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coming these obstacles and activating TAA-specific CTL responses (Fig. 1). Many new vaccines, therefore, also include autologous DCs that have been loaded with TAA (refs. 3,4,6) *in vitro*. This study instead uses the DC stimulant GM-CSF.

GM-CSF functions to promote TAA presentation by antigen presenting cells (APCs) and CTL activation by recruiting peripheral DC precursors and inducing DC maturation (Fig. 1) (ref. 6). Recent studies have also confirmed the role of CD40-mediated activation of DCs in activation of the TAA-specific CTL response[10]. Indeed, CTL-dependent rejection of B-lymphomas in mice was recently reported after treatment with an agonistic antibody against CD40 (ref. 11). Thus, GM-CSF and CD40 ligand may prove to be a winning adjuvant combination in future lymphoma, as well as other tumor vaccines.

The vaccine strategy reported by Bendandi *et al.* does not represent a particularly new approach, but its validation of therapeutic benefit, well-planned study design, and careful execution allow this study to stand as a hallmark in the field of cancer vaccine development. Consideration of the patients' disease stage cannot be ignored in determining the potential of a vaccine to achieve a beneficial therapeutic outcome. These findings suggest that in cancer therapy, early intervention with vaccine-based approaches is warranted. Furthermore, studies including specific TAAs as vaccines should carefully monitor post-vaccination immune responses. Future studies aimed at identification of TAAs for other tumor types, and additional carefully- monitored tumor vaccine trials, should lead to the development of new, more effective vaccine strategies.

1. Rosenberg, S.A. A new era for cancer immunotherapy based on the genes that encode cancer antigens. *Immunity* **10**, 281–287 (1999).
2. Vermorken, J.B. *et al.* Active specific immunotherapy for stage II and stage III human colon cancer: a randomized trial. *Lancet* **353**, 345–350 (1999).
3. Nestle, F.O. *et al.* Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nature Med.* **2**, 52–58 (1998).
4. Hsu, F.J. *et al.* Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nature Med.* **2**, 52–58 (1996).
5. Rosenberg, S.A. *et al.* Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nature Med.* **4**, 321–327 (1998).
6. Pardoll, D.M. Cancer vaccines. *Nature Med.* (suppl.) **4**, 525–531 (1998).
7. Bendandi, et al. Molecular complete remissions induced by patient-specific vaccination plus GM-CSF against lymphoma. *Nature Med.* **5**, 1171–1177 (1999).
8. Levitsky, H.I. *et.al.* Immunization with granulocyte-macrophage colony stimulating factor-transduced, but not B7.1-transduced, lymphoma cells primes idiotype-specific T cells and generates potent systemic antitumor immunity. *J. Immunol.* **156**, 3858–3865 (1996).
9. Nelson, E.I. *et al.* Tumor-specific, cytotoxic T-lymphocyte response after idiotype vaccination for B-cell, non-Hodgkin's lymphoma. *Blood* **88**, 580–589 (1996).
10. Mackey, M.F. *et al.* Dendritic cells require maturation via CD40 to generate protective antitumor immunity. *J. Immunol.* **161**, 2094–2098 (1998).
11. French, R.R. *et al.* CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. *Nature Med.* **5**, 548–553 (1999).

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Unraveling hepatitis B virus infection of mice and men (and woodchucks and ducks)

The development of liver diseases in patients suffering from chronic hepatitis B infection is determined by the nature of the host immune response. Several factors have made this immune response difficult to study, but recently developed animal models will help clear the way.

STEPHAN MENNE & BUD C. TENNANT

INFECTION OF ADULT humans with the hepatitis B virus (HBV) characteristically results in self-limited disease and in clinical recovery. Persistent or chronic infection is an outcome that usually accounts for less than 5% of adult HBV infections. Children often develop persistent infection, which later in life causes chronic hepatitis, cirrhosis of the liver, and, in some, primary hepatocellular carcinoma (HCC). Although safe and effective HBV vaccines are now available, over 300 million people worldwide are chronically infected with HBV, and these individuals have a high relative risk of developing life-threatening liver disease.

Substantial evidence now suggests that differences in outcome of HBV infection are determined by variations in the immune response. Cell-mediated immunity involving T-helper (Th) cells and cytotoxic T lymphocytes (CTL) determines whether HBV infection is followed by recovery or viral persistence (Fig. 1). During acute self-limited virus infection, patients show vigorous, polyclonal Th and CTL responses against epitopes of viral capsid and envelope proteins[1], leading to the clearance of viral DNA and envelope proteins from the serum. Patients who develop chronic HBV infection have been reported to have less-vigorous Th responses, characterized by low frequency of HBV-specific Th cells in the peripheral blood and the liver, and weak or undetectable virus-specific CTL responses[1]. A weak CTL response is also believed to cause the hepatic injury that occurs in chronically infected patients.

The immunopathogenesis of HBV infection has been difficult to investigate. HBV has a narrow host range limited to endangered animal species. Tissue culture systems used to study most other viral pathogens are not yet available for HBV. The original studies of HBV immunopathogenesis were limited to infected patients at varying stages of disease following accidental exposure, and to experimentally infected chimpanzees[1,2].

In recent years, new animal models of HBV infection have contributed substantially to our understanding of this virus[3]. The woodchuck (*Marmota monax*) and the Pekin duck (*Anus domesticus*) both have naturally occurring infections with viruses that belong to the family *Hepadnaviridae*, of which HBV is the prototype virus. Woodchucks with naturally or experimentally acquired, persistent woodchuck hepatitis virus infection develop chronic hepatitis and HCC similar to those associated with HBV infection. Duck hepatitis B virus, is not typically associated with liver disease. Although both the duck and the woodchuck have been valuable in molecular studies of viral replication and have been useful in the preclinical evaluation of prospective antiviral

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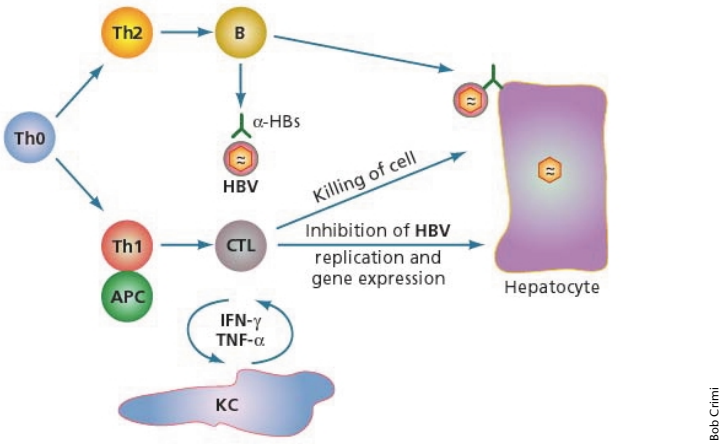

Fig. 1 Humoral- and cellular-mediated immunity during acute hepatitis B virus (HBV) infection leading to viral clearance. After infection of hepatocytes, precursor T helper cells (Th0) mature into Th1 or Th2 cells. Th2 cells amplify B cells responsible for production of virus neutralizing antibodies against HB/surface antigen (α-HBs). Anti-HBs complexes with HBV particles, facilitating removal from the circulation and preventing their attachment and uptake by hepatocytes. Th1 cells recognize viral epitopes presented by the class II MHC molecules of professional antigen-presenting cells (APC), produce cytokines, and interact with cytotoxic T lymphocytes (CTLs). CTLs identify their target cells through recognition of viral epitopes presented by class I MHC molecules of HBV infected hepatocytes. Activated CTLs can directly kill infected hepatocytes by inducing apoptosis, but also release inflammatory cytokines, especially gamma interferon-γ (IFN- γ) and tumor necrosis factor-α (TNF-α). These cytokines also activate Kupffer cells (KC) to produce additional TNF-α. Both cytokines activate antiviral pathways in hepatocytes leading to inhibition of HBV replication and gene expression. During chronic HBV infection, the immune responses are impaired and the same pathways contribute to HBV persistence, by partial inhibition of viral replication and gene expression. These events lead to incomplete clearance of HBV and hepatic injury of variable severity that leads to liver cirrhosis and hepatocellular carcinoma.

drugs for treatment of HBV infection, both species are outbred and their immune responses are just now being characterized[4,5].

Mice are not susceptible to HBV infection, but a number of lineages of mice have now been developed that carry one or more HBV transgenes. The episomal, covalently closed circular DNA (cccDNA) species, which serves as the viral transcriptional template in naturally occurring hepadnavirus infection, is not produced in these mice and transcription occurs exclusively from viral sequences integrated into chromosome DNA. Large advances in our current understanding of HBV immune pathogenesis have been based on the prodigious and creative use of transgenic mice[6].

One of the drawbacks of the HBV transgenic mouse model is that they are tolerant to HBV antigens. Thus, there are limitations to the use of transgenic mouse models in the study of the mechanisms by which the anti-HBV cellular immune response leads to liver disease. A large advance was recently made by Larkin and colleagues[7], who created a new HBV transgenic model using mice with severe combined immunodeficiency (SCID). The HBV transgenic SCID mice are not tolerant to HBV proteins, and adoptive transfer of unprimed syngeneic splenocytes results in a partial reduction in viral load and the development of chronic active hepatitis. This new model provides a unique opportunity to investigate the role of individual subsets of T lymphocytes in HBV pathogenesis and in cell-mediated viral clearance.

For some time, it was believed that the primary role of CTLs was the clearance of HBV infection by direct killing of HBV-infected hepatocytes after Th cell activation (Fig. 1). However, it has now been demonstrated that a more important effect of CTLs is the release of the antiviral cytokines gamma interferon (IFN-γ), tumor necrosis factor-α, and interleukin 2 by activated macrophages and CTLs. These cytokines inhibit viral replication and gene expression[8,9]. Such non-cytopathic antiviral mechanisms were recently demonstrated in acute experimental HBV infection in chimpanzees[10] to be responsible for the prompt hepatic clearance not only of HBV replication intermediates, but also of viral cccDNA, previously thought to have a long half-life in the liver. Similar non-cytopathic antiviral mechanisms are likely to be essential for the clearance of HBV infection in humans.

At present, approved therapy for chronic HBV infection is confined either to INF-α or the L-nucleoside analog lamivudine[11,12]. Both therapeutic approaches characteristically result in reductions in viral load, but withdrawal of treatment is associated with viral recrudescence. Attempts have been made using immunotherapy to stimulate a more robust host antiviral immune response[13,14] for treatment of chronic HBV infections. The use of the latest animal models will provide new insight into the mechanisms underlying HBV persistence and pathogenesis, and lead to improved immunotherapeutic approaches, perhaps involving a combination with antiviral drug therapy. Such advances will benefit the 300 million HBV carriers worldwide that are at high risk of developing life-threatening hepatic disease.

1. Chang, K-M., & Chisari, F.V. Immuno-pathogenesis of hepatitis B virus infection. *Clin. Liver Dis.* **3,** 221–239 (1999).
2. Chisari, F.V., & Ferrari, C. In: *Viral pathogenesis* (ed., Nathanson, N.) 745–778 (Lippincot-Raven, Philadelphia, 1997).
3. Tennant, B.C. Animal models of hepatitis B virus infection. *Clin. Liver Dis.* **3,** 241–266 (1999).
4. Vickery, K., Cossart, Y., & Dixon R. Cellular immune response of ducks to duck hepatitis B virus infection. *J. Med. Virol.* **58,** 19–25 (1999).
5. Menne, S., Maschke, J., Lu, M., Grosse-Wilde, H., & Roggendorf, M. T-cell response to woodchuck hepatitis virus (WHV) antigens during acute self-limited WHV infection and convalescence and after viral challenge. *J. Virol.* **72,** 6083–6091 (1998).
6. Chisari, F.V. Hepatitis B virus transgenic mice: Models of viral immunobiology and pathogenesis. *Curr. Top. Microbiol.* **206,** 150–173 (1996).
7. Larkin, J. *et al.* Hepatitis B virus transgenic SCID mouse model of chronic liver disease. *Nature Med.* **5,** 907–912 (1999).
8. Guidotti, L.G. *et al.* Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* **4,** 25–36 (1996).
9. Guidotti, L.G. *et al.* Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. *Proc. Natl. Acad. Sci. USA.* **91,** 3764–3768 (1994).
10. Guidotti, L.G. *et al.* Viral clearance without destruction of infected cells during acute HBV infection. *Science* **284,** 25–29 (1999).
11. Krogsgaard, K. *et al.* The treatment effect of alpha interferon in chronic hepatitis B is independent of pre-treatment variables. Results based on individual patient data from 10 clinical controlled trials. European Concerted Action on Viral Hepatitis (Eurohep). *J. Hepatol.* **21,** 646–655 (1994).
12. Dienstag, J.L. *et al.* A preliminary trial of lamivudine for chronic hepatitis B infection. *N. Engl. J. Med.* **333,** 1657–1661 (1995).
13. Pol, S. *et al.* Immunotherapy of chronic hepatitis B by anti HBV vaccine. *Acta. Gastroenterol. Belg.* **61,** 228–233 (1998).
14. Heathcote, J. *et al.* A pilot study of the CY-1899 T-cell vaccine in subjects chronically infected with hepatitis B virus. *Hepatology* **30,** 531–536 (1999).

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