diseases characterized by features of chronic inflammation. As will be discussed below, neutrophils are likely of importance for the pathology in rheumatic diseases (17).

From blood to tissue

Of importance in studies of neutrophil function, is that their main stage for carrying out antimicrobial- and/or tissue destroying activities is not in circulation, but rather in the tissues. The trek from blood to tissue is a complex process whereby the cells undergo a number of functional changes. Extravasation alludes to the process whereby neutrophils leave the blood vessels and pass through the endothelium into the tissue (**Fig. 1**).

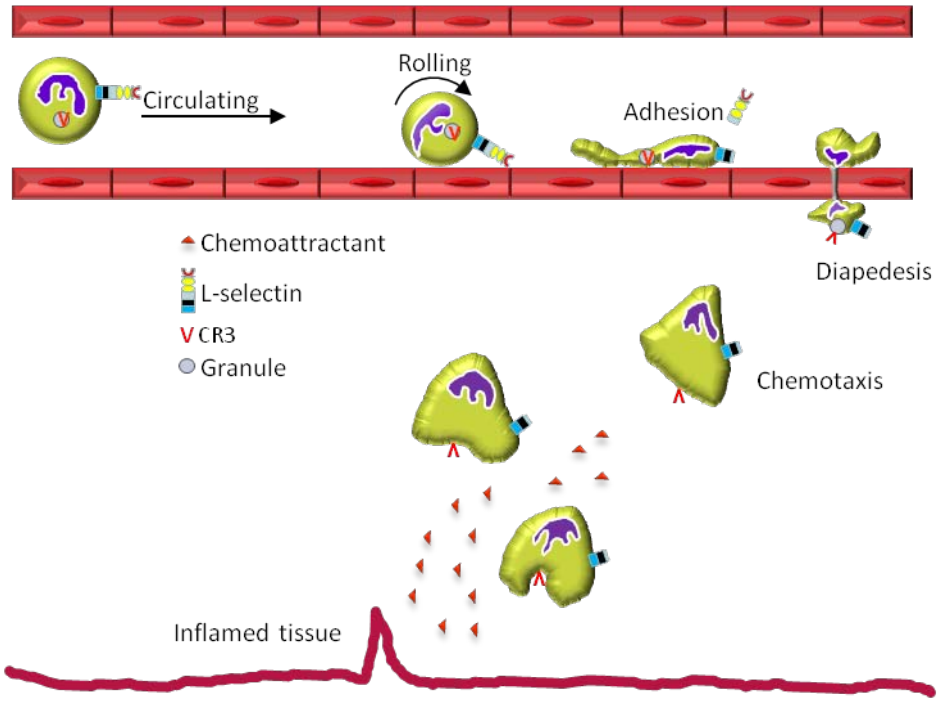


Figure 1. Neutrophil transmigration from blood to tissue*. Neutrophils sense signals from the inflamed tissues and start rolling on the endothelial surface. Interactions between the neutrophil and endothelial cells mediate neutrophil degranulation and rearrangement of cell surface receptors (e.g., cleavage of L-selectin and upregulation of CR3). The neutrophil crosses the endothelium by diapedesis and starts migrating through the tissue along chemotactic gradients towards the inflamed site.*

In circulation resting neutrophils starts to roll along the vessel wall at a rate slightly slower than that of the blood flow; this population constitutes the marginal pool (1). Neutrophils of the marginal pool are in a dynamic equilibrium with circulating cells (18). Only in the postcapillary and collecting venules does blood flow slow down sufficiently to allow optimal tethering of leukocytes to endothelial cells. Therefore, in the majority of tissues and organs, leukocyte extravasation takes place in this segment of the circulatory tree (19). However, it is obvious that leukocytes can emigrate in

vascular beds that experience considerably higher shear forces, *e.g.* in the lung alveoli and into atherosclerotic lesions of arteries (20).

Inflammatory mediators *e.g.*, chemoattractants from the inflamed tissue, induce endothelial cells of the blood-vessels to upregulate adhesion molecules (*e.g.* E-selectin and P-selectin), on their luminal surface, making them sticky for free-flowing neutrophils (21-22). During the first stages of leukocyte-endothelial interaction, L-selectin and PSGL1 are the main neutrophil receptors involved. These interact with endothelial selectins which enable neutrophils to be captured by tethering and to roll along the endothelial surface. During the transition from rolling to firm adhesion on the endothelial cell layer the neutrophil will polarize and the expression of endothelial VCAM, which is involved in the later steps of firm adhesion and diapedesis, is also increased at this point (23). Lymphocyte function-associated antigen (LFA-1) and CD11b/CD18 (CR3) on the neutrophil ensures cell arrest by binding VCAM and ICAM on the endothelium and extravasation into the tissue is initiated. The rearrangement of surface adhesion molecules is closely associated to neutrophil activation and priming (as discussed below) and both shedding of L-selectin and upregulation of CR3 and complement receptor 1 (CR1) (by degranulation) (24-27) are typical activation features that can be triggered by chemoattractants encountered during transmigration. However, this type of cellular activation may also be induced by other types of inflammatory mediators *in vivo* as well as *in vitro* (**Paper III**;(28)). Once the neutrophils have passed through the vessel, *i.e.* the endothelial layer, they will migrate towards the inflamed site via chemotaxis.

During chemotaxis, neutrophils move towards increasing concentrations of chemoattractants which are recognized by chemoattractant receptors expressed on the cell surface (described below). The chemotactic “crawling” of the cells in the inflamed tissue depends on cytoskeletal reorganization that causes directional cell movements by continuous assembling and dissociation of actin filaments, and also on interactions between neutrophil integrins and the extracellular matrix (29). In addition, by degranulation neutrophils secrete a variety of matrix degrading enzymes (*e.g.*, elastase) that could facilitate migration through extracellular matrix.

Neutrophil priming

During the passage from circulation to the tissue neutrophils undergo a number of changes, most notably represented by alterations in the flora of cell surface receptors. The change in receptor expression occurs mainly as a result of granule mobilization whereby the membranes of the various subcellular granules fuse with the plasma membrane. This is the case for *e.g.*,

complement receptors and chemoattractant receptors which surface expression are markedly increased after granule mobilizatio (30). Increased expression of chemoattractant receptors makes neutrophils increasingly responsive to stimulation by chemoattractants (as seen *e.g.*, using production of free radicals as a read out system; **Paper II**) and this cellular state is normally referred to as a primed state. Primed neutrophils typically lack surface L-selectin that is rapidly and efficiently down-regulated from the cell surface by ectodomain shedding induced by enzymatic activity of a metalloproteinase (ADAM 17) (31, 32 201). Most studies of neutrophil priming have been conducted on (resting) cells isolated from peripheral blood subsequently subjected to *in vitro* priming protocols. Frequently used *in vitro* priming agents for neutrophils are TNF and lipopolysaccharide (LPS) (33-34). Neutrophils that have encountered TNF or bacterial LPS are primed with respect to the oxidative response, but TNF or LPS *per se* does not activate the NADPH-oxidase in resting cells (35). The same principle applies for *in vivo* priming due to transmigration (**Fig. 2**). For experimental studies of *in vivo* transmigration and priming, a skin chamber technique is often employed (36). In brief, skin blisters were drawn on the volar side of the forearm by the use of suction chambers connected to a vacuum pump. The blister roofs were removed resulting in a superficial lesion of the skin. Thereafter a collection chamber was placed on the arm filled with autologuos serum. Over time chemotactic factors such as C5a and IL-8 will accumulate in this serum and attract neutrophils (36). After 24 hours the chambers were removed from the arm and the chamber fluid was collected (**Fig. 3**). This procedure allowed analyses of mediators contained in the skin chamber fluid, as well as evaluation and characterization of transmigrated neutrophils.

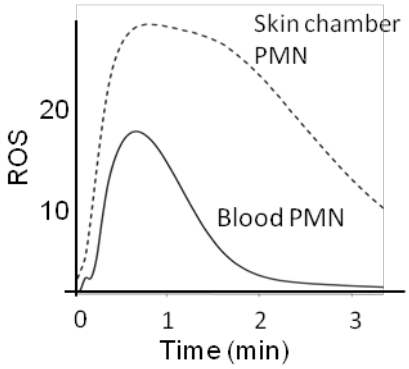
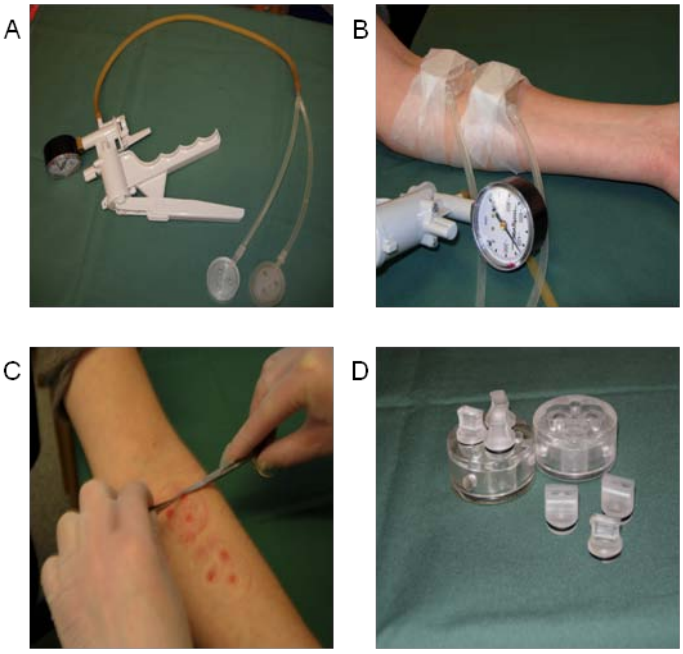


Figure 2. Transmigrated neutrophils are primed for NADPH-oxidase activation*. Purified neutrophils from peripheral blood (Blood PMN) and skin chamber neutrophils from the same donor were stimulated by WKYMVM (5X10 $^{-8}$ M). Extracellular release of superoxide anion (ROS) was monitored with use of an isoluminol-amplified chemiluminescence system.*

Figure 3.



Method for obtaining in vivo transmigrated neutrophils. *Skin chamber neutrophils were obtained according to (36). Skin blisters were induced on the volar side of the forearm of healthy volunteers by a suction-chamber connected to a portable vacuum pump (A and B). After 2 hours of continuous negative pressure, the top of the formed blisters were removed resulting in superficial lesions of the skin (C). Collection chambers with compartments corresponding to the lesions (D) were filled with autologous serum allowing neutrophils to migrate into the chamber fluid. The collection chambers were removed from the arm 24 hours later and the chamber fluid containing exudated neutrophils were aspirated (not shown).*

THE ACUTE PHASE RESPONSE

As described above, neutrophil recruitment from blood to tissue is a local manifestation of acute inflammation, but inflammation typically also triggers important systemic responses to the perceived insult, most notably the acute phase response (APR). The APR is a characteristic systemic reaction mediated by inflammatory stimuli (*e.g.* interleukin-1 (IL-1) and tumor necrosis alpha (TNF)) upon infection, direct tissue injury, or other threats to the body and it is characterized by changes in serum levels of a group of proteins called the acute phase proteins (APPs) (37-38). APPs are defined as proteins in which the plasma concentrations increase (positive) or decrease (negative) by at least 25 % during local or systemic disequilibriums (37). Several of these proteins aim to protect the body from infection and/or injury and to perpetuate homeostasis. The ratio between coagulation- and fibrinolytic APPs are balanced for formation and dissolution of fibrin clots (39) (40). C-reactive protein (CRP) and complement factor 3 (C3) are both APPs that facilitate phagocytosis by opsonisation of bacteria (41-42). In addition complement factors are APPs and activation of the complement system increases neutrophil recruitment by cleavage of complement factor 5 (C5) to the potent chemoattractant complement factor 5a (C5a) (36). The complement system can also directly kill certain microbes by the formation of bacteriolytic membrane attack complexes (43). APPs are primarily synthesized by the liver and are clinically considered as good markers of ongoing inflammation, but since most of the proteins are produced in the liver, rather than directly in the affected tissue, they usually do not help to identify the specific injured tissue(s) (44).

In case of prolonged exposure to stimulus or an inappropriate reaction to self molecules, inflammation may persist and lead to a chronic phase where the APR fails to switch off. Whether chronic elevations of some APPs have direct pathological consequences or if they are merely being biomarkers of inflammation is a matter of ongoing debate (45-46). One of the most prominent APPs is serum amyloid A (SAA), the concentration of which can increase 1000-fold in response to inflammation or infection (47). Levels of SAA may be chronically elevated in individuals with various inflammatory conditions such as atherosclerosis, diabetes, obesity, insulin resistance and above all rheumatologic diseases (48-49).

Serum amyloid A

SAA was first described in the mid seventies as a non-immunoglobulin protein purified from patients with systemic amyloidosis (50-51)and was later recognized as the precursor of reactive amyloidosis (52). The SAA proteins in

mammals are well conserved throughout evolution for at least 500 million years, indicating an important and beneficial role for survival and/or reproduction. That SAA is in fact essential is bolstered by the fact that homozygous mutations in a SAA gene resulted in prenatal lethality in mice (53).

The function of SAA was for long not clear and it was described in the literature as a protein without a function (47). Since then, SAA has been implicated in a variety of biological processes, not least in lipid metabolism (54) but also as an opsonin for Gram-negative bacteria (55) and as a proinflammatory mediator and chemoattractant (discussed below).

SAA is a family of apolipoproteins (47) with characteristic structural features (56). In circulation, SAA exists largely associated with high density lipoprotein (HDL) (54, 57), but may also be carried on very low-density lipoprotein particles, especially in circumstances where SAA levels are elevated (58) In humans, two major different forms of SAA are recognized, constitutive SAA (C-SAA) encoded by the gene *SAA4* (59) and acute SAA (A-SAA) (60) encoded by *SAA1* and *SAA2*. *SAA3* is regarded as a pseudogene, but its product has in some studies been reported to be secreted from adipose tissue and the mammary gland (61-62).

Regarding A-SAA, each 104-amino acid-residue primary product is processed to either a 103-residue or a 101-residue polypeptide by the loss of 1 or 3 amino acids from the N-terminal (47). During inflammatory conditions the concentration of A-SAA can increase quickly 1000-fold in plasma, reaching levels > 600 mg/L (~ 50 µM), with peak values usually noticed 2 days after induction (49, 63). Similar to the other APPs, SAA is predominantly produced by the liver, although extrahepatic production, *i.e.* from adipose tissue and synoviocytes, has been reported (63-64). The synthesis is largely regulated by inflammation-associated cytokines (IL-1, interleukin-6 (IL-6) and TNF), produced by endothelial cells, lymphocytes and in particular, activated monocytes and macrophages (44, 47, 65). When the inflammatory response resolves, SAA levels will decline after a few days. However, if inflammation fails to resolve, prolonged elevation of SAA levels is sustained due to overproduction of SAA, but also because the capacity of the liver to degrade SAA is believed to decrease during an APR or chronic inflammation (66).

As described above, no clear consensus exists regarding the main function of SAA, but it has been implicated as an inflammatory mediator. This view gains support from the fact that SAA is often chronically elevated in

inflammatory diseases and various investigations (described below) have also shown SAA to be directly chemotactic for monocytes and neutrophils (67-68) by binding to a surface located chemoattractant receptor. (69)

CHEMOATTRACTANTS AND THEIR RECEPTORS

For communication and recognition in a complex inflammatory environment, neutrophils are equipped with a wide variety of receptors, ranging from Toll-like receptors that recognize soluble danger signals, to immunoglobulin- and complement receptors that mediate phagocytosis. The recruitment of neutrophils to inflammatory foci is of fundamental importance for the body in response to infection and acute inflammation. This process is initiated and directed by exposure of cells to a biochemical gradient of chemoattractants, towards which the leukocytes migrate (5). Chemoattractants can be endogenous molecules such as C5a, chemokines (*e.g.*, IL-8), or lipid metabolites (*e.g.*, platelet activating factor). In addition, certain bacterial-derived substances are known to function as neutrophil chemoattractants, the best characterized being formylated peptides (*e.g.*, N-formyl-methionyl-leucyl-phenylalanine; fMLF) that are byproducts of bacterial metabolism (70-71).

The chemoattractant receptors are all G protein-coupled receptors (GPCRs) and very important for neutrophil function as they mediate both directed cell migration and trigger other neutrophil effector functions (described below). The GPCRs span the membrane seven times and transduce information to the cell interior via a heterotrimeric, guanine nucleotide-binding protein (G-protein). Receptors that are coupled to G-proteins have often been characterized with respect to their sensitivity to pertussis toxin, produced by the bacterium *Bordetella pertussis* (**Paper I** and **II**). These receptors are active in several organ systems in addition to the immune system, *e.g.* the central- and peripheral nervous system. Hence GPCRs are attractive targets for development of new drugs (72).

As mentioned above, SAA has been shown to be a chemoattractant and the chemotactic receptor claimed to be responsible for its recognition is called formyl peptide receptor 2 (FPR2), a member of the FPR family of receptors. The FPR family, all being GPCRs comprises three different chemoattractant receptors that were originally named FPR, FPR like-1 (FPRL1) and FPRL2, but the nomenclature was recently changed to FPR1, FPR2 and FPR3 respectively (73). Except for in papers I and II, which were published before the nomenclature changed, the new names will be used in this thesis. FPR1 was first cloned in 1990 from differentiated HL-60 (74) cells (a human promyelocytic leukemia cell line) and shortly after, both FPR2 and FPR3 were cloned by screening a cDNA library using FPR cDNA as a probe (75-77). FPR1 and FPR2 possess 69% identity at the amino acid level and these receptors are widely expressed by various cells of both hematopoietic and

non-hematopoietic origin (70). FPR1 and FPR2 are expressed on both neutrophils and monocytes, while FPR3 is only expressed on monocytes (70). In mice, FPR1 directly contributes to proper immune defence against bacterial infections and mice deficient in the murine FPR homologue were found to be more susceptible to infection with *Listeria monocytogenes* (78). The FPRs are also believed to be involved in the pathogenesis of several human diseases, *e.g.* Alzheimer's disease, prion disease, juvenile periodontitis, airway inflammation and HIV (79-83).

Given the multitude of chemoattractants and chemoattractant receptors that are involved in controlling inflammation, it is vital to possess adequate reagents (defined agonists and antagonists) and experimental systems in order to understand the roles played by specific molecules.

Tools for studying the FPRs –agonists and antagonists

The FPRs recognize a broad variety of ligands of exogenous and endogenous origins (81, 84). Essential for functional characterization of specific receptors and their ligands is the utilization of specific well-defined antagonist and inhibitors. Concerning the FPRs, only FPR1 and FPR2 are expressed by neutrophils and these two members are among the best studied human chemoattractant receptors with an ever expanding toolbox of specific agonists and antagonists available. The first described ligand binding FPR1 was fMLF, originally derived from growing *Eschericia coli* (85). Thereafter it has been shown that formylated peptides also from other bacteria are effective at activating phagocytes via FPR1 (86). To date, several other ligands derived from bacteria and viruses as well as endogenous substances have been described (for details; see review (70)). Well defined specific antagonist of FPR1 is Boc-MLF and Boc-FLFLF, peptides with a bulky tert-butyloxylcarbonyl (Boc) group at the N-terminus that sterically inhibits receptor activation. Another specific FPR1 antagonist is the cyclic undecapeptide Cys H (87).

In addition to being a low affinity receptor for formylated peptides, FPR2 recognizes several non-formylated peptides and is a promiscuous receptor that recognizes several different exogenous, endogenous and synthetic ligands with diverse structures (70). An established FPR2 specific agonist is the synthetic hexapeptide WKYMVM (88)which was identified by screening synthetic peptide libraries (89-90); this peptide has been used in this thesis for monitoring of FPR2 specific activities (**Paper I and II**). WRWWWW (WRW4) was the first specific antagonist for WKYMVM and thus blocks FPR2 (**Paper II**). This antagonist has later been shown to also antagonize the monocyte-specific FPR3 (91-92).

Aside from the above mentioned antagonists that all block receptor-ligand interactions there also exist inhibitors of these receptors that exert their action by blocking of intracellular signals. Phosphoinositide binding peptide (PBP10) is a ten amino acid long peptide derived from the PIP2 binding domain of gelsolin, coupled to a fluorophore rhodamin B. The presence of the fluorophore makes the peptide cell permeable and it inhibits intracellular signaling downstream of the receptor (93). PBP10 is receptor specific in that it inhibits signalling through FPR2, but not through FPR1 (**Paper I**). It should be mentioned that the inhibitory effect of PBP10 is not entirely restricted to FPR2 (**Paper II** (94)). Regardless, the ability of PBP10 to block FPR2-, but not FPR1 induced activity makes it a useful tool for determine FPR1 or FPR2 mediated neutrophil activities.

Tools for studying the FPRs –read out systems for neutrophil activation

Intracellular Ca^{2+}

When an agonist binds to a GPCR, the receptor changes conformation which in turn activates a cascade of downstream signaling molecules *e.g.* phospholipases and protein kinases (second messengers) that typically leads to the release of Ca^{2+} from intracellular stores (95). The rise in cytosolic Ca^{2+} induces the opening of Ca^{2+}-channels in the plasma membrane, which results in a sustained influx of Ca^{2+}. A Ca^{2+} transient is an early detectable event in GPCR-mediated neutrophil activation and therefore intracellular Ca^{2+} measurements are a useful read-out system for studying ligand-receptor interactions in neutrophils or transfected cell lines (**Paper I and II**).

Granule mobilization

The mobilization of granules is not just a mechanism for release of toxic material to the phagosome during phagocytosis. Cellular activation induces mobilization of granules to the plasma membrane thereby providing the surface with additional receptors (96). The granules of the neutrophils; *i.e.* azurophilic granules, specific granules, gelatinase granules and secretory vesicles are different from each other with regards to size, density, membrane content, matrix composition and they are mobilized to the cell surface or to the phagosome in a hierarchical manner (97). The secretory vesicle, the first storage organelle to be secreted during neutrophil activation, is an important source of membrane bound receptors and can be mobilized already in the circulation (98). As mentioned above, alteration of the cell-membrane due to granule mobilization represents an important activation feature (**Paper III** and **IV**).

Production of reactive oxygen species

Neutrophils are armed with a multitude of potent antimicrobial weapon systems, such as the NADPH-oxidase complex that will generate reactive oxygen species (ROS) when assembled. The oxidase complex consists of 2 membrane-bound subunits (cytochrome b; constituted by $gp91^{phox}$ and $p22^{phox}$) present in the plasma membrane and in the membranes of the specific granules, as well as at least 3 cytosolic components ($p67^{phox}$, $p47^{phox}$ and $p40^{phox}$) (99). Defects in the genes encoding subunits of the NADPH-oxidase results in phagocytes incapable of ROS production and a disease called chronic granulomatous disease (CGD). CGD patients suffer from recurring bacterial and fungal infections and a variety of inflammatory conditions (100-101). Activation of the NADPH-oxidase occurs by assembly of cytochrome b and the cytosolic components of the oxidase to the membranes, thus forming a functional electron transporting enzyme system that converts O_2 to O_2^- which can be further transformed into other ROS catalyzed by the azurophil granule enzyme myeloperoxidase (MPO).

The NADPH-oxidase can be assembled both at intracellular membranes (typically, but not exclusively, the phagosomal membrane (102)) and in the plasma membrane, generating ROS that are released from the cell (8). Traditionally, ROS (especially the extracellular) have been considered harmful and culprits of tissue damage (103). However, several studies have shown that in the absence of ROS, inflammatory disease is in fact enhanced, indicating that ROS may somehow dampen inflammatory processes (102). For example, rodents with a defect phagocytic ROS production are susceptible to develop arthritis (104-105). Regardless of function, neutrophil activation by chemoattractants via GPCRs leads to extracellular release of ROS, which is thus a good read out for cellular activation (**Paper I, II, III and IV**).

PROINFLAMMATORY EFFECTS OF SAA

Since the concentrations of APPs are so heavily raised during the APR and remain high during several inflammatory disease states, it has been argued that individual APPs in fact also drive inflammation. As such, APPs would not only be secondary to inflammation, but also a cause. Proinflammatory effects of SAA have been investigated in most part by the use of a commercially available recombinant SAA (rSAA) molecule (67-69, 106-112) that corresponds to human SAA1 except for the presence of three amino acids substitutions from SAA2. Each of the substitutions are described to exist in natural variants of SAA (113), but no naturally occurring allelic variant with identical sequence as rSAA has been described. Thus the rSAA protein is a hybrid molecule and is not identical to any of the human SAA isoforms.

rSAA was first reported as a chemoattractant for phagocytic cells that induced migration, degranulation and adhesion of human monocytes and neutrophils (68). Subsequently, the signaling pathway was shown to be pertussis toxin sensitive indicating the involvement of a GPCR (67). A few years later the receptor specificity was determined by showing that a cell line transfected with FPR2 displayed chemotactic migration towards rSAA, stating SAA as the first endogenous ligand for FPR2 (69). We have confirmed these results by showing that rSAA induced Ca^{2+} transients in HL-60 cells overexpressing FPR2 (Paper II), and the receptor specificity was further ascertained by the use of the specific FPR2 antagonist WRW4 (described above) that efficiently blocked rSAA-induced Ca^{2+} transients in these cells. rSAA also activated the neutrophil NADPH-oxidase in a pertussis-toxin sensitive manner and the response could be primed by TNF or LPS demonstrating that the responsible receptor was stored in intracellular mobilizable granules. In agreement with these result also in vivo primed neutrophils (obtained by the skin chamber technique described above) responded with a primed oxidase response (Fig. 4).

In primary neutrophils stimulated with rSAA, WRW4 however unexpectedly failed to inhibit Ca^{2+} transients (Paper II). Also the NADPH-oxidase activation by rSAA was unaffected by WRW4; this pertained to both in vitro and in vivo primed neutrophils indicating that the responsible receptor on primary neutrophils was not FPR2 (**Fig. 4**). The intracellular FPR2 inhibitor PBP10 partly blocked the NADPH-oxidase response after rSAA stimulation. The precise mechanism by which PBP10 interferes with some GPCRs has still not been determined, but it is reasonable that the signaling intracellular domains of the PBP10-sensitive receptors, i.e., FPR2

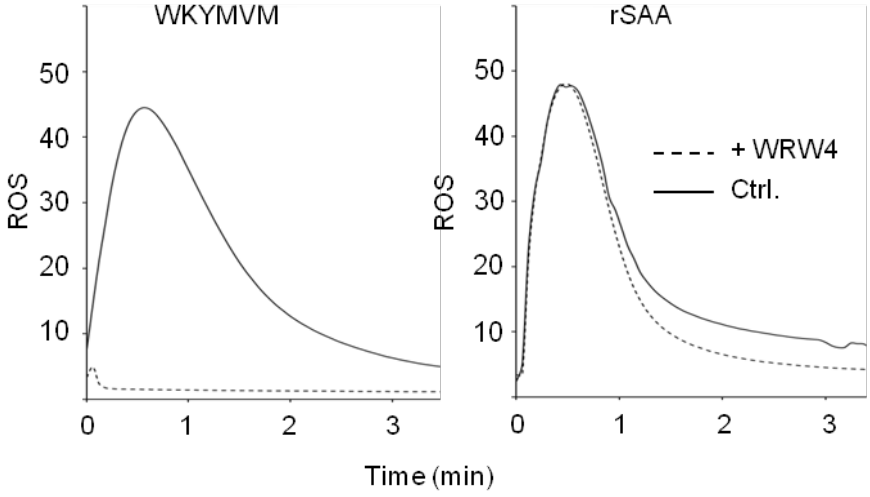


Figure 4. rSAA activates the neutrophil NADPH-oxidase through a receptor distinct from FPR2. *Skin chamber neutrophils were stimulated with WKYMVM (5X10 $^{-8}$M) or recombinant SAA (10 µM) in the presence (dotted line) or absence (solid line) of the FPR2-specific antagonist WRW4. Extracellular release of superoxide anion was monitored with use of an isoluminol-amplified chemiluminescence system.*

and the rSAA receptor are in some way structurally similar (**Paper II**). From these results we concluded that rSAA indeed activate human neutrophils, but that a GPCR receptor distinct from FPR2 is responsible (**Paper II**). In line with these findings, we have also shown that rSAA delay neutrophil apoptosis in vitro and that also this potentially proinflammatory effect was executed independently of FPR2 (114).

Aside from its suggested role as a chemoattractant and activator of phagocytes, rSAA has also been shown to possess other proinflammatory effects using a variety of non-chemoattractant receptors, most notably Toll-like receptors (TLRs) and scavenger receptors (SRs). When expressed in cervical cancer cell line (HeLa cells), TLR2 was shown to bind rSAA and subsequently activate NF-κB (115-116). TLR4 has also been proposed as an SAA receptor on primary murine peritoneal macrophages that upon stimulation with rSAA induced transcription of NO synthase (117). Two different scavenger receptors, SR-BI (118) and CD36 (119) were reported to function as endocytic rSAA receptors that trigger proinflammatory cytokine production when overexpressed in cell lines. SR-BI has also been demonstrated to mediate the cholesterol efflux function of HDL-associated SAA (120). In addition, the receptor for advanced glycation end-products (RAGE), mediated activation of NF-κB after stimulation of synovial fibroblasts with rSAA(109).

Despite lack of consensus regarding receptor specificity for rSAA, the picture has emerged of SAA as a multifunctional proinflammatory mediator that clearly activate neutrophils (**Paper II** and **III**). The majority of the studies mentioned above (including paper II) were carried out using the recombinant "consensus SAA molecule" which is not identical to the endogenous protein. It was thus of importance to study if endogenous SAA expressed during the APR was indeed proinflammatory and shared the neutrophil activating effects seen for rSAA. For this purpose we employed blood from patients with RA, that often display tremendously increased levels of endogenous SAA. Neutrophils from these patients were not activated despite the fact that they were surrounded by much higher SAA concentrations than was required to activate cells with rSAA (**Paper III**). When added to blood samples, rSAA clearly induced an activated phenotype of neutrophils, regardless of how much endogenous SAA that was present. In addition, purified acute phase SAA from human subjects neither activated neutrophils, nor stimulated cytokine production from macrophages (**Paper III**). These data provide strong evidence that endogenous SAA in circulation lacks proinflammatory activity and clearly differs from the recombinant form of the protein (**Paper III**).

A known problem with bacterial-derived recombinant proteins is contamination with bacterial products (*e.g.*, endotoxin) that could cause biological effects independent of the recombinant protein itself (45). The rSAA contained very low levels of endotoxin, however the possibility of other contaminating bacterial products can not totally be ruled out. Nevertheless rSAA activated neutrophils through a GPCR by inducing NADPH-oxidase activation and mobilization of intracellular Ca^{2+} and such activation is not mediated by endotoxin. A reason for the discrepancy between endogenous SAA in circulation and rSAA (**Paper III**) could potentially be explained by in what form the protein exists when presented to immune cells. Not only is SAA very prone to aggregation, but also to bind a variety of lipids (54). Hence proinflammatory forms of SAA could possibly be found *outside* of circulation. Since synoviocytes in rheumatic joints have been identified as an extrahepatic source of SAA (63), it has been tempting to speculate that SAA contributes directly to pathogenesis in arthritis and many investigators have argued that SAA is a proinflammatory mediator in affected joints of RA patients (67, 108, 111-112, 121-128).

ARTHRITIS

Arthritis refers to a broad spectrum of conditions involving damage to the joints. Paleontologic studies have detected destructive joint conditions in dinosaurs in fossil records, and rheumatic joint destructions has been dated back some 6500 years from investigations of North American aboriginal skeletons (129). Different etiologies can cause arthritis and consequently arthritis present in various different forms and with diverse degrees of inflammation. Roughly, the inflammation process constitutes the cornerstone that determines the diagnosis and the treatment. The most common form of arthritis is osteoarthritis where inflammation is not an obvious feature; still this condition is the most frequent cause of slow progressive disability (130). In contrast, infectious arthritis caused by bacteria is characterized by massive inflammation. One example of bacteria-induced arthritis is septic arthritis induced by *Staphylococcus aureus* which causes a rapid destruction of joint cartilage and periarticular bone (131).

A common form of arthritis usually presenting with inflammatory features is RA, affecting approximately 0.5-1% of the population with varying prevalence among different ethnicities. Caucasians are for instance more susceptible to the disease than Asians and Africans (132-133). Women present with RA more often than men, with a ratio of 3:1 (134), indicating that hormone levels are of importance (135). RA is regarded as a collection of syndromes rather than one disease; the main characteristic being symmetrical arthritis in peripheral joints (136). Clinical manifestations range from mild to rapidly progressive invasion of the synovium into the adjacent bone and cartilage resulting in erosive disease with massive tissue destruction (137). Other disease manifestations of RA can occur involving also other organs than the joints, *e.g.*, the heart, lungs, eyes, kidneys or skin; these conditions are collectively referred to as extra-articular RA (138). Reactive amyloidosis (AA-amyloidosis) is an example of extra-articular RA and is a consequence of long, pronounced inflammation and present as deposits of placque in virtually any organ of the body (139). During recent years, increased mortality in cardiovascular disease due to accelerated atherosclerosis in patients with rheumatic diseases has been frequently reported (140-141). This could also be viewed as a form of extra-articular RA and the inflammation *per se* is suggested as the risk factor overriding traditional cardiovascular risk factors (142-143).

A majority of RA patients have increased levels of rheumatoid factors (RF), *i.e.* autoantibodies, directed against the Fc portion of immunoglobulin G and serum concentration of RF has been found to correlate with disease

severity (144). Autoantibodies directed toward citrullinated peptides (anti-CCP) have also been shown to correlate with disease severity and to be useful for diagnostic and therapeutic strategies especially in RA with recent onset (145-147). Moreover anti-CCP and RF are present in the synovial tissue, implying a possible direct pathogenic role (148-149).

Synovial anatomy

Synovial joints are composed of adjacent bones with articular cartilage connected by a fibrous joint capsule. The synovium is composed of a synovial lining layer and a synovial sublining layer. The synovial lining is built up by two major types of cells; macrophage-like- and fibroblast-like synoviocytes that are developed from different progenitor cells and subsequently express different surface markers (150-152). The synovial sublining is built up of loose connective tissue traversed by blood-vessels, lymph-vessels and nerve fibers. Major cell-types of the synovial sublining are fibroblasts, macrophages, mastcells and adipocytes. In contrast to e.g, the vascular endothelium, the synovial sublining lacks a continuous basal membrane (153). The space surrounded by the synovium is the synovial cavity which is filled with hyaluronan-rich synovial fluid formed by diffusion from the blood and supplemented with mucin produced in synoviocytes. The main function of the synovial fluid is to lubricate the cartilage and facilitate articular motion (153, 154). There are two different types of bone, trabecular (spongy) bone and cortical (compact) bone and the former appears porous with much room for blood vessels and bone marrow. The location of bone marrow inside the trabecular bone creates a physical opportunity for interaction between immune cells and bone cells. In RA, the destruction of cartilage and bone may lead to a state where the inflamed synovial tissue reaches the adjacent bone marrow by breaking the cortical barrier. Thus, the bone marrow could be considered an additional compartment of importance for the disease process in RA (194).

Patophysiology of RA

In RA joints, unknown factors trigger the immune system to react against cell- or tissue antigens leading to the production and release of inflammatory mediators that induce local inflammation. Gradual alterations in the normally thin and delicate synovial membrane will arise with thickening of the synovium, *i.e.* synovial hyperplasia, due to increased cell -accumulation, -proliferation and increased angiogenesis. As inflammation proceeds, the synovium will develop and create a proliferation mass often referred to as a pannus (155-156). The pannus is an inflammatory active synovium that invade cartilage and bone with successive destruction by matrix degrading

enzymes, *e.g.* MMPs. A suggested source of MMPs is chondrocytes and synoviocytes (157), but also neutrophils are rich in MMPs suggesting that these cells could be important for the destruction (158-159) (**Fig. 5**).

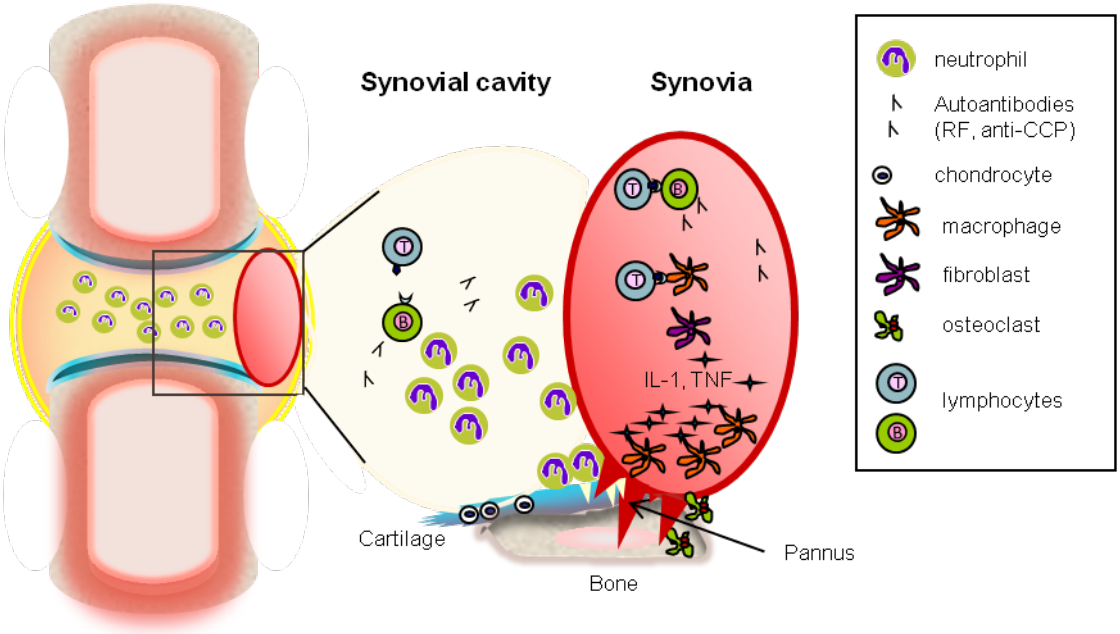


Figure 5. Schematic figure of an inflamed joint in RA*. The inflamed synovium is infiltrated by an array of immune cells; i.e. T-cells, B-cells, fibroblasts and macrophages that act in concert with inflammatory mediators like TNF and IL-1. T-cells and B-cells are found in the synovium, but also in the synovial fluid. The joint cartilage is built up by chondrocytes that cover the articular bone. A pannus is formed at the junction between synovia and cartilage that gradually degrade tissues. Neutrophils are found the pannus-cartilage junctions and in the synovial fluid, but are scarcely found in the synovium. Adapted from (213).*

The rheumatic joint cavity will be invaded by different inflammatory cells and it is not entirely clear which particular cell types that are of central importance for the initiation and progression of RA. The dominant view, however, is that the adaptive immune system plays the most important role in the pathogenesis of RA (160), but given the facts that innate immune cells such as neutrophils are often found in synovial fluid and that these are filled with potentially tissue-destroying enzymes, the adaptive immune system is not likely the full explanation. Supporting the role for adaptive immune cells in the pathogenesis of RA are mainly experimental results from animal studies that favor the hypothesis of RA being a T-cell driven disease. The T-cell population in the inflamed joints is diverse, cytotoxic T-cells, T-helper cells and T regulatory cells all occur in the synovia as well as in the synovial

fluid. B-cells are in minority in the inflamed joint, but still seem important in the pathogenesis of RA, not least as producers of autoantibodies such as RF and anti-CCP (161). RF, being present in serum and in inflamed joints, form immune complexes that in turn can activate the complement with subsequent neutrophil activation which could contribute to joint destruction.

Other immune cells *e.g.*, monocytes/macrophages, dendritic cells and fibroblasts are present in the synovial tissues and possess widespread pro-inflammatory, destructive, and remodeling capabilities (162-163). In addition stroma cells such as fibroblasts transform and proliferate in the inflamed joints and are found both at the pannus and in the synovial fluid (155-156, 164).

To halter the destructive process in the affected joints and prevent extra-articular complications, early treatment with disease-modifying anti rheumatic drugs (DMARDs) focused on repressing inflammation is required (165). In the past decade, a group of new therapeutic drugs called biological-response modifiers have remarkably transformed the field of rheumatology and dramatically improved patient outcomes (130). This is true in particular for the TNF-blockers. Blockade of TNF was first found to be effective in a mouse model of arthritis (166) and later shown to have effect also in human disease (167-168). TNF is a key cytokine in inflammation, important for host defense and immune responses, *e.g.* immune cell recruitment, -proliferation and -death just to mention a few roles (169). In RA more specific functions of TNF have been detected and it has been shown to induce matrix degradation and osteoclastogenesis (170) (171-173). TNF is also a key component in the cytokine cascade released during RA (174). Regardless of the exact mechanisms by which TNF-blockers mediate effects *in vivo*, these drugs lead to a decreased migration of leukocytes into joints and a reduction of angiogenesis (175). Given the central role of TNF and inflammation in host defense, it is not surprising that blocking of TNF can be hazardous and careful monitoring of patients are urged since the risk for infections, especially tuberculosis increases (176-178). Another treatment-strategy that has emerged lately and now challenge TNF-blockers as the number one in the treatment of RA is B-cell depletion (179). The last decade the field of rheumatology has been revolutionized by new treatments, in particular concerning RA, with several new drugs targeting multiple different cytokines or immune cells thereby highlighting the diverse nature of these diseases.

NEUTROPHILS IN RA

Among the inflammatory joint diseases, septic arthritis is the most prominent example where neutrophils have been shown to have crucial roles, both for combating the infection *per se* (180), but also for contributing to the massive tissue destruction characteristic for this disease (181). Neutrophils can also be abundant in affected joints of aseptic arthritis, especially in the synovial fluid early in disease progression or during flares of joint diseases with massive joint effusions (153). Synovial biopsies from patients with recent onset of synovitis (one week to one month) indicate their presence in the synovium, although in later stages of the disease neutrophils are scarcely found at this site (182-184). Instead, the synovial fluid is the compartment where neutrophils are primarily found later in disease. The hallmark of RA is destruction of tissues (185) and neutrophils are certainly well endowed to inflict joint damage with an arsenal of toxic compounds (186). In addition, synovial fluid neutrophils have been reported at the pannus/cartilage borders (186-188), supporting the view that these cells could in fact be of importance for RA pathogenesis.

As neutrophils are the dominant cell type in synovial fluid while often absent from the actual synovium, the question arise; how have these cells entered the synovial cavity? According to established tenets, neutrophils reach inflamed sites by transmigration from the blood through the inflamed synovium, finally reaching the synovial fluid, *i.e.* transmigration from blood to tissue. As described above, the cells found in synovial fluid would thus be expected to present with an activated phenotype and be primed (as described above) for further stimulation as is the case for *in vivo* transmigrated neutrophils obtained by the skin chamber model (189). We found that neutrophils from skin chambers showed full signs of priming (**Paper IV**) as detected by receptor exposure and NADPH-oxidase responses (**Fig. 2**). In stark contrast, we unexpectedly found neutrophils from synovial fluid to present with a relatively non-activated phenotype (**Paper IV**). These surprising results contradict several earlier studies showing the presence of activated neutrophils in synovial fluid (186, 190-192). Common for these studies is that synovial fluid neutrophils were further purified before analyses, which could possibly affect priming status in itself (24) (**Paper IV**).

In agreement with earlier studies (193), we found relatively low levels of inflammatory cytokines and chemokines in synovial fluids, compared to the exudate fluid from the skin chambers applied on healthy subjects; a pattern that was especially pronounced for IL-1, IL-8 and TNF (**Paper IV**). In contrast, SAA levels were elevated in synovial fluid as compared to exudate

fluid, but still at very moderate levels as compared with serum SAA of RA patients (**Paper III** and **IV**). This pattern is very counter-intuitive if SAA should function as a chemoattractant guiding neutrophils to the inflamed joint, since migration typically proceeds against increasing concentrations of the chemoattractant. It is possible that the SAA found in circulation differs from that in an inflamed joint and that only the latter possess proinflammatory effects. However, our results with elevated levels of SAA in synovial fluid accompanied by non-activated neutrophils argue that SAA is hardly a potent mediator in inflamed joints. This conclusion is in accordance with the fact that endogenous SAA in circulation was unable to activate neutrophils (**Paper III** and discussed above). Together, our findings argue against direct proinflammatory activities of endogenous SAA.

The unexpected presence of non-primed, *i.e.* resting neutrophils, in the synovial fluid raises several questions. First, since transmigration from blood to tissue normally prime neutrophils how do the neutrophils enter synovial fluid without getting primed? One tempting possibility is that neutrophils could reach the synovial cavity directly from the bone marrow instead of transmigrating from blood. Bone marrow has been proposed to be an additional compartment in the disease process of RA, since inflamed tissue in patients suffering of an erosive disease, *i.e.* destruction of cartilage and bone, physically connects with the bone marrow (194). Another possibility is that circulating neutrophils "leak" through synovial blood vessels without the regular activation. In fact, immature, incompletely developed blood vessels lacking periendothelial coverage have been found in RA synovia and such vessels would be leakier than mature vessels (195). Whatever route neutrophils use to reach the synovial cavity, it is clear that they reach this site in large numbers without being fully primed. It is however, still unclear what role neutrophils play in the aseptically inflamed RA joint.

Animal studies have been undertaken to clarify the importance of neutrophils in RA showing for example that initiation and progression of RA-like arthritis in mice depend on neutrophils (196). Another recent study showed that granulocyte colony stimulating factor (G-CSF) deficient mice were markedly protected from collagen-induced arthritis, the major murine model of RA (197). Moreover, neutrophil depletion attenuated already established collagen induced arthritis (CIA) demonstrating neutrophils as key effector cells that promote the progression of disease (198). It is clear that neutrophils have ability to inflict massive tissue damage also in human disease, and synovial fluid neutrophils has been suggested to phagocytose immune complexes (192) driving the release of powerful proteases.

To further highlight the role of neutrophils in inflammatory joint disease, examples from clinical situations can be used. Patients who develop Felty's syndrome, defined by RA and the development of splenomegaly and neutropenia (199) usually experience a decrease in inflammatory activity and number of swollen joints as neutropenia proceeds (200). Occasionally, if these patients need treatment with G-CSF that increase release of neutrophils from the bone marrow, it is not unusual that arthritis reoccur (201). Another example stressing the role of neutrophils in inflammatory joint disease is uncovered when studying anti-inflammatory therapies aimed at inhibiting IL-6. Blockade of IL-6 very efficiently decreases RA joint pathology and simultaneously induces prompt and potent suppression of neutrophil recruitment from the bone-marrow into peripheral blood (202).

SAA -PROINFLAMMATORY MEDIATOR OR BIOMARKER?

As mentioned above, most reports describing SAA as an active mediator of inflammation have not employed endogenous SAA, but rather the recombinant hybrid molecule. Our data, showing clear functional differences between endogenous SAA and rSAA (**Paper III** and **IV**), with the former completely lacking proinflammatory effects, strongly argues against the view that SAA is an active participant in RA and other inflammatory diseases. However, one disease where SAA is doubtlessly directly involved is AA-amyloidosis. This disease can complicate inflammatory disorders that are associated with a sustained APR; RA being the most prevalent with approximately 5 % of patients developing AA amyloidosis (203). AA-amyloidosis is characterized by extracellular deposits of SAA, forming fibrils in β-sheets (204) that gradually disrupt the structure of tissues and organs leading to progressive organ dysfunction (203). The exact mechanism of how SAA is transferred into an amyloidogenic, *i.e.* aggregated form, in humans is not known, though several mechanisms have been suggested (205-206). It should be noted that amyloid formation as detected in biopsies from adipose tissue or kidneys is not encompassed by an inflammatory reaction (*Johan Mölne, personal communication*), arguing against the idea that SAA assumes a proinflammatory conformation by aggregation. If the primary disease causing amyloidosis is appropriately treated with sustained normalization of SAA levels, amyloid deposition is halted and existing amyloid deposits often resolve (207-208). Thus prolonged presence of high levels of endogenous SAA can directly contribute to complications associated with inflammatory disease. It is important to note that this could be accomplished without SAA being an active inflammatory mediator in itself and that SAA is not the cause of inflammation, but rather a consequence.

Even if endogenous SAA lacks proinflammatory activity, the protein can still be of clinical importance as a biomarker for inflammatory disease. The ultimate biomarker should be an independent indicator and predictor of biological conditions that can be measured objectively and easy, both in health and disease. SAA has been suggested as the best marker available for the assessment of inflammatory joint disease but due to costly experimental testing, the monitoring of SAA for disease activity in RA is not a clinical routine. Compared with sedimentation rate or CRP, SAA correlates best with clinical disease activity in RA and ankylosing spondarthritis (209-210). Also, SAA was recently reported as a reliable biomarker for disease activity in Takayasu arterit, a chronic inflammatory vasculitis (211). Besides serving as a reliable biomarker in rheumatic disease, SAA has also been shown to be a biomarker in other diseases where inflammation is significant but

independent of autoimmune processes, such as different neoplastic disorders (212).

Inflammatory diseases such as RA are very complex involving both adaptive- and innate immunity in addition to numerous soluble mediators that orchestrate disease activity. SAA has been assumed to play an active part in mediating inflammation, not least in RA, but our results clearly argue against this. Instead we see elevated SAA levels as a consequence of inflammation and as such it is an important biomarker that could be used to effectively monitor disease activity.

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REFERENCES

1. Beutler B. Innate immunity: an overview. *Mol Immunol*. 2004 Feb;40(12):845-59.
2. Celsus. De Medicina. *Self published*. A.D. 25.
3. Tracy RP. The five cardinal signs of inflammation: Calor, Dolor, Rubor, Tumor ... and Penuria (Apologies to Aulus Cornelius Celsus, De medicina, c. A.D. 25). *J Gerontol A Biol Sci Med Sci*. 2006 Oct;61(10):1051-2.
4. Barreiro O, Martin P, Gonzalez-Amaro R, Sanchez-Madrid F. Molecular cues guiding inflammatory responses. *Cardiovasc Res*. 2010 Feb 4.
5. Brown C, E., editor. Cell biology of phagocyte migration. New York: Raven Press Ltd; 1992.
6. Björkman S. The splenic circulation. *Doctoral thesis*. 1947.
7. Bainton DF, Ullyot JL, Farquhar MG. The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. *J Exp Med*. 1971 Oct 1;134(4):907-34.
8. Babior BM. NADPH oxidase: an update. *Blood*. 1999 Mar 1;93(5):1464-76.
9. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002 Apr 12;296(5566):301-5.
10. Movat HZ, Cybulsky MI, Colditz IG, Chan MK, Dinarello CA. Acute inflammation in gram-negative infection: endotoxin, interleukin 1, tumor necrosis factor, and neutrophils. *Fed Proc*. 1987 Jan;46(1):97-104.
11. Andersson U, Harris HE. The role of HMGB1 in the pathogenesis of rheumatic disease. *Biochim Biophys Acta*. 2010 January - February;1799(1-2):141-8.
12. Ravetch J, Aderem A. Phagocytic cells. *Immunol Rev*. 2007 Oct;219:5-7.
13. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol*. 2006 Mar;6(3):173-82.
14. Pellme S, Mörgelin M, Tapper H, Mellqvist UH, Dahlgren C, Karlsson A. Localization of human neutrophil interleukin-8 (CXCL-8) to organelle(s) distinct from the classical granules and secretory vesicles. *J Leukoc Biol*. 2006 Mar;79(3):564-73.
15. Zhang X, Majlessi L, Deriaud E, Leclerc C, Lo-Man R. Coactivation of Syk kinase and MyD88 adaptor protein pathways by bacteria promotes regulatory properties of neutrophils. *Immunity*. 2009 Nov 20;31(5):761-71.
16. Kasten KR, Muenzer JT, Caldwell CC. Neutrophils are significant producers of IL-10 during sepsis. *Biochem Biophys Res Commun*. 2010 Jan 25.
17. Cascao R, Rosario HS, Souto-Carneiro MM, Fonseca JE. Neutrophils in rheumatoid arthritis: More than simple final effectors. *Autoimmun Rev*. 2010 Jan 7.
18. Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. *Blood*. 2008 Aug 15;112(4):935-45.
19. Colditz IG. Margination and emigration of leucocytes. *Surv Synth Pathol Res*. 1985;4(1):44-68.
20. Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. *J Exp Med*. 2006 Sep 4;203(9):2073-83.

21. Middleton J, Patterson AM, Gardner L, Schmutz C, Ashton BA. Leukocyte extravasation: chemokine transport and presentation by the endothelium. *Blood*. 2002 Dec 1;100(12):3853-60.
22. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol*. 2005 Dec;6(12):1182-90.
23. Springer TA. Adhesion receptors of the immune system. *Nature*. 1990 Aug 2;346(6283):425-34.
24. Fearon DT, Collins LA. Increased expression of C3b receptors on polymorphonuclear leukocytes induced by chemotactic factors and by purification procedures. *J Immunol*. 1983 Jan;130(1):370-5.
25. Berger M, O'Shea J, Cross AS, Folks TM, Chused TM, Brown EJ, et al. Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J Clin Invest*. 1984 Nov;74(5):1566-71.
26. Todd RF, 3rd, Arnaout MA, Rosin RE, Crowley CA, Peters WA, Babior BM. Subcellular localization of the large subunit of Mo1 (Mo1 alpha; formerly gp 110), a surface glycoprotein associated with neutrophil adhesion. *J Clin Invest*. 1984 Oct;74(4):1280-90.
27. Oxvig C, Lu C, Springer TA. Conformational changes in tertiary structure near the ligand binding site of an integrin I domain. *Proc Natl Acad Sci U S A*. 1999 Mar 2;96(5):2215-20.
28. Drost EM, MacNee W. Potential role of IL-8, platelet-activating factor and TNF-alpha in the sequestration of neutrophils in the lung: effects on neutrophil deformability, adhesion receptor expression, and chemotaxis. *Eur J Immunol*. 2002 Feb;32(2):393-403.
29. Stossel TP. On the crawling of animal cells. *Science*. 1993 May 21;260(5111):1086-94.
30. Bylund J, Karlsson A, Boulay F, Dahlgren C. Lipopolysaccharide-induced granule mobilization and priming of the neutrophil response to Helicobacter pylori peptide Hp(2-20), which activates formyl peptide receptor-like 1. *Infect Immun*. 2002 Jun;70(6):2908-14.
31. Wang Y, Herrera AH, Li Y, Belani KK, Walcheck B. Regulation of mature ADAM17 by redox agents for L-selectin shedding. *J Immunol*. 2009 Feb 15;182(4):2449-57.
32. Smalley DM, Ley K. L-selectin: mechanisms and physiological significance of ectodomain cleavage. *Journal of cellular and molecular medicine*. 2005 Apr-Jun;9(2):255-66.
33. Guthrie LA, McPhail LC, Henson PM, Johnston RB, Jr. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J Exp Med*. 1984 Dec 1;160(6):1656-71.
34. Nathan CF. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest*. 1987 Dec;80(6):1550-60.
35. Almkvist J, Faldt J, Dahlgren C, Leffler H, Karlsson A. Lipopolysaccharide-induced gelatinase granule mobilization primes neutrophils for activation by galectin-3 and formylmethionyl-Leu-Phe. *Infect Immun*. 2001 Feb;69(2):832-7.

36. Follin P, Wymann MP, Dewald B, Ceska M, Dahlgren C. Human neutrophil migration into skin chambers is associated with production of NAP-1/IL8 and C5a. *European journal of haematology*. 1991 Jul;47(1):71-6.
37. Kushner I. The phenomenon of the acute phase response. *Ann N Y Acad Sci*. 1982;389:39-48.
38. Dinarello CA. Interleukin-1 and the pathogenesis of the acute-phase response. *N Engl J Med*. 1984 Nov 29;311(22):1413-8.
39. Nilsson IM. Coagulation and fibrinolysis. *Scand J Gastroenterol Suppl*. 1987;137:11-8.
40. Sylven C, Chen J, Bergström K, Björkman L, Wallin R, Saldeen T. Fibrin (ogen)-derived peptide B beta 30-43 is a sensitive marker of activated neutrophils during fibrinolytic-treated acute myocardial infarction in man. *Am Heart J*. 1992 Oct;124(4):841-5.
41. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol*. 1983;34:141-212.
42. Volanakis JE. Human C-reactive protein: expression, structure, and function. *Mol Immunol*. 2001 Aug;38(2-3):189-97.
43. Rosado CJ, Kondos S, Bull TE, Kuiper MJ, Law RH, Buckle AM, et al. The MACPF/CDC family of pore-forming toxins. *Cell Microbiol*. 2008 Sep;10(9):1765-74.
44. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999 Feb 11;340(6):448-54.
45. Pepys MB, Hawkins PN, Kahan MC, Tennent GA, Gallimore JR, Graham D, et al. Proinflammatory effects of bacterial recombinant human C-reactive protein are caused by contamination with bacterial products, not by C-reactive protein itself. *Circulation research*. 2005 Nov 25;97(11):e97-103.
46. Bisoendial R, Kastelein J, Stroes E. In response to van den Berg et al: on the direct actions of CRP in humans. *Circulation research*. 2005 Sep 30;97(7):e71.
47. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem*. 1999 Oct;265(2):501-23.
48. Chait A, Han CY, Oram JF, Heinecke JW. Thematic review series: The immune system and atherogenesis. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? *J Lipid Res*. 2005 Mar;46(3):389-403.
49. Chambers RE, MacFarlane DG, Whicher JT, Dieppe PA. Serum amyloid-A protein concentration in rheumatoid arthritis and its role in monitoring disease activity. *Ann Rheum Dis*. 1983 Dec;42(6):665-7.
50. Linke RP, Sipe JD, Pollock PS, Ignaczak TF, Glenner GG. Isolation of a low-molecular-weight serum component antigenically related to an amyloid fibril protein of unknown origin. *Proc Natl Acad Sci U S A*. 1975 Apr;72(4):1473-6.
51. Husby G, Natvig JB. A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. *J Clin Invest*. 1974 Apr;53(4):1054-61.
52. Husby G. Classification of amyloidosis. *Baillieres Clin Rheumatol*. 1994 Aug;8(3):503-11.
53. Bult CJ EJ, Kadin JA, Richardson JE, Blake JA et al. the Mouse Genome Database 2008. http://www.informatics.jax.org

54. van der Westhuyzen DR, de Beer FC, Webb NR. HDL cholesterol transport during inflammation. *Curr Opin Lipidol*. 2007 Apr;18(2):147-51.
55. Shah C, Hari-Dass R, Raynes JG. Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. *Blood*. 2006 Sep 1;108(5):1751-7.
56. Marsche G, Frank S, Raynes JG, Kozarsky KF, Sattler W, Malle E. The lipidation status of acute-phase protein serum amyloid A determines cholesterol mobilization via scavenger receptor class B, type I. *Biochem J*. 2007 Feb 15;402(1):117-24.
57. Coetzee GA, Strachan AF, van der Westhuyzen DR, Hoppe HC, Jeenah MS, de Beer FC. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. *J Biol Chem*. 1986 Jul 25;261(21):9644-51.
58. Hu W, Abe-Dohmae S, Tsujita M, Iwamoto N, Ogikubo O, Otsuka T, et al. Biogenesis of HDL by SAA is dependent on ABCA1 in the liver in vivo. *J Lipid Res*. 2008 Feb;49(2):386-93.
59. Urieli-Shoval S, Cohen P, Eisenberg S, Matzner Y. Widespread expression of serum amyloid A in histologically normal human tissues. Predominant localization to the epithelium. *J Histochem Cytochem*. 1998 Dec;46(12):1377-84.
60. Whitehead AS, de Beer MC, Steel DM, Rits M, Lelias JM, Lane WS, et al. Identification of novel members of the serum amyloid A protein superfamily as constitutive apolipoproteins of high density lipoprotein. *J Biol Chem*. 1992 Feb 25;267(6):3862-7.
61. Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, et al. Serum amyloid A3 expression is stimulated by dexamethasone and interleukin-6 in 3T3-L1 adipocytes. *J Endocrinol*. 2004 Dec;183(3):561-7.
62. Larson MA, Wei SH, Weber A, Mack DR, McDonald TL. Human serum amyloid A3 peptide enhances intestinal MUC3 expression and inhibits EPEC adherence. *Biochem Biophys Res Commun*. 2003 Jan 10;300(2):531-40.
63. O'Hara R, Murphy EP, Whitehead AS, FitzGerald O, Bresnihan B. Acute-phase serum amyloid A production by rheumatoid arthritis synovial tissue. *Arthritis research*. 2000;2(2):142-4.
64. Sjöholm K, Palming J, Olofsson LE, Gummesson A, Svensson PA, Lystig TC, et al. A microarray search for genes predominantly expressed in human omental adipocytes: adipose tissue as a major production site of serum amyloid A. *J Clin Endocrinol Metab*. 2005 Apr;90(4):2233-9.
65. Jensen LE, Whitehead AS. Regulation of serum amyloid A protein expression during the acute-phase response. *Biochem J*. 1998 Sep 15;334 (Pt 3):489-503.
66. Gollaher CJ, Bausserman LL. Hepatic catabolism of serum amyloid A during an acute phase response and chronic inflammation. *Proc Soc Exp Biol Med*. 1990 Jul;194(3):245-50.
67. Badolato R, Johnston JA, Wang JM, McVicar D, Xu LL, Oppenheim JJ, et al. Serum amyloid A induces calcium mobilization and chemotaxis of human monocytes by activating a pertussis toxin-sensitive signaling pathway. *J Immunol*. 1995 Oct 15;155(8):4004-10.
68. Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, Bausserman LL, et al. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and

tissue infiltration of monocytes and polymorphonuclear leukocytes. *J Exp Med*. 1994 Jul 1;180(1):203-9.
69. Su SB, Gong W, Gao JL, Shen W, Murphy PM, Oppenheim JJ, et al. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J Exp Med*. 1999 Jan 18;189(2):395-402.
70. Fu H, Karlsson J, Bylund J, Movitz C, Karlsson A, Dahlgren C. Ligand recognition and activation of formyl peptide receptors in neutrophils. *J Leukoc Biol*. 2006 Feb;79(2):247-56.
71. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010 Mar 4;464(7285):104-7.
72. Le Y, Yang Y, Cui Y, Yazawa H, Gong W, Qiu C, et al. Receptors for chemotactic formyl peptides as pharmacological targets. *Int Immunopharmacol*. 2002 Jan;2(1):1-13.
73. Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, et al. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol Rev*. 2009 Jun;61(2):119-61.
74. Boulay F, Tardif M, Brouchon L, Vignais P. Synthesis and use of a novel N-formyl peptide derivative to isolate a human N-formyl peptide receptor cDNA. *Biochem Biophys Res Commun*. 1990 May 16;168(3):1103-9.
75. Bao L, Gerard NP, Eddy RL, Jr., Shows TB, Gerard C. 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*. 1992 Jun;13(2):437-40.
76. Ye RD, Cavanagh SL, Quehenberger O, Prossnitz ER, Cochrane CG. Isolation of a cDNA that encodes a novel granulocyte N-formyl peptide receptor. *Biochem Biophys Res Commun*. 1992 Apr 30;184(2):582-9.
77. Murphy PM, Ozcelik T, Kenney RT, Tiffany HL, McDermott D, Francke U. A structural homologue of the N-formyl peptide receptor. Characterization and chromosome mapping of a peptide chemoattractant receptor family. *J Biol Chem*. 1992 Apr 15;267(11):7637-43.
78. Southgate EL, He RL, Gao JL, Murphy PM, Nanamori M, Ye RD. Identification of formyl peptides from Listeria monocytogenes and Staphylococcus aureus as potent chemoattractants for mouse neutrophils. *J Immunol*. 2008 Jul 15;181(2):1429-37.
79. Chen K, Le Y, Liu Y, Gong W, Ying G, Huang J, et al. Cutting Edge: A Critical Role for the G Protein-Coupled Receptor mFPR2 in Airway Inflammation and Immune Responses. *J Immunol*. 2010 Mar 3.
80. Gwinn MR, Sharma A, De Nardin E. Single nucleotide polymorphisms of the N-formyl peptide receptor in localized juvenile periodontitis. *J Periodontol*. 1999 Oct;70(10):1194-201.
81. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. *Trends Immunol*. 2002 Nov;23(11):541-8.
82. Iribarren P, Zhou Y, Hu J, Le Y, Wang JM. Role of formyl peptide receptor-like 1 (FPRL1/FPR2) in mononuclear phagocyte responses in Alzheimer disease. *Immunol Res*. 2005;31(3):165-76.

83. Dufton N, Hannon R, Brancaleone V, Dalli J, Patel HB, Gray M, et al. Anti-Inflammatory Role of the Murine Formyl-Peptide Receptor 2: Ligand-Specific Effects on Leukocyte Responses and Experimental Inflammation. *J Immunol*. 2010 Jan 27.
84. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol*. 1994;12:593-633.
85. Schiffmann E, Corcoran BA, Wahl SM. N-formylmethionyl peptides as chemoattractants for leucocytes. *Proc Natl Acad Sci U S A*. 1975 Mar;72(3):1059-62.
86. Tempel TR, Snyderman R, Jordan HV, Mergenhagen SE. Factors from saliva and oral bacteria, chemotactic for polymorphonuclear leukocytes: their possible role in gingival inflammation. *J Periodontol*. 1970 Feb;41(2):71-80.
87. Stenfeldt AL, Karlsson J, Wennerås C, Bylund J, Fu H, Dahlgren C. Cyclosporin H, Boc-MLF and Boc-FLFLF are Antagonists that Preferentially Inhibit Activity Triggered Through the Formyl Peptide Receptor. *Inflammation*. 2007 Aug 9.
88. Dahlgren C, Christophe T, Boulay F, Madianos PN, Rabiet MJ, Karlsson A. The synthetic chemoattractant Trp-Lys-Tyr-Met-Val-DMet activates neutrophils preferentially through the lipoxin A(4) receptor. *Blood*. 2000 Mar 1;95(5):1810-8.
89. Baek SH, Seo JK, Chae CB, Suh PG, Ryu SH. Identification of the peptides that stimulate the phosphoinositide hydrolysis in lymphocyte cell lines from peptide libraries. *J Biol Chem*. 1996 Apr 5;271(14):8170-5.
90. Seo JK, Choi SY, Kim Y, Baek SH, Kim KT, Chae CB, et al. A peptide with unique receptor specificity: stimulation of phosphoinositide hydrolysis and induction of superoxide generation in human neutrophils. *J Immunol*. 1997 Feb 15;158(4):1895-901.
91. Bae YS, Lee HY, Jo EJ, Kim JI, Kang HK, Ye RD, et al. Identification of peptides that antagonize formyl peptide receptor-like 1-mediated signaling. *J Immunol*. 2004 Jul 1;173(1):607-14.
92. Shin EH, Lee HY, Kim SD, Jo SH, Kim MK, Park KS, et al. Trp-Arg-Trp-Trp-Trp-Trp antagonizes formyl peptide receptor like 2-mediated signaling. *Biochem Biophys Res Commun*. 2006 Mar 24;341(4):1317-22.
93. Cunningham CC, Vegners R, Bucki R, Funaki M, Korde N, Hartwig JH, et al. Cell permeant polyphosphoinositide-binding peptides that block cell motility and actin assembly. *J Biol Chem*. 2001 Nov 16;276(46):43390-9.
94. Bellner L, Thoren F, Nygren E, Liljeqvist JA, Karlsson A, Eriksson K. A proinflammatory peptide from herpes simplex virus type 2 glycoprotein G affects neutrophil, monocyte, and NK cell functions. *J Immunol*. 2005 Feb 15;174(4):2235-41.
95. Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P. Isozyme-selective stimulation of phospholipase C-beta 2 by G protein beta gamma-subunits. *Nature*. 1992 Dec 17;360(6405):684-6.
96. Borregaard N, Kjeldsen L, Lollike K, Sengelov H. Granules and vesicles of human neutrophils. The role of endomembranes as source of plasma membrane proteins. *European journal of haematology*. 1993 Nov;51(5):318-22.
97. Borregaard N, Heiple JM, Simons ER, Clark RA. Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation. *J Cell Biol*. 1983 Jul;97(1):52-61.

98. Borregaard N, Miller LJ, Springer TA. Chemoattractant-regulated mobilization of a novel intracellular compartment in human neutrophils. *Science*. 1987 Sep 4;237(4819):1204-6.
99. Decoursey TE, Ligeti E. Regulation and termination of NADPH oxidase activity. *Cell Mol Life Sci*. 2005 Oct;62(19-20):2173-93.
100. Holmes B, Page AR, Good RA. Studies of the metabolic activity of leukocytes from patients with a genetic abnormality of phagocytic function. *J Clin Invest*. 1967 Sep;46(9):1422-32.
101. Smith RM, Curnutte JT. Molecular basis of chronic granulomatous disease. *Blood*. 1991 Feb 15;77(4):673-86.
102. Björkman L, Dahlgren C, Karlsson A, Brown KL, Bylund J. Phagocyte-derived reactive oxygen species as suppressors of inflammatory disease. *Arthritis Rheum*. 2008 Oct;58(10):2931-5.
103. Moore AR, Iwamura H, Larbre JP, Scott DL, Willoughby DA. Cartilage degradation by polymorphonuclear leucocytes: in vitro assessment of the pathogenic mechanisms. *Ann Rheum Dis*. 1993 Jan;52(1):27-31.
104. Hultqvist M, Olsson LM, Gelderman KA, Holmdahl R. The protective role of ROS in autoimmune disease. *Trends Immunol*. 2009 May;30(5):201-8.
105. George-Chandy A, Nordström I, Nygren E, Jonsson IM, Postigo J, Collins LV, et al. Th17 development and autoimmune arthritis in the absence of reactive oxygen species. *Eur J Immunol*. 2008 Apr;38(4):1118-26.
106. Badolato R, Wang JM, Stornello SL, Ponzi AN, Duse M, Musso T. Serum amyloid A is an activator of PMN antimicrobial functions: induction of degranulation, phagocytosis, and enhancement of anti-Candida activity. *J Leukoc Biol*. 2000 Mar;67(3):381-6.
107. Koga T, Torigoshi T, Motokawa S, Miyashita T, Maeda Y, Nakamura M, et al. Serum amyloid A-induced IL-6 production by rheumatoid synoviocytes. *FEBS letters*. 2008 Mar 5;582(5):579-85.
108. Migita K, Koga T, Torigoshi T, Maeda Y, Miyashita T, Izumi Y, et al. Serum amyloid A protein stimulates CCL20 production in rheumatoid synoviocytes. *Rheumatology (Oxford, England)*. 2009 Jul;48(7):741-7.
109. Okamoto H, Katagiri Y, Kiire A, Momohara S, Kamatani N. Serum amyloid A activates nuclear factor-kappaB in rheumatoid synovial fibroblasts through binding to receptor of advanced glycation end-products. *J Rheumatol*. 2008 May;35(5):752-6.
110. Björkman L, Karlsson J, Karlsson A, Rabiet MJ, Boulay F, Fu H, et al. Serum amyloid A mediates human neutrophil production of reactive oxygen species through a receptor independent of formyl peptide receptor like-1. *J Leukoc Biol*. 2008 Feb;83(2):245-53.
111. O'Hara R, Murphy EP, Whitehead AS, FitzGerald O, Bresnihan B. Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. *Arthritis Rheum*. 2004 Jun;50(6):1788-99.
112. Vallon R, Freuler F, Desta-Tsedu N, Robeva A, Dawson J, Wenner P, et al. Serum amyloid A (apoSAA) expression is up-regulated in rheumatoid arthritis and induces transcription of matrix metalloproteinases. *J Immunol*. 2001 Feb 15;166(4):2801-7.

113. Baba S, Takahashi T, Kasama T, Shirasawa H. Identification of two novel amyloid A protein subsets coexisting in an individual patient of AA-amyloidosis. *Biochim Biophys Acta*. 1992 Dec 10;1180(2):195-200.
114. Christenson K, Björkman L, Tängemo C, Bylund J. Serum amyloid A inhibits apoptosis of human neutrophils via a P2X7-sensitive pathway independent of formyl peptide receptor-like 1. *J Leukoc Biol*. 2008 Jan;83(1):139-48.
115. Cheng N, He R, Tian J, Ye PP, Ye RD. Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J Immunol*. 2008 Jul 1;181(1):22-6.
116. He RL, Zhou J, Hanson CZ, Chen J, Cheng N, Ye RD. Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. *Blood*. 2008 Oct 24.
117. Sandri S, Rodriguez D, Gomes E, Monteiro HP, Russo M, Campa A. Is serum amyloid A an endogenous TLR4 agonist? *J Leukoc Biol*. 2008 Feb 5.
118. Baranova IN, Vishnyakova TG, Bocharov AV, Kurlander R, Chen Z, Kimelman ML, et al. Serum amyloid A binding to CLA-1 (CD36 and LIMPII analogous-1) mediates serum amyloid A protein-induced activation of ERK1/2 and p38 mitogen-activated protein kinases. *J Biol Chem*. 2005 Mar 4;280(9):8031-40.
119. Baranova IN, Bocharov AV, Vishnyakova TG, Kurlander R, Chen Z, Fu D, et al. CD36 is a novel serum amyloid A (SAA) receptor mediating SAA binding and SAA-induced signaling in human and rodent cells. *J Biol Chem*. 2010 Jan 14.
120. van der Westhuyzen DR, Cai L, de Beer MC, de Beer FC. Serum amyloid A promotes cholesterol efflux mediated by scavenger receptor B-I. *J Biol Chem*. 2005 Oct 28;280(43):35890-5.
121. Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. *Curr Opin Hematol*. 2000 Jan;7(1):64-9.
122. Furlaneto CJ, Campa A. A novel function of serum amyloid A: a potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. *Biochem Biophys Res Commun*. 2000 Feb 16;268(2):405-8.
123. Mullan RH, Bresnihan B, Golden-Mason L, Markham T, O'Hara R, FitzGerald O, et al. Acute-phase serum amyloid A stimulation of angiogenesis, leukocyte recruitment, and matrix degradation in rheumatoid arthritis through an NF-kappaB-dependent signal transduction pathway. *Arthritis Rheum*. 2006 Jan;54(1):105-14.
124. Migita K, Kawabe Y, Tominaga M, Origuchi T, Aoyagi T, Eguchi K. Serum amyloid A protein induces production of matrix metalloproteinases by human synovial fibroblasts. *Lab Invest*. 1998 May;78(5):535-9.
125. Cai H, Song C, Endoh I, Goyette J, Jessup W, Freedman SB, et al. Serum amyloid A induces monocyte tissue factor. *J Immunol*. 2007 Feb 1;178(3):1852-60.
126. Bokarewa M, Abrahamson M, Levshin N, Egesten A, Grubb A, Dahlberg L, et al. Cystatin C binds serum amyloid A, downregulating its cytokine-generating properties. *J Rheumatol*. 2007 Jun;34(6):1293-301.
127. Hatanaka E, Furlaneto CJ, Ribeiro FP, Souza GM, Campa A. Serum amyloid A-induced mRNA expression and release of tumor necrosis factor-alpha (TNF-alpha) in human neutrophils. *Immunology letters*. 2004 Jan 30;91(1):33-7.

128. Lee MS, Yoo SA, Cho CS, Suh PG, Kim WU, Ryu SH. Serum amyloid A binding to formyl peptide receptor-like 1 induces synovial hyperplasia and angiogenesis. *J Immunol*. 2006 Oct 15;177(8):5585-94.
129. Rothschild B. Contributions of paleorheumatology to understanding contemporary disease. *Reumatismo*. 2002 Jul-Sep;54(3):272-84.
130. Pisetsky DS. Rheumatology in 2006: crossroads or crisis? *Bull NYU Hosp Jt Dis*. 2006;64(1-2):9-11.
131. Bremell T, Lange S, Yacoub A, Ryden C, Tarkowski A. Experimental Staphylococcus aureus arthritis in mice. *Infect Immun*. 1991 Aug;59(8):2615-23.
132. Dunlop DD, Manheim LM, Yelin EH, Song J, Chang RW. The costs of arthritis. *Arthritis Rheum*. 2003 Feb 15;49(1):101-13.
133. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum*. 2006 Dec;36(3):182-8.
134. Oliver JE, Silman AJ. Why are women predisposed to autoimmune rheumatic diseases? *Arthritis Res Ther*. 2009;11(5):252.
135. Jochems C, Islander U, Kallkopf A, Lagerquist M, Ohlsson C, Carlsten H. Role of raloxifene as a potent inhibitor of experimental postmenopausal polyarthritis and osteoporosis. *Arthritis Rheum*. 2007 Oct;56(10):3261-70.
136. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. 1988 Mar;31(3):315-24.
137. Muller-Ladner U, Pap T, Gay RE, Neidhart M, Gay S. Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. *Nat Clin Pract Rheumatol*. 2005 Dec;1(2):102-10.
138. Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. *Ann Rheum Dis*. 2003 Aug;62(8):722-7.
139. De Beer FC, Mallya RK, Fagan EA, Lanham JG, Hughes GR, Pepys MB. Serum amyloid-A protein concentration in inflammatory diseases and its relationship to the incidence of reactive systemic amyloidosis. *Lancet*. 1982 Jul 31;2(8292):231-4.
140. Sitia S, Atzeni F, Sarzi-Puttini P, Di Bello V, Tomasoni L, Delfino L, et al. Cardiovascular involvement in systemic autoimmune diseases. *Autoimmun Rev*. 2009 Feb;8(4):281-6.
141. Shoenfeld Y, Gerli R, Doria A, Matsuura E, Cerinic MM, Ronda N, et al. Accelerated atherosclerosis in autoimmune rheumatic diseases. *Circulation*. 2005 Nov 22;112(21):3337-47.
142. Giles JT, Szklo M, Post W, Petri M, Blumenthal RS, Lam G, et al. Coronary arterial calcification in rheumatoid arthritis: comparison with the Multi-Ethnic Study of Atherosclerosis. *Arthritis Res Ther*. 2009;11(2):R36.
143. Steen KS, Lems WF, Visman IM, Heierman M, Dijkmans BA, Twisk JW, et al. High incidence of cardiovascular events in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2009 Sep;68(9):1509-10.
144. Arinbjarnarson S, Jonsson T, Steinsson K, Sigfusson A, Jonsson H, Geirsson A, et al. IgA rheumatoid factor correlates with changes in B and T lymphocyte subsets and disease manifestations in rheumatoid arthritis. *J Rheumatol*. 1997 Feb;24(2):269-74.

145. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum*. 2000 Jan;43(1):155-63.
146. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum*. 2004 Mar;50(3):709-15.
147. van Venrooij WJ, Vossenaar ER, Zendman AJ. Anti-CCP antibodies: the new rheumatoid factor in the serology of rheumatoid arthritis. *Autoimmun Rev*. 2004 Jun;3 Suppl 1:S17-9.
148. Uysal H, Nandakumar KS, Kessel C, Haag S, Carlsen S, Burkhardt H, et al. Antibodies to citrullinated proteins: molecular interactions and arthritogenicity. *Immunol Rev*. 2010 Jan;233(1):9-33.
149. Weissmann G. The pathogenesis of rheumatoid arthritis. *Bull NYU Hosp Jt Dis*. 2006;64(1-2):12-5.
150. Athanasou NA. Synovial macrophages. *Ann Rheum Dis*. 1995 May;54(5):392-4.
151. Edwards JC. Fibroblast biology. Development and differentiation of synovial fibroblasts in arthritis. *Arthritis research*. 2000;2(5):344-7.
152. Edwards JC. Synovial intimal fibroblasts. *Ann Rheum Dis*. 1995 May;54(5):395-7.
153. Tarner IH, Harle P, Muller-Ladner U, Gay RE, Gay S. The different stages of synovitis: acute vs chronic, early vs late and non-erosive vs erosive. *Best Pract Res Clin Rheumatol*. 2005 Feb;19(1):19-35.
154. Ropes MW, Bennett GA, Bauer W. The Origin and Nature of Normal Synovial Fluid. *J Clin Invest*. 1939 May;18(3):351-72.
155. Muller-Ladner U, Ospelt C, Gay S, Distler O, Pap T. Cells of the synovium in rheumatoid arthritis. Synovial fibroblasts. *Arthritis Res Ther*. 2007;9(6):223.
156. Martel-Pelletier J, Welsch DJ, Pelletier JP. Metalloproteases and inhibitors in arthritic diseases. *Best Pract Res Clin Rheumatol*. 2001 Dec;15(5):805-29.
157. Moran EM, Mullan R, McCormick J, Connolly M, Sullivan O, Fitzgerald O, et al. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumour necrosis factor-alpha, Oncostatin M and response to biologic therapies. *Arthritis Res Ther*. 2009;11(4):R113.
158. Opdenakker G, Van den Steen PE, Dubois B, Nelissen I, Van Coillie E, Masure S, et al. Gelatinase B functions as regulator and effector in leukocyte biology. *J Leukoc Biol*. 2001 Jun;69(6):851-9.
159. Gomez-Gaviro M, Dominguez-Luis M, Canchado J, Calafat J, Janssen H, Lara-Pezzi E, et al. Expression and regulation of the metalloproteinase ADAM-8 during human neutrophil pathophysiological activation and its catalytic activity on L-selectin shedding. *J Immunol*. 2007 Jun 15;178(12):8053-63.
160. Fournier C. Where do T cells stand in rheumatoid arthritis? *Joint Bone Spine*. 2005 Dec;72(6):527-32.
161. Newkirk MM, Goldbach-Mansky R, Lee J, Hoxworth J, McCoy A, Yarboro C, et al. Advanced glycation end-product (AGE)-damaged IgG and IgM autoantibodies to IgG-AGE in patients with early synovitis. *Arthritis Res Ther*. 2003;5(2):R82-90.

162. Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis. *Arthritis research*. 2000;2(3):189-202.
163. Lutzky V, Hannawi S, Thomas R. Cells of the synovium in rheumatoid arthritis. Dendritic cells. *Arthritis Res Ther*. 2007;9(4):219.
164. Fassbender HG, Gay S. Synovial processes in rheumatoid arthritis. *Scand J Rheumatol Suppl*. 1988;76:1-7.
165. Mitchell KL, Pisetsky DS. Early rheumatoid arthritis. *Curr Opin Rheumatol*. 2007 May;19(3):278-83.
166. Thorbecke GJ, Shah R, Leu CH, Kuruvilla AP, Hardison AM, Palladino MA. Involvement of endogenous tumor necrosis factor alpha and transforming growth factor beta during induction of collagen type II arthritis in mice. *Proc Natl Acad Sci U S A*. 1992 Aug 15;89(16):7375-9.
167. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum*. 1993 Dec;36(12):1681-90.
168. Feldmann M, Maini RN. Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat Med*. 2003 Oct;9(10):1245-50.
169. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ*. 2003 Jan;10(1):45-65.
170. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med*. 2001 Mar 22;344(12):907-16.
171. Feldmann M. Development of anti-TNF therapy for rheumatoid arthritis. *Nat Rev Immunol*. 2002 May;2(5):364-71.
172. Feldmann M, Maini RN. Discovery of TNF-alpha as a therapeutic target in rheumatoid arthritis: preclinical and clinical studies. *Joint Bone Spine*. 2002 Jan;69(1):12-8.
173. Schottelius AJ, Moldawer LL, Dinarello CA, Asadullah K, Sterry W, Edwards CK, 3rd. Biology of tumor necrosis factor-alpha- implications for psoriasis. *Exp Dermatol*. 2004 Apr;13(4):193-222.
174. Christodoulou C, Choy EH. Joint inflammation and cytokine inhibition in rheumatoid arthritis. *Clin Exp Med*. 2006 Mar;6(1):13-9.
175. Kerekes G, Soltesz P, Der H, Veres K, Szabo Z, Vegvari A, et al. Effects of biologics on vascular function and atherosclerosis associated with rheumatoid arthritis. *Ann N Y Acad Sci*. 2009 Sep;1173:814-21.
176. Winqvist N, Andersen PH, Lillebaek T, Björkman P, Miörner H. Demographics of tuberculosis in an emerging EU region in southern Scandinavia. *Scand J Infect Dis*. 2006;38(11-12):1033-9.
177. Bray MG, Poulain C, Dougados M, Gossec L. Frequency and tolerance of antituberculosis treatment according to national guidelines for prevention of risk of tuberculosis due to tumor necrosis factor blocker treatment. *Joint Bone Spine*. 2010 Jan 22.
178. Kim SY, Solomon DH. Tumor necrosis factor blockade and the risk of viral infection. *Nat Rev Rheumatol*. 2010 Feb 9.
179. Pollard L, Choy E. Rheumatoid arthritis: non-tumor necrosis factor targets. *Curr Opin Rheumatol*. 2005 May;17(3):242-6.

180. Verdrengh M, Tarkowski A. Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus. *Infect Immun*. 1997 Jul;65(7):2517-21.
181. Gjertsson I, Innocenti M, Matrisian LM, Tarkowski A. Metalloproteinase-7 contributes to joint destruction in Staphylococcus aureus induced arthritis. *Microb Pathog*. 2005 Feb-Mar;38(2-3):97-105.
182. Wilkinson M, Jones BS. Evaluation of needle biopsy of synovial membrane. *Ann Rheum Dis*. 1963 Mar;22:100-5.
183. Tak PP, Breedveld FC. Analysis of serial synovial biopsies as a screening method for predicting the effects of therapeutic interventions. *J Clin Rheumatol*. 1997 Aug;3(4):186.
184. Kulka JP, Bocking D, Ropes MW, Bauer W. Early joint lesions of rheumatoid arthritis; report of eight cases, with knee biopsies of lesions of less than one year's duration. *AMA Arch Pathol*. 1955 Feb;59(2):129-50.
185. Scott DL. Early rheumatoid arthritis. *Br Med Bull*. 2007;81-82:97-114.
186. Edwards SW, Hallett MB. Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. *Immunol Today*. 1997 Jul;18(7):320-4.
187. Mohr W, Westerhellweg H, Wessinghage D. Polymorphonuclear granulocytes in rheumatic tissue destruction. III. an electron microscopic study of PMNs at the pannus-cartilage junction in rheumatoid arthritis. *Ann Rheum Dis*. 1981 Aug;40(4):396-9.
188. Bromley M, Woolley DE. Histopathology of the rheumatoid lesion. Identification of cell types at sites of cartilage erosion. *Arthritis Rheum*. 1984 Aug;27(8):857-63.
189. Theilgaard-Monch K, Knudsen S, Follin P, Borregaard N. The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. *J Immunol*. 2004 Jun 15;172(12):7684-93.
190. Quayle JA, Watson F, Bucknall RC, Edwards SW. Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, Fc gamma RI (CD64): role of immune complexes and cytokines in induction of receptor expression. *Immunology*. 1997 Jun;91(2):266-73.
191. Brown KA. The polymorphonuclear cell in rheumatoid arthritis. *Br J Rheumatol*. 1988 Apr;27(2):150-5.
192. Cedergren J, Forslund T, Sundqvist T, Skogh T. Intracellular oxidative activation in synovial fluid neutrophils from patients with rheumatoid arthritis but not from other arthritis patients. *J Rheumatol*. 2007 Nov;34(11):2162-70.
193. Firestein GS, Zvaifler NJ. The pathogenesis of rheumatoid arthritis. A critical assessment of the role of autologous stimulation in the perpetuation of rheumatoid synovitis. *Rheum Dis Clin North Am*. 1987 Dec;13(3):447-61.
194. Jimenez-Boj E, Redlich K, Turk B, Hanslik-Schnabel B, Wanivenhaus A, Chott A, et al. Interaction between synovial inflammatory tissue and bone marrow in rheumatoid arthritis. *J Immunol*. 2005 Aug 15;175(4):2579-88.
195. Izquierdo E, Canete JD, Celis R, Santiago B, Usategui A, Sanmarti R, et al. Immature blood vessels in Rheumatoid synovium are selectively depleted in response to anti-TNF therapy. *PLoS One*. 2009;4(12):e8131.

196. Wipke BT, Allen PM. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J Immunol*. 2001 Aug 1;167(3):1601-8.
197. Lawlor KE, Campbell IK, Metcalf D, O'Donnell K, van Nieuwenhuijze A, Roberts AW, et al. Critical role for granulocyte colony-stimulating factor in inflammatory arthritis. *Proc Natl Acad Sci U S A*. 2004 Aug 3;101(31):11398-403.
198. Eyles JL, Hickey MJ, Norman MU, Croker BA, Roberts AW, Drake SF, et al. A key role for G-CSF-induced neutrophil production and trafficking during inflammatory arthritis. *Blood*. 2008 Dec 15;112(13):5193-201.
199. Rogers HM, Langley FH. Neutropenia associated with splenomegaly and atrophic arthritis (Felty's syndrome); report of a case in which splenectomy was performed. *Ann Intern Med*. 1950 Apr;32(4):745-54.
200. Campion G, Maddison PJ, Goulding N, James I, Ahern MJ, Watt I, et al. The Felty syndrome: a case-matched study of clinical manifestations and outcome, serologic features, and immunogenetic associations. *Medicine (Baltimore)*. 1990 Mar;69(2):69-80.
201. Stricker RB, Goldberg B. G-CSF and exacerbation of rheumatoid arthritis. *Am J Med*. 1996 Jun;100(6):665-6.
202. Nakamura I, Omata Y, Naito M, Ito K. Blockade of interleukin 6 signaling induces marked neutropenia in patients with rheumatoid arthritis. *J Rheumatol*. 2009 Feb;36(2):459-60.
203. Lachmann HJ, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, et al. Natural history and outcome in systemic AA amyloidosis. *N Engl J Med*. 2007 Jun 7;356(23):2361-71.
204. Westermark GT, Westermark P. Purification of amyloid protein AA subspecies from amyloid-rich human tissues. *Methods Mol Biol*. 2005;299:243-54.
205. Ajiro J, Narita I, Sato F, Saga D, Hasegawa H, Kuroda T, et al. SAA1 gene polymorphisms and the risk of AA amyloidosis in Japanese patients with rheumatoid arthritis. *Mod Rheumatol*. 2006;16(5):294-9.
206. Raynes JG, McAdam KP. Serum amyloid A isoforms in inflammation. *Scand J Immunol*. 1991 Jun;33(6):657-66.
207. Ahlmen M, Ahlmen J, Svalander C, Bucht H. Cytotoxic drug treatment of reactive amyloidosis in rheumatoid arthritis with special reference to renal insufficiency. *Clin Rheumatol*. 1987 Mar;6(1):27-38.
208. Tan SY, Pepys MB, Hawkins PN. Treatment of amyloidosis. *Am J Kidney Dis*. 1995 Aug;26(2):267-85.
209. Cunnane G, Grehan S, Geoghegan S, McCormack C, Shields D, Whitehead AS, et al. Serum amyloid A in the assessment of early inflammatory arthritis. *J Rheumatol*. 2000 Jan;27(1):58-63.
210. de Vries MK, van Eijk IC, van der Horst-Bruinsma IE, Peters MJ, Nurmohamed MT, Dijkmans BA, et al. Erythrocyte sedimentation rate, C-reactive protein level, and serum amyloid a protein for patient selection and monitoring of anti-tumor necrosis factor treatment in ankylosing spondylitis. *Arthritis Rheum*. 2009 Nov 15;61(11):1484-90.
211. Koga T, Nishino Y, Makiyama J, Hayashida T, Miyashita T, Izumi Y, et al. Serum amyloid A is a useful marker to evaluate the disease activity of Takayasu's arteritis. *Rheumatol Int*. 2010 Feb;30(4):561-3.

212. Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. *Cell Mol Life Sci*. 2009 Jan;66(1):9-26.
213. Klareskog L, Saxne T. Reumatologi. 2005. Studentlitteratur. Enman Y. (Ed).

POPULÄRVETENSKAPLIG SAMMANFATTNING

Samtal mellan författaren och hennes barn (deras frågor i kursiv stil) en kväll den 10 mars 2010 i Göteborg.

- Jag har skrivit en bok som sammanfattar det forskningsprojekt som jag hållit på med de senaste 6 åren.

- Va! Börjar den bli klar nu? Vad handlar den om?

- Jag arbetar ju med en typ av vita blodkroppar som heter neutrofiler, de är viktiga celler i vårt immunförsvar som skyddar oss mot främmande organismer.

- Jaha, varför är de viktiga? Vad händer om man inte har dem, eller har för lite av dem då?

- Ja, det kan vara mycket allvarligt eftersom deras viktigaste roll är att försvara oss mot infektioner.

- Hurdå försvara oss mot infektioner?

- Jo, de är fagocyter och det betyder att de kan äta upp material som kanske behöver rensas upp, t.ex. bakterier" – och på så sätt kan kroppen själv rensar upp en infektion.

-Menar du att de kan ta sig till min stortå om jag har trampat på en spik som kanske är smutsig och har bakterier på sig?

- Javisst, jag skall försöka förklara. Neutrofilerna bildas i benmärgen och släpps ut i blodet allt eftersom i stora mängder, flera miljarder neutrofiler finns i blodet hela tiden. Inte nog med det, dessutom är det en en cell som bara lever i några dagar så det betyder att det hela tiden behöver produceras nya neutrofiler i benmärgen. Om det nu är så att du trampar på en spik och får ett sår som bli svullet och rött så "larmar"ämnen från såret (bakterier och annat) att här har det skett en skada. Neutrofilerna känner av de här larmsignalerna och kan då "sniffa" sig fram till skadan med hjälp av speciella molekyler som man kallar receptorer på sin cellyta.

- Men spiken kanske inte bara sitter i blodådran, då kan väl inte neutrofilerna göra så stor nytta?

- Jo, det är sant, men neutrofilen är en fiffig cell, för den kan röra sig, typ kravla sig fram och det kallas för kemotaxi. Det betyder helt enkelt att den kan ta sig ut från blodkärlet och komma precis fram till där skadan sitter. Det kommer att samla sig en hel massa neutrofiler där och då uppstår det en inflammation. Man kan säga att detta är kroppens sätt att börja läka skadan.

- De där ämnena du pratar om, larmsignaler, vad är det för några ämnen?

- Det kan vara bakterier, men det kan också vara andra ämnen som kommer fram ur köttet då man skadat sig.

- Det är ju bra, då är det en cell som gör nytta.

- Ja, verkligen, och i de flesta fall går det bra. Men eftersom att den skall ta hand om skadliga saker, t.ex. bakterier så innebär ju det att den kan vara ganska så ”ilsken” om ni förstår vad jag menar?

- Jaha, menar du att det är en slags strid som utspelar sig kring den där spiken?

– Ja, just det, och som ni vet så är det ju så att även om man tar kål på fienden så blir det ju ofta skador på saker runt omkring. Det har ni väl märkt när ni spelar CoD?

-OK! Neutrofilen dödar t.ex. bakterien, och det är bra – men det kan bli skador runt om också!? Förresten vad har en spik i foten att göra med ditt jobb? Jag trodde att du jobbade med patienter som har ont i lederna?

-Ja, jag är reumatolog. Och nu glömmer vi spiken i foten. För inflammation, ni vet, ansamling av neutrofiler händer hos patienter med ledsjukdomar också. Man vet inte riktigt varför man får sådana sjukdomar, men, av okänd anledning uppstår inflammation i kroppen och det kan bero på att man reagerar på något inuti sig själv som man egentligen inte skulle reagera på.

- Menar du att ”spiken” kommer inifrån kroppen istället?

- Mmm.. ungefär. Nu säger vi att inflammationen är igång och det skickas ut signaler som säger till neutrofiler att röra sig mot det som är inflammerat. De i sin tur sänder ut ännu mer larmsignaler och så är karusellen alltså igång!

- Vänta lite, var det något sådant som hände när jag hade Kawasaki?

- Ja, du hade en mycket kraftig inflammation, men som tur är blev du ju helt frisk! Patienter som har ledgångsreumatism har mycket inflammation och ett av deras larmsignaler är ett protein som heter serum amyloid A. Man kan mäta väldigt höga halter av det här proteinet både i blod och i leder hos dessa patienter.

- Är det ett snällt eller ett elakt protein?

- Det var en bra fråga! Många tror att det här proteinet som vi kallar för SAA hjälper till att förvärra inflammationen just genom att påverka neutrofiler hos patienter med ledgångsreumatism.

- Ja, det tror väl du med?

- De flesta som säger det använder sig inte av det kroppsegna proteinet utan de köper något som kallas för rekombinant protein och det betyder att man framställt det konstgjort utanför kroppen i ett laboratorium. Det som är lite speciellt är att det här konstgjort framställda SAA proteinet är inte helt likt det proteinet som finns i kroppen.

- Jaha, spelar det någon roll då?

- Ja vi tror det. Det verkar som så att det konstgjort framställda proteinet framkallar mycket inflammation när man gör laborationer på det. Det jag vill säga är att det aktiverar neutrofiler mycket. Men när jag undersöker blod från patienter med ledgångsreumatism som har jättehöga nivåer av SAA så kan jag se att deras neutrofiler inte alls är aktiverade. Jag har till och med tittat på neutrofiler som kommer från ledvätska hos sådana här patienter. Neutrofilerna där var inte heller så aktiverade…är ni med..?

- *Kanske, men ta det kort en gång till.*

- OK! Vi tror att det SAA som finns i kroppen när man har en pågående inflammation mest är en larmsignal, det har ingen egen inflammatorisk effekt, till skillnad från det konstgjorda som har mycket inflammatorisk effekt.

- *OK. Vi förstår en del. Är du nöjd med boken?*

- Ja!