**Abstract** The present study used cocultures of rat dorsal root ganglia (DRG) and peritoneal macrophages to define the role of activated complement components during demyelination. The complement cascade was activated in vitro by treatment of the cultures with natural rat serum and lipopolysaccharides. Complement activation was examined by detection of the membrane attack complex of complement (MAC) with an antibody directed against rat C5-9. Detection of MAC in vitro by immunoelectron microscopy was associated with morphological changes of the myelin sheath. The sheath’s regular structure was disrupted. Myelin lamellae were split and showed signs of decompaction. These changes were followed by a selective macrophage attack on myelin sheaths resulting in demyelination. Schwann cell viability was not affected by complement activation. Axons and sensory ganglion cells also survived this attack. The specificity of the complement effect was tested in experiments using treatment regimens with natural rat serum or lipopolysaccharides alone. In these experiments, no morphological changes of the myelin sheath were observed as well as no macrophage attack on myelin.

**Key words** Dorsal root ganglia · Macrophages · Experimental demyelination · Complement · Membrane attack complex

---

**Introduction**

Mononuclear cells of the monocyte/macrophage system play a key role in myelin removal during Wallerian degeneration and immune-mediated demyelination [3, 27]. Recently, an in vitro system using rat dorsal root ganglia (DRG) cocultured with macrophages was established to investigate the role of phagocytic cells during demyelination in vitro [7]. This experimental model allows studies on the macrophage-myelin interactions as well as on events preceding the macrophage attack to myelin sheaths under controlled in vitro conditions. A first series of experiments using oxygen radicals to induce myelin and/or Schwann cell damage showed that non-resident macrophages phagocytosed only predamaged myelin.

Components of the complement system are involved in the process of myelin recognition and myelin uptake during Wallerian degeneration [4, 5] as well as during immune-mediated demyelination [11]. Complement depletion was shown to suppress the clinical expression of experimental allergic neuritis (EAN), an experimental autoimmune disease of the peripheral nervous system [9]. Activation of the classical or alternative pathway of complement results in the formation of the lytic terminal complement complex C5b-9, also referred to as the membrane attack complex (MAC) [19], the exact role of which in demyelination, however, has not yet been defined.

The present study aimed at clarifying the role of activated complement components, especially of the MAC, in the initiation of demyelination using the described coculture model of DRG and peritoneal macrophages.

**Material and methods**

Preparation and cultivation of DRG

DRG were prepared from newborn Wistar rats as described previously in detail [7]. Animals were killed under deep anesthesia and DRG were dissected, collected in Hank’s balanced salt solution (HBSS, Gibco) and incubated for 10 min in a solution containing...
0.25% trypsin (Serva, Heidelberg, Germany) and 0.05% collagenase type I (Worthington Biochemical Corporation, Freehold, USA). DRG were then suspended in Dulbecco’s minimal essential medium (DMEM, Biochrom, Berlin, Germany) containing 10% fetal calf serum (FCS, Biochrom), 200 mM L-glutamine (Biochrom), 100 IU/ml penicillin (Biochrom) and 100 µg/ml streptomycin (Biochrom) and plated onto poly-L-lysine-coated coverslips. DRG cultures were maintained for 1 week in DMEM supplemented with 10% Nu serum (Paesel and Lorei, Frankfurt, Germany), 200 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin, as described by Armati et al. [2]. After 1 week, culture medium was changed to DMEM without L-valine, containing 92 mg/l D-valine (Biochrom), 6 mg/ml glucose, 50 µg/ml L-ascorbic acid (Sigma), 10% FCS, 200 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. Cultures were given this medium weekly until the beginning of the experiments.

Preparation of macrophages

Adult Wistar rats of either sex were used. Peritoneal macrophages were harvested 4 days after intraperitoneal thioglycollate injection (2.9%, NIH, thioglycollate broth, Difco No. 0257 01) by peritoneal lavage with cold phosphate-buffered saline, pH 7.4 (PBS). In control experiments, non-thioglycollate-elicited, resting macrophages were used to exclude effects of thioglycollate injection. Macrophages were cultured in DMEM containing 10% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin and 58 mg% L-glutamine. Macrophages were tagged with latex beads with defined diameter (0.3 µm; Sigma) before their addition to DRG cultures for positive identification of the added cells within the culture.

Treatment of cell cultures

DRG were exposed to activated complement components by adding natural rat serum (NRS) and bacterial lipopolysaccharides (LPS, Sigma; 10 µg/ml), which are known to be potent activators of the alternative pathway of the complement cascade, to the culture medium. DRG cultures were exposed for 4 h before macrophages were added. Cultures exposed to NRS or LPS alone served as controls. Experiments were repeated four times.

At the end of the incubation period, Schwann cell vitality in DRG cultures was evaluated by incubation with propidium iodide, an accepted indicator for cell cytotoxicity [24]. Schwann cells were identified by their typical morphological appearance [1] and by their positive reaction with the S-100 protein antibody as described below. A minimum of 100 Schwann cells per culture dish was examined in each experiment.

Latex-tagged macrophages were added to such treated DRG cultures either immediately or 3 days after exposure to activated complement components. Macrophages were cocultured with treated and untreated DRG cultures for 3–5 days.

Immunocytochemistry

For identification of Schwann cells, DRG were incubated with a polyclonal anti-S-100 protein antibody (Dako) at a concentration of 1:100, applied for 2 h. Secondary antibody was a FITC-conjugated anti-rabbit Ig. Immunofluorescence-stained cultures were examined with a Zeiss fluorescence microscope.

Specimen preparation

In all experiments, tissue was fixed immediately in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in araldite. Semithin sections (1 µm) were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were examined with a Zeiss EM 10B electron microscope.

Immunoelectron microscopy

MAC deposition within the DRG was detected by immunoelectron microscopy using a rabbit anti-rat C5-9 antiserum [20]. The detection of C5-9 by the rabbit anti-rat antiserum has been shown to be a reliable marker of complement activation and MAC generation in tissue sections or cell cultures [20, 25]. A post-embedding method was used for immunoelectron microscopy. DRG were fixed in phosphate-buffered 4% paraformaldehyde and then embedded in LR white (The London Resin Co., Basingstoke, UK). Ultrathin sections were incubated with rabbit anti-rat C5-9 antiserum. Secondary antibody was a goat anti-rabbit Ig, conjugated with 10-nm gold particles (Amersham, Braunichweig, Germany). Treated and untreated cultures were stained for immunoelectron microscopy. The first antibody was omitted in controls.