BASIC AND TRANSLATIONAL—LIVER

Binding of Hepatitis A Virus to Its Cellular Receptor 1 Inhibits T-Regulatory Cell Functions in Humans

MOHANRAJ MANANGEESWARAN,* JÉRÔME JACQUES,* CECILIA TAMI,* KRISHNAMURTHY KONDURU,* NADIA AMHARREF,* ORESTE PERRELLA,‡ JOSE M. CASASNOVAS,§ DALE T. UMETSU,‖ ROSEMARIE H. DEKRUYFF,‖ GORDON J. FREEMAN,¶ ALESSANDRO PERRELLA,‡ and GERARDO G. KAPLAN*

*Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland; ‡VII Division of Infectious Diseases and Immunology, Hospital D. Cotugno, Naples, Italy; §Centro Nacional de Biotecnologia, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Darwin 3, Madrid, Spain; ‖Children's Hospital Boston, ¶Department of Medicine, Harvard Medical School, Boston, Massachusetts

BACKGROUND & AIMS: CD4+ T-regulatory (Treg) cells suppress immune responses and control self-tolerance and immunity to pathogens, cancer, and alloantigens. Most pathogens activate Treg cells to minimize immune-mediated tissue damage and prevent clearance, which promotes chronic infections. However, hepatitis A virus (HAV) temporarily inhibits Treg-cell functions. We investigated whether the interaction of HAV with its cellular receptor 1 (HAVCR1), a T-cell co-stimulatory molecule, inhibits the function of Treg cells to control HAV infection. **METHODS:** We studied the effects of HAV interaction with HAVCR1 on human T cells using binding, signal transduction, apoptosis, activation, suppression, cytokine production, and confocal microscopy analyses. Cytokines were analyzed in sera from 14 patients with HAV infection using bead arrays. **RESULTS:** Human Treg cells constitutively express HAVCR1. Binding of HAV to HAVCR1 blocked phosphorylation of Akt, prevented activation of the T-cell receptor, and inhibited function of Treg cells. At the peak viremia, patients with acute HAV infection had no Treg-cell suppression function, produced low levels of transforming growth factor-β, which limited leukocyte recruitment and survival, and produced high levels of interleukin-22, which prevented liver damage. **CONCLUSIONS: Interaction between HAV and its receptor HAVCR1 inhibits Treg-cell function, resulting in an immune imbalance that allows viral expansion with limited hepatocellular damage during early stages of infection—a characteristic of HAV pathogenesis. The mechanism by which HAV is cleared in the absence of Treg-cell function could be used as a model to develop anticancer therapies, modulate autoimmune and allergic responses, and prevent transplant rejection.**

Keywords: Hepatitis A Virus Cellular Receptor 1; Viral Clearance; TGF-β, Immune Regulation.

CD4+ regulatory T cells (Tregs) that express CD25+ and the forkhead box protein 3 (FoxP3) transcription factor have a significant role suppressing immune responses to self-antigens to prevent autoimmune disease and controlling the immune response to bacteria, viruses, parasites, and fungi.[1] The mechanisms by which Tregs are activated, suppressed, and modulated immune responses are not fully understood. Pathogens have evolved strategies to activate Tregs as a means to limit the immune response and prevent tissue damage, which is advantageous for the expansion and survival of obligate intracellular parasites.[1] Treg activation has been studied extensively in chronic infections of pathogens such as hepatitis B and C viruses,[1] in which it plays a significant role inducing and maintaining chronicity. A variety of functions have been proposed for Tregs in pathogenesis[1] but the mechanisms by which these cells control pathogenesis are unclear. Far less is known about the function of Tregs in acute infection. Hepatitis A virus (HAV), a *Picornaviridae* that causes acute hepatitis in human beings,[2] does not result in chronic infection and rarely causes fulminant hepatitis but induces a temporary shut-off of Treg function[3] and an increase of autoantibodies.[4,5] The mechanism by which HAV blocks Tregs without enhancing immune-mediated tissue damage or preventing viral growth is unclear. To enter the cell, HAV interacts with its cellular receptor 1 (HAVCR1),[6,7] a significant allergy and autoimmunity determinant in human beings.[8,9] HAVCR1 is expressed in T cells where it functions as a phosphatidylserine (PtdSer) receptor and T-cell co-stimulatory molecule,[8] which suggests that the HAV–HAVCR1 interaction on T cells is responsible for the long-lasting effect of HAV infection in the immune system.

Abbreviations used in this paper: AP-1, activator protein 1; FoxP3, forkhead box protein 3; HAV, hepatitis A virus; HAVCR1, hepatitis A virus cellular receptor 1; IgV, immunoglobulin V-like domain; HEK, human embryonic kidney; IL, interleukin; mAb, monoclonal antibody; mHavcr1, mouse ortholog of the hepatitis A virus cellular receptor 1; NFAT, nuclear factor of activated T cells; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PI3K/Akt, phosphatidylinositol-3-kinase/protein kinase B; PtdSer, phosphatidylserine; TCR, T-cell receptor; Teff, CD4+CD25- effector T cells; TGF-β, transforming growth factor β; Treg, CD4+CD25+ regulatory T cells.

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The poor understanding of the pathogenic process of HAV prompted us to study the consequences of the HAV–HAVCR1 interaction on human Tregs. Here we show that human Tregs constitutively express HAVCR1, and the HAV–HAVCR1 interaction directly inhibits Treg function. By shutting-off Tregs, HAV overwhelms the immune system with anti–self-responses while limiting de novo anti-HAV T-effector responses by reducing Treg production of transforming growth factor-β (TGF-β), which is needed to recruit leukocytes to the inflammation site and promote T-cell survival.[10] HAV also reduces liver damage by inducing the production of interleukin (IL)-22.[11] A complete understanding of how HAV controls and disarms Treg function could lead to the development of therapies to prevent and/or treat chronic infections, cancer, and allergic and autoimmune diseases in which Tregs play a significant pathogenic role.

Materials and Methods

Cells, Plasmids, and Virus

Stable human embryonic kidney 293 (HEK293) or acute T-cell leukemia Jurkat E6.1 (Jurkat) cell transfectants expressing human HAVCR1 or its mouse ortholog mHavcr1, Jurkat cells transiently transfected with plasmids coding for empty vector, HAVCR1, or an HAVCR1 construct containing a Y350A mutation (HAVCR1 Y350A) in the cytoplasmic tail that eliminates HAVCR1 signaling,[12] and Jurkat cells co-transfected with the earlier-mentioned plasmids and luciferase reporters under the transcriptional control of activator protein 1 (AP-1) or nuclear factor of activated T cells (NFAT)/AP-1,[13] and cytomegalovirus (pRL-CMV; Promega, Corp, Madison, WI) elements were produced as described in the Supplementary Materials and Methods section.

Cell culture–adapted strain HM175 of HAV was produced as described.[6] Cell extracts of uninfected cells were used in most experiments as negative control.

Antibodies and Reagents

Antibodies and reagents used in enzyme-linked immunosorbent assay, alanine aminotransferase assay, flow cytometry, binding assays, apoptosis analysis, and confocal microscopy are described in the Supplementary Materials and Methods section. The FACSCanto II instrument (BD, Franklin Lakes, NJ) was used for flow cytometry and analysis was performed using FlowJo software (Tree Star, Inc, Ashland, OR).

Human Blood and Plasma Samples

Blood of normal donors (National Institutes of Health Blood Bank, Bethesda, MD) and patients with acute HAV infection (Hospital D. Cotugno, Naples, Italy) at the time of hospitalization (T0) and 3–4 weeks later, after resolution of symptoms and viremia (T1) was obtained in accordance with local ethical committee approvals. Peripheral blood mononuclear cells (PBMCs) were purified using Lymphocyte Separation Medium (Mediatech, Inc, Herndon, VA). PBMCs of 9 HAV patients and 9 blood donors were cryopreserved for later use. Tregs and T effector (Teff) cells were purified from PBMCs using the Dynabeads regulatory CD4+CD25+ T-cell kit (Life Technologies Corp, Grand Island, NY) as recommended by the manufacturer. Analyses of plasma samples are described in the Supplementary Materials and Methods section.

Binding Assays

Apoptotic Jurkat cells produced by treatment with etoposide or dexamethasone were stained with 5 μmol/L 5-chloromethylfluorescein diacetate (CMFDA) (Life Technologies) (Jurkat-CMFDA cells), bound to treated or untreated stable HEK293 transfectants, and analyzed using a fluorescence microscope or plate reader as described in the Supplementary Materials and Methods section.

Quantitative Reverse-Transcription Polymerase Chain Reaction

Primers and beacons to quantitate HAV genomes[14] and human *HAVCR1* and *glyceraldehyde 3-phosphate dehydrogenase* messenger RNA were obtained from Applied Biosystems (Life Technologies, Grand Island, NY). Sample preparations, reverse transcription, polymerase chain reaction (PCR), and quantitative evaluations were performed as described in the Supplementary Materials and Methods section.

HAVCR1-Mediated Neutralization of HAV

The interaction of HAV with HAVCR1 was analyzed using a soluble-receptor neutralization assay.[15] Construction, expression, and purification of mutants of human and monkey HAVCR1 are described in the Supplementary Materials and Methods section.

Liquid Immunoarray Assays

Phosphoantibody-specific and cytokine liquid immunoarray assays were performed as suggested by the manufacturers (EMD Millipore Corp, Billerica, MA and Biolegend, Inc, San Diego, CA) and as described in the Supplementary Materials and Methods section.

Luciferase Assays

Luciferase reporter assays[16] were performed in transiently co-transfected Jurkat cells as described in the Supplementary Materials and Methods section.

Fluorescence Confocal Microscopy

HAVCR1 and T-cell receptor (TCR) co-localization[12] was analyzed by fluorescence confocal microscopy as described in the Supplementary Materials and Methods section.

In Vitro Treg Suppression Assay

In vitro Treg suppression assays[3] using purified Treg and Teff cells were performed as described in the Supplementary Materials and Methods section.

Statistical Analysis

A 2-tailed unpaired Student *t* test was used to determine differences between 2 means in analyses of cell lines. A 2-tailed paired Student *t* test was used to determine differences between 2 means in analyses involving samples from the same blood donors or HAV patients. The paired and unpaired tests were performed using GraphPad software. *P* values less than .05 were considered significant.

Results

HAV Blocks the PtdSer-Receptor Function of HAVCR1

There is no evidence that HAV can infect T cells, and wild-type HAV did not infect human T cells or a