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Regeneration of Oligodendroglia During Recovery from Demyelinating Disease

Abstract. *Infection of mice with the JHM strain of mouse hepatitis virus causes demyelination as a result of a cytolytic infection of oligodendroglia. In recovery, animals show remyelination, which could result either from surviving oligodendrocytes extending their territory or by generation of new oligodendroglia. Electron microscopic autoradiographic studies with ^{3}H-labeled thymidine demonstrate that the cells associated with remyelination are newly generated oligodendroglia.*

Demyelination in the central nervous system (CNS), the basic pathology of diseases like multiple sclerosis, can result from injury to oligodendroglial cells or to the myelin sheaths they maintain (*1, 2*). In a number of experimental models of demyelinating disease (*2–4*), recovery is associated with remyelination, a process that can occur in the face of local destruction of substantial numbers of oligodendroglia (*5, 6*). At least two mechanisms may be important in the process of remyelination. First, surviving oligodendroglia at the periphery of the lesion could expand their territory by extending processes into demyelinated regions to remyelinate the denuded axons. Second, cells in the oligodendroglial line (*7*) could undergo compensatory cellular proliferation. This study was designed to test the hypothesis that some of the oligodendroglia active in remyelination are newly generated cells.

Demyelination was produced in 4-week-old Swiss mice by intracerebral inoculation of a 10 percent suspension of suckling mouse brain containing one mean lethal dose (LD_{50}) of JHM virus (*8*) which is a neurotropic strain of mouse hepatitis virus (MHV), an enveloped, RNA corona virus. Control mice were similarly inoculated with a suspension of normal suckling mouse brain. In JHM infection, demyelination seems to result from a direct lytic effect of the virus on oligodendroglia (*9*). Multifocal demyelination is first apparent on day 3 after inoculation of the JHM virus on day 0. This pathological process is associated with death of oligodendrocytes and local inflammation. Approximately 50 percent of the mice die between days 7 and 12. By the end of the second week, active demyelination is no longer apparent. Mice surviving to the third week after inoculation show evidence of remyelination, and, at the end of 3 months, demyelinated areas are difficult to detect (*4*). In this study, [^{3}H]thymidine light- and electron-microscopic autoradiography was used to identify and characterize newly generated cells in the areas of remyelination.

Mice surviving the acute infection and control mice were given intraperitoneal injections of the DNA precursor, [^{3}H]-thymidine (specific activity 40.2 c/mmole). To cumulatively label proliferating cells (*10, 11*), two dosage schedules were used: 6 μc/g every 12 hours or 2 μc/g every 8 hours from day 13 through day 19 after inoculation. The spinal cords of experimental and control animals, fixed by perfusion at 21, 28, 35, and 49 days after inoculation, were prepared for light- and electron-microscopic autoradiography (*12*).

At each stage examined, lesions within the white matter of the spinal cord were evident. In areas of remyelination, silver grains indicating the incorporation of [^{3}H]thymidine were present in the emulsion overlying the nuclei of four types of cells: oligodendroglia (Fig. 1a), astroglia, inflammatory cells, and endothelial cells. Oligodendroglia were identified by their round nuclei containing clumps of heterochromatin, their dense cytoplasm containing many free polyribosomes, and microtubules (Fig. 1c) (*2, 7, 13*). Labeled oligodendrocytes were conspicuous in and adjacent to areas of active remyelination (Fig. 1b), but they were rarely seen

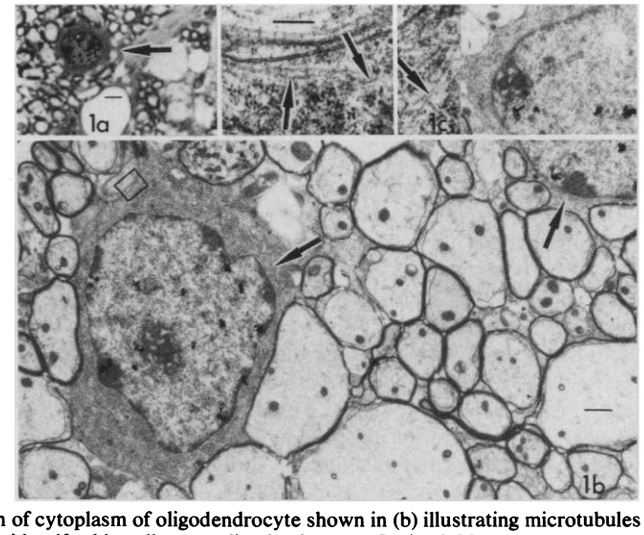

Fig. 1. (a) Light-microscopic autoradiograph of a heavily labeled oligodendrocyte (arrow) at the edge of a demyelinated area taken 28 days after inoculation. Scale, 5 μm. (b) Electron-microscopic autoradiograph of two oligodendrocytes (arrows) in a remyelinating area 28 days after inoculation. Both cells are heavily labeled as indicated by the silver grains scattered over their nuclei. Scale, 1 μm (lower right). The boxed area is enlarged in (c). (c) Portion of cytoplasm of oligodendrocyte shown in (b) illustrating microtubules (arrows), which help to identify this cell as an oligodendrocyte. Scale, 0.25 μm.

in areas away from the demyelinated foci. In some of the more actively remyelinating areas, more than 50 percent of the oligodendroglia were labeled. In control animals, labeled endothelial cells and astrocytes were rarely seen, and no labeled cells that could be unequivocally identified as oligodendroglia were seen.

These results demonstrate that cells of the oligodendroglial line can incorporate [^{3}H]thymidine during recovery from virus-induced demyelination. It is unlikely that virus infection directly stimulated nuclear incorporation of thymidine, because this RNA corona virus lacks an RNA-dependent DNA polymerase (*14*). It could be argued that [^{3}H]thymidine incorporation resulted from DNA repair. However, in experimental models in which repair was stimulated by ultraviolet irradiation, thymidine incorporation was limited even after large doses of [^{3}H]thymidine (*15*). In these experiments, the label over nuclei was moderately heavy, which indicates that the labeled oligodendroglia had passed through the synthesis (S) phase of the cell cycle during the period of [^{3}H]-thymidine administration (*11*).

Although hyperplasia of oligodendroglia has been described in both human and experimental tissues (*16*), identifying the proliferating cell populations has proved difficult. In two previous autoradiographic studies (*17*), labeled cells were interpreted to be oligodendroglia; in neither report are the identities of the proliferating cells definitively established. In developing optic nerve of the rat, oligodendroblasts are the major source of oligodendrocytes during myelinogenesis (*7*). Our cumulative labeling technique (*10*) does not allow us to identify the cells giving rise to newly formed oligodendroglia. However, in animals killed 1 hour after a pulse label of [^{3}H]thymidine (*7*), glial cells synthesizing DNA can be identified before they have had time to differentiate. This approach can be used to study the kinetics of oligodendoglial proliferation in several models of demyelinating disease and to evaluate the use of pharmacologic manipulation of proliferation.

Reappearance of oligodendroglia associated with remyelination has been described in vitro (*5*) but not well documented in vivo. Explant cultures treated with antiserums from animals with experimental allergic encephalomyelitis became demyelinated and lost oligodendroglia; withdrawal of the antiserums was associated with remyelination and reappearance of oligodendroglia (*6*). This in vivo study demonstrates regeneration of oligodendrocytes to replace those destroyed by the viral infection. Thus, in this model cell, proliferation appears to be essential to replace destroyed oligodendroglia and to permit successful remyelination in the CNS.

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Behavioral Control of Workers by Queens in Primitively Eusocial Bees

Abstract. *Queens of* Lasioglossum zephyrum, *a primitively eusocial bee, are considerably more active than workers. The queen's behavior stimulates worker activities; removal of the queen results in a marked reduction in activities of other bees. The queen not only activates workers but also directs them by a primitive recruitment behavior suggestive of tandem running of highly eusocial ants.*

Evolution of insect eusociality, in which members of a colony (workers) relinquish all or a portion of their individual fitness to assist another colony member (the queen), has attracted considerable interest (*1*). Two major areas of inquiry are (i) the consistency of apparent worker altruism with the principles of natural selection and (ii) the evolution of social integrating mechanisms. The former problem has received most of the attention while the latter remains relatively unexplored (*2*). The present report attempts to elucidate early steps toward eusociality by considering social integrating mechanisms in an incipiently eusocial species.

Table 1. Queen direction of pollen deposition by workers.

Returns	Number	Percent
Returns from foraging trips with queen leading forager to cell entrance	27	64.3
Returns from foraging trips with queen positioned at cell entrance	10	23.8
No queen involvement in pollen deposition	5	11.9
Total returns to nests	42	100.0

Solitary bees and wasps that provision nests commonly display an invarying sequence of cell construction, provisioning, egg laying, and closure, followed by a repetition of the pattern. Although some solitary species can omit parts of the sequence, it is in the social species that workers possess a large degree of behavioral plasticity. Workers of highly eusocial insects are able to engage in a variety of activities regardless of their sequence and apparently without direction from the queen (*2*). Social facilitation among workers is well documented; trail