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Inhibition of catecholamine degradation ameliorates while chemical sympathectomy aggravates the severity of acute Friend retrovirus infection in mice

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ABSTRACT

Several lines of evidence indicate that the sympathetic nervous system (SNS) might be involved in the pathogenesis and progression of retroviral infections. However, experimental data are scarce and findings inconsistent. Here, we investigated the role of the SNS during acute infection with Friend virus (FV), a pathogenic murine retrovirus that causes polyclonal proliferation of erythroid precursor cells and splenomegaly in adult mice. Experimental animals were infected with FV complex, and viral load, spleen weight, and splenic noradrenaline (NA) concentration was analyzed until 25 days post infection. Results show that FV infection caused a massive but transient depletion in splenic NA during the acute phase of the disease. At the peak of the virus-induced splenomegaly, splenic NA concentration was reduced by about 90% compared to naïve uninfected mice. Concurrently, expression of the catecholamine degrading enzymes monoamine oxidase A (MAO-A) and catechol-O-methyltransferase (COMT) was significantly upregulated in immune cells of the spleen. Pharmacological inhibition of MAO-A and COMT by the selective inhibitors clorgyline and 3,5-dinitrocatechol, respectively, efficiently blocked NA degradation and significantly reduced viral load and virus-induced splenomegaly. In contrast, chemical sympathectomy prior to FV inoculation aggravated the acute infection and extended the duration of the disease. Together these findings demonstrate that catecholamine availability at the site of viral replication is an important factor affecting the course of retroviral infections.

1. Introduction

Retroviruses comprise a large and diverse family of enveloped, positive-sense, single-stranded RNA viruses that replicate in host cells via reverse transcription. The virus family includes important human and animal pathogens that can cause a wide spectrum of serious diseases including cancer, immunodeficiency, and neurological disorders (Coffin et al., 1997). Over the past decades, research on retroviral infections has primarily focused on viral replication, transmission pathways, and anti-viral immune responses. Interestingly, several lines of evidence indicate that the sympathetic nervous system (SNS) might also be directly and/or indirectly involved in the pathogenesis and progression of retroviral infections. For example, *in vitro* studies have shown that noradrenaline (NA), the main neurotransmitter of the SNS, can accelerate the replication of human immunodeficiency virus type 1 (HIV–1) in activated human T cells (Cole et al., 1998, 2001). In line with this observation, replication of simian immunodeficiency virus (SIV) in rhesus macaques (*Macaca mulatta*) was significantly enhanced in lymph node areas adjacent to catecholaminergic varicosities (Sloan et al., 2006). Further support for a potential link between elevated SNS activity and enhanced retroviral pathogenesis comes from natural history studies reporting accelerated disease progression in HIV-infected individuals with high autonomic nervous system activity (Cole et al., 2003). However, there are also conflicting data showing inhibitory effects of NA on HIV-1 replication in viral target cells (Moriuchi et al., 2006). In addition, the influence of the SNS might change with progression of the disease. For example, the density of catecholaminergic nerve fibers in

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lymph nodes of chronically SIV-infected macaques was substantially lower compared to naïve animals (Sloan et al., 2008). Similarly, mice infected with LP-BM5 retrovirus showed a gradual loss of sympathetic nerve fibers in the spleen, the main target site of the virus (Kelley et al., 2003). Together, these data suggest that the interactions between the SNS and retroviral infections might be complex and reciprocal.

Experimental research on the putative role of the SNS during retroviral infections is limited and has thus far focused either on direct NA effects on viral replication and anti-viral immune response or on changes in sympathetic innervation of lymphoid organs during relatively late disease stages. Little is known about acute retroviral infections and alterations in NA availability at sites of viral replication. Moreover, due to the inconsistent findings of the few studies available, it is remains unclear whether the SNS-mediated effects are beneficial or detrimental with regard to retroviral pathogenesis and disease progression.

The present study employed Friend virus (FV) infection as a well-characterized model of pathogenic retrovirus infection in mice to elucidate the role of the SNS during acute retroviral disease (Hasenkrug and Chesebro, 1997). FV is a retroviral complex comprised of Friend murine leukemia virus (F-MuLV), a replication-competent but non-pathogenic helper virus, and a replication-defective but pathogenic spleen focus-forming virus (SFFV) (Kabat, 1989). SFFV encodes an envelope glycoprotein that activates the erythropoietin receptor and triggers polyclonal proliferation of erythroid precursor cells, leading within several days to the development of splenomegaly (Hoatlin and Kabat, 1995; Li et al., 1990). Depending on the genetic background of the mouse strain used, infection of adult mice with FV results in different severities of the disease. Susceptible strains progress to lethal erythroleukemia, whereas resistant mice, such as the C57BL/6 strain used herein, recover from acute splenomegaly and do not develop leukemia (Dietz and Rich, 1972; Chesebro et al., 1990). In a first set of experiments, we explored whether acute FV infection results in changes in NA availability in the spleen, which is one of the main reservoirs of FV replication and densely innervated by sympathetic noradrenergic nerve fibers. In a second set of experiments, we investigated whether experimental manipulation of NA availability by either pharmacological inhibition of catecholamine degradation or chemical sympathectomy prior to the infection affects viral pathogenesis and the severity of the disease.

2. Methods

2.1. Animals

Female C57BL/6 mice (8–10 weeks old) were used for all experiments. The animals were obtained from Harlan Laboratories (Horst, The Netherlands) and were group-housed in individually ventilated cages in a specific pathogen-free facility. Mice were maintained on a 12-h light/dark cycle (lights on at 7 AM) and had *ad libitum* access to food and water. All procedures were in accordance with the German Animal Welfare Act and were approved by the responsible state agency for animal care and use (LANUV Düsseldorf, North Rhine-Westphalia, Germany).

2.2. Friend retrovirus infection

Mice were intravenously infected with 20,000 spleen focus-forming units (SFFU) of FV complex in 0.15 ml sterile phosphate-buffered saline (PBS; Life Technologies, Darmstadt, Germany). Control mice were injected with an equal volume of PBS. The stock was prepared as a 10% spleen cell homogenate from BALB/c mice infected 14 days previously with 3000 SFFU of uncloned virus stock (Gibbert et al., 2012). Infected mice were monitored daily for sickness symptoms (e.g., lethargy, hunched posture, diarrhea, weight loss). Note that none of the FV-infected animals died because of the infection or showed overt signs of sickness such as loss in body weight.

2.3. Sample collection and cell preparation

Animals were sacrificed by cervical dislocation at the indicated time points. Spleens were aseptically removed and weighed. Approximately one third of the spleen was immediately snap-frozen and stored at −80 °C for determination of neurotransmitter concentrations. Single cell suspensions were prepared from the rest of the spleen by mechanically disrupting the tissue with a syringe plunger and a nylon cell strainer (70 μm; BD Falcon, San Jose, USA). Cells were then washed and suspended in supplemented RPMI (RPMI 1640 with GlutaMAX I, 25 mM HEPES, 10% fetal bovine serum, 5000 U/mL penicillin–streptomycin; Life Technologies). Cell concentrations were determined using a Neubauer chamber and were adjusted to 1×10^8 cells/ml. For the isolation of erythroid cells, splenocytes were labeled with mouse anti-Ter-119 MicroBeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) and positively selected by magnetic cell separation. Purity of the isolated $Ter119^+$ cells, containing mature erythrocytes and erythroid precursor cells was confirmed by flow cytometry.

2.4. Flow cytometry

Cell surface staining was performed using a standard lyse-wash protocol and the following antibodies: FITC-conjugated anti-mouse CD3 (clone 145-2C11, BioLegend, San Diego, USA), PE-conjugated anti-mouse CD4 (clone RM 4-5, BioLegend), BV421-conjugated anti-mouse CD8 (clone 53-6.7, BD Biosciences, Heidelberg, Germany), PE-Cy7-conjugated anti-mouse Ter119 (eBioscience, Frankfurt, Germany), and PerCP-Cy5.5-conjugated anti-mouse NK1.1 (clone PK136, eBioscience). Dead cells were excluded from analysis by staining with eF780-conjugated Fixable Viability Dye (eBioscience). Samples were acquired on a FACSCanto II flow cytometer (BD Biosciences) and analyzed with FACSDiva software (Version 8.0.1, BD Biosciences). Results are expressed as percentage of cells. Data on total cell numbers are not available since in all experiments part of the spleen was used for HPLC analysis.

2.5. Infectious center assay

Viral load in the spleen was quantified by using an infectious center assay (Dittmer et al., 1998). Ten-fold serial dilutions of the splenocyte suspensions were disseminated onto susceptible *Mus dunni* cells and incubated for 3 days at 37 °C and 5% CO_2. Cells were then fixed with 95% ethanol, stained with F-MuLV envelope-specific mAb 720, and developed with peroxidase-conjugated polyclonal goat anti-mouse IgG (DakoCytomation, Denmark) and aminoethylcarbazole to detect viral foci. Data are presented as number of infectious centers (IC) per 10^6 total splenocytes.

2.6. In vitro F-MuLV replication assay

Mus dunni cells (7.5×10^3/well) were infected with 25 focus-forming units (FFU) of F-MuLV and cultured for 3 days at 37 °C and 5% CO_2 in absence or presence of different concentrations of NA (Noradrenaline HCl; Sigma–Aldrich, Munich, Germany). Polybrene (8 μl/ml) was added to all cultures to enhance viral binding to target cells. After incubation, cells were fixed with 95% ethanol, stained with F-MuLV envelope-specific mAb 720, and viral foci were detected as described for the IC assay.