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Rapid isolation of highly enriched and quiescent microglia from adult rat hippocampus: Immunophenotypic and functional characteristics☆,☆☆

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Abstract

Isolation of microglia from CNS tissue provides a powerful tool to study basic microglia biology and examine the effects of in vivo treatments on microglia immunophenotype and function. Previous microglia isolation methodologies utilized whole brain. However, microglia immunophenotype varies across CNS anatomical loci, thus isolation of microglia from whole brain may obscure regional brain variations in microglia immunophenotype and function. In addition, it is unknown to what extent microglia isolation procedures alter the in situ immunophenotype and function of microglia. The present report details a procedure for the rapid isolation of microglia from discrete CNS anatomical loci and addresses the issue of whether the in situ microglia immunophenotype is significantly altered by the isolation procedure. The present microglia isolation method yielded highly enriched hippocampal microglia, which were devoid of other CNS macrophage subtypes and exhibited attributes reflecting a quiescent phenotype characteristic of microglia observed in situ under non-pathological conditions. Further, isolated microglia exhibited functional responsiveness to immunogenic stimuli ex vivo. The immunophenotypic and functional attributes of isolated microglia suggest that the isolation procedure preserves the in vivo phenotype of microglia, thus providing an experimental method with minimal procedural confounds for examining in vivo treatments on microglia ex vivo.

Keywords: Microglia; Isolation; Density gradient; Percoll; Immunophenotype; In situ; CD11b; MHC II

1. Introduction

Characterization of brain microglial function requires isolation of enriched microglia from other CNS macrophage populations such as perivascular macrophages and pericytes. Previous microglia isolation approaches have yielded enriched populations from whole brain, however utilization of whole brain may obscure regional brain variations in microglia phenotype and function. The present study developed a procedure for the rapid isolation of highly enriched, quiescent microglia from adult rat hippocampus, which are suitable for immediate functional characterization ex vivo.

Microglia comprise a subset of CNS macrophages, which display considerable phenotypic heterogeneity (Guillemin and Brew, 2004). In addition, phenotypic variation is apparent within classes of brain macrophages (Lawson et al., 1990) and the CNS microenvironment is a salient determinant of macrophage phenotype (Neumann, 2001). The brain regulatory microenvironment may vary not only between distinct anatomical regions, but also within an anatomical site depending upon the local neurochemical milieu (McCluskey and Lampson, 2000). Clearly, site-specific regulation of brain microglia and macrophage phenotype and activation state, especially under pathological conditions, introduces an additional factor contributing to the phenotypic heterogeneity of CNS macrophages. The phenotypic diversity displayed

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by CNS macrophages may also reflect a functional diversity (Streit and Graeber, 1993). Thus, the ability to isolate microglia from other CNS macrophages within discrete CNS anatomical loci would provide a powerful tool to resolve whether in vivo treatments differentially modulate CNS microglia phenotype and/or function across brain loci. However, isolation of microglia from discrete brain regions of adult rodents has not been demonstrated.

Two general methods are typically used to isolate CNS microglia. An enzymatic digestion procedure uses an enzyme cocktail to dissociate CNS tissue followed by density gradient centrifugation to isolate microglia from other CNS elements (Ford et al., 1995). A particular drawback of the enzymatic approach is the extent to which enzymatic digestion alters the immunophenotype of microglia (Ford et al., 1996). An alternate isolation procedure obviates the use of enzymes by using simple mechanical dissociation to homogenize CNS tissue (Havenith et al., 1998). Similar to the enzymatic digestion procedure, density gradient centrifugation is used to separate microglia after mechanical dissociation of tissue.

When isolating microglia, several methodological issues must be resolved prior to assessing function ex vivo. One issue is cell purity. Is the isolated microglia population sufficiently enriched to exclude a significant contribution of other cell types such as astrocytes and other brain macrophages to the functional measure? No less important is the issue of whether the immunophenotype of microglia in situ is preserved throughout the isolation procedure. Unlike previous studies, the present study compared the in situ microglia immunophenotype with that of isolated microglia to gain insight into whether the isolation procedure alters the in situ immunophenotype. Of course, whether an isolation procedure modulates microglia function is unknown given the intractability of measuring function in vivo. However, microglia immunophenotype is a strong predictor of activation state (Streit et al., 1999) and thus inferences regarding the functional state of microglia may be drawn from immunophenotype. For example, quiescent microglia are typically major histocompatibility complex (MHC) II negative and under inflammatory conditions microglia up-regulate MHC II indicating an activated immunophenotype (Kreutzberg, 1996). A separate, but related methodological issue pertains to whether or not isolated brain microglia are labile to immunogenic stimulation ex vivo. While an isolation procedure may not alter a specific microglia immunophenotype (MHC II), functional responsiveness to immunogenic stimuli may be modulated by the procedure.

The present report details a modification of a previously published method (Havenith et al., 1998) for the isolation of highly enriched hippocampal microglia, which are devoid of other CNS macrophage subtypes and exhibit attributes reflecting a quiescent phenotype characteristic of microglia observed in situ under non-pathological conditions. Further, isolated microglia exhibit functional responsiveness to immunogenic stimuli ex vivo. The immunophenotypic and functional attributes of isolated microglia suggests that the isolation procedure preserves the in vivo phenotype of microglia, which provides a basis for examining the effects of experimental treatments on microglia ex vivo.

2. Materials and methods

2.1. Animals and tissue collection

Male Sprague–Dawley rats (60–90 d; Harlan) were given a lethal dose of sodium pentobarbital and transcardially perfused with heparinized (1 U/ml) 0.9% saline for 3 min. Brain was rapidly extracted on ice and hippocampus dissected. All experimental procedures were conducted in accord with the University of Colorado Institutional Animal Care and Use Committee.

2.2. Microglia cell isolation

2.2.1. Percoll solutions

Stock Percoll (Amersham Biosciences, Uppsala, Sweden) was diluted 1:10 in sterile 10× PBS to yield 100% isotonic Percoll. One hundred percent isotonic Percoll was then diluted in 1× PBS to yield 70 and 50% isotonic Percoll. Percoll solutions were brought to room temperature (RT) prior to use.

2.2.2. Tissue processing

Whole hippocampus was immediately placed in a non-tissue culture treated 60 mm × 15 mm plastic Petri dish containing 2 ml ice cold Dulbeccos phosphate buffered saline (DPBS) supplemented with 0.2% glucose (sDPBS). Tissue was finely minced with a razor blade and transferred to a Tenbroeck (Bellco Glass, Inc., Vineland, NJ) tissue homogenizer on ice. An additional 1 ml sDPBS was used to rinse Petri dish of residual tissue and transferred to the Tenbroeck homogenizer. Tissue was gently dissociated until a homogeneous suspension appeared. Gently twisting the probe, while displacing the liquid up and down without generating bubbles reliably yielded highly viable cells. Homogenate was filtered through a 40 μM cell strainer (BD Biosciences Discovery Labware, Bedford, MA) into a 50 ml conical tube. An additional 1 ml sDPBS was passed through the filter to rinse. Homogenate was transferred to a 5 ml polystyrene tube and centrifuged at 350 × *g* for 10 min at RT. Supernatant was then aspirated.

2.2.3. Density gradient centrifugation

Homogenate was suspended in 1 ml 70% isotonic Percoll and transferred into a new 5 ml polystyrene tube. Two milliliters of 50% isotonic Percoll was gently layered on top of the 70% layer and then 1 ml 1× PBS gently layered on top of the 50% Percoll layer.

In a swinging bucket rotor, the density gradient was centrifuged at 1200 × *g* for 45 min (minimum acceleration and brake) at 20 °C.