PATHOGENESIS OF MOUSE ADENOVIRUS TYPE 1 INFECTION

by

Lisa E. Gralinski

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Microbiology and Immunology)
In The University of Michigan
2008

Doctoral Committee:

Professor Katherine R. Spindler, Chair
Professor Michael J. Imperiale
Associate Professor Nicholas W. Lukacs
Assistant Professor David Miller
Assistant Professor Mary X.D. O'Riordan

Acknowledgements

This thesis would not have been possible without the support, patience and encouragement of my family and friends. Thank you to my parents for encouraging me to follow my dreams and always believing that I can achieve them. I especially thank my husband Mike, whose unwavering support, cheering on and reminders to eat were integral to my success.

I thank members of the Spindler lab for their comradery and assistance over the years. I particularly thank my mentor Kathy Spindler for taking me on in the lab, teaching and supporting me, and allowing me to take my research in unexpected directions. I also thank Amanda Welton, Shanna Ashley and Irene Althaus for technical help and making the lab run so smoothly. Finally I truly appreciate Adina Grivej and Jenny Imperiale for making mouse harvests speed by and for helping me laugh during this last year.

My research would not have been possible without the advice and reagents from Anuska Andjelkovic-Zochowska, enabling me to culture and study the primary endothelial cells, thank you. Jason Weinberg has been a great help over the years with advice and reagents, and I greatly appreciate his help in making this thesis a reality. I also thank my thesis committee: Mike, Mary, Dave and Nick for their excellent questions suggestions over the years; they have challenged me and made my work stronger. In particular, Mike Imperiale has been a constant support and a fountain of ideas at lab

meetings. I thank Michelle Melis in the Microbiology and Immunology office for her help in keeping me covered by health insurance and making sure I knew what needed to be done in order to graduate.

Chapter 2 of this thesis ‘Mouse adenovirus type 1 infection of natural killer cell-deficient mice’ was previously published in the journal *Virology*, issue 373, pages 163-170. I thank my co-author Amanda Welton for her excellent contributions to that project and paper.

Finally I wish good luck to Tien-Huei Hsu and all future Spindler lab members!

Table of Contents

List of Figures

Figure

Abstract

Infection with mouse adenovirus type 1 (MAV-1) results in acute encephalomyelitis that is fatal in susceptible mouse strains. In the brain, MAV-1 only infects endothelial cells. We investigated factors influencing MAV-1-induced encephalitis, including the role of natural killer (NK) cells, the inflammatory response, and viral effects on the blood brain barrier (BBB).

MAV-1-infected mice depleted of NK cells had viral loads in the brain similar to those measured in mock-depleted control animals. Control and NK cell-depleted mice were able to clear MAV-1 infection to undetectable levels by 20 days post-infection. These results indicate that NK cells were not important for control of MAV-1 infection in the brain.

Brains of MAV-1-infected C57BL/6 mice showed a significant increase in leukocytes, including CD8 T cells. MAV-1 infection of C57BL/6 mice caused a dose-dependent breakdown of the BBB, indicated by dye staining of brain tissues. Breakdown of the BBB correlated with brain viral load, and was primarily due to direct effects of virus infection, because brains were permeable to dye even in the absence of inflammation. Cytotoxic inflammatory cells were not necessary for breakdown of the BBB.

A primary mouse brain endothelial cell (pMBEC) culture was used to measure direct effects of virus infection in the absence of an inflammatory response. MAV-1

infection caused a loss of transendothelial electrical resistance, which is necessary for maintaining the BBB. Tight junction proteins claudin-5 and occludin are required for the integrity of the BBB and transendothelial electrical resistance, and both proteins showed reduced cell surface expression on pMBECs following MAV-1 infection. Taken together, these results demonstrate that MAV-1 caused breakdown of the BBB and decreased barrier properties in infected endothelial cells, likely due to altered localization of tight junction proteins.

MAV-1-induced inflammation is dependent on the presence of the E3 protein products, but MAV-1-induced breakdown of the BBB did not require E3. No functional role for E3 has yet been described for MAV-1 E3. We developed a tandem affinity purification system to identify cellular proteins that interact with the major E3 protein product, E3 gp11k. Mass spectrometry analysis identified several candidate E3 gp11k-interacting proteins.