recombinant DNA and biotechnology
201 11-30-01

• finishing up the two-hybrid system (ex. of results)
• transgenic animals - why, and how
• knock-out (KO) animals - why, and how
  – with a focus on selection strategies
Interaction between complement receptor gC1qR and hepatitis C virus core protein inhibits T-lymphocyte proliferation

David J. Kittlese,1 Kimberly A. Chinense-Bullock,1 Zhi Qiang Yao,1 Thomas J. Braciale,1,2 and Young S. Hahn1,2

1Beineke Center for Immunology Research, and 2Department of Pathology, University of Virginia, Charlottesville, Virginia, USA

Address correspondence to Young S. Hahn, Box 800180, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA. Phone (804) 924-1155, Fax (804) 924-1321. E-mail: ysh5e@virginia.edu.

Received for publication May 12, 2000, and accepted in revised form October 10, 2000.

Hepatitis C virus (HCV) is an important human pathogen that is remarkably efficient at establishing persistent infection. The HCV core protein is the first protein expressed during the early phase of HCV infection. Our previous work demonstrated that the HCV core protein suppresses host immune responses, including antiviral and T-associated T-lymphocyte responses in a murine model. To investigate the mechanism of HCV core-mediated immunosuppression, we searched for host proteins capable of associating with the core protein using a yeast two-hybrid system. Using the core protein as bait, we screened a human T cell–enriched expression library and identified a gene encoding the gC1q receptor (gC1qR). gC1qR is a ligand of gC1qR and is involved in the early host defense against infection. Like C1q, HCV core can inhibit T-cell proliferative responses in vitro. This core-induced anti-T-cell proliferation is reversed by addition of anti-gC1qR Ab in a T-cell proliferation assay. Furthermore, biochemical analysis of the interaction between core and gC1qR indicates that HCV core binds the region spanning amino acids 188 to 259 of gC1qR, a site distinct from the binding region of C1q. The inhibition of T-cell responsiveness by HCV core may have important implications for HCV persistence in humans.

making transgenic animals

screening animals for transgenes by Southern blot
KO technology depends on ES cells
powerful selection strategies for KO genes
making KO animals

Transfect $\beta_2$-microglobulin gene knock-out construct into ES cells

Re-implant blastocyst into pseudopregnant female

Some offspring contain tissues (including germ cells) that derive from the injected cells

Inject ES cells into mouse blastocyst

Breed chimeric mice to generate homozygous $\beta_2$-microglobulin-deficient strain