

Differentiation of Donor-Derived Cells into Microglia after Umbilical Cord Blood Stem Cell Transplantation

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Differentiation of donor-derived cells into microglia after umbilical cord blood stem cell transplantation

Running head: Differentiation of donor cells into microglia

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Abstract

Recent studies indicate that microglia originate from immature progenitors in the yolk sac. After birth, microglial populations are maintained under normal conditions via self-renewal without the need to recruit monocyte-derived microglial precursors.

Peripheral cell invasion of the brain parenchyma can only occur with disruption of the blood-brain barrier. Here, we report an autopsy case in which cells derived from umbilical cord blood differentiated into ramified microglia in the host brain parenchyma.

Although the blood-brain barrier and glia limitans prevented invasion of these donor-derived cells, most of the invading donor-derived ramified cells were maintained in the cortical zone. This result suggests invasion of donor-derived cells occurs through the pial membrane.

Key word: microglia, differentiation, progenitor, umbilical cord blood

Introduction

Microglia, the only immunocompetent cells in the central nervous system (CNS), originate from progenitors derived from the yolk sac (1, 2). Studies have shown that after birth, microglia maintain their numbers under normal conditions by self-renewal without the recruitment of monocyte-derived microglia precursors (2, 3). Although there is currently no specific marker to fully distinguish resident microglia from monocyte-derived macrophages, some studies have shown that microglia and peripheral blood cell-derived macrophages can be distinguished by the presence of surface markers such as CCR2 and CX3CR1, even under inflammatory conditions (4, 5). Moreover, a human study showed that Y chromosome-bearing male cells cannot differentiate into microglia in the CNS of a female host after bone marrow transplantation (6). However, bone marrow stem cells and peripheral monocytes can differentiate into microglia-like cells in vitro (7, 8), suggesting that one of the most important barriers preventing the recruitment of microglial precursors from the peripheral blood is the blood-brain barrier (BBB). Indeed, irradiation or the alkylating agent busulfan can be used to disrupt the BBB, enabling the invasion and maintenance of bone marrow-derived cells in the brain parenchyma (9).

In a mouse study, stress induced the recruitment of bone marrow-derived monocytes to the brain parenchyma (10). However, the results of many other studies do not clarify the origin of microglia in human adult brain. Here, we report an autopsy case where umbilical cord blood-derived cells differentiated into ramified microglia in the host brain parenchyma.

Material and Methods

Case report

A 51-year-old Japanese woman noticed swelling of her nasal floor at the age of 48; she was diagnosed with NK/T-cell lymphoma at our hospital. She received focal radiation therapy targeting the paranasal, maxillary, and ethmoid sinuses, the parotid glands, and orbits from the right and left side (40 Gy) following three courses of DeVIC (dexamethasone, etoposide, ifosfamide, and carboplatin) chemotherapy. Additionally, she received Novalis Shaped Beam Surgery (12 Gy). After treatment, she achieved complete remission (CR); two months after chemotherapy, she received a cord blood stem cell transplant (CBSCT) from a male donor with whom there are two HLA mismatches (Donor; A*31:01, A*33:03, B*51:01, B*40:03, DRB1*08:02, DRB1*14:05, Host; A*31:01, A*02:01, B*51:01, B*40:02, DRB1*08:02, DRB1*14:05). Acute graft-versus-host disease (GVHD) was mild and well controlled by oral administration of tacrolimus. Four months after CBSCT, the patient suffered shingles of the right 12th thoracic segment and was treated with acyclovir; however, herpes zoster developed into cervical myelitis. Combined therapy with steroid pulse treatment and intravenous administration of gamma globulin was effective. Two years after CBSCT, she experienced muscle weakness and sensory disturbance of the lower extremities. Her symptoms gradually worsened although steroid pulse treatment provided slight improvement. Positron emission tomography (PET) showed relapse to the liver. Her general condition worsened and her consciousness deteriorated. Additionally she was

given a single intrathecal admission of methotrexate (15 mg) two weeks before her death. She died 29 months after CBSCT.

A general autopsy was performed 3 hours after death. The final diagnosis of the central nervous system was relapse to the spinal cord and inflammation of the left medulla with normal finding of the cerebrum and cerebellum except for a small area showing rude construction of the cortex in the left mid-frontal gyrus.

Immunohistochemistry

The brain was fixed with 10% buffered formalin. Multiple tissue blocks were embedded in paraffin, and 6- μ m sections were cut and immunostained using a Liquid DAB+ Substrate Chromogen System kit (DAKO, Tokyo, Japan) and a TMB Peroxidase Substrate kit (Vector, Burlingame, CA). The sections were counterstained with hematoxylin. Primary antibodies included rabbit polyclonal anti-Iba-1 (Wako; Osaka, Japan; 1:8000), anti-GFAP (Sigma; Tokyo, Japan; 1:10000), and anti-CX3CR1 (Abcam; Tokyo, Japan; 1:100), rabbit monoclonal anti-CCR2 (Abcam; 1:4000), and mouse monoclonal anti-HLA-A2 (MBL; Nagoya, Japan; 1:100). Appropriate antigen retrieval methods were applied to each primary antibody.

In site hybridization

We used a commercial kit to identify the Y chromosome. Briefly, 5- μ m sections from formalin-fixation paraffin-embedded blocks were deparaffinized, re-hydrated, and predigested with one drop of pepsin solution. The sections were incubated with hybridization solution containing ZytoDot CEN Yq12 Probe (ZytoVision; Bremerhaven, Germany) overnight at 37°C. The ZytoDot CISH Implementation Kit (ZytoVision) was

used to visualize the hybridization products. After staining, the products were double-stained with anti-Iba-1 antibodies.

Results

To study the migration of donor-derived cells into the central nervous system (CNS) of the host, we performed immunohistochemistry with anti-HLA-A2 antibodies that could distinguish donor-derived cells from host cells. Interestingly, donor-derived HLA-A2(+) cells were found in the cortex and around vessels (Fig. 1A-C). HLA-A2(+) cells often seemed to have accumulated in the cortical region (Fig. 1A). Very few were observed in the deep white matter. Invading HLA-A2(+) cells in the cortical region were morphologically ramified, similar to microglia (Fig. 1B). However, immunohistochemical staining of the C5 lesion with anti-HLA-A2 antibodies showed a higher number of HLA-A2(+) cells in the parenchyma (Fig. 1D, E). Moreover, HLA-A2(+) cells are morphologically round in shape or ring-shaped, but not ramified (Fig. 1D, E).

Next, we performed double staining to investigate graft cells differentiation into microglia or astrocytes. HLA-A2(+) cells stained positive for Iba-1, representative of microglia (Fig. 2A), but not GFAP specific to astrocytes (Fig. 2B). Since these results suggest the graft cells differentiated into microglia, we also performed *in situ* hybridization with anti-Yq12 probes. Although almost all cells in the sample from a male positive control showed a brown dot in their nuclei, and cells in the sample from a female negative control had no brown dot in their nuclei, only a few cells in the sample

from the patient had a brown dot in the nuclei, indicating Y chromosome-positive cells from the male donor (Fig. 2C-E). Moreover, in situ hybridization with anti-Yq12 probes following immunohistochemistry with anti-Iba-1 antibodies showed that Y chromosome-positive cells from the male donor were Iba-1-positive (Fig. 2F). CCR2 and CX3CR1 are very important to distinguish resident microglia and monocyte-originating microglia-like cells; thus, we next explored the expression of CCR2 and CX3CR1 on the HLA-A2(+) cells. About 60% of the HLA-A2(+) cells were CCR2-negative and most of the invading HLA-A2(+) cells were CX3CR1-positive (Fig. 3A-C).

Discussion

Our case showed that umbilical cord blood stem cell-derived precursors could differentiate into CX3CR1(+)CCR2(-)Iba-1(+) ramified microglia even without irradiating the brain, whereas previous clinical studies did not show donor-derived cells entering the CNS parenchyma and transformation to microglia (6).

Clinical conditions differed between our case and previous cases. First, our patient had an event of herpes zoster myelitis and relapse to the spinal cord, perhaps including meningitis of the whole brain. Moreover, an area of rude construction of the cortex in the left mid-frontal gyrus may indicate a trace of herpes zoster virus-induced encephalitis. Indeed, donor-derived cells can enter the recipient brain and differentiate into microglia after severe streptococcal meningitis (11). Bacterial meningitis often destroys the BBB and pial membrane, allows donor-derived cells to easily enter the

CNS parenchyma. Even after meningitis, we often observe abnormal meningeal findings. Massengale et al. showed that no donor-derived cells enter the CNS parenchyma even with brain injury (12). Ajami et al. also reported that infiltrating monocytes in an inflammatory site have a short life and do not contribute to the resident microglia pool (13). Although it is considered the effect of meningitis carcinomatosa due to relapse to the spinal cord, our case showed no trace of meningitis.

Second, the effect of chemotherapy and focal radiation therapy should be considered.

The fact that donor-derived cells had not invade the deep white matter of the recipient is consistent with the possibility that there was no brain irradiation, as has been shown in head-shielded mouse models (14). Indeed, we rarely observed donor-derived microglia in the deep white matter, thalamus, and basal ganglia, although we found donor-derived perivascular cells; however, inflammation of the left medulla as observed in the final diagnosis may be due to the direct effect of focal irradiation. Since the alkylating agent busulfan can cause bone marrow-derived cells to penetrate the brain parenchyma (9), DeVIC chemotherapy may also disrupt the BBB. However, donor-derived ramified cells were rare in the deep region of the brain suggesting the BBB in our patient was sufficiently intact to prevent invasion of donor-derived cells. Although Unger et al. did not provide information pertaining to irradiation and chemotherapy (6), all patients likely received aggressive chemotherapy as pretreatment before bone marrow transplantation for leukemia or lymphoma. Nevertheless, no donor-derived cells differentiated into ramified microglia. The fact that donor-derived cells did not invade the deep white matter of the recipient is considered to be consistent with the possibility

that chemotherapy had a minimal effect, as noted previously. Moreover, even if a single intrathecal admission of methotrexate could disrupt the BBB, it appears highly improbable that invading cells differentiated into CX3CR1(+)CCR2(-)Iba-1(+) ramified microglia indicating the resting form within two weeks.

In conclusion, we report an autopsy case in which cells derived from umbilical cord blood differentiated into ramified microglia in the host brain parenchyma. Although the BBB and glia limitans prevented invasion of these donor-derived cells, most of the invading donor-derived ramified cells remained in the cortical zone. This result suggests that the invasion of donor-derived cells occurs through the pial membrane.

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Figure legends

Figure 1

Immunohistochemistry of HLA-A2 with hematoxylin staining. (A) HLA-A2(+) cells accumulated in the cortex (brown cells in the circle). (B) HLA-A2(+) cells with a ramified form (arrow). (C) A HLA-A2(+) cell showing a round shape around a vessel (arrow). HLA-A2(+) cells with a round form or ring form in the C5 lesion of the spinal cord at lower magnification (D) or at a higher magnification (E).

Figure 2

Double staining of donor-derived cells by immunohistochemistry (A, B, ~~D~~, ~~E~~) and in situ hybridization (C, D, E, F). (A) Double staining of donor-derived cells with anti-HLA-A2 antibodies (brown) and anti-Iba1 antibodies (blue). The inset shows the image at higher magnification. (B) Double staining of donor-derived cells with anti-HLA-A2 antibodies (brown) and anti-GFAP antibodies (blue). The inset shows the image at a higher magnification. (C) In situ hybridization of the sample from the patient by using anti-Yq12 probes (brown dot) with the staining of nuclei by Mayer's hematoxylin solution. Arrows show Yq12(+) cells, indicating donor origin. The inset shows the image at higher magnification. (D) In situ hybridization of the sample from the positive control. The inset shows the image at a higher magnification. Arrows show Yq12(+) cells. (E) In situ hybridization of the sample from the negative control. (F) Double staining of donor-derived cells by using anti-Yq12 probes (brown dot) and anti-Iba antibodies (blue). Arrows show double-positive cells, indicating donor origin,

and arrowheads show Iba-1(+)Yq12(-) cells, indicating host origin. The inset shows the image at higher magnification.

Figure 3

Double staining of donor-derived cells by immunohistochemistry (A, C). (A) Double staining of donor-derived cells with anti-HLA-A2 antibodies (brown) and anti-CCR2 antibodies (blue). Arrows indicate CCR2(-) HLA-A2(+) cells and arrowheads indicate double-positive cells. (B) Frequency of CCR2(+) migrated cells and CCR2(-) migrated cells. The number of migrated HLA-A2(+) cells was counted in three independent sections and the frequency of CCR2(+) cells and CCR(-) cells were indicated by a bar graph. Bars indicate means \pm S.D. (C) Double staining of donor-derived cells with anti-HLA-A2 antibodies (brown) and anti-CX3CR1 antibodies (blue). Arrows show double-positive cells, indicating donor origin, and an arrowhead indicates a CX3CR1(+)HLA-A2(-) cell, indicating host origin.

Figure 1.

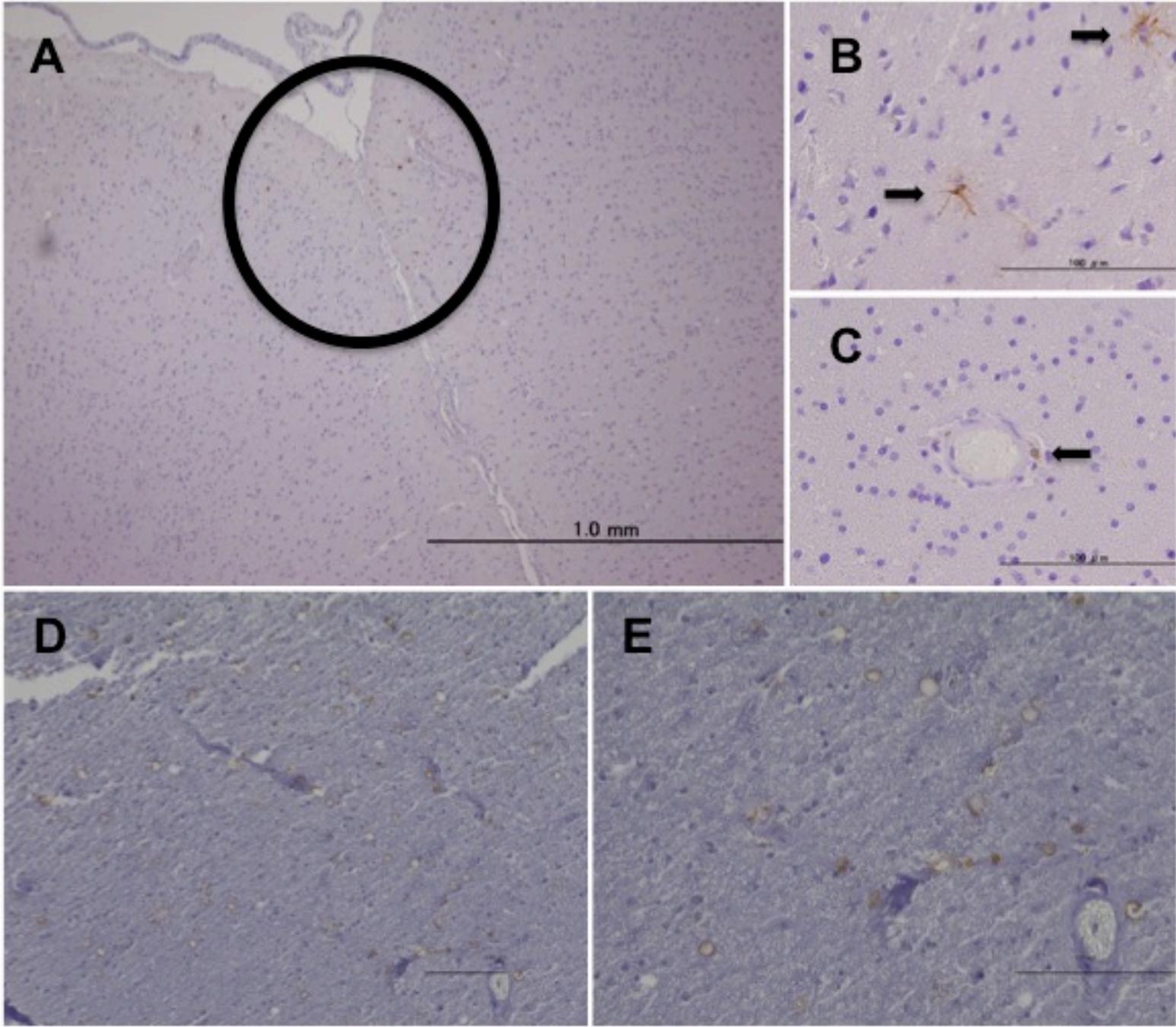


Figure 2.

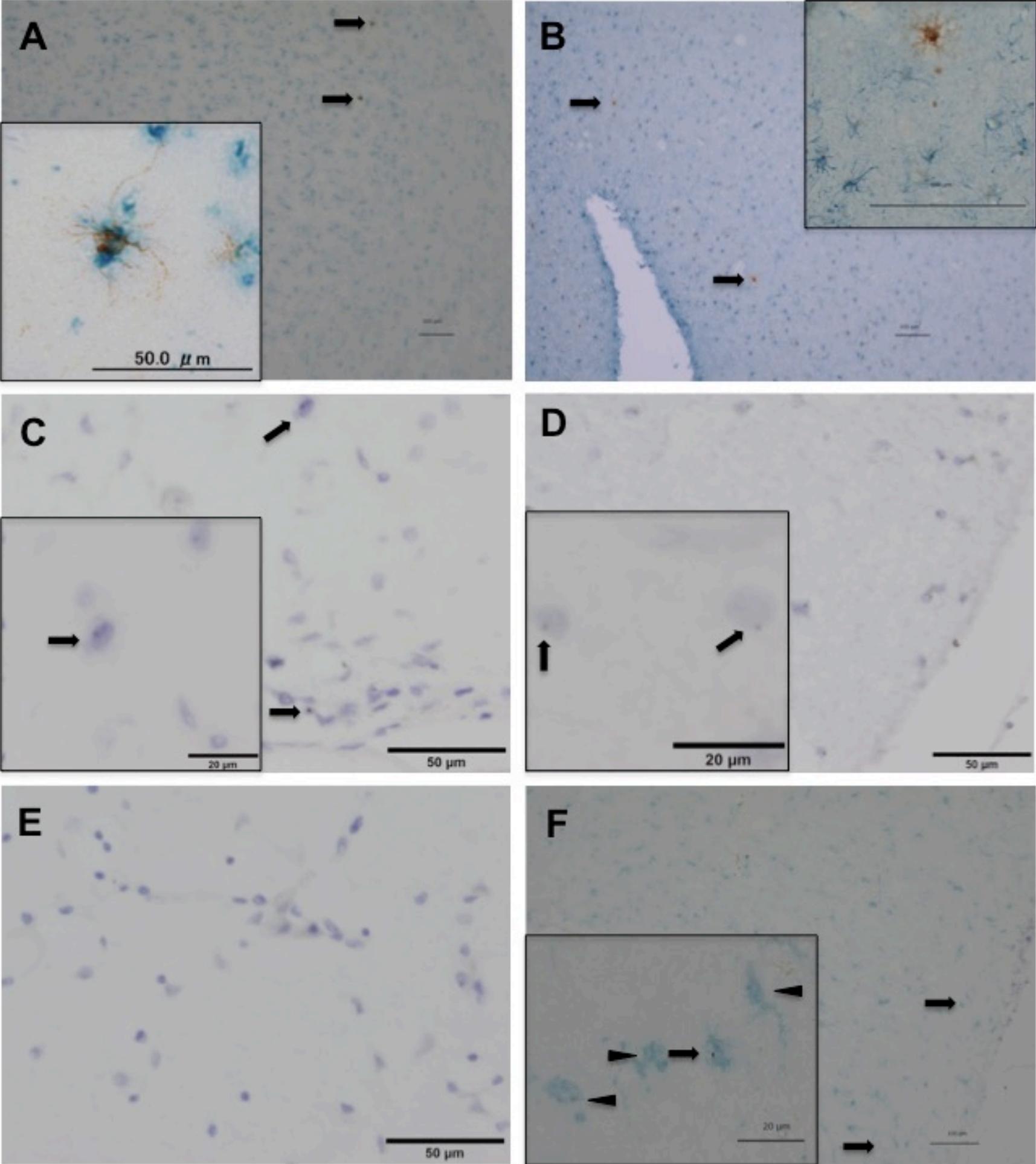


Figure 3.

