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Dysregulated Response of Follicular Helper T Cells to Hepatitis B Surface Antigen Promotes HBV Persistence in Mice and Associates With Outcomes of Patients

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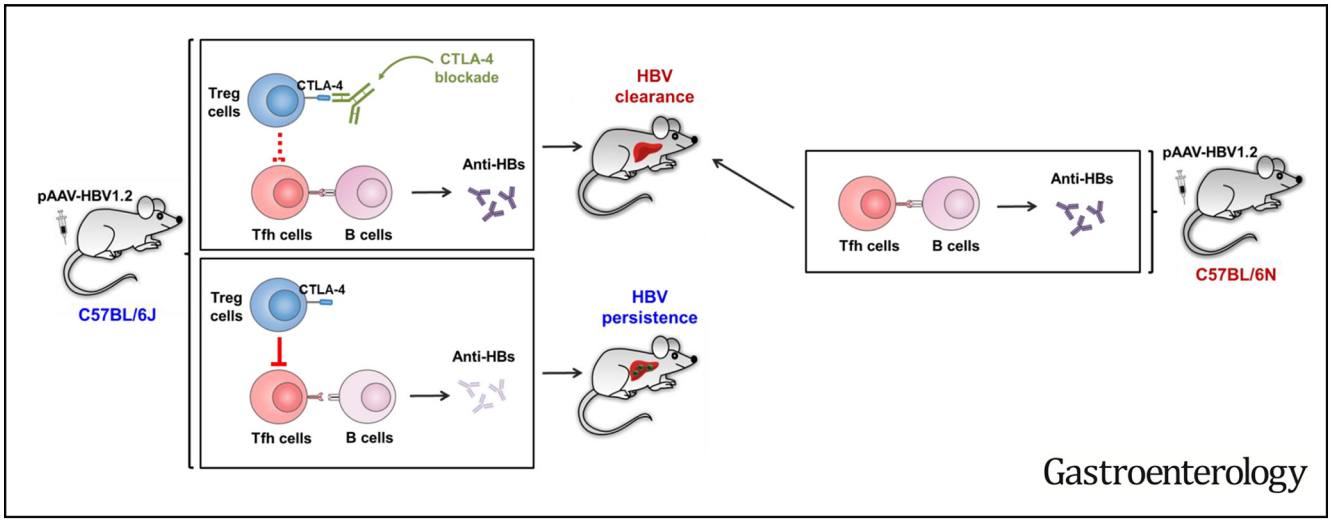

BACKGROUND & AIMS: Production of neutralizing antibodies against hepatitis B surface antigen (HBsAg) is dysregulated in patients with persistent hepatitis B virus (HBV) infection. We investigated mechanisms by which this immune response to the virus is disrupted and whether it can be restored to promote clearance of HBV. **METHODS:** Immune-competent C57BL/6N and C57BL/6J, as well as mice deficient in follicular helper T cells (Tfh-cell-deficient), B cells, or Foxp3^{+} T-regulatory cells (Treg cell deficient), were given hydrodynamic injections of pAAV/HBV1.2 plasmids. Some mice were given injections of sorted Tfh cells, pan-B cells, Treg cells, or a blocking antibody against CTLA4. Production of antibodies against HBsAg and clearance of HBV were assessed by flow cytometry, enzyme-linked immunosorbent assay, polymerase chain reaction, and immunohistochemical analyses. We obtained blood samples from patients with HBV infection and isolated Treg cells. We measured the ability of Treg cells to suppress production of interleukin 21 (IL21) in CD4^{+} T cells. **RESULTS:** Immune-competent C57BL/6N and C57BL/6J mice transfected with the plasmid encoding HBV had features of viral clearance and viral persistence observed in humans. A Tfh-cell response to HBsAg was required for clearance of HBV and was suppressed by Treg cells in mice with persistent HBV infection. Depletion of Treg cells or inhibition of Treg-cell function (with blocking antibody against CTLA4) restored the Tfh-cell response against HBsAg and clearance of HBV in mice. Impaired Tfh-cell response to HBsAg was observed in blood from patients with chronic HBV infection, responsiveness was restored by depletion of Treg cells or blocking antibody against CTLA4. **CONCLUSIONS:** In studies of HBV-infected mice and blood from patients with chronic HBV infection, we found a Tfh-cell response to HBsAg of to be required for HBV clearance, and that this response was blocked by Treg cells. Inhibiting Treg-cell activity using neutralizing antibody against CTLA4 restored the ability of Tfh cells to clear HBV infection; this approach might be developed for treatment of patients with chronic HBV infection.

Keywords: Viral Hepatitis; Immune Regulation; Neutralizing Antibody Response; Immunotherapy.

EDITOR'S NOTES

BACKGROUND AND CONTEXT

The production of antibodies against hepatitis B surface antigen (HBsAg) (anti-HBs) is dysregulated during chronic HBV infection.

NEW FINDINGS

Neutralizing anti-HBs are crucial for both circulating and intrahepatic HBV clearance, and dysregulation of the follicular T cell (Tfh cell)-dependent anti-HBs response is mediated by suppressive Foxp3$^+$ T-regulatory cells (Treg cells) during HBV persistence.

LIMITATIONS

The study does not elucidate why HBsAg-specific Treg cells are differentiated in HBV-persistent mice, and whether or not they have any direct suppressive effect on B cells.

IMPACT

This study highlights the therapeutic role of neutralizing anti-HBs- and Treg cell-targeting strategies, especially CTLA4 blockade, against chronic HBV infection.

Hepatitis B virus (HBV) results in acute or chronic infection. Chronic HBV infection is a major global public health problem that poses a risk of cirrhosis, hepatocellular carcinoma, and death.[1] Viral clearance is largely dependent on multiple mechanisms of host immune responses, including robust CD8$^+$ T-cell responses and neutralizing antibody responses, which are usually dysregulated in persistent HBV infection.[2,3] Uncovering details about the immunological tolerance to HBV and exploring the possibility to reverse the tolerance by manipulating the different pathways involved represent the most attractive challenges for a therapeutic approach to chronic HBV infection.

Antibodies that are specific for hepatitis B surface antigen (anti-HBs) have virus-neutralizing activity and limit viral spread from residual productively infected hepatocytes that are not eliminated by the CD8$^+$ T cells,[4] thus conferring protective immunity to the hosts.[5] Recent research provided new insights into understanding the therapeutic role of neutralizing antibodies against persistent HBV infection through identifying novel monoclonal antibodies (mAbs).[6,7] In addition to being primarily involved in HBV infection control, anti-HBs responses also play remarkable roles in preventing HBV reactivation.[8] In patients with chronic HBV infection, anti-HBs response is depressed. Reconstitution of anti-HBs response represents a rational therapy for chronic HBV infection. However, the mechanism underlying the dysregulated anti-HBV antibody responses in chronic HBV infection and whether it can be repaired to facilitate virus control remains largely unexplored.

HBsAg are strict T-cell–dependent antigens, and the production of anti-HBs requires aid from CD4$^+$ helper T cells.[9] Recently, a unique CD4 helper subset within the lymphoid follicle, the T follicular helper cells (Tfhs), has been specialized to aid the development of B cells into Ab-producing cells in germinal centers (GCs).[10] Many groups have found that Tfh cells are profoundly dysregulated in number and function in multiple kinds of autoimmune disease and chronic infections,[11,12] leading to aberrant protective B-cell responses, and are at least in part, responsible for disease progression. Conversely, the responses of Tfh cells to acute and chronic HBV infection are much more obscure. No specific role has been attributed to Tfh cells in the production of neutralizing anti-HBs and, most importantly, the outcome of HBV infection. Furthermore, the regulatory mechanism of Tfh-cell responses during HBV infection deserves to be explored.

Here, we investigated the contribution of anti-HBs and Tfh-cell responses to HBV clearance and explored the mechanism by which these responses are regulated. Using nontransgenic hydrodynamic transfection mouse models and peripheral blood mononuclear cells (PBMCs) from HBV-infected patients, we discovered that Tfh-cell–dependent anti-HBs responses play important roles in systemic HBV clearance, which were disturbed by T-regulatory (Treg) cells in the context of HBV persistence. Inhibition of Treg-cell activity through blockade of CTLA4 restores the anti-HBs response and results in HBV control, highlighting the potential for anti-CTLA4–based therapy in chronic HBV infection.

Materials and Methods

Mice

C57BL/6N and Balb/c mice were purchased from Charles River (Beijing, China), C57BL/6J mice were purchased from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China), Rag1$^{-/-}$ mice were obtained from Model Animal Research Center (Nanjing, China), B6.Ly5.1 (CD45.1) mice and μMT mice were generous gifts from Dr Mingzhao Zhu. CD4-Cre*Bcl6*$^{fl/fl}$ mice were generous gifts from Dr Toshitada Takemori and were crossed to C57BL/6N or Balb/c for 4 generations, Foxp3-DTR mice were generous gifts from Dr Alexander Y. Rudensky, and CD8 knockout (KO) mice were generous gifts from Dr Lilin Ye. For all experiments 6-week-old male mice were used. Mice were housed in the SPF Beijing Institute of Lifeomics animal facility. All mice experiments were approved by the Institutional Animal Care and Use Committee at the Beijing Institute of Lifeomics.

*Authors share co-first authorship.

Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; anti-HBs, antibodies specific for hepatitis B surface antigen; ASC, antibody-secreting cell; CHB, chronic hepatitis B; GC, germinal center; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IL, interleukin; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; MHC-I, major histocompatibility complex class I; PBMC, peripheral blood mononuclear cell; ROS, reactive oxygen species; Tfh-cell, T follicular helper cell; Treg cell, T-regulatory cell; WT, wild-type.

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