Respiratory burst and severity of demyelinating diseases

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"Superoxide anion" by Annika Löb Mossberg

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To Jörgen, Marina, Evelina and Elvira

”As ... cells increase their energy during activity, it might be anticipated that the respiration of leucocytes would increase during active phagocytic ingestion”

C.W.Baldridge and R.W.Gerard

Department of Physiology, University of Chicago

Abstract

Multiple sclerosis (MS) and the Guillain-Barré syndrome (GBS) are tissue-specific inflammatory diseases of the central and the peripheral nervous system, respectively. A contemporary analysis infers that these are complex autoimmune disorders. Issues remaining to be solved are the factors determining susceptibility and the extreme variability in severity displayed by these diseases. The present study examines whether this variability is related to a basal function of the innate immune defence, regulating the intensity of inflammation.

The severity of MS and GBS were evaluated by standard scoring systems, the Multiple Sclerosis Severity Score and the Medical Research Council Score. In addition, hard endpoints such as the need for intensive care unit treatment and the time to independent walking were used in the GBS, which was also evaluated for its proneness to recur.

A possible relationship between these clinical parameters and the function of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was examined. Phagocytes are endowed with this oxygen radical-forming enzyme, which reduces molecular oxygen to form several reactive oxygen species such as superoxide anion. This process, the "respiratory burst", is a pivotal part of the innate defence against invading micro-organisms. The NADPH oxidase also has a regulatory influence upon the adaptive immune system. A lack of this enzyme elicits a human disease (Chronic Granulomatous Disease) characterized by extensive spontaneous inflammation, while recent studies in animal models imply that a deficiency of NADPH oxidase activity is related to the severity of organ-specific autoimmunity.

The respiratory burst was assayed in the stationary (attack-free) phase of relapsing or slowly progressive multiple sclerosis (N = 60), in the monophasic GBS (N = 23), and in the recurrent GBS (RGBS) (N = 10), each with one age-matched healthy control analyzed simultaneously. The assay was performed by stimulating peripheral leukocytes by peptides, and measuring the amount of superoxide anion produced.

The respiratory burst was markedly weaker in the group of GBS patients with a severe course ($p = 0.0004 - 0.04$). The same relationship was found in RGBS where the severity was expressed as degree of remission ($p = 0.001 - 0.004$) and individual recurrence proneness (interval between relapses and the time from onset to the second episode, $p = 0.006 - 0.03$). An analogous relationship was also demonstrated between a weaker respiratory burst and a severe course of multiple sclerosis ($p = 0.0035 - 0.04$).

In conclusion, the present study demonstrates that the intensity of the respiratory burst markedly contributes to the extreme variability in the severity of these demyelinating disorders. The innate immune system regulates the intensity rather than the susceptibility to these diseases. While loss of this system results in spontaneous autoinflammatory disease, we found that a low function predisposes for a more severe autoimmune disease. The mechanism may be a less effective control of infection and/or of the adaptive immune system.

Original articles

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

I. **Mossberg N**, Andersen O, Nilsson S, Dahlgren C, Hellstrand K, Lindh M, Svedhem Å, Bergström T, Movitz C. Oxygen radical production and severity of the Guillain-Barré syndrome. *Journal of Neuroimmunology*. 2007; 192(1-2):186-91.

II. **Mossberg N**, Movitz C, Hellstrand K, Bergström T, Nilsson S, Andersen O. Oxygen radical production in leukocytes and disease severity in multiple sclerosis. *Journal of Neuroimmunology*. 2009; 213(1-2):131-4.

III. **Mossberg N**, Nordin M, Movitz C, Nilsson S, Svedhem Å, Hellstrand K, Bergström T, Andersson B, and Andersen O. The Recurrent Guillain-Barré syndrome. Submitted.

IV. **Mossberg N**, Andersen O, Nordin M, Nilsson S, Svedhem Å, Bergström T, Hellstrand K, and Movitz C. Leukocyte oxygen radical production determines disease severity in the recurrent Guillain-Barré syndrome. *Journal of Inflammation.* 2010 Aug 8;7(1):40.

Abbrevations

AIDP	Acute inflammatory demyelinating polyneuropathy
AMAN	Acute motor axonal neuropathy
APC	Antigen presenting cell
BBB	Blood-brain barrier
BNB	Blood-nerve barrier
CGD	Chronic granulomatous disease
CIDP	Chronic inflammatory demyelinating polyneuropathy
CNS	Central nervous system
DC	Dendritic cells
EAE	Experimental allergic (autoimmune) encephalomyelitis
EAN	Experimental allergic neuritis
EDSS	Expanded Disability Status Scale
fMLF	N-formyl-methionyl-leucyl-phenylalanine
HLA	Human Leukocyte Antigen
FPR	Formyl peptide receptor
GBS	Guillain-Barré syndrome
LPS	Lipopolysaccharide
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
MBL	Mannose binding lectin
MFS	Miller Fisher syndrome
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MPO	Myeloperoxidase
MRC	Medical Research Council
MS	Multiple sclerosis
MSSS	Multiple Sclerosis Severity Score
NADPH	Nicotinamide adenine dinucleotide phosphate
Ncf1	Neutrophil cytosolic factor
NLR	Nucleotide- binding oligomerization domain (NOD)-like receptor
PAMP	Pathogen-associated molecular pattern
PLP	Proteolipid protein
PMA	Phorbol myristate acetate
PMP	Peripheral myelin protein
PNS	Peripheral nervous system
PRR	Pattern recognition receptors
RGBS	Recurrent Guillain-Barré syndrome
RIG	Retinolic acid-inducible gene
RLR	RIG-1-like receptor
ROS	Reactive oxygen species
RRMS	Relapsing remitting MS
SPMS	Secondary progressive MS
TLR	Toll-like receptor

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Introduction

Introduction

1. The role of the innate immune system in defence against infection

Two types of immune defence are found in vertebrates, the innate and the adaptive, protecting the host from invading microbes.

The innate immune system constitutes a first-line host defence against constantly invading microorganisms, acting within hours of exposure to an infectious pathogen, while the adaptive immune response develops more slowly, over a period of days (Table 1).

The multiple receptors of the innate immune system recognizing pathogens are invariable and encoded by genes inherited through the germ line (genome-encoded) (1). The innate immune system is present from birth and lacks immunological memory.

Table 1. The characteristics of the innate and adaptive immune systems

Characteristics	*Innate immunity*	*Adaptive immunity*
Evolutionary age	Ancient, since plants and insects	Relatively new, since vertebrates
Action speed	0-4 hours	> 96 hours
Cells	Phagocytes and NK cells	T and B lymphocytes
Receptor specificity	Specific for molecular structures of a given pattern	Unique specificity for a given antigen
Receptors inherited in genome	Yes	Encoded in gene segments
Requires somatic gene rearrangement	No	Yes
Clonal distribution of specific antigen receptors on individual lymphocytes	No	Yes

In contrast, the adaptive immune system comprises highly variable antigen receptors encoded by somatic genes randomly rearranged from individual gene segments during lymphocyte development, the revolutionary discovery made by Susumu Tonegawa (2). There are millions of different antigen receptors, however each individual cell of the adaptive system expresses a number of receptors with identical specificity. After contact with its specific antigen, the stimulated cell proliferates, giving rise to a clone of cells with this unique immune reactivity, eliminating infections with increased efficiency.

These two systems are tightly linked. The cells of the innate immune system not only ingest foreign antigens; they also act as antigen-presenting cells (APC) inducing T cells to orchestrate the appropriate adaptive response. In addition, the innate immune system exerts a control over effector functions of adaptive immunity.

Surprisingly, the innate immune system presents in evolution as far back as plants and insects, while the adaptive immune system does not fully develop until the appearance of the vertebrates.

The innate immune system, assumed for decennia to be conserved and primitive, has been intensively studied during recent years, surprising us with its complex organization. At variance with the widespread concept that the innate immune system is non-specific, it can in fact recognize conserved molecular patterns such as lipopolysaccharides (LPSs) found on certain types of bacteria and distinguish them from self-molecules (1, 3, 4) (see section 7).

2. Phagocytic cells of the innate immune system

Cells of both the innate and adaptive immune systems are produced in the bone marrow by myeloid and lymphoid stem cells respectively. The myeloid lineage of immune cells including monocytes, macrophages, granulocytes and dendritic cells (DC) comprises most of the cells of the innate immune system. The granulocytes include neutrophils, eosinophils and basophils. Neutrophils are capable of enormous microbicidal activity via the production of toxic reactive oxygen species (ROS). This process is dependent on the activity of the enzyme, leukocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (see section 3) (5).

Natural killer cells (NK cells) and γδ T-cells also belong to the innate immune system despite their lymphoid origin (6).

Neutrophil granulocytes

The neutrophil is the most abundant white blood cell, its key role being to combat infection by ingesting, killing and clearance of invading pathogens and cell debris. Elie Metchnikoff, the prominent Russian immunologist, first highlighted the phagocytic and antimicrobial activity of macrophages and neutrophils (7).

To find their way to the site of infection and engulf pathogens, neutrophils are equipped with four types of granules/vesicles: *specific*, *gelatinase* and *azurophil* granules and *secretory* vesicles. The function of these organelles is to store bactericidal proteins and membrane-localized proteins such as adhesion molecules and chemoattractant receptors.

Neutrophil degranulation is induced by substances such as chemoattractants, tumor necrosis factor-alpha (TNF-α) and lipopolysaccharide (LPS) (8). The *secretory* vesicles are the most readily mobilized organelle resulting in up-regulation of chemotactic receptors and adhesion molecules. Cytokines and complement activate the endothelial cells to up-regulate P-selectin, which is necessary to initiate rolling (slowing down) of leukocytes along the blood vessel. This is followed by adhesion to the endothelium and diapedesis (extravasation), where the leukocytes pass through the endothelial cell layer. This process is orchestrated by P-selectin on the endothelial cells surface and integrins on the leukocytes (9). After passing through the endothelium, neutrophils migrate along a chemoattractant gradient to the site of infection, a process known as chemotaxis. Chemotaxis is associated with secretion of proteases degrading the extracellular matrix (10), and up-regulation of chemotactic receptors, stored in the *gelatinase* granules (11, 12). The chemoattractant gradient comprises a number of cytokines, chemokines (e.g. IL-8), bacterial products (*N*-formylated peptides), and complement (13). The *specific* granules are mobilized at the site of infection to express Fcγ- and complement receptors and inflammatory mediators such as lactoferrin, lysozyme and collagenase prior to phagocytosis.

Phagocytosis is a process of ingesting of a pathogen coated with opsonins, e.g. antibodies and complement. The binding of neutrophil Fcγ-and complement receptors to these opsonins facilitates recognition and engulfment of the microorganism into an intracellular phagosome, a plasma membrane derived vesicle (14). Phagocytosis is associated with mobilisation of *specific* and *azurophil* granules which fuse with the phagosome forming a phagolysosome. This creates the necessary conditions for bactericidal activity and subsequent killing of the engulfed microorganism (5, 11). The *azurophil* granules contain cationic proteins and defensines used to kill the bacteria, proteolytic enzymes and cathepsin G to breakdown bacterial proteins, lysozyme to break down the bacterial cell wall, and myeloperoxidase (MPO) that catalyzes the formation of highly microbicidal hypochlorous acid, chlorine, chloramines and hydroxyl radicals (14).

Monocytes and Macrophages

Monocytes circulating in the blood migrate to tissues, where they differentiate into macrophages (Greek: big eaters, from *makros* "large" + *phagein* "eat") which express a number of receptors recognizing different pathogens. They secrete proinflammatory cytokines, e.g. IL-1, IL-6, TNF-α, chemokines and acute-phase proteins such as C-reactive protein (CRP) and mannose-binding lectin (MBL). They possess crucial effector antimicrobial activity in synergy with neutrophils (13).

Thus, monocytes are highly phagocytic cells but it is less certain which granules they contain. Monocytes and macrophages are also vital APCs.

After being disregarded for many years, **the microglial cell** is now recognized as a representative cell of innate immunity, a resident macrophage of the central nervous system (CNS). There are two types of APCs in the CNS, both of myeloid origin, microglia which migrated to the CNS early in the development and perivascular macrophages which are repopulated from monocytes during adulthood (see p.25). Microglia can be activated by secretory substances or signals, associated with disease or injury, and become phagocytic cells producing their own cytokines (see p.24). The microglial cells can phagocytose myelin (15).

Dendritic cells (DC)

Dendritic cells are important APCs in peripheral tissue and are equipped with a variety of Toll-like receptors (TLRs) (see sections 6-7). The binding of microbial patterns to TLRs activates adaptor molecules such as myeloid differentiation factor 88 (MyD88), resulting in activation and maturation of DCs (4). DC activation is influenced by the type of microorganism that is recognised and the site of activation. Mature DCs express MHC II and co-stimulatory molecules such as CD80 (B7-1) and CD86 (B7-2) and start cytokine production, resulting in more potent activation of T_H cells. Thus, DCs are considered to be the most effective activator and modulator of the adaptive immune system (16) and are critical in promoting T and B cell responses, bridging the gap between innate and adaptive immunity (17, 18).

3. The phagocyte NADPH oxidase

During phagocytosis of microorganisms, phagocytes of our innate immune system increase their consumption of molecular oxygen (19). This phenomenon of sharply increased oxygen consumption, followed by a rapid generation of reactive oxygen species (ROS) is known as the “respiratory burst” (20). Baldridge and Gerald were first to report that canine neutrophils exposed to bacteria exhibited a “burst” of oxygen consumption (21).

The respiratory burst is dependent on activation of the enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that transfers electrons from NADPH to oxygen in the intraphagosomal/intracellular compartments or the extracellular compartment (22). The NADPH oxidase (NOX2 complex) consists of five subunits; two membrane-bound components, $p22^{phox}$ and $gp91^{phox}$, constituting the flavocytochrome $b_{558,}$ which are present in the membrane of the specific granules, the gelatinase granules and the plasma membrane, as well as three cytosolic subunits, $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ (Fig.1). The abbreviation “phox” is derived from the name “phagocyte oxidase”.

Upon activation, the cytosolic components translocate to the membrane-bound components to form a multi-component electron-transfer system where molecular oxygen is reduced to superoxide anion, O_2^-, by the NADPH oxidase (19, 20, 23).

This results in an intracellular production and/or an extracellular release of the highly reactive (half-life 10^{-9} s) oxygen radical, superoxide anion (Fig. 2).

$$\mathbf{NADPH + 2O_2 \rightarrow NADP^+ + 2\ O_2^- + H^+}$$

A free radical (from *radix,* meaning root) is defined as an atom or molecule that contains one or more unpaired electrons (24). This initial product of respiratory burst reacts predominantly as a reductant, where it gives up an electron and is reconverted to molecular oxygen. It can, however, act as an oxidant, where it accepts an electron and is converted to hydrogen peroxide. This reaction can occur spontaneously or be catalyzed by superoxide dismutase (SOD) (14):

$$\mathbf{2\ O_2^- + 2H^+ \rightarrow H_2O_2 + O_2}$$

The greater stability of hydrogen peroxide enables its diffusion through cell membranes leading to compromised T-and NK cell function, which induces apoptosis (25, 26).

The critical role of the NADPH oxidase in innate immunity became clear in the late 1980s, when chronic granulomatous disease (CGD) was shown to correlate with an impaired oxygen metabolism and with a genetic defect (27-29). The CGD is an autoinflammatory disease (see discussion) characterized by the failure to mount an effective immune defence against bacteria and fungi resulting in severe and recurrent infections. Genetic studies have revealed that CGD is caused by mutations in different components of the NADPH oxidase such as $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$ or $p67^{phox}$. A recent study reported the first case of CGD with autosomal recessive mutations in the gene encoding $p40^{phox}$ resulting in a defect of intracellular production of superoxide anions (30).

Superoxide anion gives rise to a variety of subsequent reactive oxygen species (ROS) such as hydrogen peroxide, toxic halides and hydroxyl radicals which are antimicrobial, but are also highly toxic for surrounding tissue upon oxidative stress (Fig. 3) (20).

Degradation of primary molecules to free radicals requires relatively high activation energy, but subsequent decomposition uses considerably less.

Fig. 1. Activation of the phagocyte NADPH oxidase
The NADPH oxidase consists of two membrane-bound components: gp91^{phox} and p22^{phox} (cytochrom b_{558}) and three cytosolic subunits: p47^{phox}, p40 ^{phox} and p67^{phox}. Upon activation, the cytosolic components translocate to membrane-bound components controlled by Rac GTPase to form a functional NADPH oxidase-complex reducing molecular oxygen to superoxide anion intracellularly or extracellularly at the expense of cytosolic NADPH. MPO from azurophil granules converts hydrogen peroxide to hypochloric acid.

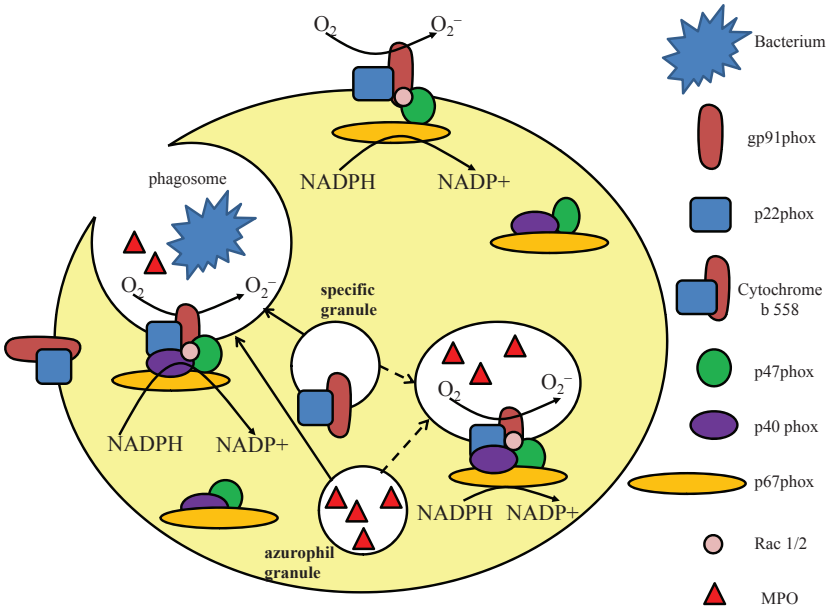
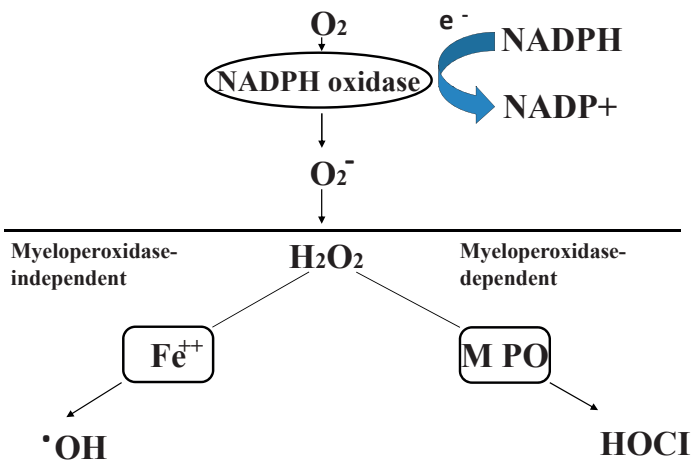


Fig. 2. Superoxide anion with Lewis electron.
The six outer shell electrons of each oxygen atom are shown in black; the unpaired electron is shown above the left oxygen atom; the additional electron conferring a negative charge is shown in red.

4. Myeloperoxidase

Myeloperoxidase (MPO) is an azurophil granule-localized protein (Fig. 1) found in both neutrophils and monocytes. In the presence of MPO, hydrogen peroxide produced by NADPH oxidase can oxidize halides to form hypochloric acid. Hypochloric acid mediates the oxidation and halogenation of cellular constituents; it is also of importance during down-regulation of auto-immune reactions. By reacting with taurine, an amino acid present in most mammalian tissues, taurine chloramines are synthesized. Taurine chloramines have been suggested to be of importance during elimination of auto-immune T-cell clones by inhibiting lymphocyte proliferation and cytokine production (31). Moreover, experimental allergic encephalomyelitis (EAE) developed in 90% of MPO-knock-out mice as compared with 33 % of wild-type mice, suggesting that MPO is protective in animals (32). Interestingly, low levels of MPO activity have been found in multiple sclerosis (MS) patients with a relapsing-remitting and a primary as well as secondary progressive course (33), while Minohara *et al* showed that upregulation of MPO correlated to disease severity in patients with opticospinal MS (34). In a recent study, demyelination in post-mortem homogenates of white matter lesions from patients with MS was associated with significantly elevated MPO activity when compared with controls (35). No MPO-associated influences have been reported in GBS patients. The *MPO* gene is located on chromosome 17q23.1, a region previously linked with MS, although, recent investigation of two polymorphisms located in the promotor region of this gene failed to reveal any association with risk of MS in a large case/control material (36).

Fig. 3. ROS generation via myeloperoxidase - dependent and - independent pathways. *Superoxid anion (O_2^-) dismutates to hydrogen peroxide (H_2O_2), in the presence of superoxide dismutase (SOD), which is further converted by MPO to hypochlorous acid and to the hydroxyl radical in the presence of iron (Fe^{++}).*



5. FPR receptors

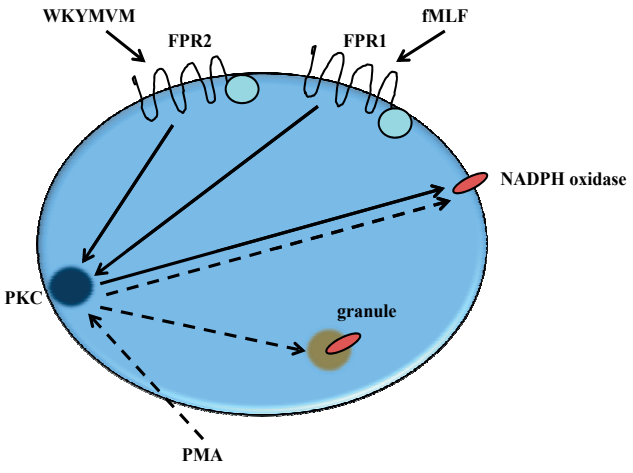
FPR receptors

One important class of chemoattractant receptors found on human phagocytes is the group of formyl peptide receptors (FPRs) (37). These receptors are seven transmembrane-spanning and G-protein coupled with an extracellular amino terminal and an intracellular carboxyl terminal. Human neutrophils express FPR1 (originally FPR) and FPR2 (originally FPR-like 1) while monocytes/macrophages also express FPR3 (originally FPR-like 2). In neutrophils the FPRs are located both in the plasma membranes and in the membranes of *secretory* vesicles, *gelatinas*e granules and *specific* granules. The new nomenclature of FPR receptors became available in 2009 and is used in this thesis (38) with the exception of **Paper I.**

Activation of the NADPH oxidase via different signalling pathways

Various natural chemoattractants such as bacterial and mitochodrial N-formylated peptides and the Helicobacter pylori derived peptide Hp(2-20) can activate different FPRs as can artificial chemoattractants such as fMLF and the synthetic peptide WKYMVM (38). The artificial analogue of bacterial *N*-formylated peptides widely used in laboratory studies is formyl-Met-Leu-Phe (fMLF) which activates the NADPH oxidase by interaction with FPR1 receptors, whereas the hexapeptide WKYMVM binds both to receptors of the FPR2 and FPR3 (Fig.4) (37).

Fig. 4. Activation of the NADPH oxidase via different signalling pathways
The signal from protein kinase C (PKC) activates the NADPH oxidase in the plasma membrane and in the granules. The peptides fMLF and WKYMVM activate PKC via FPRs, providing an extracellular NADPH oxidase response (solid line), while PMA, a membrane permeable phorbol ester, activates PKC directly, providing both an intra- and extracellular NADPH response (dashed line).



In contrast, phorbol myristate acetate (PMA) is a membrane-permeable protein kinase C (PKC) activator (39) which mimics the effects of the natural PKC activator, diacylglycerol (19). PKC is located downstream of FPRs and upstream of NADPH oxidase, and therefore activates the oxidase independently of FPRs in PMA-stimulated cells, thus providing a useful tool to investigate NADPH activation in both plasma and granule membranes. FPR1, recognizing formylated peptides synthesised during bacterial growth, can be classified as pattern recognition receptors (PRR) (40).

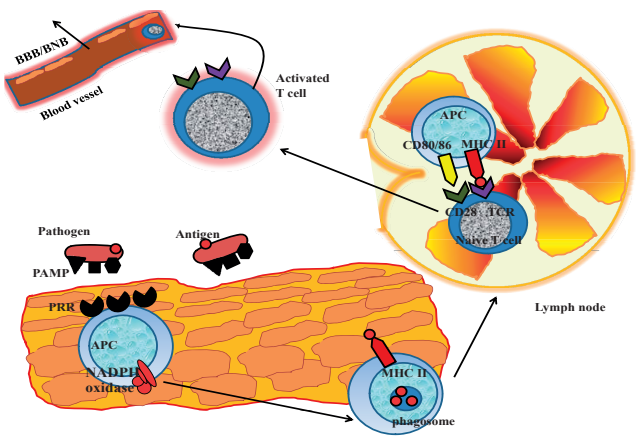
Priming

Variation among individuals to produce ROS might reflect different amounts of FPRs located on the cell surface. The extracellular release of ROS can be amplified by mobilization of receptors stored in *secretory* vesicles as well as *specific* and *gelatinase* granules to the plasma membrane, a process known as priming (8, 41, 42). The mobilization of intracellular granules to the plasma membrane is stimulated by tumor necrosis factor-alpha (TNF-α), one of the earliest cytokines produced at inflammatory sites by activated monocytes and macrophages. TNF-α influences neutrophil function mainly by binding to the type 1 TNF receptor (43). After exposure to TNF-α the cells become primed with respect to NADPH oxidase activity (44).

6. Antigen-presenting cells

Professional APCs such as DCs, monocytes and macrophages present antigen bound to a class II MHC molecule on their surfaces after phagocytosis and fragmentation of antigens (Fig.5).

Fig. 5. Antigen presentation
PRRs on APCs recognize pathogen-associated molecular patterns PAMPs on invading pathogens. After phagocytosis/ NADPH oxidase processing, APCs present peptides on MHC class II, then migrate to regional lymph nodes to present antigen to T cells. Co-stmulatory signals are needed for T cell activation. Activated T cells pass to the nervous system via the blood-brain (BBB) or blood-nerve barrier (BNB).



Immature DCs which exist in peripheral tissues and are activated by detection of antigens through pattern-recognition receptors (PRRs) such as TLRs migrate to lymph nodes. Through T cell receptors (TCR), the T cell recognizes and interacts with the MHC molecule complex on the membrane of the DC in lymph nodes, a process known as antigen presentation. Antigenic peptides bind to MHC class I or class II molecules to trigger cytotoxic activity of CD8+ T cells or activation and proliferation of naïve CD4+ T cells respectively (23). Additional co-stimulatory signal and cytokines are then produced by the APC, leading to activation of the T cell, followed by their differentiation into Th1 or Th2 cells (16).

Converting the detection of an invading pathogen by the innate immune system into an adaptive immune response requires NADPH oxidase-derived ROS followed by proteolysis of pathogen-derived antigens. Initially reduced to being solely an APC, DCs are now considered to be the essential link between innate and adaptive immune responses, because their activation is a necessary first step for the induction of adaptive immunity (6, 18, 23).

B cells are also professional APCs, but do not belong to the innate immune system.

7. Mechanisms of pathogen recognition in the innate immune system

PAMPs, PRRs

The innate immune system is genetically programmed to recognize invariant molecular patterns, pathogen-associated molecular patterns (PAMPs), which characterize a given class of micro-organism (1). This detection is accomplished via PRRs of macrophages, DCs and neutrophils (3) (Fig.5).

PRRs recognize structures common to many pathogens such as mannose-rich oligosaccharides, peptidoglycans and lipopolysaccharides in the bacterial cell walls and unmethylated CpG DNA. The PRRs are subdivided into secreted receptors such as MBL, CRP, transmembrane receptors and cytosolic classes (1). The transmembrane PRRs comprise the Toll-like receptor (TLR) family and C-type lectins, where TLRs are additionally classified into cell-surface and cytosolic receptors (45).

TLRs

TLRs were originally identified in the *fruit fly* (Drosophila) but later studies performed in mammals have led to the identification of a whole family of at least 13 receptors, allowing recognition of specific microbial components known as PAMPs (46). In mammals, 12 members of the TLR family have so far been identified (47). While cell-surface TLRs recognize extracellular PAMPs such as LPS of the Gram-negative bacteria (TLR4) and flagellin (TLR5), cytosolic (endosomal) TLRs primarily detect viral double-stranded RNA (dsRNA) (TLR3), single-stranded viral RNA (ssRNA) (TLR7) and viral unmetylated dsDNA (TLR9) (45).

Antiviral defence in mammals

Mammals possess many multifaceted antiviral defences including rapid induction of interferon, neutralizing antibody production and activation of NK- and cytotoxic T-cells.

Intracellular virus sensing has attracted much attention during recent years. Retinolic acid-inducible gene 1 (RIG-1), belonging to the RIG-1-like receptor (RLR) family, and the Nucleotide-binding oligomerization domain (NOD)-like receptor (NLRs) have been identified and classified as cytosolic PRRs (47). Unlike TLRs, most cell types express RLRs. RLR members, RIG-I and melanoma differentiation factor 5 (MDA5), recognize viral RNA (Fig.6). For instance, it has been shown that intracellular RNA of some viruses such as influenza A virus, measles, mumps and hepatitis C are detected by RLRs. Some DNA viruses such as adenovirus, herpes simplex and Epstein-Barr can trigger RLRs. This activation of RIG-1 results in interferon and proinflammatory cytokine production (48).

Activation of TLRs results in activation of the proinflammatory signalling pathway, leading to the release of transcription factors such as NF-κB, transcription of a number of proinflammatory genes and inducing T and B cell responses (46, 49). LPS may activate NADPH oxidase via TLR4 (23).

The role of the NADPH oxidase in antiviral response

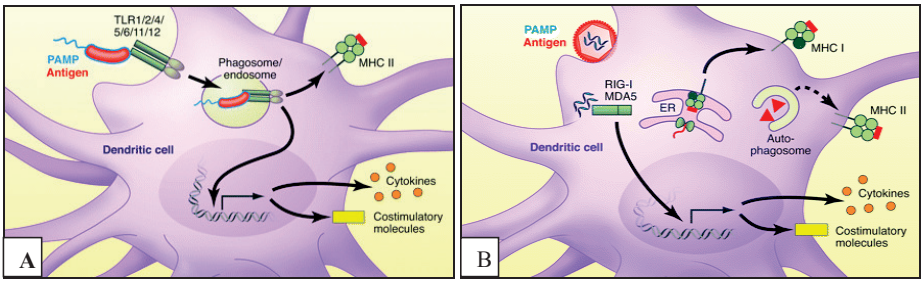
While the role of the NADPH oxidase is established in the context of phagocytosis-mediated innate defence against invading bacteria, much less is known about its role in **antiviral response**. However, several reports have illustrated the involvement of NADPH oxidase in TLR-induced signalling, suggesting that it may be involved in the innate response to viral infections. Some viruses such as respiratory syntitial virus (RSV), rhinovirus and herpes simplex virus (HSV) trigger the production of ROS by host cells, mediating apoptosis of viral pathogens (23). Furthermore, it has been shown that a monocyte-specific peptide from HSV type 2 activates NADPH oxidase to produce superoxide anion (50).

Cell-extrinsic and cell–intrinsic pathogen recognition

Two types of pathogen recognition by the innate immune system are known. Cell-extrinsic and cell-intrinsic recognition mirror either activation of transmembrane receptors by an extracellular pathogen or activation of intracellular receptors of virus-infected cells respectively. Whereas cell-extrinsic recognition (Fig. 6A) is mediated by phagocytic cells (macrophages and DCs) leading to MHC class II presentation, cell-intrinsic recognition (Fig. 6B) is mediated by intracellular sensing of viral nucleic acids, leading to MHC class I presentation via the endoplasmic reticulum (ER) pathway (45). The latter process is induced, as discussed above, through the RIG-1-like receptors (RLRs) and NLRs, which are capable of activating adaptive immunity (51). The questions why cell-intrinsic recognition is not associative i.e. PAMPS are not physically bound to their viral antigen as with cell-extrinsic

recognition and how the common origin of antigen is established in this instance, remain an enigma. Thus, it is still unclear how antigen presentation does work in virus-infected cells. Auto-phagocytosis, linked to MHC class II presentation, or phagocytosis of infected dead cells by APCs, may be one explanation.

Fig. 6. Cell-extrinsic and cell–intrinsic pathogen recognition



A*. *Cell-extrinsic recognition of pathogens. *Bacteria detected by DCs through TLRs are internalized into the phagosome where bacterial antigens are processed for presentation on MHC class II. Bacterial antigens (red) and PAMPs (blue) are present in the same phagosome, which indicates to the DC their common origin. TLR-mediated recognition of bacterial PAMPs promotes the selection of bacterial antigens for optimal presentation on MHC class II. TLR signalling also leads to the induction of co-stimulatory molecules and cytokines necessary for activation and differentiation of T lymphocytes.*
B*. *Cell-intrinsic recognition. *DCs directly infected by viruses recognize PAMPs (blue) within the cytosol via RIG-I like receptors (RLRs). Cytosolic viral proteins (red) are processed and presented on MHC class I (via the conventional endoplasmic reticulum pathway) or MHC class II (via autophagy). RLR signalling leads to the induction of co-stimulatory molecules and cytokines necessary for activation and differentiation of T lymphocytes. How the origin of antigen is established in this instance is unclear. In the case of the MHC class I pathway, this may depend on the abundance of viral antigens; for MHC class II, it may depend on targeting of viral antigens (red triangles) by the autophagy machinery.*

From Iwasaki, A. and R. Medzhitov, *Regulation of adaptive immunity by the innate immune system.* Science, 2010.**327**(5963): p. 291-5. Reprinted with permission from AAAS.

8. The role of innate immune responses and ROS in the development of autoimmune disease

The dual role of ROS

The dual role of ROS created by NADPH oxidase has been debated in several publications. ROS are traditionally believed to provoke surrounding tissue damage in several diseases including atherosclerosis, reperfusion injury and emphysema (34, 52). ROS generated locally in the CNS function as mediators of demyelination and axonal

injury, both in experimental autoimmune encephalomyelitis (EAE) and opticospinal multiple sclerosis (MS) (34, 53). ROS-induced CNS tissue damage, often in conjunction with nitrogen species with formation of peroxynitrite, may play a role in the formation of MS lesions and induce axonal degeneration (54). An ambiguity is seen concerning nitric oxide (NO) which influences severity of infection but with different outcome in different systems: In some instances its bactericidal effect dominates, inhibiting overgrowth, but in other instances its contribution to tissue damage dominates (55).

Protective role of ROS

Thus the existing pro-inflammatory dogma is being challenged as animal studies support a novel view on the regulating role of ROS in autoimmune inflammatory conditions. There is increasing evidence of the protective role of ROS in autoimmune disease (review; see (56)).

Holmdahl's group, working with an experimental rodent model, has shown that the severity of experimental arthritis is determined by a mutation on gene *Ncf1*, encoding the $p47^{phox}$ subunit of NADPH complex. The *Ncf1* allele promoting severe chronic inflammation in rodents, leads paradoxically to decreased ROS production. Moreover, myelin oligodendrocyte glycoprotein (MOG) - induced EAE in the rat model is enhanced by the mutated *Ncf1* allele associated with a lower respiratory burst (57, 58).

The *Ncf1* gene can also regulate peripheral nervous system inflammation. The protective effect of respiratory burst regulated via the *Ncf1* gene has recently been shown in an experimental allergic neuritis (EAN) rat model (59) supporting our findings in Paper I and IV.

Further support for a relationship between ROS formation and course of disease in MS stems from a recent report by Greve and co-workers showing that a deletion in one of two *Ncf1* pseudogenes is associated with early onset of MS (60).

On account of these data and our findings (Paper I, II, and IV) it would have been reasonable to examine *Ncf1* gene polymorhism in our MS and GBS patients (see Discussion). However, it has been shown that the *Ncf1* gene locus is complex with duplications in the human genome (chromosome 7) making it difficult to determine the exact genotype in a given person. Moreover, some individuals may have three functional *Ncf1* genes (61).

Explaining mechanisms of the protective role of ROS

There are mechanisms to explain the protective effects of ROS, one being a more effective clearance of pathogens (62) and others related to regulation of autoimmune T cells. T cells do not produce NADPH oxidase-derived ROS. It has been demonstrated that ROS decrease the number of reduced thiol groups on the surface of auto-reactive T cells in an experimental rat model, resulting in inhibition of T cells (see Discussion, Fig. 9). Lower ROS production leads to an increase of reduced thiol groups, resulting in for example, more severe arthritis (25).

A further explanation of ROS protective effect may be found in their abilty to suppress pro-inflammatory cytokine production (63-66).

Furthermore, ROS may act as essential signalling molecules, dampening inflammation (67).

Despite a general belief that antioxidants such as vitamin E, and C possess anti-inflammatory properties and can prevent autoimmune disease, there is no evidence for this (61, 68, 69).

On the contrary, some data suggest that respiratory burst-inducing substances could have an ameliorating effect on the course of autoimmune disease that may be applied in a therapeutic approach. For instance, phytol with a burst-inducing effect in a rat model significantly decreased arthritis severity (70). Finally, the therapeutic effect of stimulating the NADPH oxidase complex has been shown in an EAN model (59).

To summarize, the innate system with appropriate respiratory burst function can be protective by clearing the pathogens that trigger or exacerbate the disease, or by regulating presentation of antigens to T lymphocytes. Defective eradication of micro-organisms with a persisting antigen stimulation or failure in clearance of self-reactive lymphocytes may result in organ-specific autoimmune diseases.

The role of infections

Autoimmunity is characterized by an exaggerated immune response directed against both self-and microbial antigens. Generally, risk factors for autoimmunity are considered to be infection and the individual genetic background. Development of an autoimmune disease is generally prevented by central (thymic) or peripheral tolerance induced by the regulatory CD25+ T cells. A deficiency in these cells function may predispose for autoimmunity (17). Infections or overstimulation of APCs can break peripheral tolerance and induce the priming of self-reactive T cells in lymph nodes, resulting in development of autoimmune disease in a predisposed host. This issue is outside the scope of the present thesis. As one example, it was shown in mice models of $CD8^+$ T-cell mediated diabetes (71). Furthermore, some microbial products, activating innate immunity via PRR, trigger autoimmune disease in mice (72).

The role of the innate response in clearing an infection

Induced innate immune responses succeed either in clearing an infection or containing it while an adaptive immune response develops for specific pathogen recognition and generation of antigen-specific effector cells (6). A consequence of the relatively weak innate immune system could be that the more severe neurotrope infections might penetrate into the CNS (73). This may apply to triggering infections with subsequent activation of the adaptive system, characteristic for MS. A significant association between systemic infections or infectious episodes and risk of MS exacerbations (74, 75), accompanied by increased MRI activity and T cells activation has been shown (76). Another mechanism for activating the adaptive immune system could be failure to clear self-reactive lymphocytes, apoptotic cells or nuclear debris which predispose

to the development of autoimmune diseases such as systemic lupus erythematosus (62). It has been shown that defects in the innate immune system may predispose to the development of autoimmune disorders (77).

Defects in the innate immune system and autoinflammatory disease

Autoinflammatory disorders cover a group of diseases characterized by recurrent generalized inflammation in the absence of infections or autoimmune causes, absence of auto-antibodies or auto-reactive T cells and with no evidence that the process is related to auto-antigen exposure (78). Defects in neutrophil function including chemotaxis, superoxide anion production and phagocytosis were detected in Crohn´s disease (79). Mutations in nucleotide oligomerisation domains (NODs) and TLR have been described in Crohn´s disease, resulting in loss of innate immune responses (80). *NOD2* gene mutations are also known to be linked to Blau syndrome in infants, characterized by granulomatous dermatitis, arthritis and recurrent uveitis (81). The defect in the innate immune system leading to the development of autoimmunity was described in the rare X-linked syndrome known as IPEX which is characterized by immune dysfunction, polyendocrinopathy and enteropathy. IPEX syndrome results from mutations of a unique DNA binding protein gene, *FOXP3* causing generalized immune activation and T-lymphocyte infiltration with multiple autoimmune disorders (82). ROS deficient cells (aberrant intracellular production) also drive inflammatory disease in patients with SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis) despite sterile biopsies and lack of recurrent infections (83).

An inherited disorder with reduced NADPH oxidase function, chronic granulomatous disease (CGD) results not only in recurrent infections but is also associated with autoimmune diseases (29, 84). CGD is an auto-inflammatory disease and the granulomas are often sterile (see section 3 and Discussion).

The role of inflammation and ROS in the CNS

Inflammation in the CNS driven by auto-reactive T cells and activated microglia has not only detrimental but also neuroprotective effects. Some experimental studies have shown that moderate inflammation induced by autoimmune T-cells has a beneficial effect in CNS injury (85). Microglial cells may indeed clear myelin debris after activation via TLR4, increasing recruitment of oligodendrocyte progenitor cells (OPCs) that favour remyelination and confer neuroprotection. But on the other hand, microglia can promote a proinflammatory response that is detrimental for neurons resulting in demyelination and neurodegeneration. This equilibrium might depend on the expression and function of specific TLRs (86). It has been shown that production of ROS after myelin phagocytosis suppressed microglial inflammatory activation in an EAE mouse model (87).

9. Demyelination

Myelin

Myelin is a lipid-enriched membrane, enwrapping axons and is interrupted at regular intervals by nodes of Ranvier. Myelin-producing cells in the CNS are the oligodendrocytes and in the peripheral nervous system (PNS) the Schwann cells (88). Myelin is an electrical insulator that facilitates the rapid propagation of nerve impulses and modulates the maturation and survival of axons. Lipids comprise over 70% of the myelin sheath. The major characteristic of myelin lipid composition is its high cholesterol content (30%), glycolipids, galactosylceramide (GalC) and its sulfated derivative, sulfatide. Lipids are recognized by CD1 molecules and have been shown to be immunogenic (89).

Oligodendrocytes and Schwann cells comprise the major targets in the demyelinating diseases, MS and GBS. Major myelin proteins are inserted in the double lipid bilayer of myelin (review; see (90, 91)).

Blood-brain- and blood-nerve barriers

The central nervous system (CNS) is traditionally considered to be an immune privileged site, without classical lymph vessels and protected by the blood-brain barrier (BBB). Normal CNS tissue differs from other organs by its low expression of MHC class II antigens restricted to perivascular and meningeal macrophages, a part of CNS immunoprivilege (92). The BBB formed by the endothelium of capillaries, pericytes and the glia limitans, regulates diffusion of hydrophilic molecules such as plasma proteins, antibodies and complement, while leukocytes can cross this barrier (93). The compartment between the second and third layer, known as the Virchow-Robin space (perivascular space), contains perivascular APCs such as macrophages and DC. These cells are an essential link between invading encephalogenetic T cells activated in lymph nodes by peripheral APCs and oligodendrocytes. The latter are not capable of presenting target myelin antigen. While the activation and expansion of encephalogenic T cells occurs within the systemic immune compartment, these CNS-associated APCs mediate cognate antigen re-stimulation and reconfirmation, necessary for T cell entry into brain parenchyma and activation of microglial cells (94). In the absence of these APCs in perivascular spaces, T cells do not pass via the glia limitans. These findings indicate that CNS-associated APCs, strategically positioned for influencing myelin-reactive T-cell trafficking, have a regulatory role in CNS inflammation and thus in the pathogenesis of autoimmune demyelinating diseases and might be an important target for therapeutic intervention (93).

Using a novel technique of labelling leukocytes with green fluorescence and intravital two-photon imaging in an EAE rat model, a German group presented in real-time the interactive process between peripherally activated effector T cells from their first arrival, and meningeal vascular structure (95).

The blood-nerve barrier (BNB) is the barrier between the perineurium of the peripheral nerves and the endothelium of the endoneurial capillaries. Its function is similar to the BBB but ion- and anti-ganglioside antibody permeability at the BNP is higher than at the BBB. TNF-α has been demonstrated to disrupt BNB (96). However, the BNB is inefficient at nerve roots, sensory ganglions and distal nerve terminals (97).

Demyelination

Demyelination is an inflammatory process targeting myelin antigen and destroying the myelin sheath which leads to impaired signal conduction and disabled function of the affected nerves. T-cell activation, the first step of demyelination, can be initiated by systemic immunization with myelin antigens (98). EAE and EAN are simplified models of MS and GBS and confer some understanding of the mechanisms of demyelinaion. The major antigens capable of inducing EAE are myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (99). The myelin proteins P_0, P_2 and peripheral myelin protein-22 (PMP-22) can induce EAN (100).

The precise mechanisms of demyelination are not fully understood. Demyelination possibly has a common pathogenesis in the CNS and PNS. From collective evidence based on histopathological and pharmacological studies as well as on EAE and EAN models, some mechanisms have been proposed: 1) *T cell cytotoxic activity* starts with either differentiation of naïve CD4+ T cells to Th1 and Th17 auto-reactive cells by myelin antigen presented by APCs in the lymph nodes, or by the activation of pre-existing auto-reactive myelin specific T cells, probably by antigen derived from myelin or micro-organisms. The T cells cross the BBB and the BNB where they are reactivated by their cognate antigen. Later these cells activate CD8+ cytotoxic cells and NK cells. They cause direct myelin damage by secreted proinflammatory cytokines (TNF-alfa and interferon-gamma) and by perforines (resulting in cell lysis) or via the formation of Fas-Fas ligand complexes (resulting in apoptosis of targeting cells). CD4+ T cells and NK cells recruit and activate macrophages which produce metalloproteases and nitric oxide species. 2) Naïve CD4+ T cells may differentiate into Th2 cells, resulting in activation of B cells (via cytokines IL-4, IL-6 and IL-10) which produce *antibodie*s. However, since antibodies themselves are unable to cause damage as molecules, they cooperate with the innate immune system through the following two mechanisms. 3) *Complement-dependent cytotoxicity* is mediated via a process where the F_c domain of the immunoglobulin molecule activates the complement system, forming a membrane attack complex (complement factors C5-9). The latter may destroy the target cells. 4) *Antibody-dependent cellular cytotoxicity* is mediated through antibody binding to $F_{c\gamma}$ receptors on the surface of phagocytes and NK cells, leading to the blocking of nerve conduction (101-107).

There is supporting evidence for systemic activation of auto-reactive myelin specific T cells in MS. Firstly; myelin has been shown in cervical lymph nodes in MS patients (108, 109) and secondly, the presence of anti-glycolipid and anti-myelin protein antibodies in peripheral blood (110). Supporting the argument for reactivation within

the CNS compartment are the pial germinal centres observed in the progressive phase of MS (111).

Molecular mimicry

Molecular mimicry is a possible mechanism of autoimmune disease whereby micro-organisms take advantage of their antigen-similarity with the host. However, it is often used synonymously with immunological cross-reaction.

Some pathogens express protein or carbohydrate antigens that mimic host molecules which can result in cross-reactivity between pathogen epitopes and self molecules leading to the production of auto-antibody, auto-reactive naïve or effector T cells (6).

Molecular mimicry is considered to play a decisive roll in the pathogenesis of demyelinating diseases. It is supported by similarities between glycolipid moieties in *Campylobacter* and human myelin that is well-documented in GBS. These cross-reactions were found between BMP and viruses in MS. However, this situation is more straightforward in GBS.

The presence of natural auto-antibodies against a limited number of auto-antigens, including myelin, has been shown in healthy individuals (112). However, myelin antibodies in the CSF of MS patients selectively recognized specific BMP epitopes: 84-102 and 143-168 (113, 114).

Lipooligosaccharides (LOS) from the outer cell wall of *Campylobacter jejuni* serotype 19 have been shown to contain a terminal structure identical to ganglioside GM1 (see ganglioside antibodies) (115) which may be the molecular basis for mimicry in acute motor axonal neuropathy (AMAN) (116). Goodfellow and colleagues showed that anti-GD1a antibodies, via molecular mimicry, play a direct role in mediating distal motor axon pathology and are likely to be clinically relevant in the AMAN-pathogenesis. GD1a ganglioside antigens from *C. jejuni* were used to immunize a GD1a-deficient mouse to produce anti-GD1a anti-sera. These mouse antibodies and serum from a patient with AMAN, containing anti-GD1a antibodies, were shown *ex vivo* to cause a conduction block in those motor axons which contained sufficiently high levels of GD1a (117). Rabbits sensitized with LPS from *C. jejuni* O:19 strain, developed anti-GM1 IgG antibodies and showed flaccid limb weakness with pathological changes in their peripheral nerves identical to those present in AMAN (118). In addition, Koga *et al* observed cross-reactivity between serum anti-GQ1b and anti-GT1a antibodies in patients with the Miller Fisher variant of GBS following *H. influenzae* infection. He also noted that the LPS fraction from *H. influenzae* contained GT1a epitopes (119).

Molecular mimicry may also be the underlying mechanism of MS. If T and B cells become activated by micro-organisms having similar amino acid sequences to myelin, they could pass the BBB, where they may be reactivated by myelin antigens presented by APCs. Even partial homology with MHC II molecules on APC is sufficient for T cell cross-recognition (46). For instance, structural similarity between MBP and peptide sequences from EBV (120) as well as cross reactivity between myelin antigens

and EBV (EBNA1) have been reported in MS patients (121). An example of molecular mimicry has been reported in a "humanized" transgenic mouse model. A T cell receptor transferred from an MS patient to mice recognized both the Epstein-Barr virus epitope and the immunodominant MBP epitope, 85-99, presented by HLA-DR2 (122, 123). Homologies have been identified between human herpes virus-6 proteins and MBP (89). However, it has not been established whether these mechanisms are crucial for the aetiology of MS.

Myelin antibodies in demyelinating diseases

Serum antibodies were reported with varying frequency in MS patients against various myelin antigens including proteins, glycoproteins and glycolipids. The presence of anti-MOG IgG antibodies has been reported to be associated with an increased risk of developing MS that partly reflects cross-reactivity between MOG and Epstein-Barr virus nuclear antigen (124). Anti-MOG and anti-MBP antibodies were reported to have a prognostic value for patients with clinically isolated syndrome to convert to clinically definite MS (CDMS) (125). However, inconsistent results due to assay methodologies and study design question the clinical relevance of circulating antibodies recognizing myelin epitopes in MS; they may be the result of the immune response to injured tissue rather than being pathogenic (126).

Increased levels of anti-peripheral nerve myelin antibodies have been demonstrated in serum from patients with Guillain-Barré syndrome (127).

Ganglioside antibodies

Glycolipids (glycosphingolipids) are membrane lipids consisting of glycans and a lipid moiety, ceramide. Gangliosides are a subclass of glycolipids with the lipid component in the cell membrane and the sugar residue exposed on the extracellular surface. They contain one or more residues of sialic acid and are abundant in neuronal membranes (128, 129). The serum of acute-phase GBS patients frequently contains ganglioside antibodies.

Their specificity is often related to a previous infection and specific clinical phenotypes of GBS. *Mycoplasma pneumonie* infection is associated with antibodies against galactocerebroside. IgG antibodies to gangliosides, GM1, GM1b, GD1a, and GalNac-GD1a are associated with *Campylobacter jejuni* infection and with axonal forms of GBS (128). This infection may also elicit the Miller Fisher syndrome, a variant closely associated with the presence of anti-GQ1b ganglioside antibodies (130), and sensory variants with antibodies against disialylated gangliosides such as anti-GD1b (131). GBS elicited by the cytomegalovirus (CMV) tends to have GM2 antibodies (132) (see Molecular mimicry and section 10, GBS). Moreover, ganglioside location to specific sites may determine the clinical variant of GBS. Particularly, cranial motor nerves supplying the extraocular muscles have high GQ1b content.

It has been demonstrated that patients with relapsing-remitting MS may also have serum anti-ganglioside antibodies. However, there is no consensus concerning specificity, level or compartment (serum or CSF) of these antibodies (110).

10. The demyelinating diseases

1) Guillain-Barré syndrome

Background

Guillain-Barré syndrome (GBS) is a demyelinating autoimmune disease of the peripheral nervous system characterized by segmental demyelination, or in some cases axonal degeneration, and infiltration of mononuclear cells in peripheral nerves and nerve roots (133-135). The classical clinical features of this condition combined with cerebral spinal fluid findings of "albumin-cytologic dissociation" were first described in 1916 by French army neurologists Georges Guillain and Jean-Alexandre Barré along with the physiologist André Strohl (136).

The reported incidence of GBS from population-based studies in Europe varies between 1.2 and 1.9 cases per 100,000 per year. The incidence is age-dependent and approximately 1.5 times greater in men than in women (100). A survey performed 1996 in Sweden showed an incidence of 1.6 per 100,000 for all ages (137).

The clinical-neurophysiological spectrum has several patterns and extends from the most common variant (90% in Europe and North America), acute inflammatory demyelinating polyneuropathy (AIDP) to axonal forms such as acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN) and Miller Fisher syndrome. AMAN constitutes only 5-10% of patients in North America and Europe, while this variant accounts for 30-47% in Asia, South and Central America (100). Patients with AMAN usually have a more rapid progression to nadir (138).

The absolute features required for GBS (AIDP) diagnosis according to the Asbury **criteria** are progressive motor weakness of more than one limb and areflexia or proximal hyporeflexia. A number of features are strongly supportive for the diagnosis. They include the progression of neurological deficit from onset to nadir up to 4 weeks, and cerebrospinal fluid findings such as elevated CSF protein after the first week from onset of symptoms and monocyte counts/mm^3 of 10 or less (139). These criteria have been expanded with criteria of some variants of GBS such as Miller Fisher syndrome and AMAN (140).

Neurophysiological studies including nerve conduction and electromyography (EMG) are important investigations to support GBS diagnosis, to identify the GBS variants and to exclude other neurological disorders. Different neurophysiological criteria have been proposed (141).

Electrodiagnostic features which are strongly supportive for GBS diagnosis include the following: nerve conduction slowing to less than 60% of normal, conduction block, increased distal latencies and prolonged or absent F-wave latencies (139). The specificity of these basic criteria have been tested and found valid for demyelinating variants of GBS and for CIDP (142). Neurophysiological features of axonal loss in primary axonal forms (AMAN) include reduced compound muscle action potential

(CMAP) amplitudes to < 80% of the normal lower limit, absence of demyelinating features as defined above (143) and normal sensory action potentials (141). However, AMAN may show early partial motor conduction block with no further evidence of demyelination or later demyelination (144). Electromyography may show features of muscle denervation (spontaneous muscle fibre activity), which usually appears 3 weeks after onset.

Preceding infections and pathogenesis

GBS is preceded in 60-70% of cases by an infectious illness 1-2 weeks before the onset of neurological symptoms. Among these, *Campylobacter jejuni* is the most commonly identified infectious trigger, followed by viral agents such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (23%, 8% and 2% respectively) (145). Many reports have documented the occurrence of GBS shortly after operations and vaccinations (146). It has been retrospectively shown that there was a slightly increased risk of GBS development after swine influenza vaccines given in USA in 1976. The relative risk of developing GBS during the six week period after this vaccination was 7.1 times greater compared with a non-vaccinated population (147). Rabies and tetanus vaccinations have been reported to be associated with GBS whereas other conventional vaccines including "seasonal" influenza are not associated with any significant increased risk (146,148).

Campylobacter jejuni, a gram-negative bacterium in which LPS is the major component of its outer membrane, is a leading causative pathogen of infectious gastroenteritis. However only 1/1000 patients (149) and 1/3285 patients in Sweden (150) with *Campylobacter jejuni* enteritis developed GBS. Furthermore, the risk of developing GBS during the 2 month period following a symptomatic episode of *Campylobacter jejuni* infection is approximately 100 times greater than the risk in the general population (150). *Campylobacter jejuni* can cause AIDP, MFS, and AMAN. AMAN has been described as "Chinese paralytic syndrome" in Northern China (143).

The pathogenesis of GBS is not entirely clear, but the classic pathological picture with multifocal mononuclear cell infiltration and segmental demyelination throughout the PNS is considered to be the result of T-cell-mediated injury or antibody-dependent cellular cytotoxicity (ADCC) (103) (see section 9, Molecular mimicry and Ganglioside antibodies). While evidence of immunity to myelin proteins in AIDP is scarce, the ganglioside antibody-mediated attack against the nodes of Ranvier and paranodal regions has been shown to be a prominent explanation for the mechanism in AMAN and Miller Fisher syndrome (100). It has been confirmed by reports of a number of GBS cases of AMAN type, with antibodies against ganglioside GM1, after injections of gangliosides were used as treatment for stroke (151). Moreover, immunization with ganglioside GM1 induces an acute neuropathy in rabbits, histologically similar to AMAN (152). Passive transfer of anti-GM1 antibodies into the rat sciatic nerve resulted in a conduction block and features of immunoglobulin deposition at the node of Ranvier (153). The mechanism of anti-ganglioside antibodies may be related to complement-mediated disruption of sodium channels (voltage-gated Na+) at the node

of Ranvier resulting in decreased sodium current and destruction of perisynaptic Schwann cells and nerve terminals (154, 155).

However, many demyelinating variants of GBS are mediated by cellular or antibody-dependent cellular immunity, where the crucial epitopes are still unidentified (156).

Some data support an alternative view that ganglioside antibodies are not pathogenic *per se* but occur relatively late in GBS serum as a result of a secondary immune response (157).

Miller Fisher syndrome (MFS) is a variant of GBS characterized by a triad of ophthalmoplegia, ataxia and areflexia, and by an abnormal sensory function seen neurophysiologically as sensory axonal loss (158). Serum from patients with MFS has been shown to depress acetylcholine release and induce pre-and postsynaptic blockade of nicotinic acetylcholine receptor channels in cultured mouse myotubes (159).

An α-latrotoxin is a paralytic neurotoxin and a component of the black widow spider venom. An α-latrotoxin effect of anti-GQ1b antibody-complement complex resulted in a blockade of neuromuscular transmission in mice (160). It has been shown that the neurotoxic effect of these antibodies may be inhibited by immunoglobulins (161) and the complement inhibitor eculizumab in a murine model (162), which may be the rationale for clinical trials.

Microbiological and host factors influencing the risk, clinical features and severity of disease

Bacterial genetics and risk for GBS are closely linked, for example, there is a greater risk with *Campylobacter* expressing a GD1a-like epitope. It has been reported that polymorphism of a *Campylobacter jejuni* gene *cst-II* may determine different clinical features of GBS. This gene encodes an enzyme that transfers sialic acid to the lipooligosaccharide (LOS). *Campylobacter jejuni* carryng one variant of the *cst-II* allele can express GM1-like and GD1a-like LOS on its surface. These epitopes can induce anti-GM1 and anti-GD1a antibody production resulting in GBS. In contrast, an alternative variant of the *cst-II* allele promotes GT1a-like LOS expression inducing production of anti-GQ1b antibodies leading to MFS (163). Ganglioside-mimicking structures are found more frequently in neuropathy-associated *Campylobacter jejuni* strains than in strains isolated from patients with diarrhoea only (164). Sequencing studies (nucleotide sequence-based typing of 25 *C. jejuni* isolates associated with neuropathy) have revealed that certain genotypes reflecting capsular and flagellar antigens of *Campylobacter jejuni* are associated with the development of GBS (165). However, the distribution of genotypes was very heterogenous. A recent study revealed similarities between the genes involved in sialylation of LOS in GBS and non-GBS *Campylobacter* strains (166). Thus, there are more data supporting microbiological rather than host factors in determining clinical phenotypes.

However, there is indirect serological support that GBS is determined by host factors as opposed to an exclusive enteric infection. The ganglioside-like epitopes defined by

specific toxin ligands are found on all *Campylobacter jejuni* strains, isolated from both GBS and diarrhoea-only patients who did not develop anti-ganglioside antibodies (167). Epidemiological data also supports host susceptibility, with only sporadic GBS cases in outbreaks of Campylobacter enteritis. Generally, no secondary cases are found in the families of GBS patients (97).

It is poorly understood why patients develop AMAN while GM1 and GD1a gangliosides are expressed both on motor and sensory nerves, although one study has shown that GD1a epitopes recognized by antibodies are differently expressed and oriented in motor and sensory nerves (168).

It has been reported that the microbiological agent influences the severity of GBS. It tends to be severe with a preceding *Campylobacter jejuni* infection, often leading to some degree of motor sequelae (145). An initially severe sensory symptomatology with ultimate improvement has been reported to occur with a preceding CMV infection (169). Epstein-Barr virus infection has been associated with milder forms of GBS (170).

However, evidence as to whether host susceptibility factors can determine the severity of GBS is scarce.

Reduced circulating T regulatory CD4+CD25+ cell populations have been shown in acute-phase GBS patients compared with controls (171). The immunoglobulin constant region *KM* genes, which were implicated in the etiopathogenesis of several autoimmune diseases, influenced GBS risk in the Norwegian but not in the Japanese population. However, it was associated with an increased anti-GD1a ganglioside antibody production only in the Japanese population (172,173). Genetic polymorphysms in TNF region were studied in 81 Japanese patients with GBS, 14 of whom had the axonal variant. A higher frequency of a polymorphism associated with higher TNF-α secretion was found in *Campylobacter jejuni* positive GBS patients (n=43) with anti GM1 antibodies (n=36) but not in relation to disease severity (174). The relevance for Scandinavian condition is unclear and results were not replicated in another study. With relevance to the innate immune defence, functional polymorphisms in the LPS receptor CD14 and toll-like receptor TLR4 were examined in the genotypes of 242 GBS patients, but no association was found to *C. jejuni* infection or disease susceptibility (175). There was no evidence that the HLA type had any significance for the susceptibility to develop GBS (176). However, CD1 molecules are MCH-like glycoproteins specialized in capturing and presenting a variety of glycolipids to antigen-specific T cells. A sequencing study of 65 GBS patients demonstrated that susceptibility to develop GBS is associated with polymorphism of the CD1 system (177).

Few host factors have been reported as influencing the severity of GBS. The frequencies of certain haplotypes related to high activity of the mannose-binding lectin were increased in severely affected GBS patients (178). Polymorphisms in genes for macrophage-producing inflammatory mediators, matrix metalloprotease 9 (MMP-9) and TNF-α, were associated with severe weakness and poor outcome of disease, but

not with susceptibility to GBS (179). The presence of IgG1 antibodies against motor gangliosides was associated with diarrhoea, cross-reactive antibodies against LPS of *Campylobacter* and a poor prognosis, while the presence of both IgG1 and IgG3 antibodies was associated with upper respiratory tract infections (URTIs), cross-reactive antibodies against LPS of *Haemophilus influenzae* and better outcome (180). The $F_{c\gamma}$ receptors of macrophages interact with antibodies providing a link between innate and humoral immunity. An association has been shown by determining genotype frequencies between disease severity of British GBS patients and $F_{c\gamma}$ receptor III polymorphisms. The severity was assessed by dichotomization using GBS disability score (181).

To summarize, the Guillain-Barré syndrome comprises a number of clinical and serological subtypes partially related to preceding infections, while data on host susceptibility factors are scarce. In **Paper I** we found that a host factor, the individual intensity of respiratory burst, strongly contributed to the severity of GBS.

Three clinical prognostic factors, age, GBS disability scale two weeks after onset and preceding diarrhoea, have been proposed to be included in calculating outcome of GBS (the chance of walking after 6 months) according to the Erasmus GBS Outcome Scale (EGOS) (182).

Plasma exchange (PE) and intravenous immunoglobulin (IVIG) are two equally effective lines of treatment for GBS (183). Neither oral steroids nor intravenous methylprednisolone are beneficial. In combination with IVIG, intravenous methylprednisolone may hasten recovery but does not significantly affect the long-term outcome (184). Moreover, a pilot study has indicated that treatment with mycophenolate mofetil (CellCept) in addition to IVIG gave no increased effect (185).

2) Recurrent Guillain-Barré syndrome

The aim of a clinical study of RGBS was to elucidate whether RGBS represents a separate clinical entity or a chronic form of GBS with recurrences and greater severity.

RGBS was also of interest for the study of respiratory burst as an intermediate disorder (peripheral, relapsing) between MS (central, relapsing) and GBS (peripheral, monophasic).

Brief review of the literature

Guillain-Barré syndrome usually has a monophasic course. However, a relapse occurs in approximately 2-5 % of acute cases (186, 187).

Thomas *et al*. presented five cases of recurrent and chronic-relapsing polyneuritis of Guillain-Barré type, with clinical, electrophysiological and pathological features, where two of them may have been recurrent GBS. They found 14 acceptable examples of recurrent acute cases in the literature with complete or almost complete recovery between attacks (188).

Series of acute GBS relapses after long intervals have been reported (189-194). Roper and Alani reviewed 49 cases of true RGBS and reported one case of their own (195).

Recurrent Guillain-Barré syndrome (RGBS) is defined as at least two episodes of remitting and relapsing symmetric limb weakness with decreased or absent muscle reflexes or Miller Fisher syndrome. Each episode should fulfil the diagnostic criteria for GBS with regard to time from onset to peak of neurological deficit (time to nadir) of four weeks or less (139, 140), with complete or near complete functional recovery between episodes (187, 196, 197). A minimum of at least two months between episodes has been proposed (197).

The ascending phase of each episode (time to nadir) has been arbitrarily defined as lasting for less than 4 weeks in RGBS and at least 8 weeks in chronic inflammatory demyelinating polyneuropathy (CIDP) (187, 198). Nevertheless, many RGBS cases were probably concealed in early studies, being included in the 15-50 % regarded as a relapsing-remitting variant of CIDP (199-201).

The clinical characteristics of the RGBS series described in previous studies (186, 187, 196, 197, 202) are presented in Table 1 (see **Paper III**). A tendency to accumulate neurological sequelae with increasing frequency of GBS attacks has been reported (187, 196). The symptomatology of RGBS has recently been described in a material of 32 patients compared with 476 non-recurrent GBS patients based on clinical trials. RGBS was described as a subgroup of GBS where patients relapsed and were younger, had milder symptoms and more frequently, Miller Fisher syndrome. A few cases of associated autoimmunity were reported (197).

Preceding infections in RGBS

A high frequency of preceding infections was reported prior to RGBS episodes (see Table 1, **Paper III**).

Antecedent upper respiratory infections (URTI) were recorded in 10 of 19 patients with relapsing idiopathic demyelinating polyneuropathies including subacute cases without accompanying systemic disorders. Serology against common viruses was stated to be normal, however the actual number of patients who were serologically investigated was not provided. One case of rectal carcinoma was discovered (203). A case of relapsing peripheral and central demyelinating disease has been described where the first episode of the Miller Fisher type occurred after a swine influenza vaccination and an exacerbation occurred after a pneumococcal vaccination (204).

A high incidence of preceding infections which occurred in 67% of initial episodes and 85% of recurrent episodes has been reported by Grand'Maison. One patient had repeated episodes of enteritis, the majority had unspecified URTI and serology suggested a recent herpes infection in one patient. Their observed frequency of preceding infections was higher than that found by reviewing the literature, which they explained by better ascertainment (186). Common triggering events such as infection, diarrhoea and vaccination have been reported (187).

It has been discussed whether antecedent events are uniform or different in individual recurrent GBS patients (195). Wijdicks and colleagues noted a uniformity of triggering events (202), whereas other studies were unable to confirm this (186, 196) (Table 1, **Paper III**).

Differences between CIDP and RGBS

Grand'Maison and colleagues described clinical and laboratory features of 12 patients with recurrent GBS, who had in total 32 episodes with verified criteria for GBS. The RGBS as defined in this study was two or more episodes of acute idiopathic demyelinating polyneuropathies each with an onset phase lasting less than 8 weeks followed by complete or near-complete recovery, although this phase was less than 4 weeks in the majority of cases. Characteristics of RGBS, distinguishing it from CIDP, were reported as rapid onset of symptoms with subsequent complete or near-complete recovery, high incidence of an antecedent illness, lack of an apparent response to immunosuppressive therapy and normal CSF protein levels at the onset of recurrence (186).

Dyck and Arnason originally noted that weakness of respiratory and facial muscles is a frequent symptom in GBS but unusual in CIDP. Only three of their CIDP patients had preceding infections (201).

However, the diagnosis of RGBS may be difficult in cases with short intervals between episodes, since treatment-related fluctuations (TRF) can follow GBS therapy within the first two months after onset (205). Some overlap has been reported between RGBS cases and relapsing CIDP (206) and a minority of CIDP patients initially follow a course resembling GBS.

GBS and RGBS

Individual RGBS episodes were reported to be indistinguishable from GBS (196). Neurophysiological abnormalities do not differentiate RGBS from GBS episodes (186, 187, 196, 202).

The pathogenesis of recurrences in GBS is unclear. Induction by infection and abnormal autoimmunity are obviously two essential factors. A smouldering persistent inflammation in nerve tissue which resurges during the recurrence or persistent clones of B cells with a capacity to produce a pathogenic antibody, have been suggested as possible mechanisms behind RGBS (187).

3) Multiple sclerosis

A modern view on aetiology and pathogenesis

Multiple sclerosis (MS) is considered to be an organ-specific, T cell-dependent chronic autoimmune disease characterized by demyelination and axonal loss in the central nervous system (CNS) (102). The aetiology of MS remains largely unknown, but the disease is genetically complex, being associated with polymorphisms in genes of relevance to immune effector mechanisms including the HLA gene complex (207) and non-HLA genes such as the interleukin-2 receptor alpha (*IL2RA*) and the interleukin-7 receptor alpha (*IL7RA*) genes (208, 209). Sixteen genetic risk loci are known to predispose disease susceptibility (210, 211). In addition, several infectious agents have been implicated in the development of MS, such as Epstein-Barr virus (EBV), human herpes virus 6, retroviruses and *Chlamydia pneumoniae* (212). In accordance with the "hygiene hypothesis", infections early in childhood may protect against MS whereas later infections are associated with a higher MS risk (213). Vitamin D has been reported to affect the onset and course of the disease, having a protective effect against risk of developing MS (214), while a deficiency increases the risk (215). Smoking tobacco is associated with an increased risk (216). Environmental and genetic factors may interact, also leading to an increased risk. For instance, MS cases positive to HLA DRB1*1501 and antibody reactivity against the protein domain of EBV EBNA-1 are associated with a 24-fold increased risk (217). MS is thus a chronic disease with a complex pathogenic background, determined by multiple genes as well as environmental factors (104).

Inflammatory demyelination, a hallmark of acute MS lesions, has been considered to be the result of a macrophage-mediated attack on normal myelin, driven by perivascular and parenchymal auto-reactive CD4+Th1 cells following priming in the peripheral lymphatic system by an unknown self or foreign antigen (218).

In recent years, this view has been recognized as a simplification based on EAE models which may only reflect individual aspects of a multifaceted disorder (99). Firstly, lymphocytes and phagocytic infiltration may be absent in some zones of early MS lesions with extensive demyelination, which raises the question of the existence of an unidentified pre-phagocytic trigger in MS pathogenesis (218). Secondly, while MHC class I is restricted to EAE, clonally expanded CD8+ T cells comprise the bulk of lymphocyte infiltrate in MS lesions (219). Moreover, it has been reported that relapses in MS are associated with increased CD8+ T cell-mediated cytotoxicity in the CSF (220).

Additionally, increased research on the humoral immune response in MS has led to a revival of the importance of B cells and antibodies in disease pathogenesis (221) which is supported by the efficacy of treatment with the monoclonal anti-CD20 antibody, rituximab (222) in relapsing MS. It has been shown that high titres of EBNA-1 antibodies preceded the onset of MS by several years (223). Moreover, the identification of ectopic B cell follicles with germinal centres within the CNS of MS

patients with a secondary-progressive course (111), and RNA expression of EBV in these B cells (224), has gained increased attention to B cell dependent autoiummunity in MS. Since some studies have demonstrated that mutated IgG can be immunogenic, a new hypothesis of T-B cell collaboration has been proposed, explaining sustained inflammation in the absence of myelin specific T cells. According to this hypothesis, a fragment of the mutated immunoglobulin molecule presented by a B cell on MHC class II could be a T cell antigen in the absence of other antigens, and secreted immunoglobulins with the same hypervariable region after plasma cell activation could correspond to the oligoclonal CSF bands (101).

Surprisingly, it has been demonstrated that myelin auto-reactive T cells along with other leukocytes produce bioactive brain-derived neurotropic factor (BDNF) and other neurotropic factors *in vitro* and show neuroprotective effects in vivo (225).

The pathological heterogeneity of MS may reflect heterogeneous disease aetiologies. Extensive histopathological research has been carried out in recent years. Four pathological patterns were determined by Lucchinetti et al. in selected autopsy and biopsy MS tissue. To summarize, pattern 2 comprised the majority of cases (56%), was antibody-assisted cellular immunity, characterized by active perivenous lesions containing T cells, macrophages and precipitation of complement and IgG. Pattern 3 showed oligodendrocyte dystrophy and apoptosis without remyelination (24%), observed in Balo's concentric sclerosis. Pattern 1 showed cellular immunity, where the lesions were morphologically indistinguishable from those in Pattern 2, but without IgG and complement deposition (18% of patients). Pattern 4 lesions showed primary oligodendrocyte degeneration with DNA condensation in periplaque oligodendrocytes and was seen exclusively in PPMS patients (105).

Recent data has provided strong evidence for a prominent role in the pathogenesis of MS exacerbations, of the Th17 cells, a new lineage of CD4+ T cells, which produce pro-inflammatory cytokines such as IL-17, IL-21 and IL-22 (226). Microglia produces cytokine IL-23 which stimulates the Th17 cells (227). Mice lacking IL23 were completely resistant to MOG-induced EAE, and interleukin-23 rather than IL12 is the critical cytokine for autoimmune inflammation in the brain, demonstrating that IL-23 is involved in promoting and maintaining chronic CNS inflammation (228). It has been found that within the Th17 population there is a discrete population of specifically IL-23-dependent effector cells that in their EAE model exclusively contain all the encephalitogenic properties of the Th17 population. All pathogenic functions governed by effector Th17 cells are mediated by IL-23 rather than TGF-β and IL-6 (229). Moreover, IL-17-producing CD4+ cells are driven by IL23, invade the CNS initiating inflammation and play a pivotal role in the pathogenesis of EAE (230). Th17 cells are considered to be a link between innate and adaptive immunity (231).

Disease severity

The clinical course of MS varies considerably between individuals (232), and for the patient, the prognosis may be as important as the diagnosis. Certain genetic factors including HLA type have been shown to be associated with a benign or severe

outcome, but the issue is still controversial. Strategies are being discussed to determine the genetic influence on long-term disability (233). Specific clinical and MRI features at disease onset are predictive of long-term outcome (234, 235). However, these predictors are essentially parts of the disease course and not constitutional characteristics of the patients, which is the object of the present study.

The Innate immune system in MS

The importance of the innate immune system in MS pathogenesis has been demonstrated for example by the finding of activated complement deposit in MS-lesions (218). However, deletion of C3a and C5a receptors in a murin knockout model failed to protect against EAE (236).

Patients with a deletion in one of two *Ncf1* pseudogenes (see p.22) tended to have lower respiratory burst in pediatric malaria and earlier onset of MS (60).

NLRs which recognize subcomponents of bacterial cell walls such as peptidoglycan, and RLRs which sense dsRNA produced by invading viruses, belong to PRRs along with TLRs (see section 7). Polymorphisms have recently been investigated in genes coding for such PRRs as TLR-10, NOD1-2 (subfamily of NLRs), DDX58 (RIG-1) and Interferon induced with Helicase C Domain 1 (IFIH1) from RLR family. Two single-nucleotide polymorphisms (SNPs) of the *IFIH1* were associated with MS indicating that the *IFIH1* gene is a disease-associated locus in MS (237).

11. Measurement of disease severity in GBS and RGBS

In studies of GBS, functional deficit is usually assessed according to functional scales which measure the severity of neurological deficiency in acute phase and outcome of disease.

Disability in many clinical GBS trials has primarily been evaluated using a GBS disability score (238). Patients are graded on a six-point scale: 0. Healthy; 1. Minor symptoms or signs of neuropathy but capable of manual work/capable of running; 2. Able to walk without support of a stick (5 m across an open space) but incapable of manual work/running; 3. Able to walk with a stick, appliance or support (5 m across an open space); 4. Confined to bed or chair-bound; 5. Requiring assisted ventilation; 6. Death.

Another, more commonly used impairment scale, easily accessed and more sensitive to clinical rather than functional score, is the Medical Research Council (MRC) sum-score reflecting muscle strength in general (239). This score is a summation of the strength of 6 muscle groups tested on both sides ranging from 0 (paralysis) to 60 (normal strength).

0 = No visible contraction
1 = Visible contraction without movement of the limb

2 = Movement of the limb but not against gravity
3 = Movement against gravity over (almost) the full range
4 = Movement against gravity and resistance
5 = Normal

A new peripheral neuropathy activities measure, the Overall Neuropathy Limitations Scale (ONLS) was developed to assess limitations in everyday activities of patients with immune-mediated peripheral neuropathies and focuses on upper and lower limb functions (240). It is scored from 0 to 5 on the upper limb section and from 0 to 7 on the lower limb section. A score of 0 indicates no limitations whereas a score of 5 or 7 respectively, indicates no purposeful movement.

Some other acute phase and outcome measures may help to evaluate disease severity in GBS. Treatment in an intensive care unit (ICU) is an important criterium. In addition, patients requiring ventilation have a worse prognosis (241, 242). In many trials, the median time in days to recover independent walking has been used as outcome and severity measures (241). The median time to independent walking was 85 days in a North American study of the effect of plasmapheresis (243). Time to independent walking was used as a disease severity variable, at 3 months (244, 245) and 6 months (182, 246, 247). It has been reported that 20% of GBS patients were unable to walk after 6 months (146). Also, a preceding *Campylobacter jejuni* infection was associated with a more severe course of disease, slow recovery and more severe residual disability (145, 248).

In **Paper I**, the patients were, in relation to severity, dichotomized according to 1) ability to walk independently three months after onset, 2) requirement of ICU with respirator facilities , 3) mobility score according to a modified MRC scale at follow-up (239), and 4) *Campylobacter jejuni* serology (see section "Patients and controls").

We chose 3 months to independent walking as a median value, to obtain an equal number of patients in each group. Subdivision according to severity of residual symptoms at re-examination was based on a modified MRC sum score (239).

We used our modified MRC score which has been in routine use at Sahlgrenska University Hospital for several years. The following muscle groups were assessed: extension of arm; abduction/extension of the fingers; flexion of the hip; dorsal extension of the foot. 0 = normal, 1 = weak when tested against resistance (or with elevation and supination of arms), 2 = range of motion decreased with fatigue, 3 = unable to move complete range against gravity, 4 = paralysis of weaker synergy (flexion in legs), 5 = paralysis. Facial palsy, loss of position sense, sensory ataxia each =1. Loss of biceps or knee reflex each = 1. The scores are given for each half of the body. Dysarthria/dysphagia = 1. Maximum score was 51.

In **Paper III and IV**, we assessed disease severity using the MRC at follow-up and the degree of remission (complete or incomplete) for the second and last episode.

12. Measurement of disease severity in MS

Accurate, efficient measurement of disease status has become a key issue in MS clinical practice and research. The evaluation of MS disease activity/severity in clinical neurological practice and clinical studies involves a combination of neurological deficit scoring using different clinical scales (249), the measurement of annual relapse rate and progression (EDSS divided by disease duration) (250), magnetic resonance imaging (MRI) (235) and measurement of biological markers in CSF and serum (251).

Expanded Disability Status scale

The Expanded Disability Status scale (EDSS) is an important scoring scale used both clinically and in clinical trials and is the most widely used comprehensive measure of impairment in MS patients (249). It is an ordinal scale, which is up to 3.5 based on neurological evaluation of seven functional systems. The subsequent gradations are dependent on walking capacity. It ranges from 0 to 10 in half grades, where 0 is a normal function in all systems and a disability score of 4.0 indicates walking difficulties. Patients with a score of 6.0 need intermittent or unilateral constant assistance to walk 100 meters. A score of 10.0 is equivalent to death due to MS (252). However, this scale does not take into consideration duration of disease.

The multiple sclerosis severity scale

The multiple sclerosis severity scale (MSSS) adds the element of disease duration to the EDSS and is designed to provide a measure of disease severity. The MSSS is considered a more powerful measure of the individual long-term course of disease by transforming EDSS and disease duration into deciles of severity (253, 254). Thus, MSSS shows the distribution of disability in patients with comparable disease durations. For example, a patient whose MSSS is 9.0 is a fast progressor, progressing faster than 90% of patients. A patient whose MSSS is 1.0 is a slow progressor, progressing faster than just 10% of patients.

This scale is potentially superior to the non-linear EDSS for statistical evaluations, as it combines EDSS and disease duration in one normally distributed variable (255).

The weakness of this scale is that the deciles in which the results are expressed are somewhat arbitrary, as they are the deciles from a large material of MS patients with a relapsing-remitting and progressive course, collected for international genetic studies. The proportion of progressive cases probably differed from our material, collected for other purposes. The median MSSS value has earlier been used to divide patients into mild and severe cases (255, 256). It was suggested that it should be used as a reference table for future disability comparisons (253).

In **Paper II**, we used the median global MSSS value of the patient material as cut-off for measurement of disease severity. Severity was also evaluated using four dichotomous factors related to the early phase of the disease, that are reported to predict the subsequent risk of secondary progression: afferent vs. efferent symptoms, mono-regional vs. poly-regional lesions, complete vs. incomplete remission; and more or fewer than five relapses during the first 5 years of disease (234, 257). We defined a benign group having all four favourable factors and a severe group having only two or three of these factors (see section "Patients and controls").

Aims

1) The aim of this thesis was to study to what extent the respiratory burst, a pivotal function of the innate immune system, contributes to the variability in severity of autoimmune demyelinating diseases.

2) This putative relationship will be studied in the classic monophasic Guillain-Barré syndrome, in the recurrent Guillain-Barré syndrome, the relationship of which to monophasic syndrome will be analyzed, and in Multiple Sclerosis.

Materials and Methods

Patients and controls

Paper I

The patients (n = 23; 16 males and 7 females) had been treated for an acute episode of GBS at Sahlgrenska University Hospital, Gothenburg 1-5 years prior to this study. All patients fulfilled the Asbury criteria for GBS (139) and their characteristics are shown in Table 1 of **Paper I**. Healthy control subjects were the patients' spouses (n = 17) or age-matched healthy subjects (n = 6) with mean age 51 ± 17 years. Subdivision according to severity in the acute phase was performed as described in section 11.

Paper II

The study population (n = 60, mean age 51; 49 females and 11 males) were consecutively recruited outpatients at the Department of Neurology, Sahlgrenska University Hospital, Gothenburg. All patients fulfilled the McDonald criteria (258) and had neither relapsed during the previous month nor received methylprednisolone treatment during the previous 3 months. None of the patients had received mitoxantrone or natalizumab. Healthy control subjects (n = 60, mean age 52) were the patients' spouses (n = 46) or age-matched healthy subjects (n = 14) with normal neurological status, no history of neurological or other autoimmune diseases and no immunomodulatory medication. The patients were clinically examined and scored using the EDSS scale (252) which was converted to a MSSS score (253).

Two groups were defined as described in section 12 using the median global MSSS value (MSSS<2.73: less severe course, n = 30 and MSSS >2.73: more severe course, n=30) and two groups using four dichotomous factors derived from the onset phase of the disease (severe group, n = 28 and benign group, n = 30).

Demographic and clinical data of patients and controls are presented in Table 1 of **Paper II**. Thirty-eight patients had a relapsing-remitting disease course (RRMS) and 22 patients had secondary-progressive MS (SPMS). Nine patients (7 with RRMS and 2 with SPMS) were receiving interferon beta treatment at the time of sampling, and 3 patients (all with RRMS) were being treated with glatiramer acetate.

Paper III and IV

Eleven RGBS patients, defined as described in the section on recurrent GBS (mean age 49.6 years) admitted to Sahlgrenska University Hospital were included in the follow-up examination, 10 males with a mean age of 50.5 years (range 26-73) and one 41 year old female. One of these patients, with a 15 year interval between 2 episodes of GBS, was previously reported in **Paper I**. At follow-up, the patients had been free from relapse and without any immunomodulatory treatment for at least one month. All patients were treated with intravenous immunoglobulin or plasma exchange during

their episodes. The median time from the last episode to the follow-up examination was 9 months (range 1 month-12 years).

At follow-up, a neurological examination was performed by me personally, scoring according to the Medical Research Council (MRC) sum score (239). Complete or incomplete remission was recorded. Information concerning number of episodes, age at first and last episode, antecedent events, time after antecedent infections to onset of symptoms, clinical features of each episode, time to nadir, duration of the plateau phase, total episode duration, degree of remission for each episode and length of the interval between episodes, was obtained from medical records.

Blood samples for microbiological serology analyses and adaptive immunity assays were obtained and a neurophysiological examination was performed on each patient, with the exception of one male who died. This patient was not included in **Paper IV**, where we studied the remaining 10 patients, nine males and one female (mean age 52 years; range 33-73). Healthy controls were the patients' spouses (n = 5) or age-matched subjects (n = 5; mean age 52, range 33-69 years).

Coded blood samples from these 10 patients and from healthy controls were transported to the Department of Infectious Diseases, University of Gothenburg for immediate blinded analysis of NADPH oxidase and MPO activity.

Studies I-IV were approved by the Medical Ethics Committee of the University of Gothenburg and written informed consent was obtained from all participants.

Neurophysiological evaluation (Paper III)

Follow-up examination. Nerve conduction study (NCS) was carried out according to our routine methods adopted from Falck (259). Motor NCS, including an F-wave study, was performed for median and ulnar nerves bilaterally, and for the tibial and peroneal nerve on one side. Sensory NCS was performed for the median and ulnar digital nerves bilaterally and the sural nerve on one side. Electromyography (EMG) including motor-unit potential quantification (260) was performed on the anterior tibial, lateral vastus, first dorsal interosseus and biceps brachii muscles on one side. Reference values for NCS and quantitative EMG were those used routinely, with limits of $\pm$ 2 SD. To assess evidence of acute inflammatory demyelinating polyneuropathy (AIDP), data from motor NCS were evaluated using published strict criteria (142).

Retrospective evaluation: To determine the neurophysiological subtype of GBS episodes and other characteristics, all available reports and data from previous examinations were scrutinized. The same criteria for demyelinating neuropathy as in the follow-up examination were applied (142).

Virus and *Campylobacter jejuni* serology

A standardized battery of serology for diagnosis of relevant viral and bacterial infection was performed in the acute and convalescent phases of several RGBS

attacks, and at follow-up. The following analyses were performed at the Department of Virology, Sahlgrenska University Hospital, Gothenburg, Sweden: Serum IgG and/or IgM analyses for cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), mumps, measles, rubella, enterovirus, influenza A and B, parainfluenza, respiratory syncytial virus (RSV), herpes simplex virus (HSV) type 1 and 2, adenovirus, *Toxoplasma gondii*, and *Mycoplasma pneumonia.* The following serological methods were used: enzyme-linked immunosorbent assay (ELISA) of IgG and immunofluorescens (IF) of IgM antibodies against HSV (type 1 and 2), VZV and CMV; by IF assay of IgG and IgM antibodies against EBV, mumps and measles viruses as well as for *Toxoplasma gondii*; by ELISA against IgM for enteroviruses, rubella, RSV and *Mycoplasma pneumoniae*; and by complement fixation (CF) assay of antibodies against influenza A, B, and parainfluenza. IgG antibodies against adenovirus were assayed by ELISA. Criteria for positive diagnosis of these infections were either at least a four-fold titre-rise between paired samples (IgG and CF) or positive IgM at more than four-fold dilutions compared with negative controls. The assay of *Campylobacter jejuni* infection was based on DIG-ELISA utilizing an outer membrane protein of glycoprotein type. Criteria for positive diagnosis were positive IgM or IgA titres or a significant rise of consecutive IgG titres (261).

Studying adaptive immunity (Paper III)

Analyses of serum albumin, IgG, isoelectric focusing, and levels of anti-ganglioside antibodies were performed according to routine methods used at the Laboratory of Neurochemistry, Department of Clinical Chemistry, Sahlgrenska University Hospital.

Levels of anti-ganglioside serum antibodies were assessed (262). Titres ≥ 400 were outside the reference level provided by the laboratory.

Subpopulations of lymphocytes

Subpopulations of lymphocytes in blood were identified and quantified by flow cytometry (263). Analyses were carried out using a flow cytometer (FACS Calibur, BD Biosciences). Dot plots and quadrant statistics from three-colour analysis were generated by CellQuest software (BD Biosciences). The absolute number of blood lymphocytes was determined using a haematology cell counter (Sysmex K-4500; TOA Medical Electronics Co, Japan).

The cell-mediated immune response in stimulated whole blood

Heparinized blood was incubated for 7 days with phythemagglutinin pokeweed mitogen, purified protein derivative from *mycobacterium tuberculosis*, tetanus toxoid, influenza A vaccine, antigen from *Candida albicans*, CMV antigen, antigen from HSV 1 and VZV antigen, and subsequently analyzed by flow cytometry. The results are expressed as the number of CD4- or CD8-positive lymphoblasts per µL (The Flow-cytometric Assay of Specific Cell-mediated Immune response in Activated whole blood (FACSIA); (264)). Flow cytometry and FASCIA analyses were performed at the Laboratory of Immunology, Department of Medical Microbiology, Sahlgrenska University Hospital.

Isolation of leucocytes

Peripheral leukocytes (neutrophils, monocytes and lymphocytes) were isolated from heparinised venous blood by dextran sedimentation and hypotonic lysis of remaining erythrocytes (265, 266).

NADPH oxidase activity in leukocytes

To measure respiratory burst activity, luminol/isoluminol amplified chemiluminescence (CL) was used (267). In this technique, reactive oxygen species generated by the phagocyte NADPH oxidase excite the luminol/isoluminol molecules which release energy in the form of light when returning to their ground state. The advantages of this method are that it i) determines both intracellular and extracellular ROS generation ii) shows the dynamic process of superoxide anion production in time and iii) is relatively simple and iv) sensitive. The CL was measured in a Biolumat LB 9505 (Berthold Co., Wildbad, Germany) as Mcpm (10^6 counts per minute), using a reaction mixture containing leukocytes, horseradish peroxidase and isoluminol or luminol. The cells were stimulated by fMLF, WKYMVM or PMA or primed by incubation with TNF-α before stimulation with fMLF (268).

Leukocyte myeloperoxidase (MPO) activity

Leukocyte MPO activity was measured spectrophotometrically as the oxidation of 4-aminoantipyrine in the presence of hydrogen peroxide (266) and sonicated cells pre-incubated with Triton X-100. The change in absorbance was measured at 510 nm for 4 min (Perkin Elmer lambda 2). NADPH oxidase and MPO assays were partly performed by undersigned.

Statistical analysis

Demographics were compared between groups using an unpaired two sample t-test, Mann-Whitney test, and chi-square test. The statistical significance of the difference in superoxide anion production between GBS patients and controls and between GBS severity groups was determined using Student's two-sample t-test. A paired test was used between patients and controls. The influence of age, gender and *Campylobacter jejuni* serological status was evaluated using logistic regression analysis with either the fMLF- or WKYMVM-induced oxygen radical production as independent variables, and severity of disease (e.g. ICU yes/no) as a dependent variable. Superoxide anion production and MPO activity were analysed as response variables with a linear model using MS diagnosis or different MS and RGBS severity groups as predictors. The measurement day was used as a nuisance factor when analysing the superoxide anion production in Paper II and IV using one-sided research hypothesis. Reduction in time to nadir (RGBS) was calculated using a linear model with time to nadir as dependent variable, patient as factor and sequence number of episodes as covariate. For numeric severity variables the relationship to superoxide anion production was analyzed by one-sided Pearson (r) or Spearman (r_s) correlation. The logarithm of the time to the second GBS episode was used in correlation analysis. *P*-value of < 0.05 was regarded as significant.

Results

Paper I

Production of superoxide anions in clinical subgroups of GBS

Leukocytes from GBS patients with a more severe disease had a lower intensity of respiratory burst. Patients who had required ICU treatment during the acute phase produced significantly lower amounts of oxygen radicals after stimulation with the NADPH oxidase-triggering peptides fMLF and WKYMVM (see Fig.7 and **Paper I**: Fig. 1A and Table 2), compared with patients who had not required ICU treatment ($p = 0.009$). The other two independently measured severity variables, ability to walk after 3 months and MRC scale, were in strong agreement with the ICU parameter (see Table 2 - 3 and **Paper I**: Fig. 1B), and also revealed a significant correlation between diminished oxygen radical production and severity of GBS. No difference in superoxide anion production between these subgroups of GBS patients was detected after stimulation with PMA.

Fig. 7. Superoxide anion production after fMLF stimulation in patients with and without ICU treatment

Leukocytes from GBS patients were triggered with fMLF. The release of superoxide anions was measured by isoluminol/luminol-enhanced CL. Data are presented as mean peak value. The statistical significance of difference in superoxide anion production between severity groups was determined using an unpaired t-test.

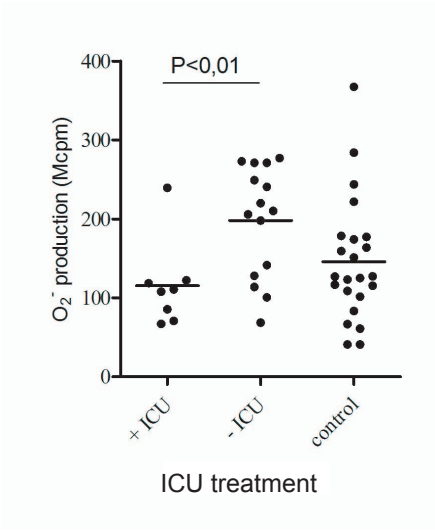


Table 2. Superoxide anion production in GBS leukocytes in relation to time to independent walking

Stimuli	O_2^- production (Mcpm)		P value
	< 3 months (n = 14)	≥ 3 months (n = 9)	
fMLF	204 ± 18.3	115 ± 17.1	0.0031**
WKYMVM	181 ± 15.4	88 ± 12.8	0.0004***
PMA	638 ± 17.5	592 ± 37.7	0.23
TNF-α + fMLF	482 ± 33.1	479 ± 41.1	0.95

Leukocytes from GBS patients were triggered with fMLF, WKYMVM and PMA. The release of superoxide anions was measured by isoluminol/luminol-enhanced CL. Data are presented as mean peak value ± SEM. The statistical significance of difference in superoxide anion production between severity groups was determined using an unpaired t-test.

Table 3. Superoxide anion production in GBS patients in relation to neurologic deficits scored by modified MRC scale

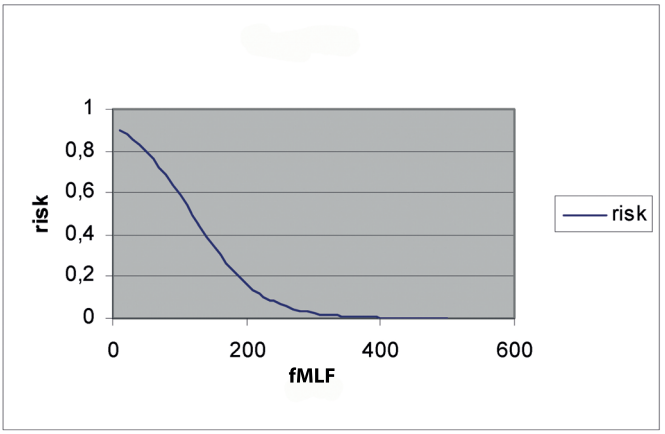
Stimuli	O_2^- production (Mcpm)		P value
	≤ 2 (n = 15)	> 2 (n = 8)	
fMLF	195 ± 19.4	121 ± 18.2	0.02*
WKYMVM	164 ± 18.2	102 ± 20.2	0.04*
PMA	623 ± 20.2	614 ± 35.0	0.8
TNF-α + fMLF	467 ± 35.6	508 ± 29.0	0.48

Leukocytes from GBS patients were triggered with fMLF, WKYMVM and PMA. The release of superoxide anions was measured by isoluminol/luminol-enhanced CL. Data are presented as mean peak value ± SEM. The statistical significance of difference in superoxide anion production between severity groups was determined using an unpaired t-test.

The risk of ICU treatment increases with lower fMLF induced superoxide anion production (Fig.8).

Fig. 8. The estimated risk function for ICU treatment

The graph shows the estimated risk of requiring ICU treatment during a previous acute phase of GBS as a function of the superoxide anion production after stimulation of leukocytes with fMLF (expressed as mean peak value), measured by isoluminol/luminol-enhanced CL in the subsequent stationary stage. The risk was calculated from a logistic regression analysis including the age and gender of the patients.



No significant differences in superoxide anion production were observed between *Campylobacter* groups (Table 4) or between the total group of GBS patients (n=23) and their respective controls for any of the activators used ($p > 0.2$, data not shown).

Table 4. Superoxide anion production in leukocytes isolated from GBS patients in relation to preceding *Campylobacter jejuni* infection

Stimuli	O_2^- production (Mcpm)		P value
	+ camp (n = 16)	- camp (n = 6)	
fMLF	157 ± 17.4	185 ±35.7	0.43
WKYMVM	125 ± 12.6	185 ± 35.7	0.053
PMA	610 ± 22.7	646 ± 36.8	0.43

Leukocytes from GBS patients were triggered with fMLF, WKYMVM and PMA. The release of superoxide anions was measured by isoluminol/luminol-enhanced CL. Data are presented as mean peak value ± SEM. The statistical significance of difference in superoxide anion production between severity groups was determined using an unpaired t-test.

The influence of age, *Campylobacter jejuni* serological status and sex was evaluated in a logistic regression analysis together with either the fMLF (FPR 1 ligand) or WKYMVM (FPR 2 ligand) induced oxygen radical production as independent variables, and severity (ICU yes/no) as dependent variable. Only fMLF- ($p = 0.03$) and WKYMVM- ($p = 0.03$) induced oxygen radical production remained significant predictor of disease severity.

Production of oxygen radicals after TNF-α induced priming

Leukocytes from clinical subgroups of GBS patients differed in their capacity to generate oxygen radicals in response to fMLF and WKYMVM. Cells primed by pre-treatment with TNF-α mobilize receptors stored in intracellular compartments, which optimizes cellular responsiveness to this triggering agent (268). To clarify whether the diminished oxygen radical production was restored by mobilization of FPR receptors, we treated leukocytes with TNF-α prior to exposure to fMLF. As shown, the priming procedure efficiently corrected the deficient oxygen radical production in patients with severe GBS and in the *Campylobacter* subgroup (sees fig.1C and D, **Paper I**).

MPO activity

MPO activity in leukocytes did not differ between patients and controls ($p = 0.94$). Neither could a difference be detected between clinical subgroups ($p = 0.99$ in ICU groups, $p = 0.88$ for *Campylobacter*, $p = 0.42$ for TTIW and $p = 0.44$ for MRC).

Paper II

Production of superoxide anion in clinical subgroups of MS

The superoxide anion production after stimulation with fMLF, WKYMVM or PMA was assessed in leukocytes isolated from MS patients with widely varying course and disease severity. Lower amounts of superoxide anion were produced after stimulation with the NADPH oxidase triggering peptides fMLF ($p = 0.04$) and WKYMVM ($p = 0.025$) in patients with a severe disease course (MSSS ≥ 2.73) (see **Paper II**: Fig. 1 and Table 2). A similar difference was detected after stimulation of leukocytes with PMA ($p = 0.0035$). Lower superoxide anion production was observed in SPMS patients compared with RRMS after stimulation with PMA ($p = 0.01$) (see **Paper II**: Table 3), and in the severe group evaluated as benign or severe by means of different predictors at onset ($p = 0.003$). A correlation between radical production and MSSS score was observed after PMA stimulation ($p = 0.04$). There was no difference in radical production between patients who received interferon beta at the time of sampling ($n = 9$) and untreated ($n = 51$) MS patients (see **Paper II**: Table 4).

No significant difference was observed in superoxide anion production between the MS patients and the controls ($p = 0.25$ for fMLF, $p = 0.24$ for WKYMVM, and $p = 0.13$ for PMA). Radical production in the control group was intermediate between the two severity groups (see **Paper II**: Tables 2 and 3). Neither age nor gender had any significant impact on superoxide anion production when analysed separately or as covariates together with severity groups.

Production of superoxide anion after TNF-α - induced priming of leukocytes

No significant difference was observed between oxygen radical formation in leukocytes isolated from MS patients and their controls ($p = 0.31$) or between patient groups defined by course and severity after priming of leukocytes with TNF-α prior to fMLF stimulation (see **Paper II**: Tables 2 and 3).

MPO activity

MPO activity in leukocytes did not differ between patients and controls ($p = 0.18$). MPO activity did not correlate with the type of course ($p = 0.27$), nor with the severity parameters ($p = 0.38$ for MSSS and $p = 0.42$ for differences in activity between benign and severe groups).

Paper III

Basic clinical data

The clinical characteristics of the 11 RGBS cases are shown in Table 2, **Paper III**. A total of 41 episodes occurred in these 11 patients (median 3 per person, range 2-7) with mean age at first episode of 35.2 years (range 21-52). The median follow-up time was 15 years (range 0.5-40 years). The median interval between successive episodes calculated for all episodes was 24 months (range 2 months – 23 years). The time to the second episode, which constitutes the diagnostic criterion for RGBS, varied between 2 months and 23 years. In five patients residual symptoms were found at the neurological follow-up examination. Residual neurological deficit at follow-up expressed as MRC score increased with the number of episodes ($r = -0.80$, $p = 0.003$). Time from onset to nadir of an episode showed a slight tendency to diminish during the course, with an average reduction of 1.2 days per episode ($p = 0.02$). Data on preceding infections are shown in Tables 2 and 3 (**Paper III**). Triggering infections occurred in all patients, in 32 of 41 episodes (78%); 24/32 episodes were preceded by upper respiratory tract infection (URTI) with individual range from 0/6 to 7/7. Autoimmune events preceding RGBS episodes were recorded in 6 episodes in 2 patients. However, in these two patients infectious complications were also present: *Campylobacter concisus* infection in the patient with ulcerative colitis and *Klebsiella* sepsis in the patient with sclerosing cholangitis (see **Paper III**: Table 2). In two of the RGBS patients a progressive course similar to CIDP supervened after many years.

By reviewing the admission diagnoses from our neurology department we ascertained a further four RGBS patients among 229 recorded GBS cases. Thus, 15 of 229 GBS cases (6.5 %) were recurrent. Moreover, we retrieved the year of recurrences for the 4 additional cases from the medical records. When these data were combined with the corresponding data from the 11 personally examined cases (see **Paper III**: Fig. 1, 1-11 and A-D), no tendency was observed for the episode frequency to wane during the average observation time of 18.4 years. Episodes continued to occur unpredictably at irregular intervals, sometimes in clusters.

Clinical characteristics of GBS vs. RGBS

The characteristics of the first 3 episodes of the RGBS cases along with the GBS patients (see **Paper I**) are listed in Table 3 (see **Paper III**). The age of patients at the first episode of RGBS (mean 35.2, $n = 11$) was significantly lower than the age at onset of monophasic GBS (mean 51.3, $n = 22$, $p = 0.009$). The mean duration of the first episode was longer in GBS (134 days) than in RGBS (69 days) ($p = 0.005$) (see **Paper III**: Table 3). Age at first episode and duration of first episode were correlated ($r = 0.4$, $p = 0.02$). When comparing duration of first episode between GBS and RGBS using multiple linear regression and adjusting for age, the mean difference was moderately reduced (from 65 to 43 days, $p = 0.18$) and the significance for age was lost ($p = 0.13$). Using a cut-off <45 days duration of a GBS episode, the positive

predictive value for a recurrent course was 6/7 (86%) and the negative predictive value was 21/26 (81%).

Neurophysiological data

As summarized in Table 4 (**Paper III**), combined follow-up examination and retrospective evaluation indicated that 9 of 11 patients had AIDP, whereas one patient had a pattern characteristic of pure motor axonal variant of GBS. The remaining patient, although he fulfilled the clinical inclusion criteria for the present study, could not be neurophysiologically classified. He did not fulfill criteria for demyelinating neuropathy, had no motor sequelae in his arms but pronounced motor axonal loss in the legs. Sensory NCS showed signs of axonal loss both in upper and lower extremities. At follow-up, four patients (three with AIDP and one with pure motor axonal variant) had complete neurophysiological recovery. One patient had evidence of demyelination and severe secondary axonal degeneration. Two patients who clinically developed a progressive course after 14 and 16 years respectively, had pronounced demyelinating neuropathy, consistent with CIDP (based on the clinical course, neurophysiologically indistinguishable from incomplete recovery from AIDP). One of them showed no evidence of axonal degeneration, while the other had signs of slight axonal loss.

Viral and *Campylobacter jejuni* serology

Standardized viral serology was available in 21 of 41 RGBS episodes in ten patients. In total, five patients had serological evidence of viral infection in nine episodes, three with CMV, one with influenza A, and one with reactivated VZV infection. The latter patient had, in addition, three probable episodes of RSV infection (see Table 2, **Paper III**). One patient had serological evidence of the CMV infection on two occasions. *Campylobacter jejuni* serology was performed in 23 of 41 episodes and was positive in fourteen episodes in six patients. Five of these episodes in two patients, all of which were IgM responses, were also associated with a positive virus serology for CMV, RSV and VZV (see Table 3, **Paper III**).

Immune deficiency

One patient had significant IgA deficiency 0.05g/L. The results from stimulation of cells with 9 different agents showed that four patients had a slightly decreased response to stimulation with PHA and two patients had a slightly reduced response to stimulation with PWM. The results from the examinations for cellular immune deficiency were otherwise normal, and only minor deviations were noted.

Titres of ganglioside antibodies

Titres of serum ganglioside antibodies at follow-up were elevated in five of ten patients: anti-sulfatide 400, anti-GA1 IgG 400, anti-GA1 IgM in 2 patients 400 and 3200, anti-GM2 IgM 400. Anti-GM2 IgM antibodies were found in the patient with serologically identified CMV infection.

Paper IV

Clinical data

The median time between the first episode and follow-up was 15 years (0.5-40 years). A total of 38 episodes occurred in these patients (median 3 per patient, range 2-7) (see **Paper IV**: Table 1). Five patients had complete recovery and 5 patients had motor residual deficit at follow-up.

Correlation between clinical and neurophysiological aspects of RGBS and respiratory burst

The superoxide anion production after stimulation with fMLF and WKYMVM was significantly lower in RGBS patients with incomplete than complete recovery after the second episode ($p = 0.0035$ for both peptides) and at follow-up ($p = 0.004$ and $p = 0.003$; **Paper IV**: Table 2 and Fig.1). Superoxide anion production correlated with MRC score at follow-up when induced by fMLF ($r_s = 0.85$, $p = 0.001$) and by WKYMVM ($r_s = 0.82$, $p = 0.002$). Lower superoxide anion production after stimulation with fMLF and WKYMVM was associated with a longer plateau phase during episodes ($r = -0.59$, $p = 0.037$ and $r = -0.58$, $p = 0.04$), a shorter median interval between episodes ($r = 0.68$, $p = 0.016$ and $r = 0.76$, $p = 0.006$) and a shorter interval to the second episode ($r = 0.58$, $p = 0.039$ and $r = 0.66$, $p = 0.02$). There was no significant difference in superoxide anion production between RGBS patients and their age-matched controls, for whom the superoxide anion production was intermediate between patients with complete and incomplete recovery ($p = 0.09$ for fMLF, $p = 0.19$ for WKYMVM and $p = 0.23$ for PMA).

Lower superoxide anion production after stimulation with fMLF and WKYMVM was associated with a longer distal motor latency ($r_s = -0.74$, $p = 0.007$ and $r_s = -0.69$ and $p = 0.015$), a lower motor nerve conduction velocity ($r_s = 0.59$, $p = 0.037$ for both peptides) and a lower amplitude ($r_s = 0.58$, $p = 0.037$ and $r_s = 0.69$, $p = 0.013$) at follow-up.

MPO activity

MPO activity in leukocytes did not differ between patients and controls ($p = 0.63$) or between severity groups of RGBS patients with complete or incomplete recovery ($p = 0.57$) (data not shown).

GBS and RGBS data in relation to respiratory burst

We compared the superoxide anion production of leukocytes isolated from 10 RGBS patients (**Paper I**) and 22 GBS patients from this study (**Paper IV**) after stimulation with fMLF, WKYMVM or PMA. The measurement day was used as a nuisance factor, and two control groups were combined for the comparison between the GBS and the RGBS material. There were no significant differences in superoxide anion production after stimulation with MLF, WKYMVM and PMA (p = 0.89) (Table 5).

Table 5. Superoxide anion production in leukocytes from RGBS and GBS patients

	O_2^- production (Mcpm)		
Stimuli	RGBS n = 10	GBS n = 22	p value, RGBS-GBS
fMLF	125 ± 23	144 ±16	0.56
WKYMVM	115 ± 22	126 ±16	0.72
PMA	563 ± 29	568 ± 21	0.89

Leukocytes isolated from RGBS, GBS and control subjects were triggered with fMLF, WKYMVM and PMA. The release of superoxide anions was measured by isoluminol/luminol-enhanced CL. Data are presented as mean peak values ± SEM comparing superoxide anion production between RGBS- and GBS patients using a one-sided test in a linear model.

Discussion

A contemporary analysis infers that the demyelinating diseases, MS and GBS, are complex autoimmune organ-specific disorders. Recent efforts to specify the antigens involved had some success, while current genetic analyses confirm the complexity of the issue. One point remaining to be resolved is the extreme variability in the severity of these diseases. This is the object of the present thesis.

A clue to the cause of this variability came from experimental findings that a polymorphism in a gene *Ncf1* coding for one protein of the NADPH oxidase, $p47^{phox}$, influenced the severity of autoimmune arthritis and EAE in rodents. The allele promoting more severe disease was associated with lower respiratory burst (57, 58). Thus, the relationship was to severity, not to susceptibility. A straightforward test would be to examine the effect of the syntenic human gene. *Ncf1* does exist in the human genome. However, because of pseudogenes and a paucity of markers, the corresponding human *Ncf1* gene has not been accessible for analysis. However, a method for analysising of the NADPH oxidase activity, in which the *Ncf1* gene codes for the $p47^{phox}$, was available. A genetic association was identified between human rheumatoid arthritis and another related gene, *Ncf4*, coding for $p40^{phox}$ protein of the NADPH oxidase complex (269). However, this allele was a tentative susceptibility factor and not relevant for disease severity.

To our knowledge, NADPH oxidase activity has not been studied in human demyelinating disease. Here we studied the correlation between the respiratory burst and the severity of demyelinating diseases. The present study aimed at constitutional factors but does not rely on molecular genetics. Rather we assessed the respiratory burst as a function of the NADPH oxidase. We assume that the respiratory burst we studied in blood leukocytes is representative for the respiratory burst in all APCs including DCs and microglia, which are antigen-presenting cells in the CNS.

The main finding in this study was the correlation between the severity of the demyelinating diseases MS, GBS and RGBS and the intensity of the respiratory burst, an early and important step in the innate immune defence. A lower respiratory burst was associated with a more severe course of disease. How can this correlation be explained?

1. More effective clearance of an infectious agent in individuals with higher production of NADPH oxidase-derived ROS during phagocytosis.

In the present scenario, the protective function, which ROS exert in infections, acts in the same way in autoimmune disease. Epidemiological features of MS and GBS have suggested that environmental factors such as infections play an important role in the etiology of these autoimmune diseases as triggering factors, particularly in genetically predisposed individuals (145, 224, 270). Perhaps identification of the etiologic agents is only partially feasible in GBS and more difficult in MS due to the latency between the initial causal infection and disease onset.

We hypothesize that a deficiency in innate immune function due to low superoxide anion production in leukocytes may result in incomplete clearing of infections and persisting antigen stimulation, followed by a protracted hyperactivity of the adaptive system. A consequence of a relatively weak innate immune system could be that the more severe neurotropic infections reach the CNS (73). Concordant with this assumption, a significant association exists between systemic infections and GBS (145, 271). A similar association was found between infections and the risk of MS exacerbations (74, 272, 273). A seasonal variation of the MS relapse rate and correlation between the number of relapses and number of common infections per month were found, confirming that infection is a risk factor for MS relapses (75).

Another reported scenario is termed *"balanced heteromorphism"*. It is easier to understand this scenario in evolutionary terms. It implies that the immune system protects against infections but at a price; high immune defence activity induces autoimmune disease. This trade-off between maximal anti-microbial and tissue-protective effects may be driven by evolution. However, the only known example is a polymorphism in the natural resistance associated macrophage protein (Nramp) which has two allelic forms in mice, resistant or susceptible. Interestingly, animals resistant to intracellular pathogens such as tuberculosis have a higher risk of developing arthritis. In the promoter for a syntenic human gene, one allele was linked to autoimmune disease and high expression (274). However, this mechanism is not compatible with our results, and a precondition is a specific antigen. This mechanism therefore seems to be related to the adaptive rather than the innate immune system.

2. The respiratory burst suppresses autoimmunity.

The mechanism could be dampening of auto-reactive T cell clones and NK cell activity (26). Taurine chloramines, which are synthesized in the presence of the halide-dependent MPO system in leukocytes, have been reported to inhibit lymphocyte proliferation and cytokine production (31). It has been proposed that phagocyte-derived radicals may serve to dampen functions of auto-reactive lymphocytes, and hence protect the host against autoimmune disease (275). An increased number of reduced thiol groups (-SH) on the T cell membrane surfaces activate auto-reactive T cells. T cell treatment with glutathione artificially increased these thiol groups on the T cell surface and lowered the threshold for T cell reactivity, resulting in increased IL-2 production and autoimmunity in a rat model. In the reverse experiment, oxidized T cells induced less severe arthritis compared with controls (Fig.9).

The lower ROS producing capacity is associated with an increased number of reduced thiol groups. Based on these findings, it has been proposed that ROS set the threshold for T cell activation and thereby regulate chronic autoimmune inflammatory diseases such as RA (25, 61).

However, we should discriminate between respiratory burst and "oxidative stress". The respiratory burst is a normal strictly regulated cellular process while "oxidative stress" may result from harmful massive ROS-mediated damage of cell structures. This occurs at the site of inflammation during the effector phase of the inflammatory

response to an invading pathogen (61). The term"*oxidative stress*" has a much wider application and gives rise to much confusion. It is usually defined as a disturbance in prooxidant-antioxidant balance favouring pro-oxidant activity, leading to potentially harmful effects such as lipid peroxidation and damage to nucleic acids (276). Mitochondrial electron transport systems (mitochondrial respiration) are one of the most important sources of ROS and oxidative stress in the context of ischemic and other tissue damage (277), but this is not within the scope of this thesis. Cellular defence against oxidative stress comprises scavengers and specific enzymes such as SOD which catalyses the dismutation of superoxide anion to hydrogen peroxide, and catalase which transforms hydrogen peroxide to water, and different repair systems such as glutatione (24). Thus, oxidative stress during the effector phases of inflammatory disease, or induced by severe infection, should be distinguished from constitutive respiratory burst possessing an immune regulatory function (61). Studies of disesases in acute destructive phases may reveal unspecific tissue damage caused by ROS. While the role of innate immune function has been increasingly studied in autoimmune diseases (17), only a few studies have investigated the production of superoxide anion. Patients with rheumatoid arthritis (RA) had higher superoxide anion production in neutrophils isolated from blood compared with controls using the same agonists as in the present study (278, 279). High superoxide anion production was also reported in neutrophils from the synovial fluid in patients with RA as compared to circulating cells (280).

The structure and function of NADPH oxidase as a central enzyme of the respiratory burst was first described after studying phagocytes from patients with CGD (28). CGD is a disease characterized by failure to produce superoxide anion due to mutations in different components of the NADPH oxidase complex resulting in predisposition to recurrent fungal and bacterial infections (27, 29). Notably, this deficient NADPH oxidase function also results in development of chronic inflammatory sterile lesions unrelated to the presence of infection (29, 281, 282).

CGD is one of an expanding list of conditions characterized by inflammatory reactions in different organs, called autoinflammatory diseases. They are characterized by unprovoked recurrent attacks of systemic inflammation with lack of auto-antibodies or auto-reactive T cells and without any evidence that this process is related to auto-antigen exposure (78). For example, the hereditary periodic fever syndromes due to mutations in the pyrin and tumour necrosis factor receptor superfamilies, both of which are intimately involved in innate immunity, are the prototype of autoinflammatory diseases (283). Infections may re-trigger inflammation, but ROS deficiency is sufficient to mediate the overexpression of inflammatory genes in a sterile environment (284). Such autoinflammatory reactions may be dependent on an association of the defect in NADPH oxidase with a defect in apoptosis (285). Human CGD leukocytes display a hyperinflammatory phenotype with increased production of proinflammatory cytokines in the absence of ROS that could be explained by the better survival of these cells (286). Thus, these ROS-deficient cells are inherently pro-inflammatory (284).

There is a day-to-day variation in the assay of NADPH oxidase activity. This problem, the variance in superoxide anion production, results from a limited number of patients (maximum of 3-4 patient-control pairs) who could be analysed per day. The strong correlation ($r = 0.6$, $p = 0.002$) between patient and matched control indicated a high day-to-day variation in phorbol myristate acetate (PMA)-stimulated cells, since PMA was prepared freshly each day to avoid degradation in water. The correlation was weaker in fMLF and WKYMVM stimulated cells. Thus, the variation was less within day than between days. Therefore, we included in the statistical analysis the measurement day as a nuisance factor to reduce the experimental variance and thereby increase the chance of identifying interesting predictors in subsequent **Papers II** and **IV**. A nuisance factor in standard statistical terminology is a factor of no interest *per se*, but still a factor that can influence the dependent variable. The inclusion of this nuisance factor improved the significance in the analysis of the effect of respiratory burst in MS, yet deleted the significance found between the severity of GBS and RGBS, probably because of the inclusion of the further differences in the analysis over time in addition to day-to-day variation.

We also studied MPO, a further enzyme in the NADPH oxidase reaction chain. In the present study no difference was observed in MPO activity between patients and controls, nor between patients with varying severity of GBS or MS. The MPO activity has been studied previously with divergent results. A low MPO activity was reported in relapsing and progressive MS (33). Another group found up-regulation of MPO in patients with opticospinal multiple sclerosis (34). The discrepancy in results might be explained by the different substrates used in these two studies since MPO is present in three distinct isoforms that vary in enzymatic activity (287, 288) and thus, possibly, also in substrate specificity.

The RGBS cases were originally included assuming them to represent a more severe course of GBS. However, this was only partially shown to be the case. RGBS was also of interest as an intermediate disorder (peripheral, relapsing) between MS (central, relapsing) and GBS (peripheral, monophasic). Therefore, we performed a clinical study of RGBS (**Paper III**) in order to elucidate whether RGBS represents a chronic form of GBS or a separate clinical entity. The main findings in our clinical study of RGBS were that there was no tendency for episode frequency to decline and clusters of episodes continued to occur. Thus, the recurrence risk seems to be highly unpredictable, and the disease does not follow a benign course converted to a progressive phase in 2/11 cases. The onset was earlier than in monophasic GBS, and the duration of the initial episode was shorter. However, the differences we found between GBS and RGBS concerning the duration of the first episode do not allow RGBS to be established as a separate disease entity. Rather, a continuum is conceivable without any definite monophasic GBS cases, only RGBS and potential RGBS cases waiting for the next episode to occur. The advantage of including of RGBS in the study of respiratory burst is that we obtain the opportunity to correlate the respiratory burst with both deficit and frequency parameters of severity.

Fig. 9. Oxygen radical induced apoptosis of auto-reactive T cells can protect neurons from demyelination

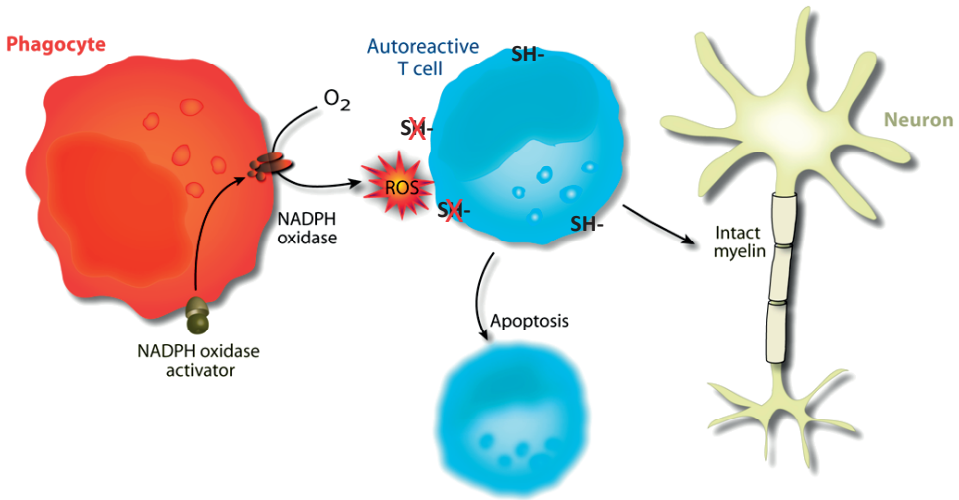
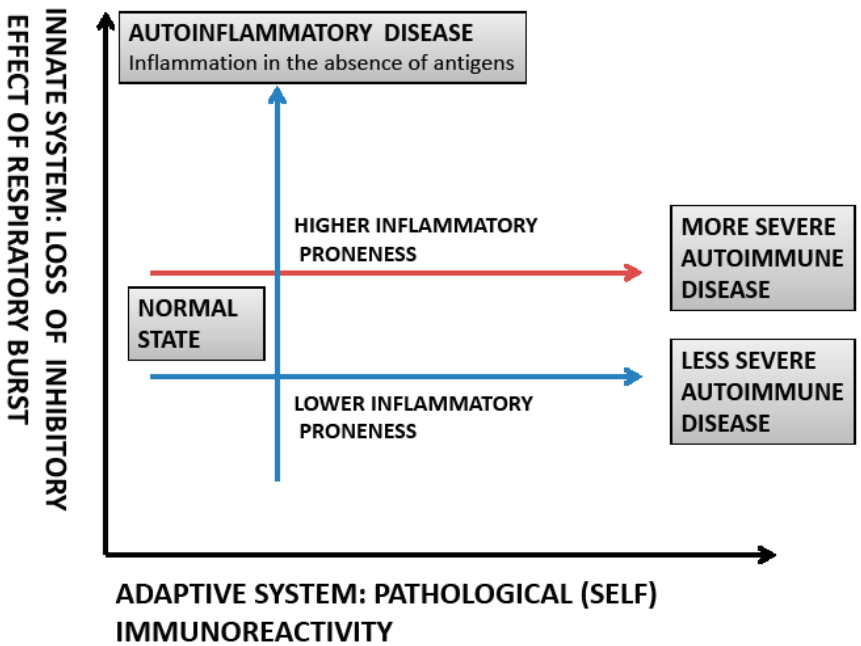


Fig. 10. Intensity of respiratory burst and severity of demyelinating disease



We make a sharp distinction between autoimmune diseases with a more or less precise known tissue antigen, related to the adaptive immune system, and autoinflammatory disorders with hyperinflammatory phenotypes, caused by defects in the innate immune system. This does not preclude that tissue damage in autoimmune diseases activates the local innate defence. The innate and adaptive immune systems interact. We suggest a hypothesis where the innate immune system with 100 % function has a dampening or braking effect upon the adaptive system via mechanisms (e.g. thiol groups) mentioned above. If the innate system is severely impaired below a certain threshold, the adaptive system will be spontaneously activated, resulting in autoinflammatory disease without any microbial challenge. According to this hypothesis, the immunoreactivity elicited by infections is usually sufficiently strong to break through the innate braking systems, mounting an adequate specific immune defence. However, infections may contribute to the development of organ- or antigen specific autoimmunity. Relative insufficiency of the innate braking system, with an intermediate function level between normal and that of autoinflammatory disease may influence the threshold level of T cells reactivity and the severity of such autoimmune processes (Fig. 10).

This hypothesis is consistent with the central findings in this thesis that the innate function influences the severity of demyelinating diseases. However, this model does not explain why this mechanism is restricted to autoimmune T cell clones. Theoretically, it could be explained by the level of activation. Subsequent studies have described lymphocytes during infection or malignancy remaining viable in spite of release of ROS due to the presence of myeloid DCs, related to their catalase activity (289).

Our clinical data suggest that a deficiency of NADPH oxidase activity, previously reported to elicit spontaneous inflammation, induces a more intense autoimmune demyelinating disease. As our study was designed to examine the patients in the stationary or slowly progressive phases, it is probable that the relationship we found between individual overall severity and NADPH oxidase activity indicates that we detected a constitutional factor regulating the disease severity rather than an activity marker. Thus, our conclusion is that the innate immune response has a constitutional protective function in GBS and MS, as low production of ROS is associated with a less effective initial innate immune response to an infectious challenge or less optimal suppression of the adaptive response. In agreement with other observations (79), we propose that a well-functioning innate immune defence has a protective role in human autoimmune diseases. The relationship between respiratory burst and human pathology might be elucidated by studies of FPRs polymorphisms, intracellular ROS production, and thiol-group expression on different cell types in GBS, RGBS, and MS.

The relationship between NADPH oxidase activity and disease severity was more obvious in GBS (**Paper I**) than MS. This may be due to a simpler infection-induced mechanism in GBS and a more complex immunopathogenesis in MS, where the role of infections is probably more remote in time. It has been shown in an experimental system that local activation of innate immunity may lead to neurodegeneration (290). In the secondary progressive phase, compartmentalization of the inflammatory process

behind the BBB occurs (111). Thus, the innate immune system may have a dual function, triggered by infections in the presymptomatic and remitting phases, but involved in neurodegeneration in the progressive phase. Differences in BBB and BNB permeability to antibodies and antigens may also contribute to less distinct results in MS. It is extremely difficult to study the local innate immune function in the human CNS.

It is impossible to study the respiratory burst in CSF cells due to insufficient numbers. However, an alternative method of studying the respiratory burst in CNS cells could be to incubate phagocytic cells of healthy controls with CSF from MS patients in order to detect a factor influencing the respiratory burst behind the BBB.

We here examined this innate function as an individual constitutive factor, which may however be modifiable by other host conditions, and may have implications for prophylactic or therapeutic intervention. The first question is whether MS and RGBS patients need to be protected from relapse-triggering infections. The next issue is whether agents that activate a weak respiratory burst such as phytol (70) may be developed for therapeutic use. Further studies are warranted to examine whether a low intensity of respiratory burst is a risk factor for other autoimmune disorders such as CIDP.

Conclusions

The present study demonstrates that low NADPH oxidase activity induces a more intense autoimmune demyelinating disease. The intensity of the respiratory burst markedly contributes to the extreme variability in severity of the demyelinating disorders. While loss of the respiratory burst results in spontaneous autoinflammatory disease, we found that decreased function predisposes for a more severe autoimmune disease. Thus, this pivotal function of the innate immune system regulates the intensity rather than the susceptibility to these diseases. The mechanism may be a less effective control of infection and/or of the adaptive immune system. As a more general conclusion, we propose that a well-functioning innate immune defence has a protective role in autoimmunity.

Svensk sammanfattning

Multipel skleros (MS) är en kronisk autoimmun sjukdom i det centrala nervsystemet (CNS) med periodiska skov som vanligtvis övergår till ständigt tilltagande bortfallssymtom som förlamningar och balanssvårigheter. Sjukdomen beror på en inflammatorisk skada på nervcellernas skyddande hölje av myelin. Behandling med bromsmediciner är effektiv under de tidiga stadierna av sjukdomen. Guillain-Barré syndromet (GBS) är den motsvarande organspecifika autoimmuna demyelinerande (myelinskadande) sjukdomen i det perifera nervsystemet (PNS), som yttrar sig som en enda akut sjukdomsperiod, och där utläkningen ofta är komplett. GBS kan recidivera i 2-5% av fallen.

Ett olöst problem utöver den grundläggande orsaken till dessa sjukdomar är den extrema variationen av svårighetsgraden. En ledtråd till denna variabilitet kom från experimentella studier och från en ärftlig sjukdom i det medfödda immunförsvaret. Det finns nämligen två delar av immunsystemet som interagerar med varandra: det innata (medfödda) immunsystemet som svarar snabbt men relativt likartat mot olika grupper av inkräktare, och det adaptiva (förvärvade) immunförsvaret som efter 1-2 veckor riktar ett specifikt svar mot en aktuell inkräktare. Medan betydelsen av det adaptiva immunsystemets reaktion mot myelin är väl etablerad som en viktig del av sjukdomsorsaken, är våra kunskaper angående det innata immunsystemets roll begränsade.

Fagocyter är utrustade med ett syreradikalproducerande enzym, NADPH-oxidaset, som omvandlar molekylärt syre till reaktiva syremetaboliter (”syreradikaler”). Sistnämnda är en viktig del av det innata immunförsvaret mot invaderande mikroorganismer. Studier i djurmodeller visade att en bristande funktion av NADPH-oxidaset kan leda till ett svårare sjukdoms förlopp. Ärftlig brist på NADPH-oxidaset hos människan ligger till grund för en sjukdom som karakteriseras av utbredda spontana inflammationshärdar i flera olika organ.

Målet med avhandlingen var att undersöka sambandet mellan intensiteten av syreradikalproduktionen och svårighetsgraden av tre demyelinerande sjukdomar, MS, GBS och recidiverande GBS (RGBS). Sjukdomens svårighetsgrad vid GBS bedömdes med tre oberoende variabler, nämligen som oförmåga att gå självständigt 3 månader efter debut, IVA vistelse (ja/nej) och en vedertagen bedömningsskala, the Medical Research Council Score (MRC) vid uppföljningen. För att avgöra om RGBS är en klinisk entitet skild från GBS, jämfördes ett antal kliniska variabler mellan GBS och RGBS. Svårighetsgraden av RGBS bedömdes med graden av tillfrisknande efter andra

och sista episoden och med frekvensen av återfall. Prognosen av RGBS och den kliniska skillnaden mellan en episod av GBS och den första episoden av RGBS studerades också för att påvisa bakomliggande faktorer för återfallsbenägenhet. Svårighetsgraden av MS bedömdes med Multiple Sclerosis Severity Score (MSSS).

Patienter med MS (N = 60), monofasisk GBS (N = 23) och RGBS (N = 10) i lugnt skede, fritt från skov samt friska åldersmatchade kontroller inkluderades i en neurologisk efterundersökning med skattning enligt kvantitativa skalor (MRC och MSSS) och blodprovtagning. RGBS-patienterna genomgick även en standardiserad neurofysiologisk undersökning.

RGBS-patienterna hade tidigare sjukdomsdebut och kortare duration av första episoden jämfört med monofasisk GBS. Duration mindre än 45 dagar av en GBS episod indicerade en högre risk för återfall. Återfallsbenägenheten minskade inte under sjukdomsförloppet, utan episoder fortsatte att uppstå oregelbundet under en observationstid på 18 år. Risken för återfall var oförutsägbar och förloppet tenderade att gå över till progressiv fas hos två patienter.

Vår laboratorieanalys gick till så att vi stimulerade NADPH-oxidaset i vita blodkroppar med substanser som härmar effekterna av mikroorganismer. Kapaciteten att producera reaktiva syremetaboliter värderades med isoluminol-luminol kemiluminiscens (CL) teknik i relation till sjukdomens svårighetsgrad.

Resultatet var att intensiteten av syreradikalproduktion var betydligt svagare hos GBS patienter med svårare sjukdomsgrad ($p = 0.0004 - 0.04$). Ett liknande samband påvisades vid RGBS när svårighetsgraden uttrycktes som graden av utläkning ($p = 0.001 - 0.004$) eller benägenheten för återfall (intervallen mellan återfallen och tiden från debuten till första återfallet, $p = 0.006 - 0.03$). Svårare MS patienter hade också lägre intensitet av syreradikalproduktion ($p = 0.0035 - 0.04$).

Sammanfattningsvis har vi visat att nivån av syreradikalproduktion i det innata immunförsvaret ger ett betydande bidrag till den uttalade variabiliteten av svårighetsgraden av de tre demyelinerande tillstånden. Det innata immunförsvaret reglerar intensiteten snarare än mottagligheten av dessa sjukdomar. Medan bortfall av denna funktion medför spontan autoinflammatorisk sjukdom, är vårt resultat att bristande syreradikalproduktion inducerar en svårare grad av autoimmun demyelinering. Mekanismen kan vara en mer effektiv infektionskontroll och/eller hämning av det överaktiva adaptiva immunförsvaret.

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