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Epitope Specificity of Th_0/Th_2 $CD4^+$ T-Lymphocyte Clones Induced by Vaccination With rHBsAg Vaccine

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Background & Aims: Different amino acid sequences of hepatitis B virus surface antigen (HBsAg) are involved in the activation of $CD4^+$ lymphocytes needed to induce an optimal antiviral function. The aim of this study was to characterize the CD4-mediated response to immunodominant HBsAg epitopes in hepatitis B virus (HBV) vaccine recipients by defining minimal sequences recognized by T cells, cytokine profiles, and HLA restriction of peptide recognition. Methods: T-lymphocyte lines and clones specific for HBsAg were isolated from the peripheral blood of subjects immunized with recombinant HBsAg and stimulated in vitro with synthetic peptides spanning the whole HBsAg sequence. Results: Four immunodominant epitopes (sequences 21–40, 136–155, 156–175, and 211–226) were identified. Using panels of truncated peptides of different length, sequences 21–28, 165–172, and 215–223 were shown to correspond to the minimal epitopes recognized by T cells. The antigen-specific T-lymphocyte proliferation was HLA class II restricted, and each peptide could be presented in association with different HLA class II determinants. Th_0/Th_2 cytokine patterns were induced on peptide stimulation. Conclusions: These results indicate the presence of at least four immunodominant epitopes within HBsAg that represent potential candidates for the design of anti-HBV synthetic vaccines.

Infection by the hepatitis B virus (HBV) stimulates cellular and humoral immune responses against HBV antigens that are responsible for the clearance of the virus and the lysis of infected hepatocytes.[1–4] An efficient immune response against HBV requires the cooperation between accessory cells, T and B lymphocytes, to induce the helper activity of $CD4^+$ cells needed for an optimal antiviral function.

Using hepatitis B envelope (HBenv) proteins purified from plasma of chronic hepatitis B virus surface antigen (HBsAg) carriers[5] and HBsAg produced by recombinant DNA technology (rHBsAg),[6,7] a very efficient anti-HBV vaccine was produced to prevent infection. The licensed yeast-derived vaccine is composed of a nonglycosylated recombinant protein of 226 amino acids corresponding to the complete HBsAg sequence.

Neutralizing antibodies are normally directed against the "a" determinant, which represents the conserved sequence among different HBV strains (HBsAg 124–137 sequence).[8,9] However, the exact role played in protection against HBV by the humoral and cell-mediated immunity to HBsAg induced by the recombinant vaccine remains only partially defined. Antibodies stimulated by the rHBsAg vaccine do not appear to block the binding of HBenv to liver cells.[10,11]

Furthermore, passive prophylaxis with anti-HBsAg antibodies in chimpanzees failed to protect the animals from HBV infection,[12,13] whereas active postexposure prophylaxis with HBsAg vaccine was protective.[14] These data suggest that the cell-mediated immunity against HBsAg should play an important role in preventing infection in HBsAg vaccine recipients.

Several HBV epitopes are involved in antibody and cell-mediated immune responses after acute infection and in vaccine recipients. Immunodominant B-cell epitopes of the HBsAg are located within the HBsAg 110–168 sequence.[15]

In patients with acute and chronic HBV infection, several epitopes recognized by $CD8^+$, HLA class I–restricted cytotoxic T lymphocytes have been identified, and some of them appear to be widely recognized in the context of the appropriate HLA haplotype, such as the sequences 18–27 and 141–151 of the hepatitis B virus

Abbreviations used in this paper: APC, antigen-presenting cell; EBV-B, Epstein–Barr virus transformed B-cell lines; FCS, fetal calf serum; HBenv, hepatitis B envelope; IFN, interferon; IL, interleukin; MAb, monoclonal antibody; mer, minimal essential residue; PBMC, peripheral blood mononuclear cell; rHBsAg, recombinant S antigen of hepatitis B virus; rIL, recombinant interleukin; SI, stimulation indices.

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core antigen,[16,17] the sequence 161–169 of HBsAg,[4] and several sequences of the HBV polymerase.[18]

$CD4^+$ lymphocytes recognize different portions of the HBenv protein. In vaccine recipients and patients with chronic hepatitis B, only one immunodominant T-cell epitope has been defined and mapped near the N terminus of HBsAg within the 19–28[19] or 19–33[20] sequences. Therefore, additional studies are needed to identify other HBsAg sequences containing important T-cell epitopes. The role of $CD4^+$ T lymphocytes in antiviral protection is of particular interest because these cells can be distinguished in subsets (Th_0, Th_1, Th_2) based on the cytokine profile synthesized after antigen stimulation.[21,22] Different HBV-specific $CD4^+$ subsets can be generated after vaccination against HBV (Th_0/Th_2 cells producing interleukin [IL]-4 and IL-5) or during chronic HBV infection (Th_1 producing interferon [IFN]-γ).[20]

Therefore, the identification of the HBV sequences responsible for the activation of $CD4^+$ helper T cells is important not only for the design of new synthetic vaccines able to prevent HBV infection but also to further characterize the mechanisms responsible for liver damage and viral clearance.

In this study, we used synthetic peptides covering the HBsAg sequence to examine the fine specificity of T-cell clones derived from subjects immunized with rHBsAg. We identified four immunodominant sequences able to stimulate the proliferation of $CD4^+$ T lymphocytes. These epitopes are located in different portions of HBsAg and can activate Th_0/Th_2 responses.

Materials and Methods

Subjects

Eleven subjects (21–42 years of age; 4 men and 7 women) belonging to laboratory staff were immunized with recombinant HBV vaccine, subtype adw2 (Engerix-B, SmithKline Beecham, Rixensart, Belgium), at a dose of 20 μg in the deltoid region at 0, 1, and 6 months. All subjects had a negative history for liver diseases, had normal biochemical test results, were negative for HBV, hepatitis C virus, and human immunodeficiency virus markers, were not taking drugs known to influence the immune response, and had high serum levels of anti-HBsAg antibodies after vaccination (geometric mean of specific antibodies, 6,288 mIU/mL; range, 1050–26,500). All subjects gave their consent to enter the study; the study protocol was approved by the Review Committee of the University of Bologna.

Reagents

Human serum was pooled from donations screened for HBV, hepatitis C virus, and human immunodeficiency virus markers. Fetal calf serum (FCS) was purchased from Biological Industries (Kibbutz Beit Haemek, Israel). Recombinant interleukin (rIL)-2 was provided by Dr. S. Ferrone (New York Medical College, Valhalla, NY) or purchased from Glaxo (Geneva, Switzerland). Phytohemagglutinin and phorbol myristate acetate were purchased from Sigma Chemical Co. (St. Louis, MO). Purified tetanus toxoid was provided by Dr. U. Avio (Biocine Sclavo SpA, Siena, Italy). [^{3}H]thymidine (sp act, 925 GBq/mmol) was purchased from Amersham International (Little Chalfont, England). Anti-CD3 monoclonal antibody (MAb) was a supernatant from cloned hybridoma cells CRL 8001 (American Type Culture Collection [ATCC], Rockville, MD). Fluorescein isothiocyanate– and phycoerythrin-conjugated anti-CD4, anti-CD8, anti-CD25 MAb, a similarly labeled control antibody and goat anti-mouse polyclonal antibody, were purchased from Becton Dickinson (Mountain View, CA). Anti-human CD30, anti-CD45RA, and anti-CD45RO MAb were purchased from Dako (Glostrup, Denmark). Murine anti-HLA MAb, sterile and without preservative for cell cultures, were anti-class I (A, B, C) (immunoglobulin [Ig] G2a from cloned hybridoma cells B9.12.1) and anti–class II DP (IgG2a from cloned hybridoma cells B7/21.2) determinants, provided by Prof. R. Accolla (Istituto di Immunologia e Malattie Infettive, University of Verona, Italy); anti–class II DR and DQ MAb were purchased from Immunothech (Marseille, France). Anti–carcinoembryonic antigen MAb was a supernatant from cloned hybridoma cells CRL 8019 from ATTC. An IFN-γ–IRMA kit (sensitivity, 0–90 IU/mL; Medgenix Diagnostic, Fleurus, Belgium) was used for the quantitation of IFN-γ; enzyme-linked immunosorbant assay kits were used for IL-2 (sensitivity, 0.1–5 ng/mL; Biokine, T Cell Diagnostics, Cambridge, MA), IL-4 (sensitivity, 0.045–3 ng/mL; Intertest-4, Genzyme, Cambridge, MA), and IL-5 (sensitivity, 0.008–5 ng/mL; Quantikine, R&D Systems, Minneapolis, MN). Gene Amp Perkin Elmer PCR kit Components for RNA reverse-transcription and complementary DNA (cDNA) amplification was purchased from Perkin Elmer (Norwalk, CT). One hundred twenty-three–base pair DNA ladder was purchased from Gibco BRL (Gaithersburg, MD). Epstein–Barr virus transformed B-cell lines (EBV-B) were purchased from ATCC or from Istituto Nazionale per la Ricerca sul Cancro (Genoa, Italy).

HBsAg and Synthetic Peptides

A recombinant yeast-derived preparation of HBsAg of subtype adw2, the same used for the vaccination, was free of preservatives and was provided by Dr. P. Voet (SmithKline Beecham).

A panel of linear 15–26 minimal essential residue (mer) peptides representing the HBsAg sequences (1–80 and 95–226) were purchased from Multiple Peptide Systems (San Diego, CA) or Biochem Immunosystems (Montreal, Canada). Synthetic peptides of 20 to 10 residues covering the 11–40, 151–185, and 207–226 sequences of HBsAg were purchased from Chiron Mimotopes (Clayton, Australia). All peptides were synthesized in accordance with the Fmoc protocol.[23] Ly-

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