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Canine microglial cells: Stereotypy in immunophenotype and specificity in function?

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Abstract

Microglial cells represent the endogenous immune system of the central nervous system (CNS). Upon pathological insults they reveal their immunological potential aimed at regaining homeostasis. These reactions have long been believed to follow a uniform and unspecific pattern which is irrespective to the underlying disease entity. Evidence is growing that this view seriously underrates microglial competence as the defenders of the CNS. In the present study, microglial cells of 47 dogs were examined ex vivo by means of flow cytometry. Ex vivo examination included immunophenotypic characterization using eight different surface markers and functional studies such as phagocytosis assay and the reactive oxygen species (ROS) generation test. The dogs were classified according to their histopathological diagnoses in disease categories (controls, canine distemper virus (CDV) induced demyelination, other diseases of the CNS) and results of microglial reaction profiles were compared. Immunophenotypic characterization generally revealed relative high conformity in the microglial disease response among the different groups, however the functional response was shown to be more specific. Dogs with intracranial inflammation and dogs with demyelination showed an enhanced phagocytosis, whereas a significant up-regulation of ROS generation was found in dogs with demyelination due to CDV infection. This strongly suggests a specific response of microglia to infection with CDV in the settings of our study and underlines the pivotal role of microglial ROS generation in the pathogenesis of demyelinating diseases, such as canine distemper.

Keywords: Canine microglia; Stereotypic versus specific reaction; Ex vivo examination; Surface molecules; ROS; Phagocytosis; Demyelination; CDV

1. Introduction

Microglial cells are the main immune effector elements of the brain (Giulian, 1987; Streit, 2002). Their cell numbers range from 5 to 20% of the entire central nervous system glial cell population (Kreutzberg, 1987; Streit, 1995). They were first described by Del Rio-Hortega (1932) as a distinct cell type within the central

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nervous system (CNS) with characteristic morphology and specialised staining characteristics.

Microglia responds to every kind of pathological event in the CNS (Giulian, 1992a) and rapidly progresses from their resting ramified state to an activated state where they can proliferate, migrate, and express surface molecules de novo or at increased levels (Streit et al., 1989). This cell population plays a critical role in host defense against invading microorganisms and neoplastic cells or otherwise altered cells, and are thought to determine the pattern and degree of central nervous system recovery or disease (Giulian, 1992b). Microglia share many properties with macrophages (Perry and Gordon, 1988), and upon activation can exert similar macrophage effector functions such as phagocytosis, modulation of T-cell response, production, and release of cytokines, chemokines, reactive oxygen (ROS), and nitrogen species (Streit and Kreutzberg, 1988; Kreutzberg, 1996; Stoll and Jander, 1999). Microglia are also capable of processing and presenting antigen by expression of MHC classes I and II (Banati et al., 1993; Gehrmann et al., 1995). These findings converge into a concept that views microglia as the local immune system of the brain (Graeber and Streit, 1990; Streit, 2002).

Examining ex vivo isolated canine microglia we found that these cells were markedly activated in demyelinating canine distemper virus (CDV) infection exhibiting a range of immunophenotypical and functional changes (Stein et al., 2004b). It was thought that these changes could be causally related to the phenomenon of demyelination, a specific lesion in the CNS. However, microglial response to CNS injury has long been believed to be uniform and stereotypic irrespective of the underlying insult (Gehrmann and Kreutzberg, 1995; Stoll and Jander, 1999; Raivich et al., 1999; Graeber et al., 2002) even though this assumption is now under intense investigation. Evidence has grown that this view substantially underestimates microglial competence in the immune surveillance of the CNS (Streit, 2002).

The aim of the current study was to evaluate whether canine microglial reaction profile is stereotypic irrespective of the underlying pathological condition or if it follows a specific scheme according to the causative insult. Therefore, the microglial profiles of the study in dogs experimentally infected with CDV (Stein et al., 2004b) were compared to the profiles found in dogs suffering from other neurological diseases. Examinations included immunophenotypical characterization and functional assays such as determination of phagocytosis activity and the generation of reactive oxygen species (ROS).

2. Materials and methods

2.1. Animals

Microglial cells from 47 dogs were included. Twenty-two of the dogs originated from a vaccine challenge experiment using the virulent CDV strain A75/17 (according to Swiss national ethical regulation 111/99/Berne/CH; previous experiment see Cherpillod et al., 2000; Stein et al., 2004b). In addition to the dogs of the vaccine challenge experiment, 25 dogs were euthanized on request of the owner because of severe diseases diagnosed by advanced imaging tools. Histopathological diagnosis was used to assign the dogs in four examination groups.

Brain material including parts of the lesion identified by advanced imaging techniques (MRI or CT) of each dog was fixed in 4% buffered formalin and processed for paraffin embedding. Sections of representative areas of brain and spinal cord were mounted on positively charged slides (Superfrost plus, Menzel-Gläser, Braunschweig, Germany) and stained with hematoxylin-eosin (HE). Based on the histopathological findings in the CNS dogs were categorized in the following examination groups (I–III; Table 1) to compare the results of immunophenotypical and functional characterization.

Group I comprised two uninfected, healthy negative control dogs with entirely normal findings in the CNS (Ia), 8 dogs which had been euthanized for severe extracranial diseases (Ib), and 13 dogs which were vaccinated and protected despite inoculation with CDV and had neither demyelinating lesions nor CDV antigen in their CNS (Ic) (Stein et al., 2004b).

Group II consisted of seven dogs with experimental demyelinating CDV infection (Vandevelde and Zurbriggen, 1995; Stein et al., 2004b) and two dogs with naturally occurring CDV induced demyelination comparable to those of the dogs of the vaccine challenge experiment.

Group III included 15 dogs with other intracranial diseases such as five dogs with intracranial tumors (IIIa), three dogs with intracranial inflammation (IIIb), two dogs with no histopathological changes but recurrent seizuring interpreted as idiopathic epilepsy (IIIc), and five dogs with other changes in the CNS such as trauma, degeneration, and malformations (IIId).

2.2. Antibodies

Monoclonal mouse antibodies (mAbs) used for immunophenotypic analyses were either canine specific