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Characterization of sequence variations in immunodominant regions of the HBV-nucleocapsid protein as a prerequisite for the development of an epitope-based vaccine

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Abstract

Background/aims: In hepatitis B virus infection, viral elimination is dependent on an efficient antiviral T cell response which is not detectable in chronic hepatitis B. Therefore, new therapeutic concepts focus on T cell activation, such as epitope-based T cell-targeted vaccines. However, with the development of peptide-based vaccines in mind, viral mutations frequently described in hepatitis B within known immunodominant helper epitopes may have an influence on peptide selection.
Methods: Mutant peptides within immunodominant epitopes (aa 1–20, aa 91–105, and aa 143–157) at position 12, 14, 93, 97, 147, 151, 153, and 155 were tested with peripheral blood mononuclear and specific clone cells for their ability to induce proliferation, produce cytokines, induce T cell receptor down-regulation or antagonize wild-type activity of the hepatitis B core antigen-specific CD4+ T cell clones.
Results: Five variants could not induce T cell proliferation or cytokine production when the variants were presented alone. Coincubation with wild-type epitopes leads to T cell activation showing that the variants do not act as T cell receptor antagonists for hepatitis B virus-specific CD4+ T cells. In contrast, five other variants and wild-type peptides stimulated CD4+ T cell proliferation and production of Th1 cytokines.
Conclusions: Our data demonstrate that frequently occurring mutations within immunodominant epitopes have rather a nonstimulatory than a strengthening effect and thus should not included in a vaccine.

Keywords: Hepatitis B; T cells; Escape mutations; HBcAg

1. Introduction

In recent years, researchers have focused on developing therapeutic vaccines that stimulate the cellular arm of the immune system, particularly CD4+ and CD8+ T cell activity against hepatitis B virus (HBV) infected cells. Several studies have emphasized the association between self-limiting acute hepatitis and multi-specific cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) responses. In contrast, chronic hepatitis is characterized by a weak or absent T cell response [1,2]. These data suggest that a vaccine capable of inducing HBV-specific CTL and HTL responses, similar in quality and magnitude to those observed in acute hepatitis, could be a safe and effective treatment.

Epitope-based vaccines represent a therapeutic approach which is based on the observation that, at least in some

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instances, the immunity evoked by natural infection (i.e. chronic disease) is far from optimal and should be improved upon. Accordingly, rational choices are made to isolate the components desired for the response. Related and crucial to these efforts are the identification of the appropriate T cell epitopes. The potential advantages of this epitope-based approach include increased safety, the opportunity to rationally engineer epitopes for increased potency and breadth, and the ability to focus immune responses on conserved epitopes. Naturally occurring immune responses do not recognize all possible epitopes, but are instead commonly focused on relatively few epitopes. For hepatitis B infection, immunodominant epitopes have been identified and an epitope-based vaccine has been developed. This peptide-based vaccine composed of a human leucocyte antigen (HLA) A2-restricted, HBV-derived epitope covalently linked to a universal HTL epitope and palmitoylated at the *N*-terminus has been developed [3]. Vaccination of normal, uninfected volunteers with this construct showed that the vaccine is highly immunogenic in humans and the magnitude of CTL responses induced was comparable to CTL responses associated with clearance of acute viral infection. A strong correlation between the induction of CTL and HTL responses was also apparent, demonstrating that HTL responses are crucial for the development or maintenance of CTL responses. Subsequent clinical studies demonstrated that while individuals chronically infected with HBV were overall hyporesponsive to immunization, nonetheless the vaccine was capable of inducing HBV-specific CTL responses in a dose-dependent fashion. Analysis of the helper T lymphocyte response in this setting revealed altered cytokine profiles (decreased γ-interferon (IFN) and Interleukin (IL)-12 secretion in favour of increased production of the Th0/Th2-associated lymphokine IL-5) and decreased responses to tetanus toxoid correlated with hyporesponsiveness to theradigm vaccination [4]. These results have implications regarding the further development of immunotherapy for chronic HBV infection and lead to the conclusion that novel vaccine approaches should also include efficient activation of CD4+ T lymphocytes. Among different strategies, i.e. combination therapy with antiviral compounds, such as lamivudine, the inclusion of well characterized HTL epitopes derived from viral proteins has been suggested. In fact, numerous epitopes within the nucleocapsid protein of HBV proved to be highly immunogenic [5] and were suggested as vaccine components. To what extent naturally occurring mutations within this region may strengthen or weaken the desired immune response and should thus be included or not included in such a vaccine is unclear. As a prerequisite for the design of new vaccines, it is necessary to examine the effect of every single mutation on T cell activation, since it may have inhibiting or enhancing consequences on the immune response. We therefore investigated in the present study the influence of frequently described naturally occurring mutations within well characterized T cell epitopes [6–9] on the quality of immune response with respect to their inclusion into a vaccine. The mutations (at positions 12, 14, 93, 97, 147, 151, 153, and 155) have been functionally characterized with wild-type-specific T cell clones and peripheral blood mononuclear cell (PBMC).

The data reported here provide evidence that these frequently described HBV variants within the nucleocapsid protein rather create nonstimulatory peptides than an enhanced immune response.

2. Material and methods

2.1. Patients

CD4+ T cell clones generated from two patients with acute hepatitis B infection and peripheral blood mononuclear cells from another patient with acute hepatitis B were studied. In all patients, direct sequencing of the virus revealed the wild-type sequence of immunodominant epitopes within hepatitis B core antigen (HbcAg) (residues aa 1–20, aa 91–105, and aa 143–157). Sequencing was performed as described elsewhere [9].

2.2. Antigens

Peptide synthesis of wild-type peptides (wt) and mutant peptides (mut) according to the virus genome have been synthesized by Chiron mimotopes, Abimed, Germany. The wild-type of known immunodominant epitopes within HBcAg (residues aa 1–20, aa 91–105, and aa 143–157) was used along with 11 variants of this wt which occur naturally in chronic hepatitis B: Wt aa 1–20, mut 12 P (mutation at position 12 with proline), mut 14 Q, wt aa 91–105, mut 97 I, mut 93 L, mut 93 T, mut 93 V, wt 143–157, mut 147 C, mut 151 C, mut 153 C, and mut 155 T. All peptides were purified to more than 95% by high-pressure liquid chromatography.

Recombinant HBcAg (rHBc) was purchased by Biogen (Geneva, Switzerland).

2.3. Generation of T cell clones

PBMC were isolated on Ficoll-Isopaque gradients (Pharmacia, Upsala, Sweden). The interphase cells were suspended in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 2% L-glutamine, 1% penicillin streptomycin, and 10% human AB serum.

Two million PBMC were stimulated with 3 mg of recombinant hepatitis B core (rHBc) per milliliter in 96 U bottom plates (Costar, Cambridge, Mass). On day 6, recombinant interleukin-2 (IL-2) was added to a final concentration of 15 U/ml (kindly provided by Boehringer, Mannheim, Germany). On day 10, cells were counted and plated at 75, 150, and 225 cells per well on a 96-well plate. A well containing close to 150 cells was chosen, and the cells