Occurrence of Oligodendrocytes within Astrocytes in Demyelinating Lesions

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Abstract. We investigated the fine structural details of the presence of apparently newly formed oligodendrocytes within reactive astrocytes in white matter lesions obtained by biopsy from seven cases (3 multiple sclerosis (MS); 3 progressive multifocal leukoencephalopathy (PML); 1 with nonspecific reactive changes next to a sarcoid granuloma). Intact oligodendrocytes were found within astrocytic cytoplasm in two acute MS lesions and also in the reactive white matter lesion. The internalized cells appeared to lie within membrane-bound vacuoles. Formation of rudimentary junctions was observed between the internalized cells and host astrocytes. Sometimes more than one oligodendrocyte was seen in the same astrocyte. Our study suggests that this newly recognized interaction between astrocytes and oligodendrocytes is not restricted to acute MS lesions and probably represents emperipolesis rather than phagocytosis. This apparently nonspecific finding may be expected in any lesion with a proliferation of astrocytes and oligodendrocytes. The precise mechanism of this phenomenon or its functional significance is not entirely clear.

Key Words: Astrocytes; Demyelination; Emperipolesis; Multiple sclerosis; Oligodendrocytes; Phagocytosis.

INTRODUCTION

In a recent light microscopic study of acute multiple sclerosis (MS) lesions in autopsy specimens, Prineas et al. (1) observed the presence of cells resembling oligodendrocytes within the cytoplasm of reactive astrocytes in some of the lesions. Subsequently, these investigators identified the internalized cells as undifferentiated oligodendrocytes by demonstrating the presence of certain specific cell markers on them and suggested that this phenomenon is restricted to acute MS lesions and represents phagocytosis of new oligodendrocytes by reactive astrocytes, which may be responsible for failed remyelination in MS (2).

We have previously described this finding briefly at the fine structural level in an acute demyelinating lesion (3). To further explore the morphologic details of this newly recognized phenomenon and to see if it is indeed restricted to acute MS, we studied different white matter lesions in biopsy specimens. In this report, we describe and analyze the fine structure of this interaction between oligodendrocytes and astrocytes and provide some new observations on its nature and possible functional implication.

MATERIALS AND METHODS

Stereotactic biopsies of white matter lesions from seven patients were selected for the study. The patients' ages ranged from 15 to 43 years and the duration of neurologic symptoms two weeks to three months. In all cases, focal white matter lesions were seen on computed tomography (CT) and magnetic resonance (MR) scans. A portion of each biopsy was embedded in paraffin and the sections were stained by hematoxylin and eosin (H&E), luxol fast blue (LFB)-PAS and Bielschowsky methods. The remaining tissues were immersed in 2.5% buffered glutaraldehyde solution shortly after removal and were embedded in resin. One micron thick sections were stained with toluidine blue. Selected blocks of tissue were prepared for electron microscopic (EM) study. Serial sections were obtained from several blocks of tissue to a variable depth.

RESULTS

Based on the histologic study, the lesions were classified as follows: acute demyelinating, probably MS (three cases); progressive multifocal leukoencephalopathy (PML) (three cases); reactive changes in white matter adjacent to a sarcoid granuloma (one case).

The MS lesions appeared hypercellular and displayed extensive demyelination with relative preservation of axons. In addition to a variable number of lipid-laden macrophages and reactive astrocytes, a large number of small cells, presumably oligodendrocytes, were present in two lesions. The other lesion appeared relatively older with more prominent reactive astrocytes and fewer oligodendrocytes. The PML lesions showed destruction of myelin with many macrophages and a variable number of reactive astrocytes. The oligodendrocytes were reduced in number. Some of the oligodendrocytes showed enlarged nuclei containing papovavirus virions. The white matter sample next to the granuloma showed rarefaction and partial demyelination with a slightly increased number of oligodendrocyte-like cells and many reactive astrocytes.

Inclusions of cells resembling oligodendrocytes within the cytoplasm of the reactive astrocytes were seen mostly in the relatively more acute primary demyelinating lesions in two biopsy specimens. Only an occasional internalized oligodendrocyte was seen in the reactive white
Fig. 1A & B. Oligodendrocytes (arrows) in reactive astrocytes in MS lesions surrounded by a halo. The cell in B might represent deep indentation of astrocytic cytoplasm from outside. Resin embedded sections stained with toluidine blue. × 700.

Fig. 2. Low power view showing oligodendrocytes in MS lesions occupying: a—mid-portion of an astrocyte; b—periphery of an astrocytic cell body; c—an astrocytic process. (a) indicates astrocytic cytoplasm, (b) oligodendrocyte and (n) astrocytic nucleus. × 3,000.
matter and none in the older MS or in the PML lesions. With the light microscope, these internalized cells with small round nuclei were seen within the cytoplasm of reactive astrocytes and were usually surrounded by a halo (Fig. 1A). In many instances, it was difficult to ascertain whether the cells were situated within astrocytic cytoplasm or whether they indented the astrocytes from outside (Fig. 1B). The intracellular oligodendrocytes could be identified more easily in resin-embedded sections than in the routine paraffin sections.

Electron microscopic study demonstrated this phenomenon in the MS lesion clearly and also much more frequently than one could anticipate from the light microscopic observations. The internalized cells were seen infrequently in the reactive white matter. Intact oligodendrocytes were seen occupying different regions of the astrocytic cytoplasm (Fig. 2). The majority of the cells appeared to be situated at the periphery of the astrocyte separated from the extracellular space by a thin rim of cytoplasm (Figs. 2, 3). The internalized cells usually had smooth surfaces which looked viable and displayed dark cytoplasm containing various organelles including abundant rough endoplasmic reticulum (ER) and microtubules but no filaments. They conformed to the morphology of oligodendrocytes described in acute MS lesions (3–5). These cells were separated from the astrocytic cytoplasm by a clear space and appeared to lie within giant vacuoles (Fig. 3). This space was extremely narrow in some areas and often rudimentary junctional devices were seen between the closely apposed membranes of the internalized oligodendrocytes and the host astrocytes (Fig. 3). Similar junctions were also seen between juxtaposed astrocytes and oligodendrocytes. Rarely, two oligodendrocytes were seen in the same astrocyte, each occupying a separate vacuolar compartment. The reactive astrocytes containing oligodendrocytes also displayed variable amounts of lipid droplets and sometimes even myelin debris in their cytoplasm indicating phagocytic activity of these cells as has been described previously (6).

We attempted to study several internalized oligoden-
OLIGODENDROCYTES IN REACTIVE AstrocyTES

Fig. 4. An oligodendrocyte in a MS lesion showing partial investment by pseudopod-like astrocytic processes (p). Arrow indicates a junction between the oligodendrocyte (o) and the astrocyte (a) shown in inset. Nu indicates astrocytic nucleus. × 9,000; inset × 20,000.

drocytes on serial sections and were able to trace some of the cells only to a depth of 10 to 15 microns. The cytoplasmic rim of the astrocyte around these oligodendrocytes was always found to be continuous in the deeper sections. The entire body of the internalized cells could not be traced on serial sections.

Pseudopod-like slender astrocytic processes were often seen around oligodendrocytes extending to a variable distance, sometimes approaching closely to one another (Figs. 4, 5). Occasional junctions were seen between the astrocytes and the partially internalized oligodendrocytes (Figs. 4, 5). On a rare occasion, both partially and completely internalized oligodendrocytes were seen in the same cell (Fig. 5). Oligodendrocytes were frequently seen to indent the cytoplasm of the adjacent astrocytes to a variable depth. Sometimes an oligodendrocyte seemed to lie in a recess presumably formed as a result of deep invagination of the astrocytic cytoplasm (Fig. 6).

DISCUSSION

The small cells within astrocytes observed in this study closely resembled lymphocytes at the light microscopic level. However, the fine structural morphology of these cells was identical to that described in newly formed oligodendrocytes in acute MS lesions (3–5). In addition, the formation of rudimentary junctions between these cells and astrocytes was helpful in distinguishing them from lymphocytes. As mentioned earlier, the identity of these cells as newly generated nonmyelinating oligodendrocytes has also been established by the demonstration of certain specific cell markers on them (2). In addition to several antigens characteristically seen in immature oligodendrocytes, these cells were found to express the carbohydrate epitope present on the family of cell adhesion molecules recognized by the monoclonal antibody HNK-1 (2, 7). This finding, apparently detectable only during the early
developing phase of oligodendrocytes, seems particularly interesting in view of our observation of frequent junction-like devices between them and reactive astrocytes, which are not known to occur between the mature forms of these glial cells. This interesting feature has not received much attention in the past and its significance is not known.

Acute hypercellular MS lesions which are known to contain a large number of newly proliferated oligodendrocytes and astrocytes (3–5, 7) might be expected to provide an ideal setting for this unusual interaction between these cells. Indeed, most of the internalized cells in our study were found in the acute MS lesion, and none were found in the older MS or in the FML lesions which contained a reduced number of oligodendrocytes. Furthermore, albeit rare, the presence of oligodendrocytes in astrocytes in the reactive white matter in one of our cases indicates that this finding is not restricted to MS lesions and probably represents a nonspecific phenomenon rather than an immunologically-mediated response between astrocytes and oligodendrocytes during the evolution of acute MS lesions as has been postulated (2). Most of the oligodendrocytes in our study were found to occupy the periphery of the astrocytes separated from the extracellular space by attenuated cytoplasmic rims. The intracellular location of such cells was often not possible to ascertain with the light microscope. This may explain why this finding went unrecognized in the past. Moreover, the internalized oligodendrocytes can easily be mistaken for small lymphocytes in routine histologic preparations as might have been the case in a previous report in which inclusion of lymphocytes was described within the cytoplasm of reactive astrocytes in edematous white matter adjacent to a neoplasm (8).

In this fine structural study of a large number of internalized oligodendrocytes, we found no evidence of cel-
ular destruction or degeneration as would be expected if this process indeed represented phagocytosis. Alternatively, this finding might be considered to be akin to emperiplois, a term that was coined by Humble et al in 1956 (9) to describe the presence of lymphocytes within malignant cells. This phenomenon has since been observed in various other types of cells and is now broadly defined as an active penetration of one cell by another, which remains intact (10–16). Thus, from the morphologic standpoint, the presence of intact oligodendrocytes within astrocytes seems more likely to represent emperiplois rather than phagocytosis with eventual destruction of the internalized cells as has been suggested by Prineas et al (1, 2).

In a morphologic study of tissue sections, it is obviously difficult to establish the sequence of events leading to the inclusion of oligodendrocytes within astrocytes. Based on various sectional profiles in the relationship between these cells in our study, two possible mechanisms might be suggested to account for this finding. First, the oligodendrocytes could have been internalized by extension and subsequent fusion of pseudopod-like astrocytic processes around them analogous to that seen in endocytosis. Second, an oligodendrocyte indenting deeply into an astrocytic cell body from outside could appear to be within a cytoplasmic vacuole if sectioned on a certain plane (see Fig. 6). Our limited study of several internalized oligodendrocytes by serial sectioning was not sufficient to draw any firm conclusion as to whether these cells were completely sequestered within the astrocytes. This important issue also remains unresolved in emperiplois despite detailed morphologic analysis of this process (10). For instance, serial sections of megakaryocytes containing granulocytes in one study confirmed their intracytoplasmic location (11), while another study in which horseradish peroxidase was used as a tracer demonstrated that the vacuoles containing the apparently internalized cells freely communicated with the extracellular space indicating that these cells probably occupied a deep recess in the host cell cytoplasm (16). Thus, the precise mechanism

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by which a viable cell seemingly occupies the cytoplasm of another cell remains unclear.

The functional significance, if any, of this unusual astrocyte-oligodendrocyte interaction especially with regard to the question of remyelination in MS lesions must remain a matter of conjecture at this time. As indicated before, our findings do not support the assumption that reactive astrocytes in early MS lesions are responsible for phagocytosis and destruction of newly generated oligodendrocytes, thus impeding remyelination. Failure of adequate remyelination in MS has long been attributed to the investment of the demyelinated axons resulting in a physical barrier between naked axons and oligodendrocytes. This study suggests another possible mechanism by which reactive astrocytes could adversely affect remyelination by partial or complete sequestration of new oligodendrocytes during the early stage of the lesions. To confirm this hypothesis it is essential to determine whether a significant number of oligodendrocytes are sequestered in this manner in acute MS lesions. Such a quantitative assessment was not possible in this study because of limited tissue samples in a small number of cases. Another important question in this regard is whether the oligodendrocytes remain sequestered within the astrocytes indefinitely or whether their apparent intracytoplasmic location is a transient phenomenon as seems to be the case in emperipolysis (10, 11). Obviously, further investigation is needed before any biological significance can be assigned to this newly recognized interaction between oligodendrocytes and astrocytes.

REFERENCES

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