Antibodies to the Hepatitis B e Antigen (HBeAg) Can Be Induced in HBeAg-Transgenic Mice by Adoptive Transfer of a Specific T-Helper 2 Cell Clone

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Production of antibody to hepatitis B e antigen (HBeAg); i.e., anti-HBe antibody, in HBeAg-transgenic mice is believed to be mediated by T-helper 2 (Th2) cells. Injection of an HBeAg-specific Th2 clone into HBeAg-transgenic H-2k mice induced anti-HBe antibody production, confirming the function of Th2 cells in this model system.

Recent studies with a mouse model of the hepatitis B e (HBe) antigen (HBeAg) expressed as a transgene have indicated that the degree of immunological tolerance to the HBeAg transgene is dependent on the major histocompatibility complex and suggest that mainly T-helper 2 (Th2)-like T cells preferentially evade tolerance induction (6, 7, 9, 10). An endogenous anti-HBe antibody response in H-2k HBeAg-transgenic mice can be induced by immunization with linearized monomeric HBeAg or a synthetic Th-cell site, residues 129 to 140. The response is characterized by production within 2 to 3 weeks of an anti-HBe autoantibody consisting exclusively of the immunoglobulin G1 (IgG1) isotype and a hepatitis core antigen (HBCAg)- or HBeAg-specific Th-cell population producing predominantly Th2-like cytokines on in vitro recall (10). It has been proposed that an imbalance between HBeAg-specific Th-cell subsets may be present in humans with chronic hepatitis B virus infections (4, 5) and that the CD4+ T-cell responses to HBcAg and HBeAg play a pivotal role in clearing the infection (1, 3). We were therefore interested in the functional characterization of HBeAg-specific Th2-like cells.

HBeAg-transgenic mice of the H-2k haplotype were obtained by repetitive backcrossing of B10 (H-2b) HBeAg-transgenic mice (7) with C3H mice (H-2k). C3H and CBA (H-2k) mice were purchased from Harlan-Sprague Dawley or B&K Universal, Sollentuna, Sweden.

Mice were immunized with particulate recombinant HBcAg (rHBcAg), rHBeAg, or linearized, alkylated, monomeric HBcAg containing residues 1 to 150 (P16; 15). To study the induction of anti-HBe antibodies in transgenic mice, blood samples were collected on a weekly basis following immunization or adoptive transfer.

All enzyme immunoassays (EIAs) for detection of murine anti-HBe antibodies were performed essentially as described previously (9, 12, 13). In brief, HBeAg was used at 10 μg/ml in 50 mM sodium carbonate buffer, pH 9.6, to coat 96-well microtiter plates by incubation overnight at 4°C. Serial dilutions of sera were then incubated on the plates for 60 min. Bound murine serum antibodies were indicated by either rabbit anti-mouse IgG (Sigma Chemical Co., St. Louis, Mo.) or rabbit anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Sigma), followed by a peroxidase-labelled goat anti-rabbit IgG.

To study the Th-cell phenotype in vitro, groups of four mice were injected subcutaneously with 10 μg of HBcAg emulsified in Freund's complete adjuvant (CFA) in the hind foot pads or at the base of the tail. Nine to 11 days later, the mice were sacrificed and the spleens and draining lymph nodes (LNs) were harvested. Single-cell suspensions were prepared in Click's medium and plated in microplates at 6 × 10^5 cells per well for proliferation assays and at 8 × 10^6 cells per well for cytokine assays (9, 14). Supernatants were removed after 24 h for determination of interleukin 2 (IL-2), and after 48 h for determination of IL-4, IL-6, and gamma interferon (IFN-γ). The plates for T-cell proliferation assay were incubated for 96 h with the addition of 1 μCi of [3H]thymidine ([3H]TdR; Amer sham) for the final 16 h. The labelled cells were harvested onto cellulose filters, and the level of [3H]TdR incorporation was determined by a liquid scintillation β counter.

The presence of cytokines was determined by previously described (9, 14). In brief, the presence of IL-2 in supernatants was determined by proliferation of the IL-2-sensitive NK′/A cell line. The presence of IL-4 was determined by proliferation of the IL-4-sensitive CT.4S cell line, and the presence of IFN-γ was determined by a sandwich EIA (Pharmingen, San Diego, Calif., or Endogen, San Diego, Calif.). IL-2, IL-4, and IL-6 were also determined by sandwich EIA (Endogen).

To characterize the phenotype of H-2k-restricted HBeAg-specific Th cells, groups of three to five C3H or CBA mice were primed with 10 μg of rHBCAg/CFA and 10 days later the draining LNs were harvested. In vitro recall of the in vivo-primed HBeAg-specific Th cells revealed the presence of a mixture of Th1 and Th2 cells since IL-2, IL-6, and IFN-γ were routinely detected (Fig. 1). Thus, polyclonal H-2k-restricted, HBeAg-specific LN responses consist of both Th1 and Th2 cells when rHBcAg is used as the immunogen.

An HBeAg-specific Th2 clone was produced from C3H (H-2k) mice. Six mice were immunized by injection of the foot pads with 10 μg of rHBcAg/CFA and 10 days later the popliteal LNs were harvested and a single-cell suspension was prepared. Bulk cultures of 10^7 LN cells were cultured for 10 days with 10 μg of rHBeAg per ml. The cells were then pooled and cloned at a density of 0.1 cell per well with irradiated (1,000 rads) syngeneic spleen cells as antigen-presenting cells (APCs). The cultures were maintained by addition of IL-2 (10 U/ml)

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and rHBcAg (10 μg/ml) on a weekly basis. Growing clones were expanded by standard techniques. Five HBc/eAg-specific clones were obtained, one of which was stable for 2 months (clone CNT7). The cytokine phenotype of the CNT7 clone was characterized by using irradiated syngeneic APCs. The CNT7 clone produced high levels of IL-4 and little or no IL-2 (data not shown) or IFN-γ (Fig. 2). This is consistent with a Th2 phenotype (11). HBeAg-transgenic mice of the H-2k haplotype do not spontaneously produce anti-HBe antibodies, indicating a certain degree of tolerance or anergy toward the transgene product. Anti-HBe antibody production could be induced by immunization with P16, indicating a low degree of transgene

5x10⁴ CNT7 cells and APC 10⁶ per well

FIG. 2. Proliferative capacity (a) and cytokine phenotype (b and c) of H-2k-restricted, HBc/eAg-specific Th-cell clone CNT7. A total of 5 x 10⁴ CNT7 cells were added to wells containing 10⁶ irradiated (1,000 rads) syngeneic APCs and cultured for 48 h. Values are given as described in the legend to Fig. 1.
tolerance (Fig. 3). P16 does not induce anti-HBe antibodies in wild-type mice (9, 10). Thus, endogenous HBcAg-specific Th cells are primed by exogenous P16 whereas antibody production is driven by endogenous HBeAg (8).

To analyze the ability of the HBc/eAg-specific Th2 clone to induce anti-HBe antibody production in HBcAg-transgenic mice, additional experiments were performed. The CNT7 clone was expanded and adoptively transferred intraperitoneally (i.p.) into HBcAg-transgenic H-2k mice, and anti-HBe antibody production was monitored. All mice produced anti-HBe within 1 to 2 weeks of transfer (Fig. 3). This provides the functional evidence that HBc/eAg-specific Th2 cells are key mediators of anti-HBe serum conversion in transgenic mice.

A series of studies have characterized the murine recognition of HBc/eAg in both wild-type and HBcAg-transgenic mice. Several factors influence the immunological response to HBc/eAg. First, the major histocompatibility complex of the immunized host can affect the Th subset phenotype of the activated T cells, as well as the degree of HBc/eAg-specific T-cell tolerance in the transgenic host. It has been suggested that HBc/eAg-specific Th2-like cells evade tolerance (10). However, the transgenic-specific Th2 phenotype seems to vary in different transgenic models (2). To further test whether HBc/eAg-specific Th2 cells are able to elicit anti-HBe antibody production in vivo, we produced an HBc/eAg-specific Th2-cell clone. This clone induced anti-HBe seroconversion in HBcAg-transgenic mice in the absence of an exogenous source of HBcAg- or HBcAg-specific B cells. Thus, HBc/eAg-specific Th2 cells are key mediators of anti-HBe antibody production in vivo. It has been proposed that in acute hepatitis B virus infection, a strong HBcAg- and/or HBcAg-specific Th1-cell response may be vital for viral clearance (1, 3, 4). One may hypothesize that HBc/eAg-specific Th2 cells, which did not cause elevations in murine alanine aminotransferase levels (data not shown), promote mainly humoral responses and are thus possibly less efficient at mediating viral infection.

REFERENCES


