

virtually all their patients were receiving immunomodulatory agents at the time of infliximab dosing may have diminished infusion reaction rates as was recently suggested in another paper (2).

The report by Stephens *et al.* (1), along with two others in the literature (3, 4), brings to 116 the total number of pediatric patients with Crohn's disease receiving infliximab reported to date. Most assuredly, many thousands of children have been treated, but we only have data on a small fraction making it difficult to ascertain the likelihood of uncommon short-term or long-term complications.

There are compelling reasons why the future must bring us controlled data on the use of newer (and for that matter older) therapies in children. It is not clear if dosing guidelines developed in adults can be applied to children. Children may metabolize drugs very differently, and extrapolating a mg/kg dose from adults incurs risk of underdosing or overdosing. Adverse events in children may not be predicted based upon experience in adults. Children also pose some different clinical problems than adults, particularly with respect to the effect of disease and therapy on growth. Growth failure is a serious complication of Crohn's disease in the pediatric population. Even small amounts of corticosteroids can slow linear growth velocity suggesting that aggressive corticosteroid-sparing treatment regimens need to be considered early in the course of certain patients. The recent observation that the use of 6-mercaptopurine at diagnosis in moderate-to-severe Crohn's disease in children may decrease total corticosteroid exposure over the first 2 years of treatment was an important one (5). It has also suggested a potential role of infliximab in the early treatment of selected patients who present with growth failure and/or osteopenia where the use of prednisone may be fraught with increased hazards.

These concerns raise further questions. What is the role of infliximab in the treatment of Crohn's disease in children? Should it be used as salvage therapy after "conventional" therapy including 5-aminosalicylate agents, corticosteroids, immunomodulators, and nutritional support has failed? Is it bridge therapy in the corticosteroid-dependent or corticosteroid-refractory patient while the clinician awaits a response to immunomodulators such as azathioprine or 6-mercaptopurine, which may take months to be effective? Should it be used at diagnosis in those with severe disease instead of corticosteroids? Are there genetic markers that can help us identify patients more likely to have a beneficial effect from this drug (and thereby decrease expense and potential toxicity)? Most importantly, are we acquiring data that will allow us to determine if infliximab is changing the natural history of Crohn's disease in children? We need to know if these children grow better, have a better quality of life, fewer hospitalizations, less surgery, and perhaps more benign courses as adults. We also need to develop methodologies to follow children receiving infliximab as well as other immunomodulatory agents into adulthood to examine the still unresolved question of malignancy risk. Does the use of immunomodulatory and/or biological therapy promote cancer decades down the line?

So where do we go from here? Although the report by Stephens *et al.* (1) further allays our short-term concerns about infliximab therapy in children, the days of single-center reports of modest numbers of patients receiving new therapies must eventually come to an end. This will only be possible with financial support from industry and lay organizations that need to actively promote multicentered data collection along with prospective controlled trials of new therapies for children with IBD. Product development should include pediatric studies early in the process, not as an afterthought. Kids can get hand-me-down clothes, but they shouldn't have to get hand-me-down science.

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## REFERENCES

1. Stephens MC, Shepanski MA, Mamula P, et al. Safety and steroid-sparing experience using infliximab for Crohn's disease at a pediatric inflammatory bowel disease center. *Am J Gastroenterol* 2003;98:104-111.
2. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: The ACCENT I randomised trial. *Lancet* 2002;359:1541-9.
3. Hyams JS, Markowitz J, Wyllie R. Use of infliximab in the treatment of Crohn's disease in children and adolescents. *J Pediatr* 2000;137:192-6.
4. Kugathasan S, Werlin SL, Martinez A, et al. Prolonged duration of response to infliximab in early but not late pediatric Crohn's disease. *Am J Gastroenterol* 2000;95:3189-94.
5. Markowitz J, Grancer K, Kohn N, et al. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. *Gastroenterology* 2000;119:895-902.

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## Cytokine Gene Polymorphisms in Chronic Hepatitis B: A Step Up the Immunology Ladder

Chronic hepatitis B virus (HBV) infection is a major global health problem with an estimated 300 million people chronically infected worldwide (1). In endemic areas, chronic HBV is a leading cause of death because of the development of liver failure and liver cancer (1). In the United States, an estimated 1 million Americans have chronic HBV and approximately 5000 patients die of HBV-related liver disease each year (2). Hepatitis B is parenterally transmitted via contact with contaminated blood, saliva, and semen (1). Individuals with an inadequate primary immune response to hepatitis B are at increased risk of developing chronic HBV. Age is the strongest host feature associated with chronic

infection with 90% of infants, 20–50% of children, and 5–10% of adults developing chronic HBV after exposure (3). In addition, immunocompromised patients including HIV coinfecting individuals and organ transplant recipients are at increased risk of developing chronic HBV. Viral factors such as hepatitis B surface antigen “a” determinant mutants, precore and core-promoter mutants, and HBV genotypes have also been implicated in the risk of developing chronic HBV, but the role of these factors is not well established (4).

Clearance of HBV requires a coordinated innate and adaptive humoral and cell-mediated immune response. Cytokines are soluble polypeptide molecules that mediate cell-to-cell communication and regulate the intensity and duration of the immune response. Cytokine response profiles from T helper cells are classified as Th1 responses that enhance cellular immunity (interferon [IFN]- $\gamma$ , interleukin [IL]-2, tumor necrosis factor [TNF]- $\alpha$ ) or Th2 responses that enhance humoral immunity (IL-4, IL-5, IL-10). With acute self-limited HBV infection, most HBV DNA molecules are rapidly cleared in the incubation phase because of a vigorous, multifaceted, polyclonal immune response to hepatitis B surface, core, and polymerase antigens (5). Under these circumstances, a proinflammatory Th1 response with high serum and intrahepatic levels of IFN- $\gamma$  and TNF- $\alpha$  inhibit HBV gene expression and replication and lead to the rapid destruction and clearance of infected hepatocytes by both cytopathic and noncytolytic mechanisms (5, 6). After recovery from acute infection, HBV-specific cytotoxic T cells as well as neutralizing antibodies to hepatitis B core antigen and surface antigen persist for decades and provide long-lasting immunity (7). However, low levels of HBV may be detected in the blood using sensitive polymerase chain reaction-based assays (7, 8).

Chronic HBV infection is defined as persistence of serum HB surface antigen for more than 6 months after exposure. Patients with chronic HBV typically have a weak, narrowly focused intrahepatic and systemic immune response to HBV antigens (9, 10). In these patients, lower levels of IFN- $\gamma$  and TNF- $\alpha$  are produced in response to HBV antigens compared with patients with acute self-limited infection (11). Patients with perinatally acquired HBV who have high HBV DNA but normal serum aminotransferase levels seem to have “immune tolerance” to HBV antigens. This immunological hyporesponsiveness may be caused by viral inhibition of T helper cells or depletion of effector T cells (6, 10). These patients rarely develop progressive liver disease during the immune tolerant phase but are at increased risk of developing liver cancer. Other patients with chronic HBV and elevated serum aminotransferase levels seem to have a weak but nonclearing immune response to HBV antigens and are at risk of developing progressive liver disease. Studies that identify the mediators of variability in host immune response to HBV may provide insight not only into the pathogenesis of disease but also potential targets for treatment.

Genetic susceptibility to chronic HBV infection may reside in variability in host recognition, cytokine, or antigen

presenting and processing genes. Previous studies have demonstrated that variability in human leukocyte antigen class I and class II genes may be seen in patients with acute resolving HBV compared with those with chronic HBV (12). However, these findings are not likely to be clinically useful because the relative risk conferred by possession of a specific allele is low (2- to 4-fold), and the frequency of the preferred allele in the general population is low (13). Furthermore, associations of HLA polymorphisms with the severity of liver disease and the response to treatment have not been demonstrated. However, because the worldwide burden of disease from chronic HBV is so extensive, further studies of host genetic factors that influence the immune response are warranted.

It is in this context that the work of Ben-Ari *et al.* examining the role of cytokine gene polymorphisms in chronic HBV is presented in this issue of the Journal (14). Previous studies have shown that the maximal capacity of cytokine production varies among individuals and correlates with single nucleotide polymorphisms in the promoter region of various cytokine genes (15). Furthermore, cytokine gene polymorphisms have been associated with liver disease severity in patients with viral hepatitis (16, 17). In this study, the investigators set out to determine if the frequency of five cytokine gene polymorphisms was significantly different in 77 patients with varying severity of chronic HBV infection compared with 48 uninfected controls. Contrary to prior reports, the authors did not find a significant association between TNF- $\alpha$  polymorphisms at position -308 and -174 and the presence of chronic HBV (18). In addition, there was no significant association between IL-6, transforming growth factor- $\beta$ , and IL-10 polymorphisms and the presence of chronic HBV. However, a significantly greater frequency of the A/A IFN- $\gamma$  polymorphism at position 874 was noted in chronic HBV (65%) patients compared with uninfected controls (37%) ( $p = 0.003$ ). Although the authors did not measure serum or intrahepatic levels of IFN- $\gamma$ , prior studies have demonstrated that individuals with the A/A 874 polymorphism tend to have lower levels of IFN- $\gamma$  production (19). Therefore, the study results are consistent with the hypothesis that a defective proinflammatory Th1 cytokine response may lead to the development of chronic HBV (6).

In reviewing the results of this study, one must keep several potential limitations in mind. Firstly, the authors do not present information on the presumed route of infection in the study patients. These data are important because one may not be able to detect a significant difference in a host genetic factor if the majority of patients were infected as infants. In addition, the authors selected uninfected patients as well as previously exposed patients as controls. Because unexposed patients remain at risk of acquiring chronic HBV, the inclusion of a mixed control group limits the ability to compare the polymorphism results to the patients with established chronic HBV. Ideally, a control group for a disease-association study should consist of age- and gender-matched controls previously exposed to HBV at a sim-

ilar age and via a similar route to the patients with chronic infection. However, because it is very difficult if not impossible to identify large cohorts of control patients in nonendemic countries, our understanding of the role of cytokine gene polymorphisms in chronic HBV may be limited. In addition, because cytokine gene expression may be altered at the transcriptional, translational, and secretory level, further studies of cytokine expression *in vivo* will be necessary to determine the contribution of reported disease associations with cytokine polymorphisms. Lastly, the small number of patients included in this study and the absence of data on the prevalence of the cytokine gene polymorphisms in the general population may lead to a type II error.

Notwithstanding the above caveats, the findings of this study and others may have important scientific implications if they are confirmed in large, population-based studies. For example, if the IFN- $\gamma$  polymorphism is proven to be a significant risk factor for developing chronic HBV, the potential to enhance host IFN- $\gamma$  production may prove useful as a therapeutic intervention. Although previous work has demonstrated that administration of *s.c.* IFN- $\gamma$  is less effective and more toxic than IFN- $\alpha$  in patients with chronic HBV (20), other approaches of enhancing Th1 cytokine responses may prove useful. For example, administration of therapeutic peptide vaccines may enhance host immune response to HBV antigens *in vivo* (11). Recent studies with lamivudine have shown that reducing HBV replication and viral antigen load can improve Th1 cytokine response profiles and cytotoxic T-cell responses in some patients with chronic HBV (21, 22). Therefore, combining an antiviral and immunomodulatory agent together may prove useful. Although we are uncertain if useful information is up the ladder at the cytokine gene promoter level or down the ladder at the level of protein expression, further studies defining the clinical and biological relevance of cytokine gene polymorphisms in chronic HBV are warranted. A multifaceted approach using laboratory animals to identify candidate genes, twin studies to establish causality, and disease-association studies may help us unravel the mystery. Stay tuned.

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## REFERENCES

- Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: A global perspective. *Vaccine* 1999;17:1730-3.
- Margolis HS, Alter MJ, Hadler SC. Hepatitis B: Evolving epidemiology and implications for control. *Semin Liv Dis* 1991;11:84-92.
- Tassopoulos NC, Papaevangelou GJ, Sjogren MH, et al. Natural history of acute hepatitis B surface antigen hepatitis in Greek adults. *Gastroenterology* 1987;92:1844-50.
- Mayerat C, Mantegani A, Frei PC. Does hepatitis B virus genotype influence the clinical outcome of HBV infection? *J Viral Hepatitis* 1999;6:299-304.
- Webster GJ, Reignat S, Maini MK, et al. Incubation phase of acute hepatitis B in man: Dynamic of cellular immune mechanisms. *Hepatology* 2000;32:1117-24.
- Chisari FV. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995;13:29-60.
- Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients, recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996;2:1104-8.
- Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001;19:65-91.
- Lohr H, Gerken G, Schlicht H, et al. Low frequency of cytotoxic liver-infiltrating T lymphocytes specific for endogenous processed surface and core proteins in chronic hepatitis B. *J Infect Dis* 1993;168:1133-9.
- Bertoletti A, D'Elia MM, Boni C, et al. Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997;112:193-9.
- Livingston BD, Alexander J, Crimi C, et al. Altered helper T lymphocyte function associated with chronic hepatitis B virus infection and its role in response to therapeutic vaccination in humans. *J Immunol* 1999;162:3088-95.
- Thursz MR, Kwiatkowski D, Alsopp CEM, et al. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N Engl J Med* 1995;332:1065-9.
- Thursz M. MHC and the viral hepatitis. *Quart J Med* 2001;94:287-91.
- Ben Ari Z, Mor E, Kfir B, et al. Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am J Gastroenterol* 2003;98:144-150.
- Bidwell JL, Wood NA, Morse HR, et al. Human cytokine gene nucleotide sequence alignments. *Eur J Immunogenet* 1998;25:83-265.
- Gewaltig J, Mangasser-Stephan K, Gartung C, et al. Association of polymorphisms of the transforming growth factor-B1 gene with the rate of progression of HCV induced liver fibrosis. *Clin Chim Acta* 2002;316:83-94.
- Powell EE, Edwards-Smith CJ, Hay JL, et al. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000;31:828-33.
- Kohler T, Kruger A, Gerken G, et al. A tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) promoter polymorphism is associated with chronic hepatitis B. *Clin Exp Immunol* 1998;111:579-82.
- Pravica V, Asderakis A, Perrey C, et al. In vitro production of IFN- $\gamma$  correlates with CA repeat polymorphism in the human IFN- $\gamma$  gene. *Eur J Immunogenet* 1999;26:1-3.
- DiBisceglie AM, Rustgi VK, Kassianides C, et al. Therapy of chronic hepatitis B with recombinant human alpha and gamma interferon. *Hepatology* 1990;11:266-70.
- Boni C, Bertoletti A, Penna A, et al. Lamivudine treatment can restore T-cell responsiveness in chronic hepatitis B. *Br J Clin Invest* 1998;102:968-75.
- Boni C, Penna A, Ogg GS, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: New perspectives for immune therapy. *Hepatology* 2001;33:963-71.

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