2 INTRODUCTION

2.1 Selenium and selenoproteins

In 1818 the Swedish physician and chemist Jons Jacob Berzelius discovered selenium when he fractionated the waste of sulphuric acid plant in Sweden. He named it selenium after Selene, the Greek goddess of the moon. It is closely related to the element tellurium (Te; tellus is earth in Latin). For a long period selenium was known only for its toxicity and as a possible potent carcinogen. In 1943 Moxon and Rhian reviewed the selenium poisoning problem (1): the toxic reactions of high intake of selenium include acute Se poisoning, chronic ("alkali disease") and acute ("blind staggers") livestock poisoning. Only in 1957 the significance of selenium to animal health was documented by Schwarz and Foltz (2): they changed the perception of Se and provided strong evidence for a beneficial and essential role of this element. They discovered that selenium was required in the diet of mammals to prevent liver necrosis.

The review of Levander (3), which provides a global view of the role of selenium in human nutrition and health, described two human diseases related to a low selenium intake:

- Keshan disease, endemic cardiomyopathy (4)
- Kashin-Beck disease, osteoarthritis deformans endemica (5)

These diseases were identified in an area of China noted for its low soil content of selenium; in fact selenium enters the food chain via plants, which take it up from the soil.

Now the implicit importance of selenium to human health has been recognised universally.

Many diseases in different medical fields and clinical conditions seem to be ameliorated by selenium supplementation (6):

- Immune function (selenium deficiency is accompanied by loss of immunocompetence) (7, 8)
- Viral infection (9)

- Reproduction (selenium is essential for male fertility, being required for testosterone biosynthesis and the formation and normal development of spermatozoa) (10)
- Mood (in a study in the USA, selenium deprivation led to depressed mood and high dietary selenium appeared to improve mood) (11, 12)
- Endocrine system (thyroid function) (13)
- Cardiovascular disease (14)
- Oxidative-stress condition (15)
- Cancer (16, 17)
- Aging process
- Diabetes

2.1.1 Characteristics of selenocysteine

In the periodic table selenium belongs to group VIA, which includes other elements such as oxygen, sulfur, and tellurium with which selenium shares many properties. Selenoproteins contain selenium in the form of selenocysteine (Sec), a cysteine (Cys)-analogue with a selenium atom replacing the sulfur atom in Cys. Between selenium and sulfur there are close similarities but also considerable differences in terms of their chemistry and biochemistry. Thus, the Sec residue exhibits different characteristics compared with a Cys residue. In Table 1 some characteristics for Sec and Cys are listed (18). A major difference is a significantly lower pK_a value of the selenol group in Sec (pK_a = 5.2) relative to that of the thiol group of Cys (pK_a = 8.3). As a consequence, at physiological pH, selenols in selenocysteine-containing proteins are normally present as selenolates (anionic form), whereas thiols in cysteine-containing proteins are typically protonated. This makes Sec significantly more reactive than Cys.

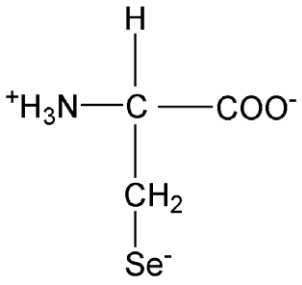
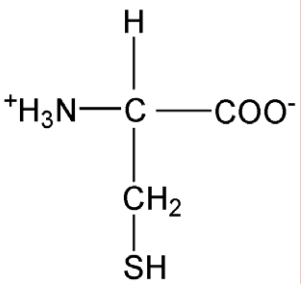
Characteristic feature	Selenocysteine (Sec; U in one-letter code)	Cysteine (Cys; C in one-letter code)
Structure		
pK_a	5.2	8.3
Redox potential	- 488 mV	-233 mV
Codon(s)	UGA	UGU, UGC

Table 1 Properties of the amino acids selenocysteine and cysteine (adapted from L. Johansson et al. (18)).

2.1.2 Synthesis of selenoproteins in eukaryotes.

Most of the selenium in animals is associated with proteins, where selenium is incorporated as selenocysteine (Sec, the 21st proteinogenic amino acid) in specific selenoproteins. In addition to the incorporation as selenocysteine, selenium can substitute sulfur in methionine, forming selenomethionine, which can be incorporated non-specifically into proteins. Selenium can also be tightly bound by some proteins, known as selenium-binding proteins (19).

All the characterized selenoproteins except selenoprotein P (20) contain single Sec residues that are located in enzyme-active sites and are essential for their activity. In fact, when Sec is replaced with Cys, the catalytic activity of a selenoenzyme is drastically reduced (21).

The details of the mechanism of selenoprotein synthesis differ between species, in particular between pro- and eukaryotes (22). Selenocysteine is incorporated into at least 25 human proteins at specific UGA codons that are decoded by the selenocysteil tRNA (Sec-tRNA$^{[Ser]Sec}$). Compared with other mammalian tRNAs, this tRNA is special because the Sec biosynthesis takes place on the tRNA after it is aminoacylated with serine (23). Sec-tRNA$^{[Ser]Sec}$ is the longest eukaryotic tRNA sequenced to date (90 nucleotides in length). Many additional novel features of Sec-tRNA$^{[Ser]Sec}$ transcription were reported and reviewed by Hatfield in 1999 (24).

Besides Sec-tRNA$^{[Ser]Sec}$ and the UGA codon in selenoprotein mRNA, there are several other factors that are required for the donation of Sec to protein and determine the specific function of UGA as Sec in eukaryotes:

- the Sec insertion sequence (SECIS element) (25)
- the SECIS-binding protein 2 (SBP2) (26)
- the Sec-specific elongation factor (eEFsec) (27).

The SECIS element, a complicated stem-loop structure, is located far away from the selenocysteine UGA codon, in the 3´untranslated regions (3´-UTRs) of all eukaryotic selenoproteins genes. By contrast, in prokaryotic selenoproteins SECIS sequences are present immediately downstream of the Sec-encoding UGA codon.

Eukaryotes assemble a stable insertion complex, consisting of the Sec codon, the Sec-tRNA$^{[Ser]Sec}$, the eEFsec and the SBP2 (Figure 1). SECIS elements function by recruiting SBP2 to form a tight SECIS-SBP2 complex and may be also be firmly

associated with ribosomes. Besides binding to the SECIS element and ribosomes, SBP2 binds the eukaryotic elongation factor eEFsec, which in turn recruits selenocysteine-carrying tRNA and inserts Sec into nascent polypeptides in response to UGA codons.

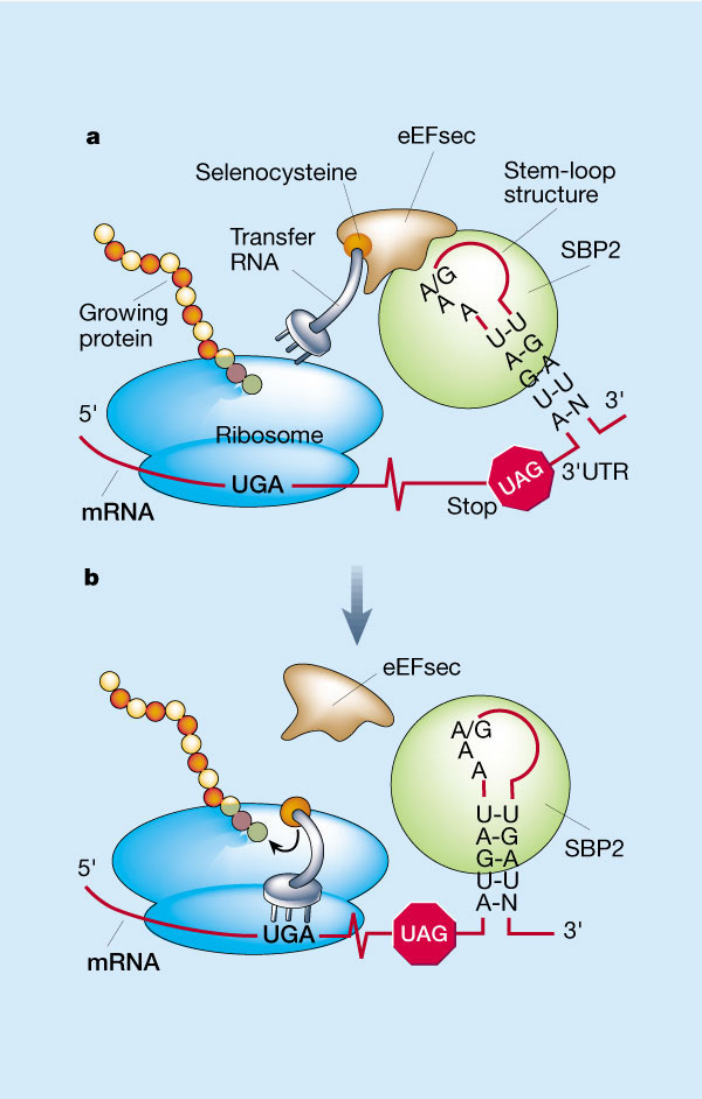


Figure 1 Selenoprotein synthesis in eukaryotes (adapted from Atkins and Gesteland (28)). The ribosome moves along the mRNA, deciphering the nucleotide sequence and making a protein according to the encoded amino-acid sequence. The nucleotide sequence UGA normally specifies that the ribosome should stop the translation. But sometimes this stop codon can be redefined, so that selenocysteine, the twenty-first amino acid, is incorporated instead. A) a “stem loop” structure (SECIS element) in the downstream, untranslated region of the mRNA binds to a protein called SBP2. SBP2 in turn binds to the eEFsec protein, which itself has recruited the transfer RNA carrying selenocysteine. B) the selenocysteine-bound tRNA is then delivered to the UGA for incorporation into the growing amino-acid string that constitutes the newly created protein.

2.1.3 Mammalian selenoproteins

Information on full sets of selenoproteins (selenoproteome) is essential for understanding the biology of selenium. The vital importance of selenoproteins was demonstrated by knock-out mice lacking the Sec-tRNA$^{[Ser]Sec}$ gene, which resulted in early embryonic lethality (29). 29 eukaryotic selenoproteins have been identified, however between 30 and 50 mammalian selenoproteins are expected to exist. Kyriakopoulos and Behne detected more than 30 selenoproteins by means of ^{75}Se pulse labelling of rats and 2D gels electrophoresis (30). Recently, Zhang et al (31) identified 310 prokaryotic selenoprotein genes using a computational approach. Table 2 shows a list of currently known mammalian selenoproteins: most of them are, or at least seem, to be involved in antioxidative defence and redox metabolism (32). Five of these are glutathione peroxidases and will be described later.

Many questions about their role in the organism remain to be answered, but new methodologies that allow the recombinant expression of these selenoproteins will be of advantage for elucidating each specific function.

Selenoproteins	Tissue distribution	Localization in the brain	Functions	Refs.
Cytosolic glutathione peroxidase (Gpx1)	various tissues including the brain	microglia, activated astrocytes, low in neurons	antioxidant	(33)
Gastrointestinal glutathione peroxidase (Gpx2)	gastrointestine and liver	-	antioxidant	(34)
Plasma glutathione hydroperoxidase (Gpx3)	kidney, plasma	-	plasma antioxidant	(35)
Phospholipid hydroperoxide glutathione peroxidase (Gpx4)	various tissues included the brain	neurons and astroglia	antioxidant	(36)

Sperm nuclei glutathione peroxidase (snGpx)	testis, spermatozoa	n.t.	antioxidant, protamine condensation	(37, 38)
Human Gpx6	olfactory epithelium, bowmans gland	n.t.	antioxidant?	(39)
Thioredoxin reductase 1 (TRx1)	various tissues including the brain	glia and neurons	multiple roles, redox control, drug metabolism	(21),(40)
Thioredoxin reductase 2 (TRx2)	various tissues including the brain	n.t.	multiple roles	(41, 42), (43)
Thioredoxin reductase 3 (TRx3)	various tissues	n.t.	multiple roles	(44)
Thyroid hormone deiodinase 1 (DI1)	mainly in the thyroid, liver, kidney and pituitary		catalyses the deiodination of T4 to T3	(45),(46)
Thyroid hormone deiodinase 2 (DI2)	mainly in the brain, brown adipose tissue, pituitary and placenta	mainly glial	catalyses the deiodination of T4 to T3	(47),(48), (49)
Thyroid hormone deiodinase 3 (DI3)	mainly in the CNS, placenta and skin	neurons	catalyses the deiodination of T4 to T3	(47)
Selenophosphatase synthetase 2 (SPS2)	various tissues	n.t.	required for selenoprotein synthesis, catalyses the production of selenophosphatase	(50),(51)
15-kDa Selenoprotein (Sel15)	various tissues, brain	n.t.	unknown	(52),(53)
18-kDa Selenoprotein (Sel18)	various tissues	n.t.	unknown	(30)
Selenoprotein I (SelI)	various tissues	n.t.	unknown	(39)
Selenoprotein H (SelH)	various tissues including the brain	n.t.	unknown	(39)
Selenoprotein K (SelK)	various tissues including the brain	n.t.	unknown	(39)
Selenoprotein M (SelM)	various tissues including the brain	n.t	unknown	(54)

Selenoprotein N (SelN)	skeletal muscle, liver, brain, heart, stomach	n.t	unknown	(55),(56)
Selenoprotein O (SelO)	various tissues	n.t.	unknown	(39)
Selenoprotein P (SelP)	liver is main source of plasma SelP; ubiquitous	neurons, glia	Se transport, antioxidant?	(57),(58)
Selenoprotein R (SelR, MrsB, SelX)	various tissues including the brain	n.t.	R-methionine sulfoxide reductase	(59, 60)
Selenoprotein S (SelS)	various tissues	n.t.	unknown	(39)
Selenoprotein T (SelT)	various tissues	n.t.	unknown	(61)
Selenoprotein V (SelV)	only testis	n.t.	unknown	(39)
Selenoprotein W (SelW)	mainly in the skeletal muscle, heart muscle, brain, testis and spleen	neurons	antioxidant?	(62),(63)
Selenoprotein Y (SelY)(DI2)	heart and brain	n.t.	unknown	(39)
Selenoprotein Z (SelZ)(TRx2)	various tissues including the brain	n.t.	unknown	(64)

Table 2 Mammalian selenoproteins and their distribution in the central nervous system. All currently known selenoproteins with at least one selenocysteine in their amino acid sequence are shown (adapted from Schweizer (65)). Abbr. n.t., not tested.

2.1.4 Selenoproteins in the brain

Several selenoproteins have been identified in human and rodent brain, but systematic studies are still lacking. Ulrich Schweizer reviewed recent findings on selenoproteins in the brain in his paper "Selenium and brain function: a poorly recognized liaison" (65). The available information about the cell-type expression patterns of selenoproteins in brain are summarized in Table 2.

2.1.4.1 Priority position of the brain in selenium retention and selenoprotein biosynthesis

The brain is a privileged organ with reference to selenium supply and retention.
Certain tissues and proteins appear to have priority on the available selenium supply, in fact Se depletion in the organism leads to a characteristic hierarchy of the remaining levels of individual selenoproteins within different organs. The Se ranking order in different organs when selenium intake is limited is as follows (66):

brain > testis > thymus > thyroid > heart > liver, kidney, lung, muscle

The brain is one of the last tissues affected by insufficient Se intake (67), whereas liver kidney and lung show a pronounced reduction of selenoprotein expression in selenium deficiency. The fact that no increased mortality could be observed in rats fed a selenium-deficient diet for 16 generation (19), while knocking out the selenocysteyl-tRNA results in early embryonic lethality, might be due to the maintenance of essential selenoproteins in the priority tissues such as the brain. Furthermore, during Se deficiency, the ratio of methylated $tRNA^{[Ser]Sec}$ and nonmethylated forms decreases, but not in the brain, indicating brain-specific regulation of selenoprotein biosynthesis (68).

2.1.5 The selenoenzyme glutathione peroxidase

The classical or cytosolic glutathione peroxidase (Gpx1) was the first mammalian selenoprotein to be identified and was described by Flohé in 1973 (33). Gpx1 (EC 1.11.1.9) is the most abundant member of the family of selenoproteins and plays an important role in the defence mechanisms of mammals against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate (Figure 2).

Four distinct species of glutathione peroxidase have been identified in mammals, the classical cellular enzyme (Gpx1), the gastrointestinal tract enzyme (Gpx2), the extracellular plasma enzyme (Gpx3) and the phospholipid hydroperoxide metabolizing enzyme (Gpx4). Very recently a new human Gpx6 gene was identified by a computational screening (references are listed in Table 2).

All glutathione peroxidases utilize glutathione as thiol substrate and reduce hydrogen peroxide and alkyl hydroperoxides, but their primary structures are poorly related, and it has been shown that they are encoded by different genes and have different enzymatic properties.

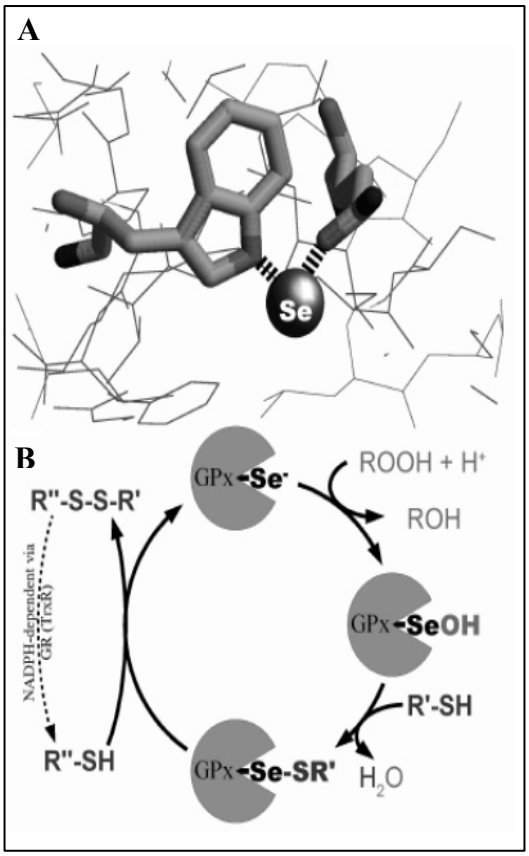


Figure 2 A) The catalytic triad of Gpx1. The selenium is hydrogen-bonded to the imino group of a tryptophan residue and the amido group of a glutamine. B) General reaction scheme of glutathione peroxidase. The selenolate form of the enzyme is oxidized by H_2O_2 or alkyl hydroperoxide to a selenenic acid derivative that is stepwise reduced by RSH, typically glutathione. Adopted from Ref. (69).

The individual glutathione peroxidases are not equally distributed. They display preferences for certain tissues: the Gpx1 is ubiquitously expressed, Gpx2 is exclusively expressed in the gastrointestinal tract, Gpx3 is mainly expressed in tissues in contact with body fluids and Gpx4 is highly expressed in the testis.

Besides the tissue hierarchy in case of selenium deficiency (as briefly described in 2.1.4.1), a hierarchy for individual selenoproteins has also been found (66, 70). Compared to other selenoproteins, glutathione peroxidase enzyme levels fall further and more rapidly upon selenium deficiency. A study in 1995 examined the tissue-specific regulation of Gpx1, Gpx4 and DI1 in the heart, liver and thyroid of rats fed a diet with changing concentration of selenium (71). They found differential regulation of the three mRNAs within and between the organs, demonstrating both that

mechanisms exist to channel Se for the synthesis of a definite selenoprotein and that there is a tissue-specific regulation of selenoprotein mRNAs.

Another study with H4 hepatoma cells showed that in selenium deficiency, the stability of translated mRNA was an important factor in the regulation of selenoprotein levels. It was demonstrated that selenium depletion had a selective effect on the stability of Gpx1 and Gpx4 mRNAs: Se deficiency had no effect on the Gpx4 mRNA stability, but decreased the stability of the Gpx1 mRNA (and therefore the protein synthesis) (72). Vice versa, Gpx1 reacts very quickly to Se resupplementation in mice (73).

The ranking of glutathione peroxidase was made as follows (74):

Gpx2 > Gpx4 > Gpx3 = Gpx1

Although the ability of Gpx1 to protect against oxidative damage is well known, its exact role under normal conditions and in disease states has not been clearly defined. Recently, knock-out mouse models allowed to find out the function of the enzyme. The transgenic mouse studies confirmed the importance of Gpx1 and its role in oxidative stress. Mice with genetic inactivation of Gpx1 (knock out, $Gpx1^{-/-}$) were apparently healthy and fertile indicating a limited role of Gpx1 during normal development and under normal physiological conditions (75). In addition, with regard to the brain, they did not show a spontaneous neurological deficit. In contrast, $Gpx1^{-/-}$ mice are highly susceptible to elevated levels of oxidant stress. In particular, these mice are more vulnerable to brain ischemia and neurotoxins, implicating a role of Gpx1 in the protective mechanism against these challenges (76).

2.2 Microglia: immunocompetent cells of the brain

One of the basic principles of neurobiology is that glia cells provide metabolic, structural and trophic support to neurons. For instance, oligodendrocytes wrap axons and facilitate nerve impulse conduction. Astrocytes take up glutamate and protect neurons from excitotoxicity. Microglia form the first line of defence in emergency situations in the CNS. They are the resident immune cells of the central nervous system.

Microglia are ubiquitous in the CNS parenchyma and constitute approximately 5-20 % of the total glia population. In the normal uninjured adult brain, the cells are referred to as resting microglia to distinguish them from the activated or reactive microglia that are encountered after brain injury.

Microglia can be present at least in three identifiable states (Figure 3):

- Resting (ramified) microglia, as in the normal and non-pathological state.
- Activated (reactive) microglia, which occur in pathological states but are non-phagocytic.
- Phagocytic microglia that appear as rounded brain macrophages.

Figure 3 Morphology of microglia. From left to right, transformation of resting microglia into activated cells which in their end state resemble phagocytic cells. Adapted from Ref. (77).

2.2.1 Historical background

Prior to the twentieth century, the nervous system was considered to be composed of two types of cells: nerve cells (*first element*) and neuroglia (*second element*, interstitial cells of the nervous system).
When Cajal in 1913 introduced an improved staining technique for neuroglial cells, he recognized other non-nervous cells that were distinct from astrocytes. He called them the *third element* of the CNS.
Cajal´s third element received further classification into oligodendrocytes and microglia by del Rio Hortega, the Spanish neuroanatomist, who conducted the first systematic studies on this cell type (78). Microglia cells had been previously described by F. Nissl and W. Ford Robertson but del Rio-Hortega is rightly considered the "father" of the microglia. Many of his observations are still valid.
Microglia were viewed by del Rio Hortega as distinct from all other neuroglial cells, because his studies showed them to be of mesodermal origin and to have phagocytic capabilities. The origin of microglia has been a longstanding controversial issue, but it is now generally accepted that they are ontogenetically related to cells of the mononuclear phagocyte lineage, unlike all other cell types in the CNS (79).

2.2.2 Activation of microglia and secondary cell death

Activation of glia, a process termed reactive gliosis, has been observed in numerous pathological states of the central nervous system, like brain infections, neurodegenerative diseases, inflammation, ischemia and normal aging. A hallmark of such changes is the activation of microglia cells that produce neurotoxic factors such as cytokines, reactive oxygen intermediates and nitric oxide, which contribute to neuronal injury (Figure 4).

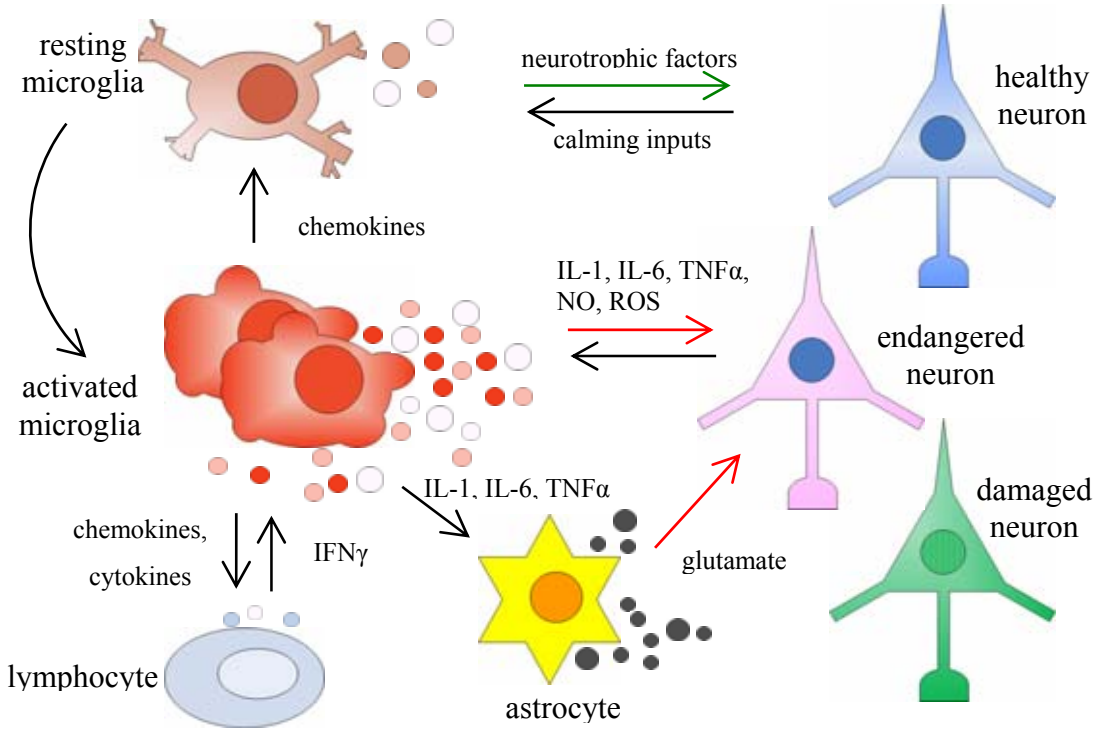


Figure 4 Scheme illustrating some major routes of communication between microglia and other resident or invading cells. Healthy neurons may inform microglia about their normal activity via calming inputs. Resting microglia or microglia at a low degree of activation (alerted microglia) could support neuronal function and survival by production of neurotrophic factors. Upon transformation due to a challenge by danger signals, activated microglia may not only attract leukocytes via chemokine synthesis, but also produce cytokines and other factors (NO, reactive oxygen intermediates) with potential toxicity for neurons and activating or aggravating potential for astrocyte involvement and further microglial recruitment. Modified from Ref. (80).

Microglia become activated in response to even minor pathological changes in the CNS or alterations in their microenvironment. For that reason G.W. Kreutzberg defined them as "a sensor for pathological events in the CNS" (77).

Glial activation is thought to be protective via destruction of pathogens, removal of debris, and promotion of tissue repair; however, excess activation can be deleterious. Numerous research groups showed that inflamed glia have the capacity to kill co-cultured neurons (in vitro) and during pathological state of the CNS (in vivo). The mechanisms have been suggested to include the release of NO, reactive oxygen species, glutamate and cytokines.

Bal-Price and Brown showed that nitric oxide produced by activated microglia inhibits neuronal mitochondrial respiration that might contribute to neuronal cell death by massive influx of Ca^{2+} caused from glutamate release in response to the

inhibition of neuronal respiration. They concluded that inhibitors of iNOS could completely prevent necrotic cell death caused by activated glia (81).

Brain damage, such as in excitotoxicity and stroke, leads to primary neuronal destruction which is potentiated by late-occurring secondary cell death response. This secondary neuronal cell death is mediated primarily by activated microglial cells as described in Figure 5.

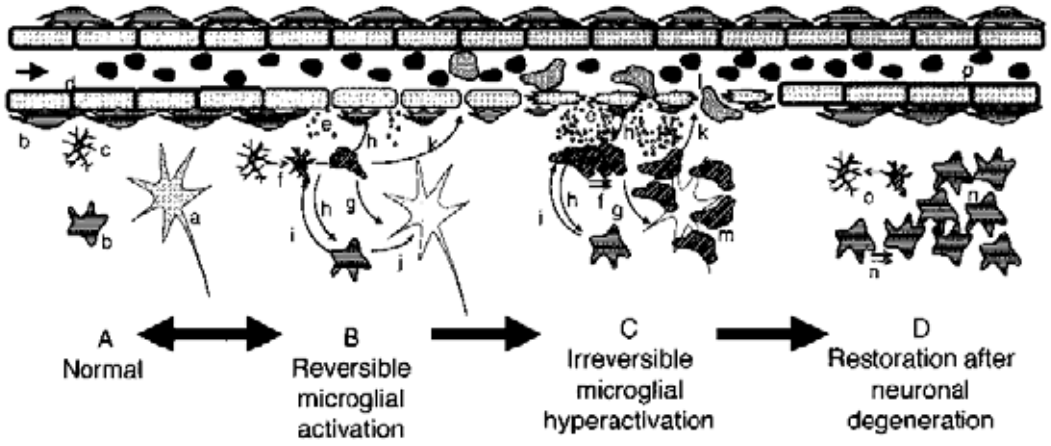


Figure 5 Mechanism for neuronal degeneration by microglial activation (adopted from Nakamura (82)). Stage A—Normal—a: Neurons. b: Astrocytes play a role in the maintenance of blood-brain barrier (BBB) impermeability and in the functions of neurotransmitter transport and metabolism around synaptic sites. c: Microglia (in the so-called ramified shape) possess many fine multiple-branched processes in the normal state *in situ.* d: Endothelial cells construct the BBB forming tight junctions with each other. Stage B—Reversible microglial activation—e: Leaking of serum factors promotes microglial activation. f: Microglia start to proliferate, changing their morphology into an amoeboid shape. g: Activated microglia produce substances causing neuronal injury, such as TNF a, NO, and O_2-. h: Activated microglia also produce various cytokines, such as IL-1ß and IL-6, affecting astrocyte functions. i: Astrocytes produce various factors, such as MIP1α. j: Astrocytes produce neuroprotective factors such as NGF. k: A microglial cytokine, such as TNF- a, affects endothelial cells, causing impairment of the BBB. Stage C—Irreversible microglial hyperactivation—Responses e and k in stage B become significant in a synergistic manner; consequently responses f, g, and h are no longer controlled by physiological regulation. l: Invasion of blood cells occurs through breakdown of the BBB. m: Activated microglia and invading monocytes (macrophages) injure neurons. Stage D—Restoration after neuronal degeneration—n: Astrocytes proliferate to occupy the postneuronal space (gliosis or glial scarring). o: Microglial activation is terminated and the morphology returns to the ramified type. p: Capillary endothelium and surrounding astrocytes are repaired and BBB functions are restored.

In ischemia, microglial activation was detected shortly after the ischemic insult and preceded histologically detectable neuronal damage in the hippocampus (83),

suggesting that the microglial activation is not only a response to the neuronal damage, but also a cause of neuronal damage.

2.2.3 Microglia and diseases

The activation of microglia is an important event which occurs in many degenerative diseases, especially in Alzheimer's disease, Parkinson's disease and prion diseases.

2.2.3.1 Microglia activation in Alzheimer's disease (AD)

Several lines of evidence indicate that microglial activation may be involved in the pathogenesis of Alzheimer's disease (AD). Alzheimer's disease leads to a progressive deterioration of cognitive function with loss of memory; it is characterized by extracellular plaques of amyloid-ß (Aß) peptide aggregates and intracellular neurofibrillary tangles. The involvement of microglia in the progression of AD and other age-related neurodegenerative conditions was suggested in 1989 by Griffin WST and collaborators (84). They proposed a glial cytokine-mediated immunological process, with overexpression of cytokines that would result in Alzheimer-type neuropathological changes. Since then many studies have followed the idea that neurodegeneration in AD is mediated in part by a neuroinflammatory response of microglial cells to Aß protein (85). Microglia may lead to cellular damage not only via the production of cytokines, but also via the generation of ROS products, as was demonstrated in studies where oxidative stress generated by microglia was responsible for cellular injury (86). This has led to clinical trials with nonsteroidal anti-inflammatory drugs and with scavenger agents for ROS. The local inflammatory response and microglial activation in AD has become the focus of a therapeutic approach towards AD. However, microglia have neuropathic as well as neuroprotective actions. Recently another therapeutic strategy (immune stimulation) has received more attention: the idea is that vaccination with Aß peptides will cause microglia to more efficiently phagocitose amyloid deposits removing what it is thought by many to be the direct cause of neurodegeneration (87-89).

Treatment with either anti-inflammatory drugs (reduction of the inflammatory process) or immunization (stimulation of the inflammatory process) might be both

helpful. It is still uncertain whether the neuroprotective or the neurotoxic properties of microglial activation observed in AD are of more significance.

2.2.3.2 Microglia activation in Parkinson's disease (PD)

PD is characterized by a progressive and selective destruction of the nigrostriatal dopaminergic system that is important in the regulation of body movements. The detection of elevated levels of proinflammatory cytokines and evidence of oxidative stress-mediated damage in post-mortem PD brains strongly suggested the involvement of microglia in the degenerative process. In the substantia nigra (SN) region the dopaminergic degeneration is most prominent; this region is particularly rich in microglia and it is known that the dopaminergic neurons in the SN have a reduced antioxidant capacity, rendering them more vulnerable to oxidative stress. Therefore the activation of microglia represents a risk factor that may trigger the cascade of events leading to a progressive degeneration of dopaminergic neurons.

2.3 Oxidative stress and neurodegenerative diseases

All aerobic organisms are susceptible to oxidative stress simply because reactive oxygen species (ROS) are produced by mitochondria during respiration. Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them (90).

ROS are defined as molecular entities that react with cellular components, resulting in detrimental effects on their function. ROS include free radicals (containing highly reactive unpaired electrons), such as superoxide ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$) and hydroxyl radical ($OH^{\cdot}$), and other molecular species, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$).

All these species are redox-active and can interact with cellular components nearby, such as proteins, lipids and DNA. The oxidation of proteins can have damaging effects, such as the disruption of the active site of enzymes or disturbance in the conformation of structural proteins. Fortunately, organisms have molecules at their disposal, referred to as antioxidants, which help them to resist oxidation.

The brain is considered particularly sensitive to oxidative damage for many reasons, e.g. it has a high content of easily peroxidisable fatty acids; the brain requires very high amounts of oxygen per weight unit (about 20 % of the total amount used in humans); and it is not very rich in antioxidant protective defences (91).

The CNS is highly susceptible to damage by a variety of biological agents. Furthermore neuronal cells do not renew themselves so their gradual reduction throughout a lifetime is inevitable. This includes virtually all CNS functions.

Oxidative stress is involved in many neurological diseases and processes, such as Alzheimer's disease, amyotrophic lateral sclerosis, autoimmunodeficiency syndrome (HIV infection), Down's syndrome, epileptic seizures, head trauma, inflammation, ischemia/reperfusion, Parkinson's disease, schizophrenia, viral infection etc. (92).