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SJL bone marrow-derived macrophages do not have IRF3 mutations and are highly susceptible to Theiler's virus infection

Kyung-No Son[a], Zhiguo Liang[a], Howard L. Lipton[a,b,*]

[a] Departments of Microbiology-Immunology, University of Illinois at Chicago, Chicago, IL, USA
[b] Departments of Neurology & Rehabilitation Medicine, University of Illinois at Chicago, Chicago, IL, USA

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ABSTRACT

It is well known that SJL mice are susceptible to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease while C57BL6 (B6) and B10 mice are resistant, and H-2^s on a B10 background (B10.S) contributes modestly to susceptibility. A recent study linked two IRF3 non-conservative mutations in SJL compared to B10.S mice to resistance to TMEV infection of SJL peritoneal-derived macrophages, an observation of practical interest in light of the central role of IRF3 transcription factor in the type I interferon (IFN) response. However, we did not find these non-conservative mutations among SJL, B10.S, B6 and B10 mice in the IRF3 amino acid sequence, and show SJL bone marrow-derived macrophages infected with TMEV exhibit increased virus RNA replication and infectious virus yields as well as greater IL-6 production than C57Bl strain (including B10.S) cultures.

Theiler's murine encephalomyelitis virus (TMEV) persists in the CNS of susceptible mice, resulting in immunopathologic demyelination and providing a relevant model for multiple sclerosis. After intracerebral infection, brief logarithmic virus growth, largely in neurons, is followed by virus persistence in microglia/macrophages, and to a lesser extent, in oligodendrocytes and astrocytes (Brahic et al., 1981; Rodriguez et al., 1983; Schlitt et al., 2003; Zheng et al., 2001). Because of its cytolytic nature, TMEV requires continuous virus replication and cell-to-cell spread to persist in cell culture or an animal host. *In vitro* analyses have demonstrated that TMEV replicates in differentiated macrophages but not in monocyte precursors or in activated macrophages (Clatch et al., 1990; Jelachich et al., 1999; Schlitt et al., 2003), inducing apoptosis in the macrophages through the intrinsic apoptotic pathway (Jelachich et al., 1995; Jelachich et al., 1999; Ohara et al., 2002; Son et al., 2008; Stavrou et al., 2011; Tsunoda et al., 1997). By contrast, infection of other somatic cell lines, such as BHK-21, leads to programmed necrosis (necroptosis) or nonspecific necrosis (Yildiz Arslan et al., 2016). Virus-induced apoptosis provides a means of virus attenuation and protected virus spread within apoptotic bodies, which are subject to phagocytosis by uninfected cells. Recently, we found that TMEV-infected macrophages undergoing apoptosis in culture also release microvesicles that display virions in single file on their surface that are resistant to neutralization (*In Preparation*).

Susceptibility to TMEV persistent infection varies among inbred mouse strains and is controlled by multiple genes (Brahic et al., 2005). H2 class I genes have a major effect on viral persistence, particularly resistance imparted by the H-2D gene on a C57BL/6 (B6) or C57BL/10 (B10) background, and linked to acute virus clearance (Azoulay-Cayla et al., 2001). Thus, the severity of demyelinating disease depends in part on the ability to control virus replication acutely, that may lead to a lower setpoint prior to persistence.

IL-6, a pleiotropic cytokine prominently involved in inflammation, was initially reported to mediate host immunity to viral infections and was referred to as IFN-β2 (Sehgal et al., 1988). Since then, IL-6 was shown to control chronic lymphocytic choriomeningitis virus infection in mice by enhancing virus-specific T follicular helper cells and antibody responses (Harker et al., 2011). IL-6 promoter activity in human monocytes was reported to be NFκB-dependent (Tuyt et al., 1999), and expression in synoviocytes is regulated by IRF3 (Sweeney et al., 2010). However, IL-6 expression in dengue virus-infected mice was shown to be independent of IRF3 and IRF7 (Chen et al., 2013). The exact signaling pathways regulating IL-6 expression in virus infections remain to be fully elucidated.

Moore et al. (2012) reported greater IL-6 expression in TMEV (DA strain)-infected thioglycolate-elicited B10.S peritoneal macrophages than in susceptible SJL macrophages, suggesting that IL-6 is critical for virus clearance, and that its expression level in macrophages affects virus persistence by controlling acute infection. Moreover, those investigators identified three polymorphisms in IRF3 of SJL mice, two of which result in missense mutations in the DNA binding domain and in

* Correspondence to: Department of Microbiology-Immunology, MC 790, University of Illinois at Chicago, 835 South Wolcott, Chicago, IL 60612-7344, USA.
E-mail address: hlipton@uic.edu (H.L. Lipton).

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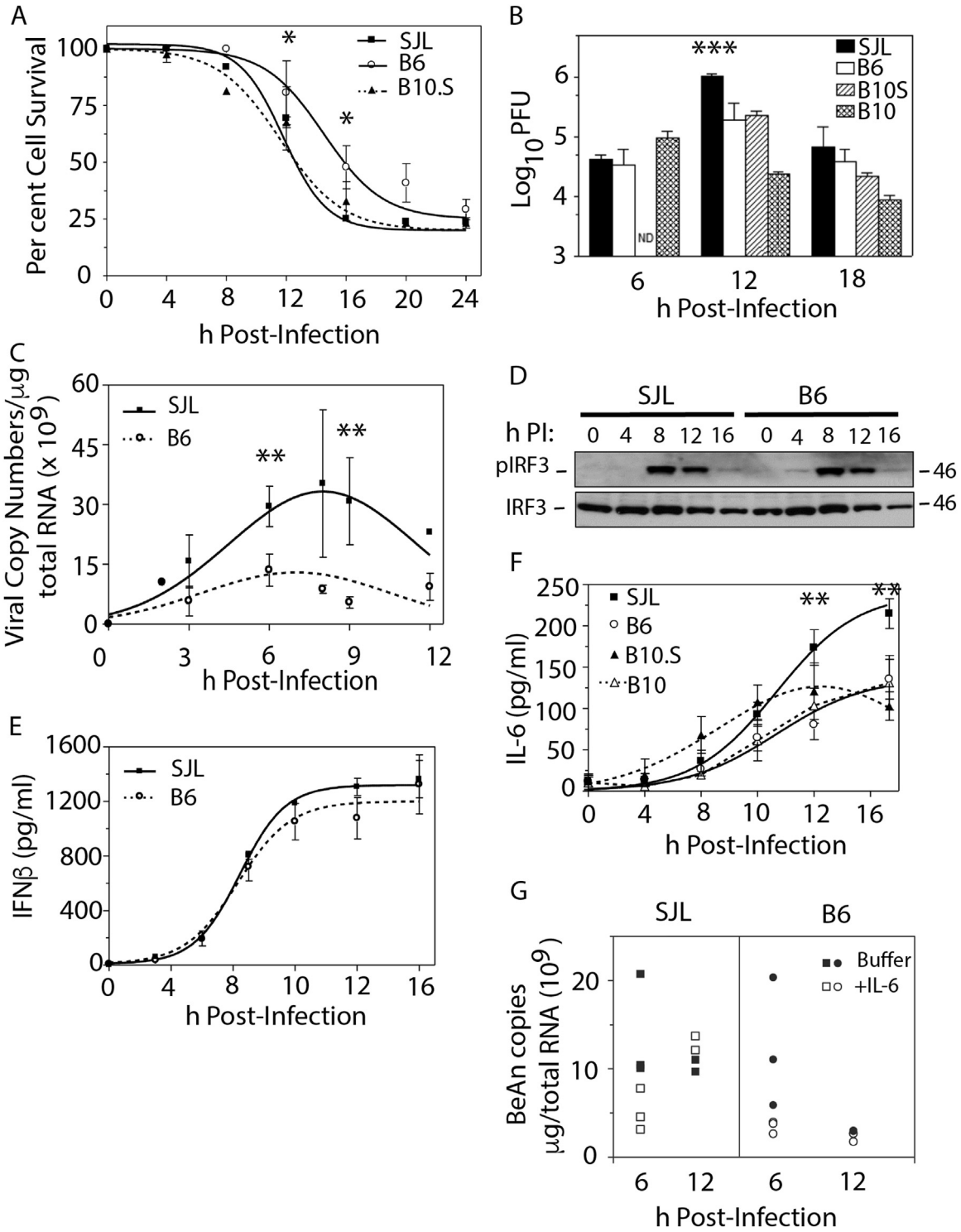

Fig. 1. BeAn virus infection (moi = 10) and pathogenetic properties of BM-derived macrophages from susceptible SJL and resistant C57BL strains of mice, including with the $H2^s$-isotype, seeded in 6-well plates with 1×10^6 cells per well. Results are representative of at least 3 experiments. A. Temporal profile of cell death due to apoptosis in infected macrophages from SJL and B10.S mice showed no differences (mean ± sd). B. Kinetics of infectious BeAn virus growth determined by standard plaque assay, showed higher virus titers at 12 h pi in SJL macrophages compared to the three B6 strains (mean ± sd, ***, $p < 0.001$). C. Kinetics of virus RNA replication in cultures, expressed as virus RNA copy equivalents per µg of total RNA determined by real-time RT-PCR, showing higher copy numbers in SJL macrophage cultures at 6, 9 and 12 h pi than in B6 cultures (mean + sd; *, $p < 0.05$; **, $p < 0.01$). D. Immunoblot analysis of activation of the major transcription factor IRF3 showed similar temporal profiles of phosphorylated IRF3 in SJL and B10 cultures. E. Similar pg amounts of IFNβ were produced in infected SJL and B6 macrophage cultures determined by ELISA (mean ± sd). F. Increased levels of IL-6 were produced at later times pi (12 and 16 h pi) in macrophages derived from SJL than C57BL mouse strains (mean ± sd; **, < 0.02). G. A trend of reduced virus RNA copy numbers were observed in both infected SJL and B6 macrophage cultures at 6 h pi when IL-6 (10 ng/ml) was added vs PBS for 30 min before incubation and after virus adsorption. The values were obtained from 2 culture replicates on different days.