Down-regulation of membrane glycoprotein in amoeboid microglia transforming into ramified microglia in postnatal rat brain

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Summary

The present study describes the ultrastructural localization and labelling pattern of lectin in different microglial cell phenotypes in the postnatal rat brain using the isolectin, GSA I-B4. The nascent round and amoeboid microglial cells (round cells and cells displaying short processes) were labelled at their cytoplasmic membrane and the membrane of the subplasmalemmal vacuoles. In the course of their transformation into ramified forms with age, dense lectin labelling was observed successively at different sites in the differentiating cells. The most striking feature was the staining of the Golgi saccules on the trans face, the trans tubular network and associated vesicles and vacuoles in the 'intermediate' ramified microglia (ramified cells bearing thick and long processes and those with thin and long processes). The vacuoles with accumulated reaction products were closely associated with many microtubules extending into the cytoplasmic processes. At the surface, the lectin-labelled vacuoles and vesicles appeared to fuse with the membrane and their contents communicated with the exterior. In the advanced or most differentiated ramified microglial cells (cells bearing attenuated processes), the lectin staining at all the above mentioned sites became diminished. In conclusion, in the transformation of the round microglia into their ramified derivatives, the glycoconjugates at the cytoplasmic membrane are progressively reduced. It is postulated from this study that the down-regulation of the glycoconjugates of the microglial plasma membrane is due primarily to their internalization during endocytosis. This process would trigger a de novo galactosyl protein synthesis and/or modification at the trans Golgi saccules and trans tubular network probably in an attempt to degrade the internalized membrane glycoproteins or to replenish the consumption of the membrane glycoconjugates.

Introduction

The origin of ramified microglial cells has been a much debated issue (Ling, 1981; Ling & Wong, 1993). Using silver impregnation (Rio-Hortega, 1932), autoradiography (Imamoto & Leblond, 1978), histochemical (Murabe & Sano, 1982; Kaur et al., 1984; Fujimoto et al., 1987) and immunohistochemical studies (Perry et al., 1985; Perry & Gordon, 1987; Ling et al., 1990, 1991; Leong & Ling, 1992), it has been demonstrated that they are derived from the transformation of amoeboid microglial cells (AMC), a transient population of phagocytes in the developing brain (Ling, 1981; Leong & Ling, 1992). The above mentioned methods for the marking of AMC and their derivative cells, however, failed to demonstrate the dynamics of membrane activity that may be associated with the metamorphic transformation. Examples of cellular dynamics may include the turnover of membrane antigens or receptors expressed by the cells. Recently, an HRP-conjugated lectin (GSA I-B4), derived from *Griffonia simplicifolia* that binds α-D-galactosyl terminals, has been shown to mark specifically the AMC and ramified microglial cells in rodent nervous tissues (Streit & Kreutzberg, 1987; Kaur et al., 1990; Wu et al., 1992, 1993). With the lectin-labelling method, our previous morphometric study has shown that the diverse morphological forms of lectin-labelled microglial cells represent the different stages or degrees of differentiation of the cell type in growing rat brain. An interesting feature of our study was the gradual diminution of the lectin staining when the nascent round cells transformed into the mature ramified microglial cells (Wu et al., 1992). The significance of this phenomenon remained unclear, although it was suggested that the glycoconjugates at the plasma membrane of the lectin-labelled cells were down regulated. It was further postulated that this may be

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related to some functional activities associated with the membrane, e.g. endocytosis, during the transformation of the phagocytic AMC into ramified microglia. Since our earlier study was conducted at the light microscopic level, it remains to be ascertained whether there are any demonstrable changes associated with the regressive labelling for lectin of the cell type. The present study extended our previous findings by examining the lectin-labelled AMC and their derivative cells at the ultrastructural level. Arising from this, a possible intracellular routing of surface glycoconjugates coupled with synthesis of galactosyl protein is identified during microglial cell differentiation.

Materials and methods

Twenty Wistar rats from newborn (P0) to 15 days of age (P15) were used in this study. Following anesthesia with an intraperitoneal injection of 7% chloral hydrate, the rats were perfused with a mixed aldehyde solution composed of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 8 h at 4°C. The motor cortex including its underlying corpus callosum at the level of the optic chiasma was cut serially into coronal sections 40 or 60 μm thick with a vibratome. In order to exclude the staining of endogenous peroxidase in lysosomes, the sections were preincubated in 0.5% H2O2 for half an hour before the application of the lectin. After a brief pre-wash with 0.1 M cacodylate buffer, free-floating sections were incubated in a lectin solution of GSA I-B4-HRP (Sigma, L5391; 0.025 mg/ml−1 of 0.05 M Tris-buffer saline (pH 7.4) with 0.1% Triton X-100) overnight at 4°C. Following the incubation, the sections were reacted with diaminobenzidine. For light microscopy, 40 μm thick sections treated with lectin were collected on slides and counterstained with 0.5% thionin. For electron microscopy, 60 μm thick lectin-treated sections were postfixed in 1% OsO4 in 0.1 M cacodylate buffer. The sections were then dehydrated in a graded series of alcohol and embedded in Araldite mixture. Ultrathin sections were double stained with lead citrate and uranyl acetate and examined in a JEOL 1200 EX electron microscope.

For controls, the sections were pretreated with 0.1 M melibiose (6-O-α-D-galactopyranosyl-D-glucose; Sigma, M5500), which blocks the attachment of the lectin to α-D-galactosyl terminals. Microglia in the controls were unlabelled.

In the developing brain tissues, the external morphology of the lectin-labelled microglial cells varied with the degree of differentiation (Fig. 1a–e) as described in our previous study (Wu et al., 1992, 1993). We had established earlier that these diverse morphological forms of labelled cells represent different stages or degrees of differentiation (Wu et al., 1992). In the present study, the identification of the various morphological forms of microglial cells was first made in vibratome sections. In addition to their characteristic features, their preferential site of distribution and staining intensities also facilitated their ultrastructural identification. In the postnatal (P4–P13) brain tissues, the round cells and cells with short processes tended to concentrate in the central portion of the developing corpus callosum. These cells displayed the overall morphology of AMC as described by Ling (1981). The ramified cells with long extending thick processes were readily recognized in the peripheral zone of the growing corpus callosum, while the ramified cells bearing long and thin processes were found predominantly in the intermediate zone of cerebral cortex. From P15 onwards, only ramified cells displaying attenuated processes were observed (Wu et al., 1992, 1993). After the identification of the cell types at light microscopic level, a minimum of fifty cells of each phenotype were examined ultrastructurally. The result derived from each cell type were consistent and clearly reproducible.

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Fig. 1. Photomicrographs of various morphological forms of GSA I-B4-labelled cells in eight-day-old rat brain. Labelled cells vary from round cell (a) to cells bearing short stout processes (b), thick and long processes (c), thin and long processes (d) and attenuated processes (e). The blood vessels (*) are also labelled by GSA-I-B4-HRP. (a, b) × 280; (c–e) × 460.

Fig. 2. A round, amoeboid microglial cell in the corpus callosum of an eight-day-old rat. Intense lectin reactivity occurs at its cytoplasmic membrane (arrowheads), vesicles (arrows) and subplasmalemmal vacuoles (V); other organelles including phagosomes and Golgi apparatus (G) are not labelled. N, nucleus; × 12 000.

Fig. 3. Portions of round, amoeboid microglial cells showing lectin-labelled surface invaginations (arrows). The cytoplasmic vacuoles are weakly stained or unlabelled with lectin (a, V). In (b), arrowheads indicate the fusion of lysosomes (L) with vacuoles (V). A vacuolar residual body (**) appears to be labelled. Eight-day-old rat; (a) × 24 000, lead citrate only; (b) × 30 000.

Fig. 4. A microglial cell with short stout processes. The cell is labelled at its plasma membrane (arrowheads) and invaginations (arrowhead, see area outlined, inset), subplasmalemmal vacuoles (SV) and several cytoplasmic vacuoles (CV) away from the surface. Reaction products are also localized in a few small vesicles near the Golgi sacculles (arrows, inset). Eight-day-old rat; × 13 000; inset, × 28 000.

Fig. 5. An intensely labelled microglial cell with a long thick process is closely apposed to the surface of an unlabelled neuron (N). Arrowheads indicate sites of plasma membrane labelling. Note the dense lectin reactivity in the Golgi sacculles (G) and the wide distribution of stained vesicles (small arrows) in the process (P). Eight-day-old rat; × 9000.

Fig. 6. Portion of a labelled microglial cell with long and thick processes. Note the intense labelling in trans sacculles of the Golgi apparatus (TS), the trans tubular network (TTN) and associated vesicles (arrowheads). C, centriole. Eight-day-old rat; × 24 000.