CONCISE COMMUNICATION

IgG Subclass Distribution of Hepatitis B Surface Antigen Antibodies Induced in Children with Chronic Hepatitis B Infection after Interferon-α Therapy

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The IgG subclass distribution of antibody to hepatitis B surface antigen (anti-HBs) was investigated in 19 children with chronic active hepatitis B infection who showed a complete serological seroconversion after interferon-α therapy. Determinations were done 6 and 12 months after treatment. Our results showed no selectivity in anti-HBs synthesis among IgG subclasses. All 4 IgG isotypes were involved in the response, with similar percentage contributions, on average, of IgG1 (35%), IgG3 (27%), and IgG4 (28%), followed by IgG2 (10%). IgG4 became the second most dominant isotype at the end of observation. These results are in contrast to those found after natural seroconversion, in which anti-HBs was highly restricted to neutralizing IgG1 and IgG3, with only a minor contribution from IgG2 and IgG4. It is postulated that analysis of the specific profiles of IgG subclasses may be of value for the estimation of the therapeutic efficacy of recombinant interferon-α used and may be helpful in choosing more-effective treatment.

Chronic infection with hepatitis B (HB) virus can occur in children at any age and may lead to chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma during childhood or adulthood [1]. Suppression of viral replication and modulation of the immune responses can prevent disease progression and decrease the severity of liver damage. Thus, the development of an effective therapy against HB virus infection seems to be a high priority. Interferon (IFN)-α, a molecule that combines antiviral properties with the capacity to modulate the cellular immune response by its effects on the cytokine network [2], was proposed for the treatment of chronic HB 20 years ago [3]. Although its therapeutic use as an antiviral drug has increased significantly in recent years, a complete seroconversion to antibody to HB e (anti-HBe)/antibody to HB surface antigen (anti-HBs) rarely occurs.

The antibody response to viral envelope antigens (i.e., anti-HBs) plays a critical role in removing free viral particles from circulation in the bloodstream, in limiting virus spread in the host, and in protection against perinatal infections [4, 5]. Serum titers of total anti-HBs generally are used as criteria for the immune response to HB surface antigen (HBsAg). However, several studies have confirmed that the most effective humoral response to many viral protein antigens is provided by neutralizing antibodies of IgG [6]. This isotype of immunoglobulins consists of 4 subclasses, each encoded by a separate C-region gene and endowed with unique biological functions that are important for an efficient humoral response to a given pathogen. In adults, antibody responses to viral protein antigens mainly are restricted to IgG1, IgG3, or both; IgG2 generally is stimulated by carbohydrate antigens, whereas IgG4 most likely reflects a chronic antigenic stimulation and has been associated with immediate-type hypersensitivity [7]. Several studies in animal models and in humans have shown that the selection of a proper isotype is important for complete viral clearance from the host and that cytokines mainly are responsible for this process. Therefore, the monitoring of specific IgG subclass profiles after antiviral treatment, compared with natural virus infection, may give an insight into the mechanisms that drive antibody production in both conditions.

Although the mechanisms of IFN-α actions have been studied widely in chronic HB viral infection, humoral response to HBsAg at the IgG subclass level has not been investigated. The aim of our study was to assess whether anti-HBs antibodies...
induced in children with chronic active HB, by IFN-α therapy, have the same IgG subclass distribution as in those recovering from natural infection; whether any particular changes of specific response occurred over a period <12 months after completion of therapy; and whether any association exists between the IgG subclass profiles of anti-HBs antibodies and the age or sex of the children studied.

Patients and Methods

Patients. Serum samples were collected from 19 children (8 girls and 11 boys; median age, 3.33 years; range, 2–13 years) with chronic active HB infection who showed seroconversion to anti-HBs after antiviral therapy with recombinant IFN-α (rIFN-α; Intron A, Schering-Plough, Kenilworth, NJ). The rIFN-α was given subcutaneously at a dose of 3 MU 3 times a week for 20 weeks. Serological markers of HB virus were tested during therapy and then at 6 and 12 months after completion of treatment. The markers were tested with commercially available IMx AUSAB kits (Abbott Laboratories, Chicago, IL). Only children who were HBsAg negative, HB e antigen (HBeAg) negative, or anti-HBs, anti-HBe, or anti–HB core antigen (anti-HBc) positive 6 months after therapy were included in the study.

Serum samples also were collected from 30 age-matched children (10 girls and 20 boys; median age, 4 years; range, 4 months–14 years) who showed complete serological (HBsAg, HBeAg, anti-HBs*, anti-HBc*, anti–HB core antigen (anti-HBc)) and biochemical (normalization of alanine aminotransferase and bilirubin levels) recovery from acute HB at 6–18 months after the onset of jaundice.

Assays. To exclude possible deficiencies of total serum immunoglobulins, IgG, IgA, and IgM were determined by nephelometry (Array 360 System; Beckman Instruments, Brea, CA). IgG subclasses were measured by ELISA according to a method described elsewhere [8].

The quantitative determination of total anti-HBs was performed by microparticle enzyme immunoassay with IMx AUSAB kits (Abbott Laboratories). The geometric means of antibody titers (GMTs) were calculated for each set of serum samples. IgG anti-HBs subclasses and IgM anti-HBs were determined by an ELISA standardized according to the general principles of solid-phase immunoassay; the detailed protocol has been described elsewhere [9]. Levels of IgM anti-HBs were measured in those serum samples in which IgG anti-HBs was not detected.

Statistical analysis. Comparisons between groups were performed with the Kolmogorov-Smirnov nonparametric test. The differences in the frequency of specific subclasses were analyzed by the χ² test, and individual results were compared by nonparametric sign tests for dependent samples. GMT values were accompanied by 95% confidence intervals (CIs). A level of P < .05 was considered significant.

Results

In all children studied, the concentration of total serum immunoglobulins and IgG subclasses were within the normal range for their ages [8].

Total anti-HBs titers. Six months after IFN-α therapy, 18 (94.7%) of 19 children we studied had detectable levels of anti-HBs in the IgG class, with a GMT of 121 IU/L (95% CI, 52–279 IU/L), and their titers generally rose during the next 6 months to a GMT of 173 IU/L (95% CI, 36–829 IU/L) at 12 months (P = .05). Nevertheless, both mean levels of anti-HBs were ~2.1–1.5-fold lower than those found in convalescent serum samples after acute HB (GMT, 258 IU/L; 95% CI, 132–505 IU/L; figure 1A, 1B). One child, who tested positive only for IgM anti-HBs at 6 months, later tested positive for IgG at 12 months, whereas 2 patients with detectable IgG anti-HBs at 6 months (14 and 211 IU/L of total anti-HBs, respectively) tested negative for IgG anti-HBs 6 months later. Moreover, 1 child, an 8-year-old girl in whom the total level of anti-HBs was 350 IU/L at 6 months, again tested positive for HBsAg at the end of the study.

IgG subclass profiles of anti-HBs. In the group of children who recovered from acute HB, the response to HBsAg showed high selectivity, engaging mainly 2 IgG subclasses; IgG1 was present in 96.7% of serum samples and contributed, on average, 63% of the total IgG response, and IgG3 was present in 33.3% of serum samples and contributed 24.3% to the IgG response. Together, both isotypes contributed, on average, 87.3% of the total IgG response and were present simultaneously in 30% of serum samples. The frequency and percentage contributions of specific IgG2 (6.7% and 3.4%, respectively) and IgG4 (16.7% and 9.3%, respectively) to the total IgG anti-HBs response were low (figure 2A).

In contrast, the analysis of anti-HBs response at 6 months in children treated with IFN-α showed a high variability in the composition of individual profiles. The predominant subclass was IgG1 (88% of positive serum samples), but its percentage contribution to the total IgG response was, on average, much lower than that in convalescent serum samples after acute HB.
and was similar to those who seroconverted spontaneously and after interferon-α (IFN-α) therapy. (35% vs. 63%, respectively; \( P < .05 \)) and was similar to those for IgG4 (28%) and IgG3 (27%), followed by IgG2 (10%; figure 2B). Specific IgG2 was positive in 24%, IgG3 in 53%, and IgG4 in 47% of serum samples. In 23% of patients, anti-HBs was found in all IgG subclasses, and in the remaining patients specific profiles showed different compositions (IgG1 + IgG3, IgG1 + IgG2 + IgG3, IgG1 + IgG4, etc.).

One year after therapy, the activity of IgG1, IgG2, and IgG4 had increased in comparison with values found at 6 months, but the differences were not statistically significant. The percentage contribution and frequency of particular IgG subclasses in the response to HBsAg did not change significantly with time, except for IgG4, which rose from 47% to 63% of positive serum samples and, in 50% of these, became the most represented isotype, contributing ~45%–77% of the total IgG anti-HBs response at the end of the follow-up. There were statistically significant differences in the percentage contribution of specific IgG1 (\( P < .05 \)), IgG2 (\( P < .01 \)), and IgG4 (\( P < .05 \)) in the total IgG anti-HBs response when compared with natural seroconversion. There were no significant relations among any kind of specific subclass composition and the age or sex of children studied.

**Discussion**

Our study of anti-HBs antibody distribution among IgG subclasses in 19 children with chronic active HB showed that complete seroconversion to IgG anti-HBs, induced by IFN-α, was not a sustained phenomenon in ~15.8% of them. These observations confirm recent findings that seroconversion to anti-HBs is not associated invariably with sustained clearance of HB virus [10].

Many studies have provided evidence that, in addition to multiple types of effector cells, antibodies to HBsAg of IgG isotype with neutralizing activity are required for complete viral clearance from the host [4]. It also has been shown by us and by others [11] that synthesis of anti-HBs in convalescent serum samples is highly restricted to IgG1 and IgG3. In fact, both isotypes are responsible for several important biological activities, such as cooperation with monocytes or macrophages, complement-mediated lysis, opsonization and phagocytosis, and antibody-dependent cellular cytotoxicity [7]. Moreover, their presence is associated strongly with Th1 cytokines interleukin (IL)–2, IFN-γ, and tumor necrosis factor–β, which are involved in cell-mediated defense against intracellular viral particles and in promotion of memory IgG responses [12]. Therefore, activation of Th1 response and induction of specific antibodies in the IgG1 or IgG3 subclasses is most important for effective virus neutralization [13]. Thus, we wished to discover if the use of rIFN-α for the treatment of children with chronic HB infection stimulated the same profile of specific antibodies among IgG subclasses as the virus does alone.

In contrast to spontaneous seroconversion, the distribution of anti-HBs among those IgG subclasses induced by IFN-α differed significantly. There was no characteristic selectivity of isotypes involved in the response, and all subclasses, with a wide spectrum of individual diversity in their composition, were found. In most of the children, specific IgG1 or IgG3 antibodies did not exceed 50% of total IgG response, which suggests the presence of a weak immune response to the virus. The most unexpected finding was the high frequency and percentage contribution to the response by specific IgG4. Antibodies of this subclass do not activate the complement system, have a low affinity to Fcγ receptors, and are dominant components of circulating immune complexes present in patients with chronic HB infection [14]. Moreover, IgG4 is up-regulated by the Th2 cell cytokine IL-4, which in turn inhibits IFN-γ synthesis. Thus, a high concentration of antibodies with low biological activity, which may compete with more effective antibodies for the same or neighboring domains and is the effect of strong Th2 activation, seems to be an undesirable phenomenon during antiviral therapy. In fact, we found a high level of activity of IgG4 anti-HBs in 3 children who lost antibodies of the IgG isotype 1 year after treatment.

Many factors may influence the development of anti-HBs in IgG subclasses, the most important being age and current status of the immune system [15]. Naturally age-delayed maturity of IgG2 and IgG4 subclass synthesis observed in children <5 years of age might promote stimulation of a more selective IgG1 or IgG3 response, as we found in a study of vaccinated children [9]. However, this was not the case in the patients in this study, because the same diversity of IgG subclass composition was found in children both younger than (13 patients) and older than (6 patients) 5 years of age. Thus, it may be assumed that the wide diversity of specific IgG subclass profiles and the high participation of biologically ineffective IgG4 anti-HBs is attributable to the unselective modulatory effect of rIFN-α.
These observations prompted us to determine whether the presence of circulating anti-HBs was associated with complete clearance of HB virus. In fact, by using the PCR amplification assay, we found HB virus DNA in 35.7% of serum samples studied to date, despite the presence of anti-HBs, but with a low activity of specific IgG1 and IgG3 (data not shown).

In conclusion, our results showed a weak and nonselective modulatory effect of rIFN-α on the cellular immune response in children with chronic HB. It may be postulated that the evaluation of specific profiles of IgG subclasses may be a simple predictive marker for stability of IFN-α therapy, as well as being useful in the development of more-effective therapeutic strategies.

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References