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Original Article

A Double Staining Technique for Simultaneous Demonstration of Astrocytes and Microglia in Brain Sections and Astroglial Cell Cultures[1]

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We developed a double staining technique for simultaneous demonstration of astrocytes and microglial cells in histological brain sections and cell cultures. The procedure included a histochemical stain specific for microglial cells and an immunocytochemical stain specific for astroglial cells, with postponement of the final visualization of the staining products until both reactions had been performed. First, microglial cells were specifically but invisibly labeled by histochemical reaction for nucleoside diphosphatase (NDPase). Then the astroglial cells were labeled by performing the first parts of the immunocytochemical reaction for glial fibrillary acidic protein (GFAP). Finally, in a series of intervening steps, the NDPase reaction product was visualized and stabilized by treatment with ammonium sulfide and silver nitrate, while the 1-naphthol basic dye method was used to visualize the GFAP immunoreactive product. As an end product, the NDPase-positive microglial cells were brown and the GFAP-reactive astroglial cells blue. The two types of glial cells were clearly distinguishable in vibratome sections of rat brain tissue and in primary astroglial cell cultures, and we never observed cells that stained for both NDPase and GFAP. When the GFAP antibody was replaced by the OX-42 antibody, which recognizes microglial cells and macrophages, double staining of microglial cells was observed. The staining protocol has wide applications in studies of the functional interactions between microglial and astroglial cells in the normal brain and in different pathological states with neuronal or axonal degeneration, just as it can be used for experimental studies in cell cultures. (*J Histochem Cytochem 39:561-568 1991*)

KEY WORDS: Glia; Histochemistry; Immunohistochemistry; Double staining technique; Cell cultures; Central nervous system; Nucleoside diphosphatase; Glial fibrillary acidic protein; OX-42-positive microglial cells.

Introduction

Ninety years after the first description of microglial cells in the central nervous system (Robertson, 1900) their exact function is still not well established. In the past, microglial cells were regarded as resting scavenger cells, which, in certain circumstances, could react and become phagocytic (Rio Hortega, 1932). Now, there is increasing evidence that microglial cells, which in the normal adult brain appear as ramified, resting microglial cells and during development or after injury appear as ameboid cells, serve several important functions (Perry and Gordon, 1988; Streit et al., 1988). Besides a possible role in relation to synaptic transmission (Murabe and Sano, 1982a), microglial cells are involved in immune reactions and repair in the central nervous system (CNS), apparently in close interaction with astroglial cells. Microglial cells may act as antigen-presenting cells, since Class II antigens of the major histocompatibility complex (MHC) have been demonstrated on their cell surface (Konno et al., 1989; Hickey and Kimura, 1988; Steiniger and vander-Meide, 1988; Hayes et al., 1987). In addition, astrocytes have been proposed as antigen presenting cells on the basis of observations of expression of MHC antigens in vitro (Frei et al., 1987; Wong et al., 1984) and in vivo (Frank et al., 1986; Hofman et al., 1986; Hickey et al., 1985). In relation to brain injury, it moreover appears that microglial cells can trigger astrocyte proliferation by release of cytokines such as interleukin-1 (Giulian, 1987; Giulian et al., 1986; Giulian and Lachman, 1985) and other growth factors (Giulian, 1987; Giulian and Baker, 1985). Ameboid microglial cells present in the developing brain can also release growth factors, influencing astroglial proliferation and differentiation (Giulian et al., 1988). In vitro studies have shown that astrocyte-derived interleukin-3-like substances can stimulate microglial proliferation (Frei et al., 1986). The exact functional relations between microglial

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and astroglial cells are by no means clarified, but observations such as those cited here clearly point to a close interrelationship between the two cell types. A technique allowing specific visualization of both astroglial and microglial cells in the same histological sections and cell cultures would therefore be of great help. Astroglial cells are usually demonstrated by immunocytochemical staining for glial fibrillary acidic protein (GFAP), an intermediate filament protein (Raff et al., 1979; Bignami et al., 1972). Other antibodies that distinguish between different astroglial cell subpopulations have also been used (Miller et al., 1986; Miller and Raff, 1984). Visualization of microglial cells has been more of a problem. Earlier, the study of these cells had to rely on the use of the classical silver impregnation methods developed by Rio Hortega (1919). During the last 10 years, however, enhanced interest in microglial cells has stimulated investigators to find microglial markers to overcome the problems inherent to the silver stains. One of the better techniques for selective staining of microglial cells is the histoenzymatical demonstration of nucleoside diphosphatase (NDPase) (Glenn et al., 1989; Schnitzer, 1989; Castellano, 1987; Fujimoto et al., 1987; Castellano et al., 1984; Murabe and Sano, 1982a,b; Vorbrodt and Wisniewski, 1982). Other recent techniques have involved the use of lectin markers (Kreutzberg et al., 1989; Suzuki et al., 1988; Yamamoto et al., 1988; Streit and Kreutzberg, 1987; Mannoji et al., 1986) and immunocytochemical staining with macrophage/microglial cell-specific antibodies (Tillotson and Wood, 1989; Perry and Gordon, 1987; Matsumoto and Ikuta, 1985; Matsumoto et al., 1985). In the present study we present a reliable method for simultaneous and specific demonstration of microglial and astroglial cells in brain sections and cell cultures by combining the histochemical demonstration of NDPase with immunocytochemical visualization of GFAP. In addition, we tried a combination of NDPase staining and immunocytochemical staining with the microglia marker OX-42 to double stain microglial cells.

Materials and Methods

Preparation of Brain Sections

Twelve Wistar rats, weighing 180–200 g, were deeply anesthetized with sodium pentobarbital and perfused transcardially for 20 min with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) with 5% sucrose. Sucrose was added because in another ultrastructural study this was shown to improve the structural preservation of the tissue (Castellano et al., 1989). The brains were then removed and immersed in the same fixative for 2 hr at 4°C before being sectioned at 30 μm on an Oxford vibratome. The sections were cut in the frontal plane, collected in cacodylate buffer (pH 7.4) with 7.5% sucrose, and rinsed for 2 hr at 4°C with several changes. During all steps in these and the following procedures special care was taken to prevent any drying of the sections.

Preparation of Cell Cultures

Primary astroglial cell cultures were prepared according to a modification of the method outlined by Hertz et al. (1985). Proliferating astroblasts were obtained from the brain of newborn C57 B1/6 mice. Under aseptic conditions, the neopallium was cleared of meninges and blood vessels and dissociated in a modified Eagle's medium (Gibco; Grand Island, NY). The suspension was then filtered through an 80-μm nylon mesh and transferred as a single cell suspension to 35-mm culture dishes (Nunc), using 1.5 ml medium per dish and four dishes per brain. The cultures were incubated at 36.5°C in an atmosphere containing 5% CO_2. The culture medium (a modified Eagle's MEM with 20% horse serum supplemented with vitamins, amino acids, and 2.5 mM glutamine) was changed every third day. After 1 week, the concentration of horse serum was changed to 10%. After a further 2 weeks 0.25 mM dibutyril cyclic AMP (Sigma, St Louis, MO; Cat. No. D-0627) was added for another 2 weeks to induce structural differentiation of the astroglial cells (Hertz et al., 1985). For the rest of the 6–7-week culture period normal medium with 10% HS was used.

At the end of the culture period the cultures were immersed for 2 hr at 4°C in the fixative described above. The cultures were then carefully washed with several changes of cacodylate buffer (pH 7.4) at 4°C before staining. Again, special care was taken at every step to avoid any drying out of the samples.

Double Labeling Technique

Simultaneous demonstration of microglial and astroglial cells was achieved through three sequential steps: (a) incubation for histochemical demonstration of NDPase activity; (b) incubation for immunocytochemical detection of GFAP; and (c) visualization of the histochemical and the immunocytochemical reaction products for light microscopy.

Histochemical Reaction for Microglial NDPase. Incubation for histochemical demonstration of NDPase activity was carried out as earlier reported (Castellano, 1987). In brief, 25 mg of the sodium salt of inosine 5'-diphosphate (Sigma; Cat. No. I 4375) were dissolved in 7 ml of distilled water, followed by addition of 10 ml of 0.2 M Trizma buffer (Sigma) (pH 7.4), 5 ml of 0.5% $MnCl_2$, and 3 ml of 1% $Pb(NO_3)_2$ in this order. The solution was then filtered and used immediately. After transfer to the solution the brain sections were incubated for 5–25 min at 38°C and then rinsed (three times for 10 min) in cacodylate buffer (pH 7.4). Staining of the astroglial cell cultures was carried out in the same way by adding the incubation medium to the petri dishes after removal of the buffer. Some sections and cell cultures were immediately treated with ammonium sulfide to visualize the reaction product (see below) and thereby check the histochemical reaction. Otherwise, the sections and cultures continued to the next step in the procedure, the immunocytochemical reaction for astroglial GFAP.

As control for the histochemical reaction, some sections and cultures were incubated in medium lacking the substrate inosine 5'-diphosphate.

Immunocytochemical Reaction for Astroglial GFAP. The brain sections and astroglial cell cultures were washed in 0.05 M Tris-buffered saline (TBS), pH 7.4, with 1% Triton X-100 (three times for 15 min) and then incubated in 10% fetal calf serum (FCS) for 30 min at room temperature. The incubation with the primary rabbit anti-GFAP antibody (Dakopatts; Glostrup, Denmark) was performed overnight at 4°C in a 1:2400 dilution in TBS with 10% FCS. After washing (three times for 15 min) in TBS with 1% Triton X-100, the sections and cell cultures were incubated at room temperature for 60 min with an anti-rabbit Ig–biotinylated antibody (Amersham; Poole, UK) in a 1:300 dilution in TBS with 10% FCS. After washing in TBS with 1% Triton X-100 (three times for 15 min), the samples were incubated for 60 min at room temperature in a 1:70 dilution of avidin labeled with peroxidase type VI (Sigma) in TBS with 10% FCS. Finally, the samples were washed in TBS with 1% Triton X-100 (twice for 15 min) and in TBS (twice for 15 min).

As a control, some sections and cultures were processed without incubation in the primary GFAP antibody.

Visualization of the Histochemical and Immunocytochemical Reaction Products. From this stage the routine histochemical staining for NDPase consisted of treatment of the incubated sections and cultures with ammonium sulfide and silver nitrate solutions to visualize and stabilize the ini-

tial and at this stage invisible lead phosphate reaction product by conversion into brownish-black silver sulfide.

For the routine immunocytochemical staining for GFAP, a corresponding visualization of the avidin-bound horseradish peroxidase by a histochemical procedure was the next step. To achieve successful and differentiated double staining the final steps of the two staining techniques did, however, have to be executed in a certain sequence. We also chose the 1-naphthol basic dye method of Mauro et al. (1985) for demonstration of the horseradish peroxidase in the immunocytochemical staining for GFAP, because this gave a blue HRP reaction product which was easily distinguished from the brownish-black NDPase staining.

The final steps of the double staining procedure then were as follows:

1. Immersion of sections and glial cell cultures in a 1-naphthol solution with 0.01% hydrogen peroxide for 15 min at room temperature. The naphthol solution was freshly prepared by dissolving 50 mg of 1-naphthol (Sigma) in 0.5 ml ethanol and adding this, together with 10 ml 1% ammonium carbonate, to 89.5 ml of 0.05 M TBS, pH 7.4. The solution was filtered before addition of the hydrogen peroxide.
2. Three 10-min washes in TBS, followed by immersion of samples in 2% ammonium sulfide for 2 min at room temperature, and three washes for 10 min in TBS.
3. Immersion of samples in 0.05% Azur A in 0.05 M TBS, pH 8.0, for 30 min, followed by a wash in TBS, pH 7.4, with continuous stirring for 1 hr and several changes of TBS.
4. Finally, after two 2-min washes in distilled water, immersion of sections and cell cultures in 1% silver nitrate for 1 min and two washes in distilled water.

The Vibratome sections were mounted on gelatin-coated slides, dehydrated, and coverslipped with Dammar resin. In the petri dishes the astroglial cell cultures were coverslipped with glycergel mounting medium (Dakopatts).

Double Labeling with NDPase and OX-42

To test the specificity of NDPase staining as a microglial marker, some sections were briefly (5 min of incubation) reacted for NDPase demonstration and then reacted immunohistochemically using the OX-42 antibody, which has been proposed as a specific marker for microglial cells in the rat nervous system (Perry and Gordon, 1987; Robinson et al., 1986). The double staining procedure was performed essentially as described above, except that the GFAP antibody was replaced by OX-42 (Seralab; Crowley Down, UK) used in a 1:600 dilution, and that the secondary antibody correspondingly was exchanged with an anti-mouse Ig–biotinylated antibody (Amersham) used in a 1:200 dilution. As control for the NDPase reaction, some sections were incubated without substrate. As control for the OX-42 immunocytochemical staining the primary OX-42 antibody was omitted.

Results

The present double staining procedure combined enzymatic histochemistry with immunocytochemistry for simultaneous demonstration of microglial NDPase activity and astroglial GFAP immunoreactivity in brain sections and tissue cultures. In brain sections the same principle was used to demonstrate microglial co-localization of NDPase activity and OX-42 immunoreactivity.

Vibratome Sections from Rat Brain

In the brain sections the double staining procedure enabled us to distinguish two populations of cells: microglial cells with a brownish-black staining precipitate corresponding to the location of the NDPase activity (Figures 1–4) and astroglial cells with a blue GFAP immunoreactive staining (Figures 2–4). In addition, cells lining the blood vessels (endothelial cells and some perivascular cells) also reacted for NDPase (Figures 1–4). All NDPase-positive cells in the gray and the white matter of all brain areas examined were negative for GFAP. The NDP-positive microglial cells had an elliptical or elongated irregularly shaped cell body with irregular branching processes, covered with small thorny processes. In the gray matter the microglial cell processes covered a sphere with an approximate diameter of 70–80 μm. There was no overlap between the spheres from the individual cells, which seemed to exclude neighbors from their territory (Figure 1 and 2). Some microglial cell bodies were found in association with blood vessels, but most cells had no apparent relation to blood vessels, although their processes were often found near capillaries. Astroglial cells, positive for GFAP immunostaining, were located in between the NDPase-reactive microglial processes (Figures 2 and 3). The GFAP-reactive astroglial processes did not display any particular arrangement with respect to the microglial processes. The processes of the two cell types thus seemed neither to seek contact nor to avoid each other.

Brain sections reacted for both NDPase and OX-42 showed that both stains were localized in the same type of cells. In these sections all microglia-like cells accordingly displayed both a brown and a blue color in relation to the plasma membrane (Figure 5).

Primary Glial Cell Cultures

In primary mouse astroglial cell cultures, NDPase and GFAP double staining revealed the presence of several NDPase-positive cells among the GFAP-positive astroglial cells (Figures 6 and 7). Two main populations of NDPase positive cells were distinguished. One population consisted of ramified cells, referred to as ramified microglia. The other consisted of small non-ramified cells, referred to as ameboid microglia. Neither of the two NDPase-positive cell populations stained for GFAP. The ramified microglial cells (Figure 6) displayed distinctive NDPase reactivity at the plasma membrane, but the reaction was always lighter than that observed in ameboid microglia (see below). The ramified cells differed in shape and size. Some were elongated, others were stellate, but they all had some processes. These processes were sometimes further branched, with small protrusions resembling the spiny processes of the ramified microglial cells in normal adult brain. In the cultures, the NDPase-positive ramified cells were found scattered among the astroglial cells, with no apparent clustering. The cell bodies and processes of the ramified microglial cells appeared in close contact with the cell bodies and processes of the astroglial cells, and it was often possible to see how the microglial processes surrounded and contacted the astroglial cells (Figure 6). The ameboid microglial cells (Figure 7) always showed strong NDPase reactivity, apparently associated with the plasma membrane. These cells were round or elongated and usually without processes. Sometimes they had one or two thick non-ramified processes with thin filopodia. In the cultures, the ameboid cells were usually located on top of the astroglial cell layer, while the ramified microglia mingled directly with the astroglial cells (see above).

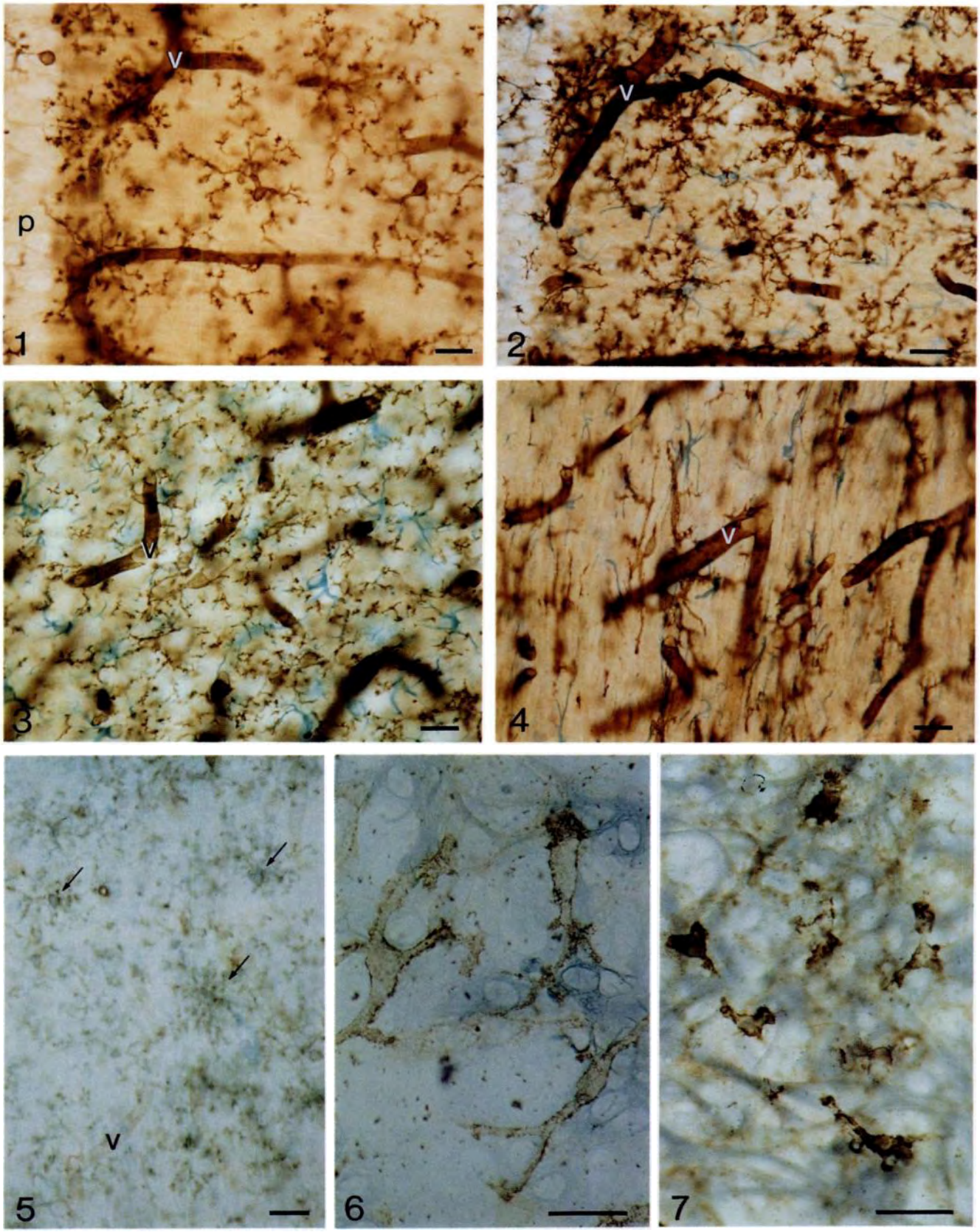

Figure 1. Microglial cells stained for NDPase in stratum radiatum of the rat hippocampal CA1 region. Endothelial cells in blood vessels (v) are also stained. Note that pyramidal cells (p) are unstained. Bar = 20 μm.

Figure 2. Simultaneous demonstration of microglial cells (brown NDPase staining) and astrocytes (blue GFAP immunoreactivity) in stratum radiatum of the rat hippocampal CA1 region. v, vessel. Bar = 20 μm.

Discussion

Technical Considerations

We have described a method for simultaneous demonstration of astroglial and microglial cells, which can be applied to Vibratome sections of brain tissue as well as primary cell cultures. The double staining was accomplished by employing the histochemical staining for NDPase as a specific marker for microglial cells together with immunohistochemical demonstration of GFAP, an intermediate filament protein specific for astroglial cells. By experience, we found it essential to perform the incubation part of the NDPase staining before the immunohistochemical reaction for GFAP because Triton X-100, used to facilitate tissue penetration of antibodies in immunohistochemical stains, would otherwise eliminate the enzymatic NDPase activity on the microglial plasma membrane. The histochemical incubation for NDPase was accordingly done as the first step. The inorganic phosphate released from the substrate inosine 5'-diphosphate by the enzyme NDPase was precipitated as lead phosphate, a white salt not visible by light microscopy. The lead phosphate was later converted to visible brown lead sulfide by treatment with ammonium sulfide, before a final immersion in silver nitrate resulted in the formation of an even more insoluble and visually distinct brownish-black precipitate of silver sulfide. Since treatment with ammonium sulfide reduced the GFAP antigen reactivity, we postponed this treatment (and the subsequent addition of silver nitrate) until after the immunocytochemical reactions with the primary and secondary antibodies.

For GFAP immunoreactivity we chose to use 1-naphthol combined with the basic dye Azur A as chromogen instead of the more commonly used diaminobenzidine. The reason was that the reaction product of the 1-naphthol basic dye (1-NBD) method (Mauro et al., 1985) had a strong blue color (Figures 2–4), which differed more clearly from the brownish-black reaction product of the histochemical NDPase staining than the brown diaminobenzidine product. The 1-NBD method basically consisted of two steps. First, the 1-naphthol was oxidized in the presence of hydrogen peroxide. Then the basic dye Azur A was bound to the oxidation product, resulting in an intense blue color. If the 1-NBD staining was completed with the addition of Azur A before the first step in the NDPase visualization with ammonium sulfide, there was significant loss of the blue color. Although this could to some extent be reinstated by repeating the application of Azur A, we usually postponed the treatment with Azur A until after the initial visualization of the NDPase reaction by ammonium sulfide.

In this study, the double staining procedure was primarily used to demonstrate and distinguish two separate cell types, microglial and astroglial cells. The procedure can, however, in principle be adapted for simultaneous demonstration of NDPase-positive microglial cells and all other immunocytochemically detectable antigens besides GFAP. As an example of this, we combined NDPase staining and OX-42 immunocytochemistry, demonstrating the presence of both of these markers on microglial cells (Figure 5).

Histochemical Demonstration of NDPase as a Microglial Marker

The presence of NDPase activity as a specific microglial marker in brain sections requires some comment. This enzyme, which catalyzes the hydrolysis of nucleoside diphosphates as well as of thiamine pyrophosphate (Sano et al., 1988; Barchi and Braun, 1972; Yamazaki and Hayaishi, 1968), has been demonstrated histochemically in cells with the characteristic morphology of microglial cells (Schnitzer, 1989; Castellano, 1987; Fujimoto et al., 1987; Castellano et al., 1984; Murabe and Sano, 1982a,b; Vorbrodt and Wisniewski, 1982), but it has also been found in neurons (Vorbrodt and Wisniewski, 1982; Shantaveerapa and Bourne, 1965; Goldfisher, 1964), astrocytes (Castellano et al., 1984; Vorbrodt and Wisniewski, 1982), and oligodendrocytes (Castellano et al., 1988,1989; Vorbrodt and Wisniewski, 1982). It should be noted, however, that histochemical localization of NDPase to the plasma membrane is restricted to microglial cells. No studies have thus far reported that NDPase was present in the plasma membrane of neurons, astrocytes, and oligodendrocytes. Those studies that have reported the presence of histochemical NDPase activity in different cytoplasmic localizations in neurons and glial cells used frozen sections or sections cut by a tissue chopper after the tissue had been embedded in agar. When the NDPase reaction is performed on Vibratome sections and drying of the sections is carefully prevented, the NDPase staining is restricted to blood vessels and the plasma membrane of microglial cells. In the present material, consisting of Vibratome-cut brain sections and astroglial cell cultures, NDPase staining of neurons, astrocytes, and oligodendrocytes was not observed (Figure 1). Performed in this way, the histochemical staining for the NDPase accordingly is a very good and reliable marker for microglial cells. In agreement with this, sections reacted for both NDPase and the OX-42 antibody showed double staining of all microglia-like cells (Figure 5).

Figure 3. Double staining for NDPase and GFAP, showing microglial cells (brown) and astrocytes (blue) in the rat neocortex. v, vessel. Bar = 20 μm.

Figure 4. Simultaneous demonstration of microglial cells (brown) and astrocytes (blue) in the rat corpus callosum after double staining for NDPase activity and GFAP immunoreactivity. v, vessel. Bar = 20 μm.

Figure 5. Microglial cells (arrows) in rat hippocampus, double labeled by NDPase staining (brown) and OX-42 immunoreactivity (blue). v, vessel. Bar = 20 μm.

Figure 6. Primary astroglial cell culture double stained for NDPase and GFAP, showing ramified microglial cells (brown) and astrocytes (blue) at the same focus level in the culture. Bar = 20 μm.

Figure 7. Primary astroglial cell culture with NDPase-positive ameboid microglial cells (brown) on top of GFAP-reactive astroglial cells (blue, and at a different focus level). Bar = 20 μm.

Double Staining of Astroglial and Microglial Cells

In the last 10 years, much interest has been devoted to finding specific antibodies for microglial cells. Based on the view that microglial cells are resident macrophages in the CNS, most attempts have been directed to find macrophage-related antigen expression on microglial cells. To test the specificity of such microglial markers, double staining with the astroglial marker GFAP has sometimes been tried on paraffin or frozen sections. One of the first antibodies reported to mark microglial cells was the monoclonal antibody Mac-1 raised against mouse macrophages (Matsumoto and Ikuta, 1985; Matsumoto et al., 1985). Double immunocytochemical staining for GFAP and Mac-1 on frozen sections from postnatal cold-injured mice revealed the presence of Mac-1-positive cells in the white matter, which at the same time were GFAP negative (Matsumoto et al., 1985). However, the authors were not able to visualize Mac-1-positive microglia-like cells in the brain gray matter. Double staining of human brain tissue for GFAP and EBM/11, which was another monoclonal antibody raised against macrophages (Esiri and McGee, 1986), revealed that GFAP-positive cells in some cases also showed a weak diffuse reaction for EBM/11 (Woodroofe et al., 1986). Provided that this staining is specific, astrocytes may accordingly, under certain circumstances, express macrophage antigen properties, just as they can exert phagocytotic activity (Sturrock, 1988; Noske et al., 1982; Fulcrand and Privat, 1977). Transformation of cells of astroglial lineage into macrophage-like cells has, for example, been reported in organotypic cultures of spinal cord tissue (Kusaka et al., 1986). Specific binding of certain lectins has also been used to visualize microglial cells (Kreutzberg et al., 1989; Suzuki et al., 1988; Yamamoto et al., 1988; Streit and Kreutzberg, 1987; Mannoji et al., 1986). Simultaneous demonstration of astroglial and microglial cells in paraffin sections has thus been accomplished by combining GFAP immunostaining and the binding of the B4 isolectin from *Griffonia simplifolia* (GSA-IB4) to microglial cells (Streit and Kreutzberg, 1987). Simultaneous demonstration of such lectin binding and immunocytochemical staining for phosphotyrosine was used recently by Tillotson and Wood (1989) to verify that the phosphotyrosine immunoreactivity in the brain was specifically confined to microglial cells.

Presence of Microglial Cells in Astroglial Cell Cultures

The use of NDPase staining in combination with GFAP immunoreactivity on primary astroglial cell cultures enabled us to identify two types of NDPase-positive but GFAP-negative cells in these cultures. From other ongoing studies of mixed brain cell cultures, where the astroglial differentiation with cyclic AMP was omitted, we know that astroglial cells also lack NDPase activity without cyclic AMP stimulation. Based on differences in morphology, two types of NDPase positive cells were identified in the astroglial cell cultures. They were ramified microglia (Figure 6) and ameboid microglia (Figure 7). In most cultures the ramified cells were more frequent than the ameboid ones. In addition, their localization in relation to the astroglial cells was distinctly different. The ramified cells were thus located among the astroglial cells, whereas the ameboid microglial cells usually were located on top of the other cells. They were clearly located in a separate focus level when examined in the microscope (Figure 7). The relationship between the two types of NDPase-positive cells is not clear, but the presence of what appeared as intermediate forms suggested that transformations between the two might occur. This would be in agreement with studies supporting the view that the ramified microglial cells of the adult brain are derived from the ameboid microglial cells of the developing brain (Murabe and Sano, 1982b; Ling, 1979; Immamoto and Leblond, 1978). In vitro studies have supported this by showing that addition of retinoic acid or dimethylsulfoxide to ameboid microglial cell cultures induced the cells to form processes and lose their phagocytic properties (Giulian and Baker, 1989).

Concluding Remarks

We have presented a reliable method for simultaneous demonstration of astroglial and microglial cells by successfully combining NDPase histochemistry for microglial cells with astroglial GFAP immunoreactivity in Vibratome brain sections and cell cultures. With increasing amounts of data pointing to intimate functional relations between microglial and astroglial cells (Frei et al., 1986; Tedeschi et al., 1986; Giulian and Baker, 1985; Giulian and Lachman, 1985), the procedure should have wide applications for combined studies of these cells in the normal brain, during development and in different pathological conditions, including experimental studies of neuronal and glial cell cultures.

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