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REVIEW ARTICLE

The Role of Macrophages in Wallerian Degeneration

Wolfgang Brück, M.D.

Department of Neuropathology, University of Göttingen, Germany

The present review focuses on macrophage properties in Wallerian degeneration. The identification of hematogenous phagocytes, the involvement of cell surface receptors and soluble factors, the state of activation during myelin removal and the signals and factors leading to macrophage recruitment into degenerating peripheral nerves after nerve transection are reviewed. The main effector cells in Wallerian degeneration are hematogenous phagocytes. Resident macrophages and Schwann cells play a minor role in myelin removal. The macrophage complement receptor type 3 is the main surface receptor involved in myelin recognition and uptake. The signals leading to macrophage recruitment are heterogenous and not yet defined in detail. Degenerating myelin and axons are suggested to participate. The relevance of these findings for immune-mediated demyelination are discussed since the definition of the role of macrophages might lead to a better understanding of the pathogenesis of demyelination.

Introduction

The macrophage represents the most differentiated cell of the mononuclear phagocyte system. This system includes monoblasts and promonocytes in the bone marrow, peripheral blood monocytes, as well as tissue macrophages, all of which derive from a myeloid stem cell in the bone marrow where they differentiate to monocytes upon the effects of different growth factors (3,115). Monocytes leave the bone marrow into the peripheral blood where they circulate for 60 to 70 hours representing approximately 5-20% of the circulating leukocytes. They are the largest cells of the peripheral blood with a diameter of 15-20 µm. As a reaction to different stimuli, monocytes migrate into various organs or tissue systems and finally differentiate to resident tissue macrophages. The resident macrophage population does not represent a constant population, but rather is permanently exchanged by newly recruited blood monocytes, except microglial cells, which form a stable cell population in the central nervous system (61). Only a minor proportion of the tissue macrophages regenerates by cell division.

The central function of mononuclear phagocytes is the stimulation and regulation of immunoreactions as well as cytotoxic activity (81). Their most evident function is phagocytosis (31). Macrophages possess a range of different surface receptors which allow them to interact with numerous partners (46). Furthermore, they possess an extensive secretory activity including the secretion of toxic oxygen radicals, cytokines, enzymes and arachidonic acid metabolites (90). Another central function of these cells is the presentation of antigens. They also serve as immunosuppressive cells and stimulate regeneration and repair in the nervous system by the induction of trophic factors (e.g., nerve growth factor [NGF]) (16,93).

Wallerian degeneration is one of the most elementary and common reactions of the nervous system which occurs when the continuity of a nerve fiber is interrupted through traumatic, toxic, degenerative, ischemic or metabolic events (Table 1). The phenomena occurring after transection of a nerve fiber were first described by Augustus Waller (123). Wallerian degeneration concerns the axon, the myelin sheath and the myelin-forming cell, e.g., the Schwann cell in the peripheral nervous system (51). Axonal structures break down, and the nerve fiber loses its capacity to conduct action potentials. The Schwann cells proliferate as early as the first week after nerve transection and form the so-called Büngner bands which induce regeneration by connecting the dissected nerve stumps (51). The myelin sheaths break down into the characteristic myelin ovoids (Fig. 1a and b). The Schwann cell actively retracts the cytoplasm from the myelin sheath (10,51) and downregulates the mRNA synthesis for myelin proteins (112).

The identification of the myelin-removing cell during Wallerian degeneration has long been a matter of debate. Many different cell types have been

Corresponding author:
Dr. Wolfgang Brück, Institut für Neuropathologie, Georg-August-Universität, Robert-Koch-Str. 40, 37075 Göttingen, Germany; Tel.: x49-551-396622; Fax: x49-551-398472; E-mail: wbrueck@med.uni-goettingen.de

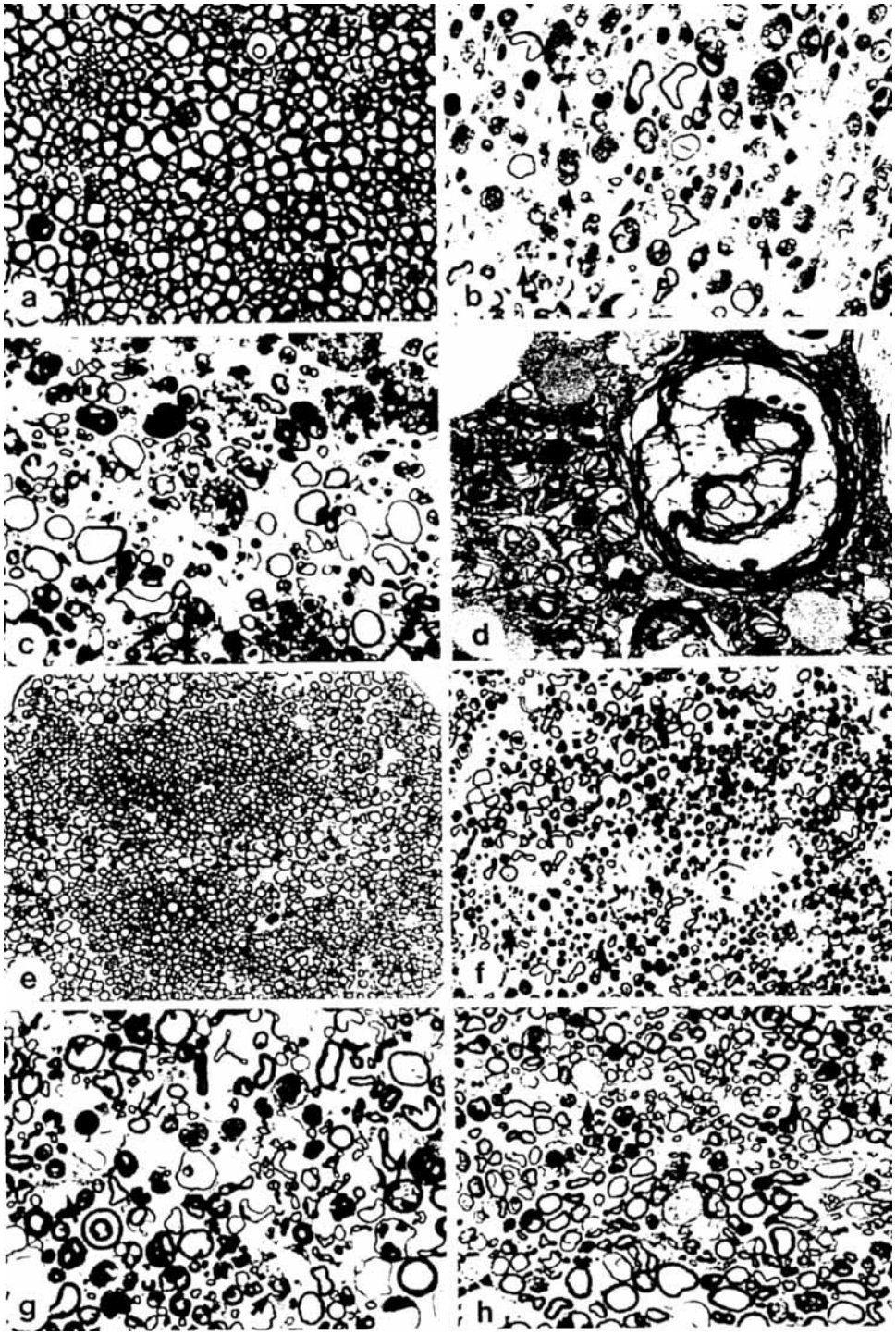

Figure 1. A. Non-transsected sciatic nerve with intact myelin sheaths and axons. **B.** Wallerian degeneration *in vivo*. Sciatic nerve 6 days after transection shows myelin loss and many myelin-removing macrophages (arrows). **C.** Wallerian degeneration *in vitro*. The cultured nerve segment is infiltrated by numerous macrophages which ingest the degenerating myelin sheaths. **D.** Ultrastructurally, the invading macrophages contain myelin debris in their cytoplasm. **E.** Wallerian degeneration in C57Bl/Ola mice. Myelin and axons are intact and there are no invading macrophages 6 days after transection. **F.** Treatment with C12MDP-containing liposomes. There are many preserved myelin sheaths and only few invading macrophages (arrows) after nerve transection. **G.** Treatment with anti-CR3 antibody *in vitro*. The myelin-removing capacity of the invading macrophages (arrows) is completely abolished. **H.** Wallerian degeneration *in vitro*. Nerves and macrophages cultured in the presence of C3-deficient serum. The invading phagocytes (arrows) do not ingest myelin.

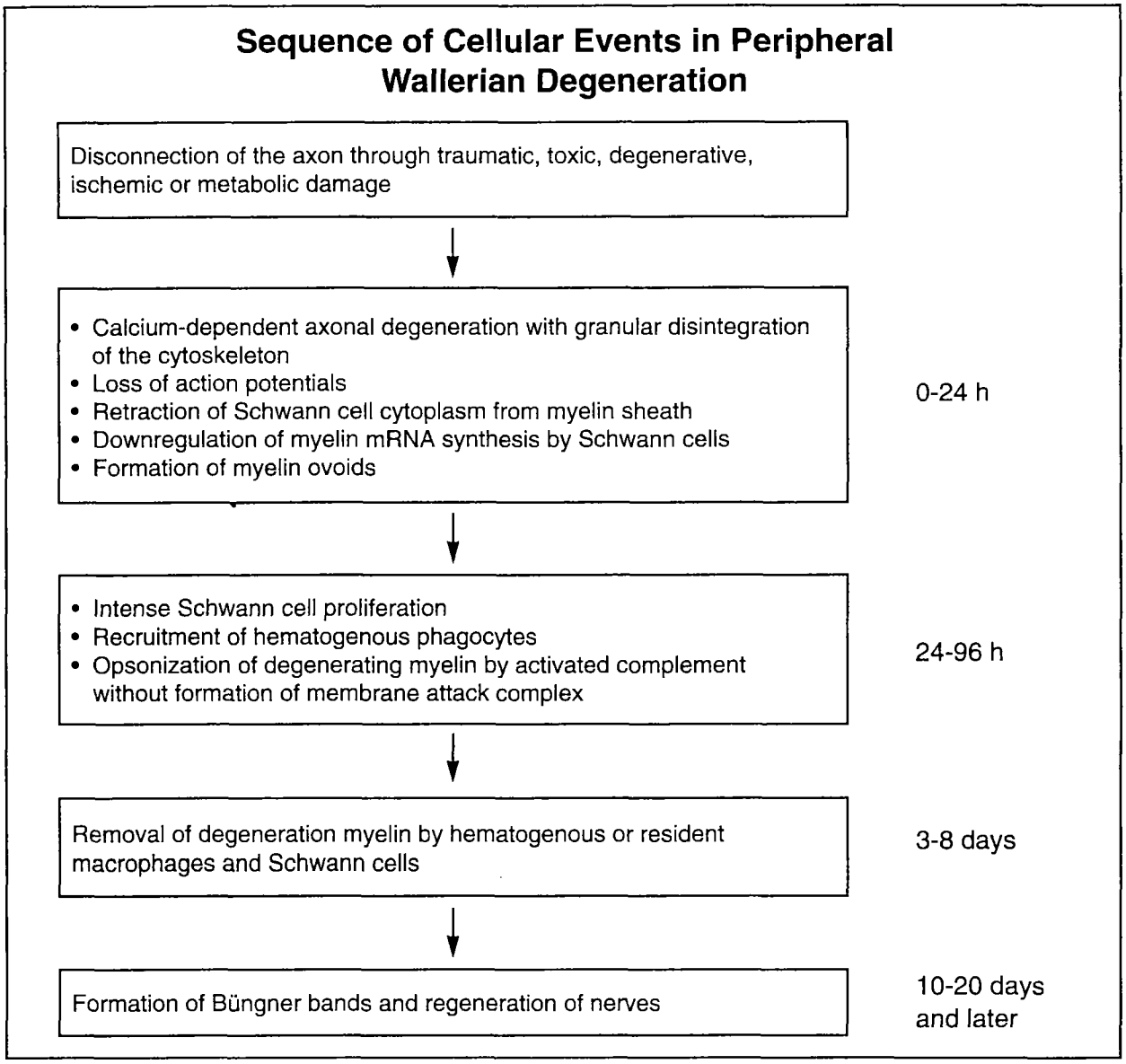

Table 1.

suggested to be responsible for myelin removal in this process: Schwann cells, resident or hematogenous macrophages, endoneurial fibroblasts or mesenchymal cells (103). Different experimental approaches have been undertaken to clarify this question. Using Millipore diffusion chambers Beuche and Friede (10) identified non-resident cells of the mononuclear phagocyte system as the main effector cells in myelin phagocytosis during Wallerian degeneration. This hypothesis was confirmed in a series of follow-up experiments. Immunocytochemistry identified the myelin-removing cells as Mac-1- and Fc-receptor positive (12,101,102). The establishment of a cell culture model allowed a detailed investigation of the signals and events which lead to macrophage invasion into degenerating nerve as well as of the macrophage properties during myelin removal (32,54) (Figs. 1c and d). Schwann cells were also shown to possess myelin phagocytosing capacities (13,36,86,107).

The present study aims at reviewing experiments which have been performed to identify cellular and soluble factors involved in myelin removal to define the various activation and differentiation properties of macrophages during myelin digestion as well as to describe the different chemotactic signals that are involved in Wallerian degeneration. The knowledge of these processes may lead to better understanding of the pathogenesis of segmental demyelination in the peripheral and central nervous

system in which mononuclear phagocytes are also essentially involved.

The Identification of the Myelin-Removing Cell during Wallerian Degeneration

The role of hematogenous cells during Wallerian degeneration *in vivo* has been studied using different experimental approaches. The best indication for an involvement of this cell type is probably provided by the mouse mutant C57Bl/Ola. In this mouse strain there is a very sparse and retarded macrophage invasion after nerve transection without any indication of myelin removal (84) (Fig. 1e) compared to wild type mice in which numerous myelin-removing phagocytes are identified (Figs. 1b and d). Antibodies to the macrophage complement receptor type 3 (CR3) as well as a non-specific depletion of macrophages by silica dust also caused a reduction of myelin removal which was due to a decreased cell invasion (12,84,89).

These *in vivo* experiments were hampered by the fact that the circulating monocyte population could not be selectively eliminated. The intravenous injection of dichlormethylene diphosphonate (Cl_2MDP)-containing liposomes allows a selective and temporary elimination of macrophages from the spleen and liver as well as of monocytes from the systemic circulation (65,116,117,118). This selective monocyte depletion technique was used to clarify the role of hematogenous cells during Wallerian degeneration (24). The depletion of macrophages from the peripheral blood by Cl_2MDP-containing liposomes caused a significantly retarded myelin removal (Figs. 1b and f) which was due to a significantly decreased cell invasion into the degenerating nerve stumps (24). Compared to untreated control animals, monocyte-depleted mice showed a certain degree of myelin digestion which suggests that a resident cell population within the peripheral nerves is also involved in myelin uptake. A delayed injection of Cl_2MDP-containing liposomes one or two days after nerve transection was significantly less effective in reducing macrophage influx. This means that the main part of the hematogenous cells invade the degenerating nerves during the first 24-48 hours after transection and that the already invaded cell population is not affected by the treatment. Injected liposomes do not seem to pass the blood-nerve barrier.

These investigations confirmed early *in vivo* experiments which suggested an important role of hematogenous cells during myelin removal (12,84). Similar observations were made during a radiation-induced macrophage reduction (96). In these experiments, two different phases of myelin removal were suggested, the first of which depends on Schwann cell activity and the second in which macrophages are involved. Earlier studies already implied that the macrophage population which participates in myelin removal is composed of resident and hematogenous cells (17). The resident macrophage population in the peripheral nerve compromises approximately 2-9% of all endoneurial cells, and this cell population undergoes a significant and rapid turnover (49,120).

Schwann cells are also involved in myelin removal (36,107). They were shown to proliferate intensively after nerve transection and to digest myelin ovoids (30,71,80). Schwann cells even express antigens during Wallerian degeneration which are usually almost exclusively found in tissue macrophages (5). They also seem to adapt a macrophage phenotype during Wallerian degeneration (98). In conclusion, newly recruited hematogenous macrophages play an essential role in myelin uptake during Wallerian degeneration forming the main myelin-removing cell population while under normal conditions the contribution of the resident cells is minor.

The Role of Macrophage Receptors and Soluble Components

Recognition and uptake of particles are important functions of macrophages. A range of different receptors are expressed on the macrophage cell surface (46). In phagocytosis two different basic mechanisms have to be distinguished. The opsonin-dependent phagocytosis is mediated via the Fc receptor or complement receptors of the macrophage. Opsonin-independent phagocytosis can be mediated by carbohydrate receptors (105).

The established *in vitro* model of Wallerian degeneration was used to investigate the role of carbohydrate-specific cell membrane receptors during the recognition and uptake of myelin (21). A range of different simple or complex carbohydrates as well as carbohydrate-splitting enzymes were tested for their capacity to interfere with myelin uptake by macrophages. Many of the investigated carbohydrates impaired the migration of macrophages into the degenerating nerve segments. A dramatic effect was seen when the cultures were exposed to the enzyme L-fucosidase. The myelin-removing capacity of macrophages was completely abolished whereas their capacity to phagocytose carbon or latex particles was unimpaired. Cell migration in these experiments was not disturbed (21).

The myelin sheath of the peripheral nerves possesses a high carbohydrate content within the myelin glycoproteins with which the L-fucosidase treatment could interfere. A competitive blockade of fucose receptors using fucose did not impair myelin removal. It thus remains unresolved whether the effect of L-fucosidase can be attributed to the blockade of a carbohydrate-dependent opsonin-independent myelin removal (21). Another possibility might be that L-fucosidase impairs other recognition

Summary of Macrophage Functions in Wallerian Degeneration

Invasion	• Expression of adhesion molecules (Mac-1, LFA-1[12,101]) (migration is blocked by the 5C6 antibody directed to the macrophage CR3[84]) • Interaction with ICAM-1 expressed on endothelial cells[27] • Reaction to chemotactic stimuli arising from degenerating axonal and myelin structures or from serum complement (fails in C57Bl/Ola mice or in the absence of serum complement[19,23,50,53,84])
Phagocytosis	• Receptor-mediated phagocytosis of opsonized myelin via the macrophage CR3[17,22,23,25] (phagocytosis is blocked by anti-CR3 antibody and in the absence of complement) • Participation in opsonization by synthesis of complement components[23] (immunocytochemical evidence) • No involvement of the macrophage Fc receptor (depletion of immunoglobulins or anti-FcR antibody does not affect myelin removal[22,55])
Secretion	• Interleukin-6[97] • Phospholipase A[92] • Apolipoprotein E[44,89] • Lysozym[121] • TNF-α[109] • No induction of a respiratory burst[20]
Regeneration	• Induction of trophic factors (e.g., NGF[16,93]) (impaired regeneration in C57Bl/Ola mice with disturbed macrophage recruitment)

Table 2.

mechanisms. There is a fucose-containing surface glycoprotein which regulates the function of the macrophage complement receptor (48). The lymphokine which activates the macrophage complement receptor type 3 (CR3) for phagocytosis binds to a fucose-containing glycoprotein. Pretreatment of macrophages with L-fucosidase prevented the CR3-dependent phagocytosis. The selective blockade of myelin uptake by L-fucosidase may be due to a specific interaction of this enzyme with a cell membrane receptor.

Further experiments elucidated the essential role of CR3 in myelin removal. The macrophage Fc receptor does not seem to play a role in myelin removal since Wallerian degeneration was not impaired in the absence of immunoglobulins (55). The macrophage CR3 is built of two non-covalently linked subunits with molecular weights of the α chain of 160 kDa and the ß chain of 95 kDa (78). The α subunit is also designated as CD11b, the ß subunit as CD18. The antibody Mac-1 recognizes selectively the α chain (CD11b) of CR3 from humans and mice (9). The main function of the CR3 is the uptake of particles which are opsonized by complement components, especially by the component C3 (75).

Cocultures of macrophages and degenerating nerve segments were treated with the antibody Mac-1. Numerous macrophages invaded the degenerating nerves in these experiments (Fig. 1g). These cells revealed many fat droplets in their cytoplasm, uptake of myelin, however, was not observed (22). Further experiments proved that these cells are non-resident and invaded the nerves. Similar results were obtained when cobra venom factor was used to scavenge the complement components within the medium or when the nerves and macrophages were cultured in C3-deficient serum (23) (Fig. 1h).

In vivo experiments confirmed the important role of the CR3 in myelin removal (23). The application of the antibody Mac-1 after nerve transection caused a significant reduction of myelin phagocytosis. The 5C6 antibody which recognizes a different epitope of the macrophage CR3 caused a significant inhibition of cell migration in Wallerian degeneration (84). The complement component C3 was detected at the surface of degenerating myelin sheaths by immunoelectronmicroscopy, indicating that myelin is opsonized by complement during Wallerian degeneration. Comparable results were obtained when the translation of the α or ß chain of the CR3 was

blocked by antisense oligonucleotides (25). Myelin removal by macrophages was significantly impaired in these experiments.

The question arises as to how complement is activated under these circumstances. Different experiments showed that isolated myelin initiates an activation of the alternative as well as the classic pathway of complement (73,74,119). Anti-myelin antibodies were also shown to cause complement activation (72). Besides the complement component C3 antibodies to myelin proteins and normal serum are capable of opsonizing myelin and thus facilitate uptake by macrophages (43,113). In conclusion, complement components play an important role in the opsonization of myelin and the macrophage CR3 is essentially involved in myelin uptake. The functional role of antibodies during myelin opsonization and complement activation is not yet clarified in detail.

Macrophages possess a range of different immunological functions besides phagocytosis. These include the secretion of proteins, enzymes and cytokines (90). A series of experiments aimed at clarifying the effects of two important macrophage-derived cytokines, tumor necrosis factor α (TNF-α) and interleukin-1 (17). The treatment of macrophages and degenerating myelin sheaths with TNF-α caused a significant reduction of myelin uptake. This effect was reversible by utilizing anti-TNF-α antibodies. Immunofluorescence studies revealed a significant reduction of CR3 expression by macrophages which was probably the cause of the reduced myelin phagocytosis.

The described effects of TNF-α are highly variable. A known property is the modulation of surface molecules as observed in the present experiments (28,63). Immunocytochemically, TNF production by macrophages in the *in vitro* experiments was not detectable whereas in the *in vivo* situation TNF-positive macrophages were recognized (109). An effect on Schwann cells by TNF-α was not observed as has already been described by others (87). This is in contrast to the central nervous system in which TNF-α leads to selective oligodendrocyte damage (104). In summary, a modulation of surface receptors may affect the process of myelin removal.

Macrophage Activation

Macrophages possess a range of different functions during immunoregulation which include the secretion of many mediators. Macrophages themselves are targets of such mediators and are able to perform different effector functions after stimulation, one of these being the secretion of oxygen radicals. One of the most potent activators of macrophages is interferon-γ which activates a broad spectrum of macrophage functions (100) and also has the greatest potency to stimulate oxygen radical production compared to other cytokines (88,91).

In a series of experiments the effect of recombinant interferon-γ on oxygen radical production, macrophage migration and myelin removal was analyzed (20). The oxygen radicals produced were measured by luminol-dependent chemoluminescence (1). The experiments showed that cultivated macrophages can be activated to produce oxygen radicals at any time of the culture period. When macrophages were activated with interferon-γ at the start of the experiments, a complete inhibition of macrophage migration was observed. The application of interferon-γ at the later stage of the experiments had no effect on myelin removal although oxygen radical production was induced. Myelin phagocytosis itself did not induce chemoluminescence. These experiments show that myelin removal by macrophages occurs independent of oxygen radical production and that a macrophage activation by interferon-γ does not lead to an increased myelin uptake. This observation is of high significance since the uptake of particles via the macrophage CR3 is known to happen independent of an oxygen radical production, thus supporting the hypothesis of an important role of the macrophage CR3 in myelin removal (124).

Oxygen radicals do not seem to be involved in myelin removal during Wallerian degeneration. There are, however, other substances which are produced by macrophages under these conditions. These include interleukin-6, phospholipase A, apolipoprotein E and lysozym, which is recognized as a marker for active phagocytosis (44,89,92,97,121). Furthermore, macrophages show an increased expression of their scavenger receptor (8).

Macrophage Recruitment

The mechanisms and signals leading to migration of non-resident cells into degenerating nerves have not yet been clarified in detail. Concerning these phenomena, the mouse mutant C57Bl/Ola or C57Bl/WLD is of extraordinary interest. This mouse strain shows a very sparse and retarded macrophage invasion after nerve transection which is independent of age (33,82,84,94). Axons remain intact in the distal nerve stumps and the myelin sheaths do not show any signs of degeneration (Fig. 1e). The time course of Wallerian degeneration in wild-type C57Bl mice has been characterized in a range of experiments (10,20,22,23) (Figs. 1a and b). A comparative analysis of the behavior of degenerating C57Bl and C57Bl/Ola nerves in the presence or absence of non-resident cells aimed at identifying chemotactic signals which induce macrophage recruitment (19). Peripheral nerves of C57Bl/Ola mice did not show any signs of axonal or myelin degeneration after nerve transection *in vivo* and macrophage invasion was very sparse compared to C57Bl nerves (Figs. 1b and c). The characteristic proliferation of Schwann cells seen in wild

type mice after transection was lacking in C57Bl/Ola nerves. Surprisingly, the obvious differences between both mice strains were not evident under cell culture conditions (19). The properties of both nerves could not be distinguished. Axonal as well as myelin degeneration combined with a massive macrophage invasion was observed when culturing nerves and macrophages of both mice strains.

A range of mediators such as Schwann cells, myelin or serum components were suggested to have chemotactic properties during Wallerian degeneration. The lack of macrophage recruitment in C57Bl/Ola mice is due to an intrinsic property of the nerves and not due to a defect of circulating macrophages (95). The gene responsible for the delayed Wallerian degeneration in these mice was recently localized on mouse chromosome 4 (85). Serum components are involved in macrophage recruitment. In the absence of the serum complement component C3 no macrophages invaded degenerating nerves (23). Antibodies to the macrophage CR3 reduced cell recruitment as well as myelin uptake by macrophages (23,84). The expression of adhesion molecules on macrophages as well as on endoneurial or perineurial endothelial cells also seems to be involved in the process of macrophage invasion (own observations). Recent observations demonstrate an upregulation of ICAM-1 and VCAM-1 expression on endothelial cells in the distal transected nerve stump (27). Degenerating myelin is another factor which participates in the induction of macrophage migration into degenerating nerves (50). Its role in cell recruitment was suggested to succeed that of axoplasmic degeneration because non-myelinated nerve fibers show a reduced cell infiltration compared to myelinated nerve fibers. This hypothesis is supported by experiments in C57Bl/Ola nerves in which a demyelination was induced by the injection of lysophosphatidylcholine. In these experiments a massive macrophage recruitment was observed while the axons remained intact (53).

The most remarkable observation in our experiments was the axonal and myelin degeneration of cultured C57Bl/Ola nerves in the *in vitro* model of Wallerian degeneration. This is in contrast to earlier *in vitro* experiments in which axonal degeneration was shown to be absent in these nerves (95). Axonal degeneration was even detectable in the absence of non-resident macrophages. This means that the disintegration of axonal structures is not induced by invading cells. This hypothesis was confirmed in recent experiments in which axonal degeneration was shown to be a calcium-dependent intrinsic axonal process (42). The sequence of events after axotomy involves a calcium influx into the axon followed by an activation of calcium-dependent effector molecules which degrade the axonal cytoskeleton (39). This observation is of striking importance since the role of macrophage invasion and axonal degeneration for nerve regeneration are still a matter of debate (14,15,16,29). An efficient degeneration seems to be a prerequisite for a successful regeneration (111).

Earlier experiments suggested that the lack of cell recruitment in C57Bl/Ola mice contributes to the delayed axonal degeneration (84). Recent experiments in contrast confirmed that the delayed axonal degeneration is an intrinsic property of the axon and does not depend on Schwann cells or invading phagocytes (41). In summary, the stable Schwann cell-myelin-axon unit does not seem to release chemotactic signals for macrophages. A disturbance in one or more components of this unit is sufficient to induce macrophage recruitment into the peripheral nervous system. Soluble factors participate in the induction of hematogenous cell invasion into degenerating nerves. In contrast to the peripheral nervous system, Wallerian degeneration in the central nervous system occurs significantly slower (40,50). There is a profound difference in the macrophage response between the peripheral and central nervous systems (4). The reasons for these differences have not yet been clarified. Resident factors within the injured central nervous system seem to inhibit macrophage migration (62). The lack of adhesion molecule expression on CNS endothelial cells may also contribute to the delayed macrophage invasion (27). There is no doubt that hematogenous macrophages form the cell pool which is responsible for myelin removal in the central nervous system (110).

Relevance for Immune-Mediated Demyelination

Mononuclear cells of the monocyte/macrophage system are also essentially involved in the effector phase of inflammatory demyelinating diseases of the central and peripheral nervous systems (7,26,34,52,108). Depletion experiments using Cl_2MDP-containing liposomes have been clarifying their role in experimental allergic neuritis and experimental allergic encephalomyelitis, which are animal models for Guillain-Barré syndrome and multiple sclerosis (66,68).

The macrophage CR3 plays an extraordinary role in the adhesion and the recruitment of inflammatory cells. An anti-CR3 antibody was shown to inhibit cell migration during inflammatory reactions (99). Complement components, as well as CR3, are also important in myelin uptake during immune-mediated demyelination. Depletion of complement during experimental allergic neuritis led to a significant reduction of cell invasion as well as demyelination and suppressed the development of clinical symptoms (35,122).

Complement components are also essentially involved in experimental allergic encephalomyelitis.

Complement depletion by cobra venom factor reduced demyelination (79). Antibodies to different epitopes of the rat CR3 were also capable of suppressing the development of experimental allergic encephalomyelitis (45,64). The complement component C3 was detected in multiple sclerosis plaques as well as in peripheral neuropathies (60,83). All these investigations confirm the role of complement component and of the macrophage CR3 during myelin removal.

There is an intense interaction of macrophages and T-lymphocytes in immune-mediated demyelination resulting in completely different patterns of macrophage activation when compared to Wallerian degeneration (59). Cytokines, such as TNF-α, oxygen radicals or arachidonic acid metabolites, are important factors involved in the pathogenesis of these diseases (56,57,58,108,109).

The interaction of lymphocytes, soluble cytokines and macrophages was studied in an *in vitro* model of xenogeneic peripheral nerve rejection (18). There was a massive tissue rejection observed when degenerating rat nerve segments were cocultivated with a mouse macrophage population which has been sensitized to rat peripheral nerve. The tissue rejection was clearly distinguishable from the basic myelin phagocytosis during Wallerian degeneration. The sensitized population was composed of T-cells and macrophages. Depletion experiments revealed a dependence of the rejection from the presence of T-lymphocytes in the culture medium. Antibodies against a range of cytokines including IL-2, IL-3, IL-4, IL-6 and interferon-γ identified these cytokines as the main effector molecules during rejection. Antibodies to the macrophage CR3 and to class II major histocompatibility antigens also blocked the tissue rejection.

These investigations confirmed previous experiments which showed that mouse peritoneal macrophages do not induce allogenic nerve rejection in the absence of the lymphocytes (11). The *in vitro* model used in the experiments allowed a detailed analysis of the interactions between T-lymphocytes and macrophages. Different *in vivo* models also revealed lymphocytes and macrophages as the main components of the inflammatory infiltrate during the rejection of transplanted peripheral or central nervous tissue (2,37,38). Macrophages are the main effector cells during rejection (77). Their effector functions are induced by a range of mediators (69). The cytokines which have been shown to be essential in the *in vitro* model have also been identified as important mediators during acute graft-versus-host reaction as well as during autoimmune demyelination (6,67,70,106,114).

The interaction between macrophages and T-lymphocytes is regulated by a network of cytokines which induce different effector functions of macrophages. These mediators may induce an increased function of the macrophage CR3 which is essentially involved in tissue rejection (47). There are similarities to immune-mediated demyelination because in both models there is an intense interaction between T-lymphocytes and macrophages which is regulated through a panel of mediators (56,59). A permanent antigen presentation is necessary to support the immunological effector phase during tissue rejection and during autoimmune demyelination (76). This model allows detailed analysis of the interaction between T-cells and macrophages in a defined *in vitro* system.

Conclusions

Mononuclear cells of the monocyte/macrophage system are the main effector cells in myelin removal during Wallerian degeneration (Table 2). A range of different factors participates in this complex process. Intrinsic properties of the peripheral nerve as well as systemic factors are involved. The role of hematogenous monocytes could clearly be demonstrated by using the liposome depletion technique. Myelin recognition and uptake is dependent on the macrophage complement receptor type 3 as well as on serum complement components. The process of Wallerian degeneration can be modulated by different factors such as cytokines. In more complex immunological situations such as tissue rejection, there is an intense interaction between macrophages and lymphocytes which is regulated by a range of soluble factors. Knowledge of macrophage properties in Wallerian degeneration may provide insights into the pathogenesis of demyelinating disease processes in which these cells are massively engaged.

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References

1. Allen RC, Loose LD (1976) Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. *Biochem Biophys Res Comm* 69: 245-252
2. Ansselin AD, Pollard JD (1990) Immunopathological factors in peripheral nerve allograft rejection: quantification of lymphocyte invasion and major histocompatibility complex expression. *J Neurol Sci* 96: 75-88
3. Auger MJ, Ross JA (1992) The biology of the macrophage. In: *The Macrophage*, Lewis CE, McGee JO (eds.), pp. 1-74, Oxford University Press: Oxford, England.
4. Avellino AM, Hart D, Dailey AT, MacKinnon M, Ellegala D, Kliot M (1995) Differential macrophage responses in the

peripheral and central nervous system during Wallerian degeneration of axons. *Exp Neurol* 136: 183-198

5. Azzarelli B, Woodburn R, Olivelle S, Kimbro S, Siakotos A, Taylor M, Lee C-H, Yen M, Paulsrud J (1993) The A-1 antigen: a novel marker in experimental peripheral nerve injury. *J Comp Neurol* 337: 353-365
6. Baker D, O'Neill JK, Turk JL (1991) Cytokines in the central nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. *Cell Immunol* 134: 505-510
7. Bauer J, Ruuls SR, Huitinga I, Dijkstra CD (1996) The role of macrophage subpopulations in autoimmune disease of the central nervous system. *Histochem J* 28: 83-97
8. Bell MD, Lopez-Gonzalez R, Lawson L, Hughes D, Fraser I, Gordon S, Perry VH (1994) Upregulation of the macrophage scavenger receptor in response to different forms of injury in the CNS. *J Neurocytol* 23: 605-613
9. Beller DI, Springer TA, Schreiber RD (1982) Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J Exp Med* 156: 1000-1009
10. Beuche W, Friede RL (1984) The role of non-resident cells in Wallerian degeneration. *J Neurocytol* 13: 767-796
11. Beuche W, Friede RL (1985) Millipore diffusion chambers allow dissociation of myelin phagocytosis by non-resident cells and of allogenic nerve graft rejection. *J Neurol Sci* 69: 231-246
12. Beuche W, Friede RL (1986) Myelin phagocytosis in Wallerian degeneration of peripheral nerves depends on silica-sensitive, bg/bg-negative and Fc-positive monocytes. *Brain Res* 378: 97-106
13. Bigbee JW, Yoshino JE, De Vries GH (1987) Morphological and proliferative responses of cultured Schwann cells following rapid phagocytosis of a myelin-enriched fraction. *J Neurocytol* 16: 487-496
14. Bisby MA, Chen S (1990) Delayed Wallerian degeneration in sciatic nerves of C57BL/Ola mice is associated with impaired regeneration of sensory axons. *Brain Res* 530: 117-120
15. Brown MC, Perry VH, Hunt SP, Lapper SR (1994) Further studies on motor and sensory nerve regeneration in mice with delayed Wallerian degeneration. *Eur J Neurosci* 6: 420-428
16. Brown MC, Perry VH, Lunn ER, Gordon S, Heumann R (1991) Macrophage dependence of peripheral sensory nerve regeneration: possible involvement of nerve growth factor. *Neuron* 6: 359-370
17. Brück W, Brück Y, Friede RL (1992) TNF-α suppresses CR3-mediated myelin removal by macrophages. *J Neuroimmunol* 38: 9-18
18. Brück W, Brück Y, Maruschak B, Friede RL (1994) Macrophages properties during peripheral nervous tissue rejection *in vitro*. *J Neuropathol Exp Neurol* 53: 51-60
19. Brück W, Brück Y, Maruschak B, Friede RL (1995) Mechanisms of macrophage recruitment in Wallerian degeneration. *Acta Neuropathol* (Berl) 89: 363-367
20. Brück W, Friede RL (1989) Activation of macrophages by recombinant interferon-gamma has no effect on myelin phagocytosis but hinders invasion of nerves in organ culture. *J Neuroimmunol* 25: 47-55
21. Brück W, Friede RL (1990) L-Fucosidase treatment blocks myelin phagocytosis by macrophages *in vitro*. *J Neuroimmunol* 27: 217-227
22. Brück W, Friede RL (1990) Anti-macrophage CR3 antibody blocks myelin phagocytosis by macrophages *in vitro*. *Acta Neuropathol* (Berl) 80: 415-418
23. Brück W, Friede RL (1991) The role of complement in myelin phagocytosis during PNS wallerian degeneration. *J Neurol Sci* 103: 182-187
24. Brück W, Huitinga I, Dijkstra CD (1996) Liposome-mediated monocyte depletion during Wallerian degeneration defines the role of hematogenous phagocytes in myelin removal. *J Neurosci Res* 46: 477-484
25. Brück W, Maruschak B, Brück Y (1995) CD11b and CD18 antisense oligonucleotides suppress myelin removal by macrophages. *E J Pathol* 1.3: 953-03
26. Brück W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, Lassmann H (1995) Monocyte/macrophage differentiation in early multiple sclerosis lesions. *Ann Neurol* 38: 788-796
27. Castano A, Bell MD, Perry VH (1996) Unusual aspects of inflammation in the nervous system: Wallerian degeneration. *Neurobiol Aging* 17: 745-751
28. Chang RJ, Lee SH (1986) Effects of interferon-gamma and tumor necrosis factor α on the expression of an Ia antigen on a murine macrophage cell line. *J Immunol* 137: 2853-2856
29. Chen S, Bisby MA (1993) Long-term consequences of impaired regeneration on facial motoneurons in the C57BL/Ola mouse. *J Comp Neurol* 335: 576-585
30. Clemence A, Mirsky R, Jessen KR (1989) Non-myelin-forming Schwann cells proliferate rapidly during Wallerian degeneration in the rat sciatic nerve. *J Neurocytol* 18. 185-192
31. Coleman DL (1986) Regulation of macrophage phagocytosis. *Eur J Clin Microbiol* 5: 1-5
32. Crang AJ, Blakemore WF (1986) Observations on Wallerian degeneration in explant cultures of cat sciatic nerve. *J Neurocytol* 15: 471-482
33. Crawford TO, Hsieh S-T, Schryer BL, Glass JD (1995) Prolonged axonal survival in transsected nerves of C57BL/Ola mice is independent of age. *J Neurocytol* 24: 333-340
34. Dijkstra CD, De Groot CJA, Huitinga I (1992) The role of macrophages in demyelination. *J Neuroimmunol* 40: 183-188
35. Feasby TE, Gilbert JJ, Hahn AF, Neilson M (1987) Complement depletion suppresses Lewis rat experimental allergic neuritis. *Brain Res* 419: 97-103
36. Fernandez-Valle C, Bunge RP, Bunge BM (1995) Schwann cells degrade myelin and proliferate in the absence of macrophages: evidence from *in vitro* studies of Wallerian degeneration. *J Neurocytol* 24: 667-679
37. Finsen BR, Sørensen T, Castellano B, Pedersen EB, Zimmer J (1991) Leukocyte infiltration and glial reactions in xenografts of mouse brain tissue undergoing rejection in the adult rat brain. A light and electron microscopical immunocytochemical study. *J Neuroimmunol* 32: 159-183
38. Finsen BR, Sørensen T, González B, Castellano B, Zimmer J (1991) Immunological reactions to neural grafts in the central nervous system. *Restorative Neurol Neurosci* 2: 271-282
39. George EB, Glass JD, Griffin JW (1995) Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J Neurosci* 15: 6445-6452
40. George R, Griffin JW (1994) Delayed macrophage responses and myelin clearance during Wallerian degeneration in the central nervous system: the dorsal radiculotomy model. *Exp Neurol* 129: 225-236

41. Glass JD, Brushart TM, George EB, Griffin JW (1993) Prolonged survival of transsected nerve fibers in C57BL/Ola mice is an intrinsic characteristic of the axon. *J Neurocytol* 22: 311-321
42. Glass JD, Schryer BL, Griffin JW (1994) Calcium-mediated degeneration of the axonal cytoskeleton in the Ola mouse. *J Neurochem* 62: 2472-2475
43. Goldenberg PZ, Kwon EE, Benjamins JA, Whitaker JN, Quarles RH, Prineas JW (1989) Opsonization of normal myelin by anti-myelin antibodies and normal serum. *J Neuroimmunol* 23: 157-166
44. Goodrum JF, Novicki DL (1988) Macrophage-like cells from explant cultures of rat sciatic nerve produce apolipoprotein E. *J Neurosci Res* 20: 457-462
45. Gordon EJ, Myers KJ, Dougherty JP, Rosen H, Ron Y (1995) Both anti-CD11a (LFA-1) and anti-CD11b (MAC-1) therapy delay the onset and diminish the severity of experimental allergic encephalomyelitis. *J Neuroimmunol* 62: 153-160
46. Gordon S, Perry VH, Rabinowitz S, Chung L-P, Rosen H (1988) Plasma membrane receptors of the mononuclear phagocyte system. *J Cell Sci Suppl* 9: 1-26
47. Griffin FM, Jr., Griffin JA (1980) Augmentation of macrophage complement receptor function *in vitro*. II. Characterization of the effects of a unique lymphokine upon the phagocytic capabilities of macrophages. *J Immunol* 125: 844-849
48. Griffin FM, Jr., Mullinax PJ (1984) Augmentation of macrophage complement receptor function *in vitro*. IV. The lymphokine that activates macrophage C3 receptors for phagocytosis binds to a fucose-bearing glycoprotein on the macrophage plasma membrane. *J Exp Med* 160: 1206-1218
49. Griffin JW, George R, Ho T (1993) Macrophage systems in peripheral nerves. A review. *J Neuropathol Exp Neurol* 52: 553-560
50. Griffin JW, George R, Lobato C, Tyor WR, Yan LC, Glass JD (1992) Macrophage responses and myelin clearance during Wallerian degeneration: relevance to immune-mediated demyelination. *J Neuroimmunol* 40: 153-166
51. Griffin JW, Hoffman PN (1993) Degeneration and regeneration in the peripheral nervous system. In: *Peripheral Neuropathy*, v Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds.), pp. 361-376, WB Saunders Company: Philadelphia
52. Griffin JW, Stoll G, Li CY, Tyor W, Cornblath DR (1990) Macrophage responses in inflammatory demyelinating neuropathies. *Ann Neurol* 27: S64-S68
53. Hall SM (1993) Observations on the progress of Wallerian degeneration in transsected peripheral nerves of C57BL/Wld mice in the presence of recruited macrophages. *J Neurocytol* 22: 480-490
54. Hann Bonnekoh PG, Scheidt P, Friede RL (1989) Myelin phagocytosis by peritoneal macrophages in organ cultures of mouse peripheral nerve. A new model for studying myelin phagocytosis *in vitro*. *J Neuropathol Exp Neurol* 48: 140-153
55. Hann PG, Beuche W, Neumann U, Friede RL (1988) The rate of Wallerian degeneration in the absence of immunoglobulins. A study in chick and mouse peripheral nerve. *Brain Res* 451: 126-132
56. Hartung H-P, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, Toyka KV (1992) Inflammatory mediators in demyelinating disorders of the CNS and the PNS. *J Neuroimmunol* 40: 197-210
57. Hartung H-P, Schäfer B, Heininger K, Stoll G, Toyka VK (1988) The role of macrophages and eicosanoids in the pathogenesis of experimental allergic neuritis. *Brain* 111: 1039-1059
58. Hartung H-P, Schäfer B, Heininger K, Toyka KV (1988) Suppression of experimental autoimmune neuritis by the oxygen radical scavengers superoxide dismutase and catalase. *Ann Neurol* 23: 453-460
59. Hartung H-P, Toyka KV (1990) T-cell and macrophage activation in experimental autoimmune neuritis and Guillain-Barré syndrome. *Ann Neurol* 27 (Suppl.): S57-S63
60. Hays AP, Lee SSL, Latov N (1988) Immune reactive C3d on the surface of myelin sheaths in neuropathy. *J Neuroimmunol* 18: 231-244
61. Hickey WF, Vass K, Lassmann H (1992) Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. *J Neuropathol Exp Neurol* 51: 246-256
62. Hirschberg DL, Schwartz M (1995) Macrophage recruitment to acutely injured central nervous system is inhibited by a resident factor: a basis for an immune-brain barrier. *J Neuroimmunol* 61: 89-96
63. Hoffman M, Weinberg JB (1987) Tumor necrosis factor-α induces increased hydrogen peroxide production and Fc receptor expression, but not increased Ia antigen expression by peritoneal macrophages. *J Leukocyte Biol* 42: 704-707
64. Huitinga I, Damoiseaux JGMC, Döpp EA, Dijkstra CD (1993) Treatment with anti-CR3 antibodies ED7 and ED8 suppresses experimental allergic encephalomyelitis in Lewis rats. *Eur J Immunol* 23: 709-715
65. Huitinga I, Damoiseaux JGMC, van Rooijen N, Döpp EA, Dijkstra CD (1992) Liposome mediated affection of monocytes. *Immunobiol* 185: 11-19
66. Huitinga I, van Rooijen N, De Groot CJA, Uitdehaag BMJ, Dijkstra CD (1990) Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages. *J Exp Med* 172: 1025-1033
67. Jadus MR, Wepsic HT (1992) The role of cytokines in graft-versus-host reactions and disease. *Bone Marrow Transplant* 10: 1-14
68. Jung S, Huitinga I, Schmidt B, Zielasek J, Dijkstra CD, Toyka KV, Hartung H-P (1993) Selective elimination of macrophages by dichlormethylene diphosphonate-containing liposomes suppresses experimental autoimmune neuritis. *J Neurol Sci* 119: 195-202
69. Kelso A (1989) Cytokines: structure, function and synthesis. *Curr Opin Immunol* 2: 215-225
70. Kennedy MK, Torrance DS, Picha KS, Mohler KM (1992) Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. *J Immunol* 149: 2496-2505
71. Komiyama A, Novicki DL, Suzuki K (1991) Adhesion and proliferation are enhanced *in vitro* in Schwann cells from nerve undergoing Wallerian degeneration. *J Neurosci Res* 29: 308-318
72. Koski CL (1990) Characterization of complement-fixing antibodies to peripheral nerve myelin in Guillain-Barre syndrome. *Ann Neurol* 27 (suppl): S44-S47
73. Koski CL, Sanders ME, Swoveland PT, Lawley TJ, Shin ML, Frank MM, Joiner KA (1987) Activation of terminal components of complement in patients with Guillain-Barre syndrome and other demyelinating neuropathies. *J Clin Invest* 80: 1492-1497

74. Koski CL, Vanguri P, Shin ML (1985) Activation of the alternative pathway of complement by human peripheral nerve myelin. *J Immunol* 134: 1810-1814

75. Lambris JD (1988) The multifunctional role of C3, the third component of complement. *Immunol Today* 9: 387-393

76. Lamont AG, Sette A, Fujinami R, Colón SM, Miles C, Grey HM (1990) Inhibition of experimental autoimmune encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for IA^s molecules. *J Immunol* 145: 1687-1693

77. Lassner F, Schaller E, Steinhoff G, Wonigeit K, Walter GF, Berger A (1989) Cellular mechanisms of rejection and regeneration in peripheral nerve allografts. *Transplantation* 48: 386-392

78. Law SKA (1988) C3 receptors on macrophages. *J Cell Sci Suppl* 9: 67-97

79. Linington C, Morgan BP, Scolding NJ, Wilkins P, Piddlesden S, Compston DAS (1989) The role of complement in the pathogenesis of experimental allergic encephalomyelitis. *Brain* 112: 895-911

80. Liu HM, Yang LH, Yang YJ (1995) Schwann cell properties: 3. C-fos expression, bFGF production, phagocytosis and proliferation during Wallerian degeneration. *J Neuropathol Exp Neurol* 54: 487-496

81. Löms Ziegler-Heitbrock H-W (1989) The biology of the monocyte system. *Eur J Cell Biol* 49: 1-12

82. Ludwin SK, Bisby MA (1992) Delayed Wallerian degeneration in the central nervous system of Ola mice: an ultrastructural study. *J Neurol Sci* 109: 140-147

83. Lumsden CE (1971) The immunogenesis of the multiple sclerosis plaque. *Brain Res* 28: 365-390

84. Lunn ER, Perry VH, Brown MC, Rosen H, Gordon S (1989) Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. *Eur J Neurosci* 1: 27-33

85. Lyon MF, Ogunkolade BW, Brown MC, Atherton DJ, Perry VH (1993) A gene affecting Wallerian nerve degeneration maps distally on chromosome 4. *Proc Natl Acad Sci USA* 90: 9717-9720

86. Meador-Woodruff JH, Yoshino JE, Bigbee JW, Lewis BL, Devries GH (1985) Differential proliferative responses of cultured Schwann cells to axolemma and myelin-enriched fractions. II. Morphological studies. *J Neurocytol* 14: 619-635

87. Mithen FA, Colburn S, Birchem R (1990) Human alpha tumor necrosis factor does not damage cultures containing rat Schwann cells and sensory neurons. *Neurosci Res* 9: 59-63

88. Murray HW, Spitalny GL, Nathan CF (1985) Activation of mouse peritoneal macrophages *in vitro* and *in vivo* by interferon-gamma. *J Immunol* 134: 1619-1622

89. Müller HW, Minwegen P (1987) Nonresident macrophages in peripheral nerve of rat: effect of silica on migration, myelin phagocytosis, and apolipoprotein E expression during Wallerian degeneration. *J Neurosci Res* 18: 222-229

90. Nathan CF (1987) Secretory products of macrophages. *J Clin Invest* 79: 319-326

91. Nathan CF, Prendergast TJ, Wiebe ME, Stanley ER, Platzer E, Remold HG, Welte K, Rubin BY, Murray HW (1984) Activation of human macrophages. Comparison of other cytokines with interferon-gamma. *J Exp Med* 160: 600-605

92. Paul JA, Gregson NA (1992) An immunohistochemical study of phospholipase A2 in peripheral nerve during Wallerian degeneration. *J Neuroimmunol* 39: 31-48

93. Perry VH, Brown MC, Gordon S (1987) The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J Exp Med* 165: 1218-1223

94. Perry VH, Brown MC, Lunn ER (1991) Very slow retrograde and Wallerian degeneration in the CNS of C57BL/Ola mice. *Eur J Neurosci* 3: 102-105

95. Perry VH, Brown MC, Lunn ER, Tree P, Gordon S (1990) Evidence that very slow Wallerian degeneration in C57BL/Ola mice is an intrinsic property of the peripheral nerve. *Eur J Neurosci* 2: 802-808

96. Perry VH, Tsao JW, Fearn S, Brown MC (1995) Radiation-induced reductions in macrophage recruitment have only slight effects on myelin degeneration in sectioned peripheral nerves of mice. *Eur J Neurosci* 7: 271-280

97. Reichert F, Levitzky R, Rotshenker S (1996) Interleukin 6 in intact and injured mouse peripheral nerves. *Eur J Neurosci* 8: 530-535

98. Reichert F, Saada A, Rotshenker S (1994) Peripheral nerve injury induces Schwann cells to express two macrophage phenotypes: phagocytosis and the galactose-specific lectin MAC-2. *J Neurosci* 14: 3231-3245

99. Rosen H, Gordon S (1987) Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells *in vitro* and inflammatory cell recruitment *in vivo*. *J Exp Med* 166: 1685-1701

100. Russell SW, Pace JL (1987) The effects of interferons on macrophages and their precursors. *Vet Immunol Immunopathol* 15: 129-165

101. Scheidt P, Friede RL (1987) Myelin phagocytosis in Wallerian degeneration. Properties of millipore diffusion chambers and immunohistochemical identification of cell populations. *Acta Neuropathol* (Berl) 75: 77-84

102. Scheidt P, Waehneldt TV, Beuche W, Friede RL (1986) Changes of myelin proteins during Wallerian degeneration *in situ* and in millipore diffusion chambers preventing active phagocytosis. *Brain Res* 379: 380-384

103. Schubert T, Friede RL (1981) The role of endoneural fibroblasts in myelin degradation. *J Neuropathol Exp Neurol* 40: 134-154

104. Selmaj KW, Raine CS (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Ann Neurol* 23: 339-346

105. Sharon N (1984) Carbohydrates as recognition determinants in phagocytosis and in lectin-mediated killing of target cells. *Biol Cell* 51: 239-246

106. Smith SR, Terminelli C, Kenworthy-Bott L, Phillips DL (1991) A study of cytokine production in acute graft-vs-host disease. *Cell Immunol* 134: 336-348

107. Stoll G, Griffin JW, Li CY, Trapp BD (1989) Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol* 18: 671-683

108. Stoll G, Hartung H-P (1992) The role of macrophages in degeneration and immune-mediated demyelination of the peripheral nervous system. *Adv Neuroimmunol* 2: 163-179

109. Stoll G, Jung S, Jander S, van der Meide P, Hartung H-P (1993) Tumor necrosis factor-α in immune-mediated demyelination and Wallerian degeneration of the rat peripheral nervous system. *J Neuroimmunol* 45: 175-182

110. Stoll G, Trapp BD, Griffin JW (1989) Macrophage function during Wallerian degeneration of rat optic nerve: clear-

ance of degenerating myelin and Ia expression. *J Neurosci* 9: 2327-2335

111. Tanaka K, Zhang Q-L, Webster Hd (1992) Myelinated fiber regeneration after sciatic nerve crush: morphometric observations in young adult and aging mice and the effects of macrophage suppression and conditioning lesions. *Exp Neurol* 118: 53-61

112. Thomson CE, Mitchell LS, Griffiths IR, Morrison S (1991) Retarded Wallerian degeneration following peripheral nerve transection in C57BL/6/Ola mice is associated with delayed down-regulation of the P_0 gene. *Brain Res* 538: 157-160

113. Trotter J, DeJong LJ, Smith ME (1986) Opsonization with antimyelin antibody increases the uptake and intracellular metabolism of myelin in inflammatory macrophages. *J Neurochem* 47: 779-789

114. Troutt AB, Kelso A (1992) Enumeration of lymphokine mRNA-containing cells *in vivo* in a murine graft-versus-host reaction using the PCR. *Proc Natl Acad Sci USA* 89: 5276-5280

115. Unanue ER (1989) Macrophages, antigen-presenting cells, and the phenomena of antigen handling and presentation. In: *Fundamental Immunology,* Paul WE (ed.) pp. 95-115, Raven Press Ltd.: New York

116. van Rooijen N (1989) The liposome-mediated macrophage 'suicide' technique. *J Immunol Methods* 124: 1-6

117. van Rooijen N, Kors N, v.d.Ende M, Dijkstra CD (1990) Depletion and repopulation of macrophages in spleen and liver of rat after intravenous treatment with liposome-encapsulated dichloromethylene diphosphonate. *Cell Tissue Res* 260: 215-222

118. van Rooijen N, Sanders A (1994) Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods* 174: 83-93

119. Vanguri P, Shin ML (1986) Activation of complement by myelin: identification of C1-binding proteins of human myelin from central nervous tissue. *J Neurochem* 46: 1535-1541

120. Vass K, Hickey WF, Schmidt RE, Lassmann H (1993) Bone marrow-derived elements in the peripheral nervous system. An immunohistochemical and ultrastructural investigation in chimeric rats. *Lab Invest* 69: 275-282

121. Venezie RD, Toews AD, Morell P (1995) Macrophage recruitment in different models of nerve injury: lysozyme as a marker for active phagocytosis. *J Neurosci Res* 40: 99-107

122. Vriesendorp FJ, Flynn RE, Pappoila MA, Koski CL (1995) Complement depletion affects demyelination and inflammation in experimental allergic neuritis. *J Neuroimmunol* 58: 157-165

123. Waller A (1850) Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibers. *Phil Trans R Soc Lond (Biol)* 140: 423-429

124. Wright SD, Silverstein SC (1983) Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J Exp Med* 158: 2016-2023