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Role of peripheral immune response in microglia activation and regulation of brain chemokine and proinflammatory cytokine responses induced during VSV encephalitis

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ABSTRACT

We report herein that neuroinvasion by vesicular stomatitis virus (VSV) activates microglia and induces a peripheral dendritic cell (DC)-dependent inflammatory response in the central nervous system (CNS). VSV neuroinvasion rapidly induces multiple brain chemokine and proinflammatory cytokine mRNAs that display bimodal kinetics. Peripheral DC ablation or T cell depletion suppresses the second wave of this response demonstrating that infiltrating T cells are primarily responsible for the bimodal characteristics of this response. The robust infiltrate associated with VSV encephalitis likely depends on sustained production of brain CCL19 and CCR7 expression on infiltrating inflammatory cells.

1. Introduction

Many lethal human pathogens (e.g. rabies, Ebola, and Hendra viruses) can be found in the Order Mononegavirales. These viruses can also have economic impacts because of their ability to infect poultry and cattle (Lamb and Parks, 2007; Lyles and Rupprecht, 2007; Planz et al., 2009; D'Agostino et al., 2012b). Vesicular stomatitis virus (VSV) is a member of the Vesiculovirus genus in the Rhabdoviridae family, one of the four families in the Order Mononegavirales and is the prototypic virus of this family that includes rabies virus. The genome of VSV encodes only five major proteins that nonetheless provide sufficient information to infect a broad range of host cells, shut down cellular protein synthesis and produce productive infections (Das et al., 2008). VSV introduced systemically induces a rapid and potent type I interferon (IFN) response essential for survival (Barchet et al., 2002). Components of the adaptive immune response (antibody, T cells) appear around 6–8 days post infection (PI) and eliminate infectious VSV from peripheral tissues. In the absence of adaptive immunity mice invariably succumb to infection (Thomsen et al., 1997). Unexpectedly, VSV antigen remains in peripheral tissues for almost two months PI despite efficient clearance of infectious VSV from the host (Turner et al., 2007).

A single intranasal application of VSV results in infection and viral replication in olfactory neurons with transmission of this neurotropic virus to the olfactory bulb (OB) via the olfactory nerve (Reiss et al., 1998). VSV replicates invasively in the OB penetrating deeper layers of the OB (Reiss et al., 1998) and progresses caudally reaching the hindbrain around day 8 (Huneycutt et al., 1994). CNS invasion by VSV does not go unnoticed as Rig-1 (retinoic acid like receptor-1 (Rieder and Conzelmann, 2009)) and toll-like receptor-7 (TLR7) (Lund et al., 2004; D'Agostino et al., 2012a) signaling pathways are engaged resulting in astrocyte and microglia activation and a subsequent astrocytosis and microgliosis (Steel et al., 2009). Neutrophils are the initial inflammatory cell seen in the OB around 1 day PI (Chen et al., 2001) suggesting rapid chemokine production, a view supported by elevated CCL1 and CXCL10 transcripts in the brain detected one day PI (Ireland and Reiss, 2006). Around 6–8 days PI a robust mixed cellular infiltrate dominated by neutrophils, T cells, macrophages and to a lesser extent DCs accumulates in the brain parenchyma (Bi et al., 1995; Ciavarra et al., 2006; Steel et al., 2008, 2009). A number of studies have demonstrated that inhibition of VSV replication, caudal penetration and survivability are dependent on

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both innate and adaptive effector mechanisms (Huneycutt et al., 1993; Thomsen et al., 1997; Komatsu et al., 1999). It has previously been suggested that VSV encephalitis is T cell-independent although this view has not been critically tested during either the acute (6–8 days PI) or recovery (10–14 days PI) phases of VSV encephalitis (Frei et al., 1989; Nansen et al., 2000).

The observed activation and expansion of microglia in encephalitic brains are consistent with prior studies in autoimmune and inflammation models suggesting that microglia represent key regulatory cells in the innate and adaptive immune response (Pope et al., 1998; Marques et al., 2006). Furthermore, microglia are reported to up regulate MHC class II and CCR7 at the onset of symptoms and progression of experimental allergic encephalomyelitis collaborating the view that microglia develop into antigen presenting cells (APCs) with migratory potential in the inflamed CNS (Dijkstra et al., 2006). However, under basal conditions the brain parenchyma also contains a trace population of CD11c+ cells that may represent antigen-presenting DCs. Most of these cells reside in the juxtavascular parenchyma and not in the perivascular spaces. Interestingly, their cellular processes extended into the glia limitans that may allow for presentation of antigens to extravasated T cells in the perivascular spaces (D'Agostino et al., 2012a). CD11c+ cells can also be found in the meninges and choroid plexus and at this location they constitutively express MHC class II antigens, unlike parenchymal DCs and microglia (Anandasabapathy et al., 2011; D'Agostino et al., 2012a). In the VSV encephalitis model, both innate and adaptive immune responses were markedly impaired by prior conditional ablation of peripheral DCs (Steel et al., 2009), whereas conditional depletion of peripheral macrophages did not alter VSV encephalitis (Steel et al., 2010). Interestingly, selected depletion of brain perivascular macrophages (bPVMs) inhibited anti-viral immunity and survival (Steel et al., 2010). Thus the precise role these various cell types play in the CNS innate and adaptive anti-viral immune response is poorly defined and remains a contentious issue.

In this study, we describe phenotypic changes on microglia isolated from encephalitic brains that argue against these cells functioning as professional APCs although microglia are clearly activated by VSV. The robust infiltrate characteristic of the acute phase of VSV encephalitis reflects the rapid (24 h PI) global induction of transcripts encoding multiple chemokines and proinflammatory cytokines. Multi-parameter flow cytometric analysis confirmed that activated microglia expressed two CC receptors (CCR4, CCR9) that could contribute to their activated state. In contrast, inflammatory cells were devoid of all tested chemokine receptors with the exception of CCR7. Kinetic analysis revealed that VSV-induced a bimodal chemokine and proinflammatory cytokine mRNA response with a major peak on day 3 followed by a second weaker induction on 7 days PI. Ablation of peripheral DCs or T cells markedly inhibited global chemokine and cytokine transcript levels on 7 days PI. Thus, infiltrating T cells represent not only anti-viral effector cells but may also function to sustain the inflammatory response until infections virus is cleared from the CNS.

2. Materials and methods

2.1. Mice and virus infection

Diphtheria toxin (DT) receptor transgenic (DTRTg) mice (C.FVB-Tg(Itgax-DTR/EGFP)57Lan/J, Jackson Laboratories Bar Harbor, ME) were bred to C57BL/6 mice (B6, Jackson Laboratories). Transgenic F1 mice were used in all DC depletion studies and are referred to as DTRTgF1 mice. 2m-knock out mice (strain B6.129P2-b2m) were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in our animal facility in sterile microisolator cages with sterile food, water and bedding. Wild-type VSV-Indiana strain, provided by Dr. Philip Marcus, University of Connecticut, was grown and assayed as previously described (Marvaldi et al., 1977). Virus was grown in confluent monolayers of Vero cells and virus titers determined by standard plaque assays (Sekellick and Marcus, 1979). VSV was introduced into the brain via intranasal application of 5 μl/nostril with 5×10^4 PFU VSV (Barna et al., 1996). All experiments were performed in accordance with federal guidelines and under an Institutional Animal Care and Use Committee-approved protocol.

2.2. Cell isolation and depletion

Brains were excised from perfused (30 ml cold PBS) mice, individually homogenized, pooled and then subjected to discontinuous Percoll centrifugation to enrich for microglia and leukocytes as previously described (Steel et al., 2009). Single cell suspensions of peripheral organs (spleen, draining cervical lymph nodes (CLNs), lung) were scrubbed through 40 μm nylon mesh cell strainers. Erythrocytes were lysed as necessary using BD PharmLyse Ammonium Chloride lysing reagent (Becton Dickinson, Carlsbad, CA). In vivo ablation of DCs was achieved in DTRTgF1 mice with DT following an established protocol (Ciavarra et al., 2006). Depletion of CD4+ cells was achieved in ß2m-knock out mice (CD8-deficient mice). Groups of age-matched mice were treated with anti-mouse CD4 (clone YTS 191, Bio X Cell, Inc., West Lebonon, NH) to deplete their CD4+ T cells, or with rat IgG2b isotype control (clone LTF-2, Bio X Cells, Inc.). Mice received 100 μg of anti-CD4 or isotype control in sterile PBS by intraperitoneal injection on days −3, −1, +1, +3 and were euthanized on day +7.

2.3. Multiparameter flow cytometry

Unless indicated otherwise, monoclonal antibodies (mAbs) against cell surface antigens were purchased from eBioscience (San Diego, CA). Cells were stained and washed in flow cytometry wash buffer (PBS supplemented with 1% goat serum and 0.1% sodium azide). The following mAbs were used in this study: CD11b, clone M1/70; CD45, clone 30-F11; MHC II, clone M5/114.15.2; CD11c, clone N418; CD4, clone GK1.5; CD8α, clone 53–6.7; CD45R, clone RA3-6B2; MHC I, clone 34-1-2S; PD-1, clone J43; CD115, clone AFS98; CD40, clone 1C10; CCR1, clone 643854; CCR2, clone 475301; CCR4, clone 2G12; CCR7, clone 4B12; CCR9, clone CD-1.2; CXCR3, clone CXCR3-173; and CD80, clone 16-10A1. Fluorophore conjugates varied based on staining profiles used. Acquisition of 20–200,000 events was performed using a Becton Dickinson (San Diego, CA) FACSCalibur using FlowJo software (Tree Star, Ashland, OR). Non-specific binding in the absence of additional Fc block was previously evaluated and did not affect staining patterns. To determine the absolute number of microglia and infiltrating leukocytes in the CNS, a leukocyte gate was first defined for these cells based on forward and side scatter characteristics. Preliminary studies verified that these gated cells were CD45+ and viable (≥95%propidium iodide and annexin V negative). The percentage of microglia ($CD45^{low/int}$) or infiltrating blood cells ($CD45^{high}$) within this gate was then used to calculate cell recoveries. All gates and quadrants were established with the use of appropriate isotype controls.

2.4. RNA profiles

Whole brains were removed following perfusion and homogenized in cold PBS. A volume equivalent of 20 mg was removed from each brain homogenate and RNA isolated using the QuickGene RNA tissue kit SII (RT-S2) from FUJIFILM Corporation. RNA profiles were characterized by real-time PCR on RNA isolated either from individual animals or pooled RNA depending on the experiment using a commercial inflammatory cytokine array (SABiosciences, catalog number PAMM-011).