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Immunology

Interleukin 12 suppresses autoantibody production by reversing helper T-cell phenotype in hepatitis B e antigen transgenic mice

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ABSTRACT **Helper T (Th) cells are classified as Th_1 or Th_2 cells by virtue of cytokine secretion and function as mediators of cellular or humoral immunity, respectively. Cytokines also regulate the differentiation of Th cells. For example, interleukin (IL)-12 promotes Th_1 and suppresses Th_2 cell development, suggesting that IL-12 may be useful therapeutically in Th_2-mediated autoimmune and allergic disorders. Therefore, the effect of systemic IL-12 treatment on *in vivo* autoantibody synthesis in hepatitis B e antigen (HBeAg)-expressing transgenic mice, which is dependent on self-reactive Th_2 cells, was examined. Low-dose IL-12 significantly inhibited autoantibody production by shifting the Th_2-mediated response toward Th_1 predominance. Additionally, previous studies suggest that a predominance of HBeAg-specific Th_2-type cells may contribute to chronicity in hepatitis B virus infection. Therefore, IL-12 may also prove beneficial in modulating the HBeAg-specific Th response to favor viral clearance in chronic hepatitis B virus infection.**

Distinct subsets of regulatory $CD4^+$ helper T (Th) cells designated as Th_1 and Th_2 cells have been defined (1). The Th subsets are largely defined by unique patterns of cytokine secretion such that Th_1 cells produce interleukin (IL)-2, interferon γ (IFN-γ), and tumor necrosis factor β (TNFβ), whereas Th_2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 (2). The differences in cytokine secretion account for the functional dichotomy between Th_1 cells, which mediate cellular immunity, and Th_2 cells, which enhance humoral immunity. In addition to mediating effector functions, cytokines appear to exert a dominant influence on the differentiation of T cells into the Th_1 or Th_2 subset. For example, IL-4 is essential for the differentiation of Th_2 cells and blocks development of Th_1 cells (3, 4), whereas IL-12 enhances Th_1 cell development and diminishes Th_2 cell induction largely through stimulation of IFN-γ production (5, 6). Furthermore, cytokines also influence the isotype of antibody produced. Human and murine IgE and murine IgG_1 responses are promoted by IL-4, and IFN-γ enhances murine IgG_{2a} responses (7).

The clinical significance of distinct Th_1 and Th_2 phenotypes first emerged from studies of parasitic infections. These studies revealed that specific pathogens may be more effectively controlled by a predominant Th_1- or Th_2-type immune response (8–11).

These previous studies suggest that, in addition to the treatment of parasitic infections, IL-12 may be useful therapeutically in antibody-mediated autoimmune disorders and allergies in which Th_2-like responses may dominant (12, 13). We utilized a transgenic (Tg) model of autoantibody production to examine the effects of IL-12 on an autoimmune process (14). In this model, hepatitis B e antigen (HBeAg)-expressing Tg mice on a specific major histocompatibility complex (MHC) background can be induced to produce autoantibody (anti-HBe) by the injection of a T-cell recognition site that fails to elicit T-cell tolerance in these mice. Hemizygous Tg31e mice secrete HBeAg into the circulation at a concentration of 10 ng/ml (15). Th-cell recognition of HBeAg is focused on residues 120–131 in $H\text{-}2^s$ mice and on residues 129–140 in $H\text{-}2^b$ mice. HBeAg-specific T-cell tolerance in Tg31e mice is epitope specific and MHC dependent. For example, the Th cells of B10.S-Tg31e mice ($H\text{-}2^s$) are tolerant to HBeAg, whereas in B10-Tg31e mice ($H\text{-}2^b$) at least a proportion of 129–140 specific Th cells have evaded tolerance-inducing mechanisms. These autoreactive Th cells are neither deleted nor anergized, because they can be activated *in vivo* by a single injection with 0.5–50 μg of the 129–140 peptide into B10-Tg31e mice (16). Such Th-cell activation results in the cognate interaction of 129–140-specific Th cells with HBeAg-specific B cells (which are not tolerant in this model) and high-titer IgG autoantibody (anti-HBe) production occurs [see Fig. 1*A*; control treated with phosphate-buffered saline (PBS)]. Autoantibody production is maximal 3 weeks after the 129–140 injection and slowly declines thereafter. Serum HBeAg levels decline in parallel with increased autoantibody production due to immune complex formation (see Fig. 1*B*; PBS control). The aim of this study was to examine the effects of rIL-12 treatment on the induction of this autoantibody response, on established autoantibody production, and on the phenotype of the Th cells regulating the autoimmune response.

MATERIALS AND METHODS

Tg Mice. The Tg mouse lineage designated B10.S-Tg31e, which expresses HBeAg (9–13 ng/ml) in the serum, was produced as described (15). The F_1-Tg31e and B10-Tg31e mice were produced by breeding to B10.S-Tg31e mice homozygous for the HBeAg transgene.

rHBeAg and Synthetic Peptides. An *Escherichia coli*-derived rHBeAg corresponding in sequence to serum-derived HBeAg encompassing the 10 precore amino acids remaining after cleavage of the precursor and residues 1–149 of hepatitis B core antigen (*ayw* subtype) was provided by Florian Schödel (Max Planck Institute for Biochemistry, Munich). The presence of the 10 precore amino acids prevents particle formation, and this rHBeAg preparation is recognized efficiently by HBeAg-specific monoclonal antibodies (mAbs) but displays little hepatitis B core antigenicity (17). Synthetic peptides derived from the HBeAg sequence representing residues 120–131 and 129–140 were synthesized by the Merrifield solid-phase method in the peptide laboratory of the R. W. Johnson Pharmaceutical Research Institute (La Jolla, CA) and were provided by G. B. Thornton.

Serology. HBeAg was measured in diluted Tg mouse sera by commercial ELISA (HBeEIA; Abbott) and rHBeAg was used as a standard. Anti-HBe IgG antibody was measured in murine sera by an indirect solid-phase ELISA using rHBeAg-coated wells as described (16). The IgG isotype was determined by

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Abbreviations: Th, helper T; IL, interleukin; IFN-γ, interferon γ; Tg, transgenic; HBeAg, hepatitis B e antigen; r-, recombinant; mAb, monoclonal antibody; PLN, popliteal lymph node; SN, culture supernatant.

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using IgG isotype-specific second antibodies in the ELISA. Anti-HBe-positive sera were confirmed in the commercial anti-HBe ELISA (Abbott). The data are expressed as antibody titer, representing the reciprocal of the highest dilution (log_4) of serum required to yield a reading 3 times that of preimmunization sera.

Adoptive Transfer of Autoantibody Production in HBeAg Tg Mice. Non-Tg donor mice were immunized in the hind footpads with 100 μg of the T-cell peptide sites 120–131 or 129–140 emulsified in complete Freund's adjuvant (CFA) or CFA alone, and draining popliteal lymph node (PLN) cells were harvested after 10 days and cultured with peptide 120–140 (0.2 μg/ml) or purified protein derivative (PPD) for an additional 3 days. *In vitro* activated PLN cells (9×10^6) were then transferred into sublethally irradiated (500 R) HBeAg-expressing Tg recipient mice (14). Sera were collected before and at various times after adoptive transfer and analyzed for HBeAg (autoantigen) and anti-HBe (autoantibody) by ELISA.

Cytokines and Cytokine Assays. Murine rIL-12 was provided by the Genetics Institute, Cambridge, MA. Synthetic IL-4 corresponding to amino acids 24–140 of murine IL-4 was generously provided by Ian Clark-Lewis (Biomedical Research Center, University of British Columbia, BC, Canada). To measure cytokines, antigen-specific culture supernatants (SNs) were harvested at 24 hr for IL-2 determination and at 48 hr for IL-4 and IFN-γ determinations. IL-2 was measured by the ability of SN to stimulate proliferation of the IL-2- and IL-4-sensitive NK-A cell line in the presence of mAb 11B11 specific for IL-4. IL-4 was measured by the ability of SN to stimulate proliferation of the IL-4-sensitive CT.4S cell line (generously provided by William Paul, National Institutes of Health, Bethesda). IFN-γ was measured by two-site ELISA using mAb HB170 and a polyclonal goat anti-mouse IFN-γ (Genzyme). To normalize cytokine measurements, results were expressed as the reciprocal of the minimal concentrations of Ag (1/min. Ag conc.) necessary to elicit measurable (3 times background) amounts of the various cytokines.

RESULTS

***In Vivo* Treatment with IL-12 Suppresses Autoantibody Production in B10-Tg31e Mice.** Early treatment [i.e., IL-12(1) group] of B10-Tg31e mice with rIL-12 (1 μg per day) on days 0–4 (peptide 129–140 was injected on day 0) resulted in significant inhibition of autoantibody production from week 2 through week 14 (Fig. 1*A*). The minimum rIL-12 required to significantly inhibit autoantibody production when given during this early time frame was a total dose of 2.0 μg (data not shown). Initiation of the rIL-12 treatment at a later time [days 8–12; IL-12(2) group] was less effective than early treatment but nevertheless inhibited autoantibody production by approximately 16-fold from week 2 through week 7 (Fig. 1*A*). However, autoantibody production was inhibited to the greatest degree (a 256-fold or greater reduction in IgG autoantibody titer relative to the control from week 2 through week 7) when 1 μg of rIL-12 per day was administered on days 0–4 and 8–12 [IL-12(1+2) group]. Note also that serum autoantigen levels were significantly and persistently reduced only in the untreated PBS control group (Fig. 1*B*). The apparent elevations in HBeAg concentrations observed in the IL-12 treatment groups may be due to aggregation of HBeAg by levels of autoantibody insufficient to neutralize the detection of HBeAg or may relate to the IgG isotype of the autoantibody, which differs among these groups (see below). These results indicate that systemic treatment with relatively low doses of rIL-12 (2.0–10 μg, total dose) can significantly inhibit autoantibody production *in vivo* and that the time the IL-12 is administered relative to the T-cell activation event is important.

Autoantibody IgG Isotype Is Modulated by IL-12 *in Vivo*. Because inhibition of autoantibody production was most likely

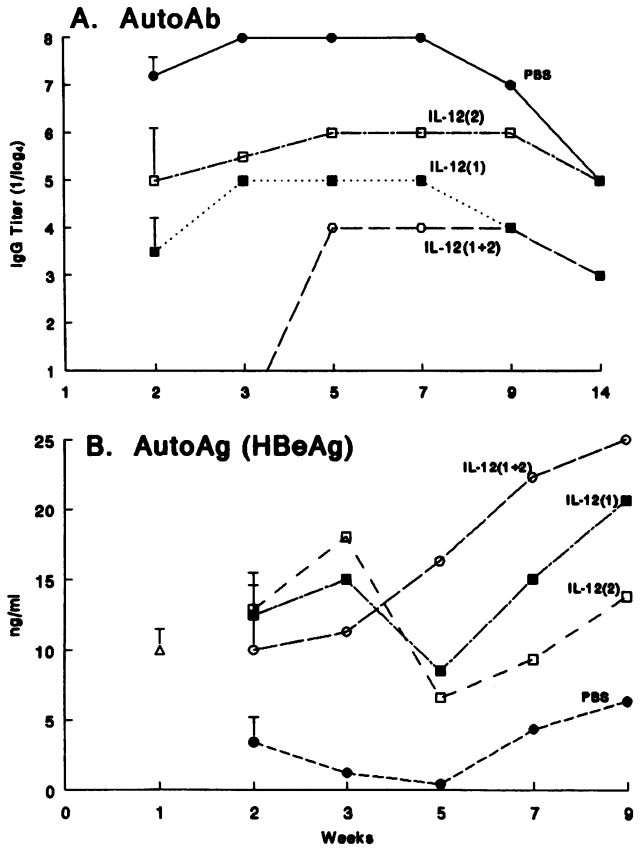

FIG. 1. *In vivo* autoantibody production and autoantigen (HBeAg) levels in IL-12-treated and control (PBS) B10-Tg31e mice. Groups of nine B10-Tg31e mice each were treated with murine rIL-12 (1 μg per day) on days 0–4 [IL-2(1)], days 8–12 [IL-12(2)], or days 0–4 and 8–12 [IL-12(1+2)] or were given PBS only, and injected with the HBeAg-derived T-cell peptide 129–140 (50 μg) on day 0. Sera were collected and analyzed from individual mice at week 2, and therefore sera were pooled and autoantibody (*A*) and autoantigen (*B*) levels were determined by ELISA. At 2 weeks, all IL-12 treatment groups demonstrated significantly reduced autoantibody production and elevated serum HBeAg levels as compared with the PBS control group ($P < 0.01$, Student's *t* test); thereafter, sera were pooled. The triangle represents the serum concentration of HBeAg in unmanipulated B10-Tg31e mice.

related to the ability of IL-12 to influence Th phenotype, we next examined the IgG isotype distribution of the autoantibodies. As shown in Fig. 2, in untreated B10-Tg31e mice (PBS control) the autoantibody response is composed predominantly of the IgG_1 isotype, no IgG_{2a} autoantibody was produced, and IgG_{2b} autoantibody was produced at low level early (week 3) but disappeared by week 7. In striking contrast, the predominant autoantibody IgG isotypes in IL-12(1)-treated mice were $IgG_{2a} \geq IgG_{2b}$ and the IgG_1 isotype was significantly reduced relative to the PBS control group. Although initiation of IL-12 treatment during week 2 [IL-12(2)] inhibited total autoantibody production, this delayed treatment regimen "permitted" all three IgG isotypes to be produced and the IgG_1 response remained superior. In the treatment group that received IL-12 during weeks 1 and 2, IgG_1 autoantibody was totally suppressed and only late-onset and low-titer IgG_{2a} and IgG_{2b} autoantibody was detected (Fig. 2). The autoantibody IgG isotype profile of IL-12(1+2)-treated mice represents a complete reversal of the isotype pattern of untreated mice with respect to IgG_1 and IgG_{2a}. Given the positive influence of IL-4 produced by Th_2 cells on IgG_1 secretion and the positive influence of IFN-γ produced by Th_1 and natural killer (NK) cells on IgG_{2a} secretion (7), the autoantibody isotype profiles strongly suggest that a predominate Th_2-mediated autoanti-

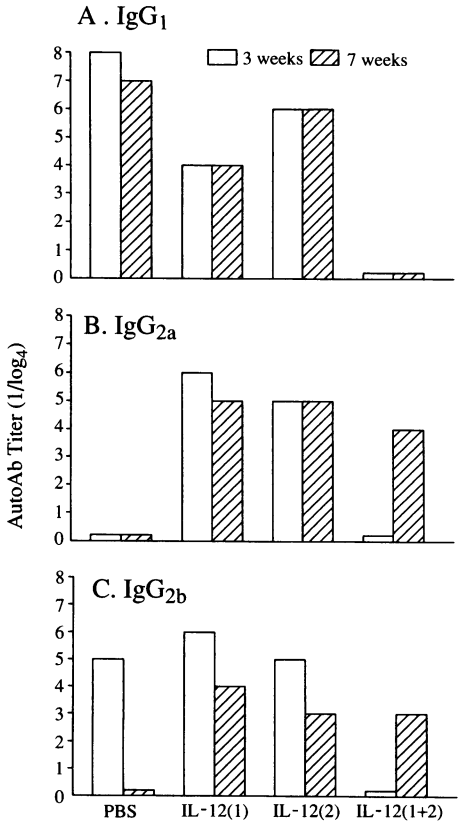

FIG. 2. Effect of *in vivo* rIL-12 treatment on autoantibody IgG isotype. Sera from the rIL-12-treated B10-Tg31e groups or PBS control B10-Tg31e mice described in Fig. 1 were collected at weeks 3 (open bars) and 7 (hatched bars) after injection of peptide 129–140. The IgG isotype distribution of the autoantibodies was determined by using IgG isotype-specific second antibodies in the ELISA. Autoantibody titer is expressed as a reciprocal of the highest dilution ($\log_4$) of serum required to yield an A_{492} reading 3 times that of preimmunization sera.

body response was shifted toward Th_1 predominance by *in vivo* IL-12 treatment.

***In Vivo* Treatment with IL-12 Modulates Autoantigen-Specific Cytokine Production.** To more directly examine the effect of IL-12 treatment on autoreactive Th cell phenotype, B10-Tg31e mice induced to produce autoantibody by injection with peptide 129–140 were either treated with IL-12 (1 μg per day) on days 0–4 or untreated (PBS only), and HBeAg and 129–140-specific Th cell cytokine production was measured (Table 1). The *in vivo* IL-12 treatment completely inhibited the 10-day autoantibody response. The HBeAg-specific Th cells derived from B10-Tg31e mice treated with IL-12 *in vivo* required a 125-fold higher *in vitro* concentration of HBeAg and a 25-fold higher concentration of peptide 129–140 to elicit IL-4 production as compared with control HBeAg-specific Th cells, whereas HBeAg-specific IL-2 production and IFN-γ production were approximately equal (Table 1). The lack of HBeAg-specific IFN-γ induction by IL-12 treatment suggests that modulation of the level and IgG isotype distribution of autoantibody by IL-12 may be due more to inhibition of IL-4 production than to up-regulation of IFN-γ production. The lack of HBeAg-specific IFN-γ induction by IL-12 in B10-Tg31e mice may reflect the fact that HBeAg and peptide 129–140 elicit predominantly Th_2-like responses in B10 mice (18); furthermore, preliminary evidence suggests that the HBeAg-specific Th cells which survive tolerance/deletion mechanisms in B10-Tg31e mice are primarily of the Th_2 phenotype (23).

***In Vivo* Treatment with IL-12 Can Suppress Established Autoantibody Production.** Because IL-12 treatment was most effective when given early with respect to autoreactive Th cell activation, it was of interest to determine if IL-12 treatment would affect established autoantibody production. Therefore, B10-Tg31e mice were injected with peptide 129–140 to elicit the autoantibody response, and, starting at week 3 (i.e., maximal autoantibody production), groups of Tg mice were either treated with IL-12 (1 μg every other day) for a 4-week period (total IL-12 dose of 14 μg) or given PBS as a control (Fig. 3). In the context of ongoing autoantibody production, IL-12 had minimal effects during the first 2 weeks of treatment (i.e., 4-fold reduction in autoantibody titer); however, after 4 weeks of treatment the IL-12 group demonstrated a 64-fold lower IgG autoantibody titer as compared with the control group. Two weeks after IL-12 administration was stopped a 16-fold difference in autoantibody titer was observed, and by week 14 the IL-12-treated and control groups demonstrated equivalent autoantibody levels (Fig. 3*A*). The serum HBeAg levels were inversely related to autoantibody production in both groups, and serum HBeAg concentrations were significantly higher in the IL-12 treatment group at weeks 7 and 9 (Fig. 3*B*). These results indicate that *in vivo* treatment with IL-12 can inhibit an established autoantibody response; however, the effects of IL-12 treatment are somewhat muted and delayed once autoantibody production has become established, and suggest that continuous IL-12 treatment may be required to suppress ongoing autoantibody production.

Table 1. Effect of IL-12 on antigen-specific cytokine production *in vivo*

Treatment (*in vivo*)	Autoantibody, titer	Antigen (*in vitro*)	Cytokine, 1/min. Ag conc. IL-2	IL-4	IFN-γ
PBS	1:5120	HBeAg	1.0	125	5.0
		129–140	1.0	1.0	0
IL-12	0	HBeAg	5.0	**1.0**	5.0
		129–140	0.2	**0.04**	0

Groups of three B10-Tg31e mice were treated either with five 1.0-μg doses of IL-12 or with PBS on days 0–4, and all were injected with peptide 129–140 (50 μg in incomplete Freund's adjuvant) on day 0. Spleens were harvested on day 10, and pooled spleen cells were cultured (8×10^6 per ml) with various concentrations of HBeAg (0.0003–5.0 μg/ml) or peptide 129–140 (0.003–50 μg/ml). Culture SNs were collected for IL-2, IL-4, and IFN-γ determination. The data are expressed as the reciprocal of the minimal antigen concentration (1/min. Ag conc.) required to elicit measurable (3 times background in the absence of antigen) amounts of each cytokine.

IL-12 Inhibits and IL-4 Enhances the Ability of Th Cells to Transfer Autoantibody Production. To confirm the effect of IL-12 treatment on Th phenotype and autoantibody production a second method was employed. Autoantibody production in HBeAg-expressing Tg mice can also be elicited by adoptive transfer of peptide-primed Th cells into sublethally irradiated Tg recipient mice (14). This method allows for the manipulation and analysis of different Th cell specificities. For example, we compared HBeAg peptide 120–131-primed Th cells (IA^s-restricted) with HBeAg peptide 129–140-primed Th cells ($I\text{-}A^b$- restricted) for the ability to transfer functional T-cell help for autoantibody production *in vivo*, and we examined the effects of the cytokines IL-2, IL-4, and IL-12 on these responses. In this analysis non-Tg (+/+) (B10 × B10.S)F_1 donor mice were immunized with either 120–131 or 129–140, and 10 days later draining PLN cells were harvested and cultured *in vitro* with the HBeAg-derived composite peptide antigen 120–140 for 3 days in the presence of IL-2, IL-4, IL-12, or no cytokine and then transferred (9×10^6 cells) into irradiated F_1-Tg31e (e/+) recipient mice, which were then analyzed for anti-HBe antibody production (Fig. 4). Because treatment with IL-2 and with no cytokine supplement yielded similar results, the control data are not shown. Although peptide

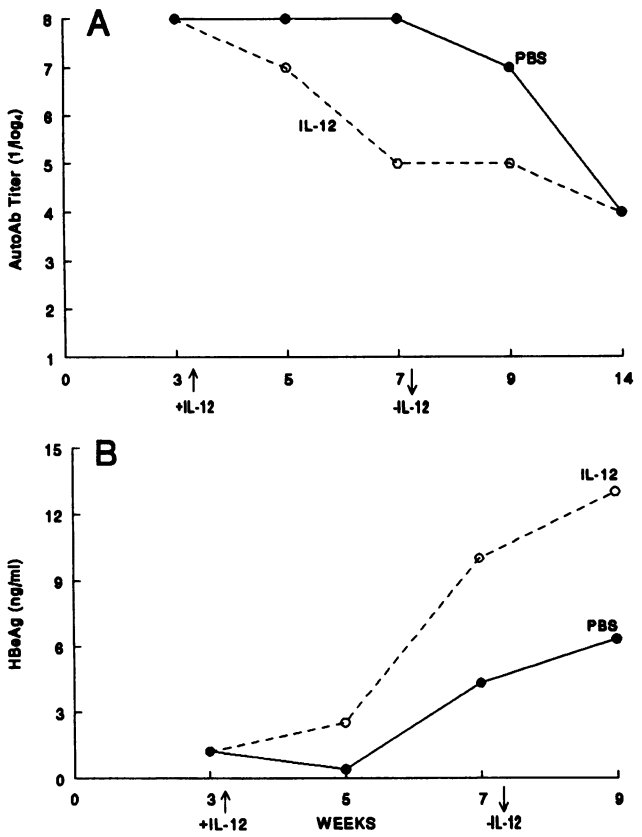

FIG. 3. Effect of *in vivo* rIL-12 treatment on established autoantibody production. Two groups of five B10-Tg31e mice were injected with peptide 129–140 (50 μg). Starting 3 weeks later, after autoantibody production was maximal, one group was treated every other day with rIL-12 (1 μg per dose) for a period of 4 weeks (total IL-12 dose of 14 μg) (○), whereas the other group was given PBS only (●). Sera were collected, pooled, and analyzed for levels of autoantibody (*A*) and autoantigen (*B*) by ELISA.

120–131 elicits strong HBeAg-specific T-cell proliferative responses and efficient IL-2 and IFN-γ production (18), it primes T-cell helper function for antibody production relatively poorly even in the presence of IL-2 during the culture phase (Fig. 4 *Upper*). In contrast, peptide 129–140 elicits weak HBeAg-specific T-cell proliferative responses and poor IL-2 and IFN-γ production (18), yet primes Th cell function very efficiently. Transfer of 129–140-primed Th cells into F_1-Tg31e recipient mice resulted in high titer autoantibody production which reduced serum HBeAg levels (Fig. 4 *Lower*). These results are consistent with classification of 120–131-specific T cells as Th_1-like and of 129–140-specific T cells as Th_2-like (18). Addition of IL-4 to the 3 day *in vitro* culture had no significant effect on 129–140-specific Th cells. This most likely is due to sufficient endogenous secretion of IL-4 by 129–140-specific Th cells. However, exogenous IL-4 corrected the "defect" in the ability of 120–131-specific Th cells to transfer T-cell help for autoantibody production. Reciprocally, *in vitro* addition of IL-12 to 120–131-primed T cells had relatively little effect on their ability to transfer Th cell function, whereas addition of IL-12 to 129–140-primed T cells *in vitro* significantly diminished their ability to transfer Th cell function *in vivo* (Fig. 4). It appears that the Th_1-like 120–131-specific response can be shifted towards a Th_2-like response by IL-4, and reciprocally the Th_2-like 129–140-specific response can be shifted towards a Th_1-like response by the addition of IL-12 to the *in vitro* stimulation culture. To confirm this possibility, IgG isotype analysis was performed on the autoantibodies produced as a result of these adoptive transfers.

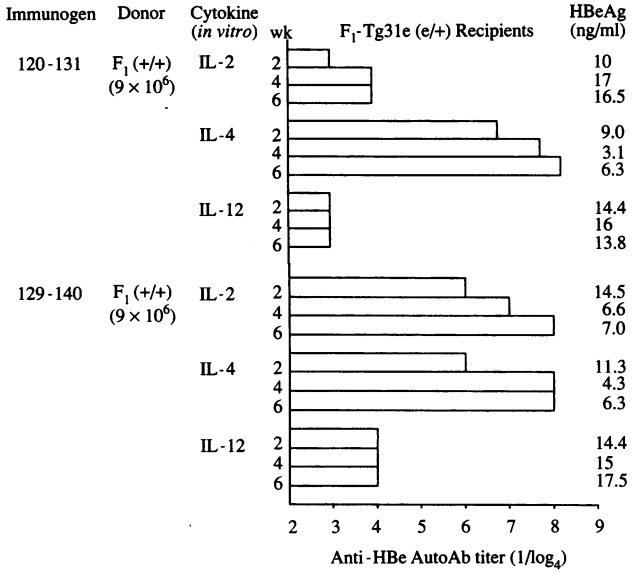

FIG. 4. Exogenous cytokines can alter the ability of Th cells to transfer autoantibody production in F_1-Tg31e mice. Non-Tg donor F_1(+/+) mice were primed in the hind footpads with 100 μg of the synthetic T-cell peptide 120–131 (*Upper*) or 129–140 (*Lower*) emulsified in complete Freund's adjuvant. Draining PLN cells were harvested and cultured *in vitro* with the HBeAg-derived composite peptide 120–140 (0.2 μg/ml) for 3 days in the presence of rIL-2 (20 units/ml), synthetic IL-4 (20 μg/ml), or rIL-12 (0.1 μg/ml). *In vitro* activated T cells (9 × 10^6) were transferred into five sublethally irradiated (500 R) HBeAg-expressing F_1-Tg31e (e/+) recipient mice. Sera were collected from recipient mice before transfer and 2, 4, and 6 weeks after adoptive transfer and were analyzed for IgG anti-HBe (autoantibody) and serum HBeAg concentration (autoantigen) by ELISA.

***In Vitro* Cytokine Treatment of Th Cells Modulates Autoantibody IgG Isotype Profile.** As shown in Fig. 5 *Upper*, the only 120–131-specific T cells that efficiently transferred help for autoantibody production were those supplemented *in vitro* with IL-4. The autoantibody produced by F_1-Tg31e recipients after the transfer of IL-4-treated 120–131-specific Th cells was exclusively of the IgG_1 isotype, indicative of Th_2-mediated helper function. The levels of IFN-γ present in the SNs of the *in vitro* cultures were also determined, and they are expressed as the highest dilution of SN to yield a positive result in a murine IFN-γ-specific ELISA. The presence of IL-4 during the *in vitro* culture of 120–130-specific Th cells significantly suppressed antigen-specific IFN-γ production measured in the SN (1:3), whereas IL-12 induced IFN-γ production (1:2100) relative to the IL-2- and IL-4-supplemented cultures. Note that 120–131-specific Th cells cultured in the presence of IL-12 elicited autoantibodies of the IgG_{2a} and IgG_{2b} isotypes exclusively upon transfer into F_1-Tg31e mice. In the case of 129–140-primed Th cells, IgG_1 autoantibody was predominant regardless if the Th cells were cultured in the presence of IL-2, IL-4, or without cytokine prior to transfer (Fig. 5 *Lower*). However, addition of exogenous IL-12 to the culture phase resulted in significant inhibition of *in vivo* IgG_1 autoantibody production (a 256-fold decrease in titer relative to IL-2 and IL-4 treatment) upon adoptive transfer of 129–140-specific Th cells into F_1-Tg31e mice with a concurrent increase in autoantibody of the IgG_{2a} and IgG_{2b} isotypes. The decrease in autoantibody of the IgG_1 isotype and increases in the IgG_{2a} and IgG_{2b} isotypes observed after IL-12 treatment correlated with the IFN-γ level in the culture SN, suggesting that the effects of IL-12 treatment were mediated through IFN-γ. These results indicate that regardless of the Th subset predisposition of HBeAg-specific Th cells, the presence of the cytokines IL-4 and IL-12 during a brief 3-day *in vitro* stimulation culture can

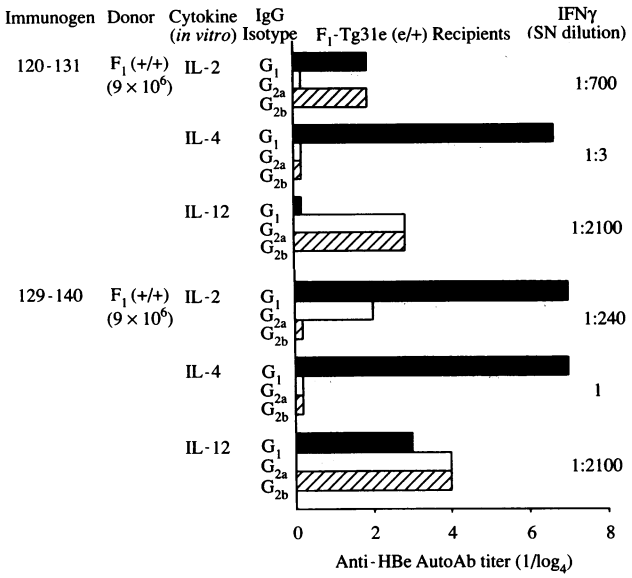

FIG. 5. Effect of exogenous cytokines on autoantibody IgG isotype. Sera from F_1-Tg31e recipient mice adoptively transferred with either 120–131-primed Th cells (*Upper*) or 129–140-primed Th cells (*Lower*) were collected before transfer and 4 weeks after adoptive transfer and were analyzed for autoantibody of the IgG_1, IgG_{2a}, and IgG_{2b} isotypes by ELISA using isotype-specific second antibodies. The 120–131-primed and 129–140-primed Th cells were derived from non-Tg $F_1(+/+)$ donors and cultured *in vitro* with peptide 120–140 and IL-2, IL-4, or IL-12 as described for Fig. 4. Additionally, SNs were collected at 48 hr for determination of IFN-γ concentration. The relative IFN-γ concentrations are expressed as the highest dilution of SN to yield a positive result (3 times background) in a murine IFN-γ-specific ELISA.

dramatically influence the functional Th phenotype of these cells. This is somewhat surprising, since it is assumed that the influence of cytokines on Th subset differentiation requires the presence of the cytokine during the antigen priming event and not 10 days later (2). This suggests that precursor HBeAg-specific Th cells still capable of differentiating into Th_1 or Th_2 cells are present in the draining lymph node 10 days after *in vivo* priming, or small numbers of the subdominant Th subset are present but require exogenous cytokine for their expansion, or least likely, that mature Th cells of a given phenotype can be converted to the alternative phenotype.

DISCUSSION

These studies revealed that relatively low-dose systemic treatment with rIL-12 can significantly suppress autoantibody production *in vivo*. The mechanism of autoantibody inhibition appears related to the ability of IL-12 to enhance Th_1-associated cytokine production (IFN-γ) and inhibit Th_2-associated cytokine production (IL-4). Therefore, the large number of autoimmune and allergic disorders that are believed to be mediated by Th_2-type cells may be amenable to treatment with IL-12. Furthermore, the observations that ongoing autoantibody production was susceptible to inhibition by IL-12 and that incubation of mature Th with IL-12 *in vitro* shifted a Th_2-like response to a Th_1-like response suggest the possibility that IL-12 treatment may be beneficial in established autoimmune or allergic disease states. Of course, the inherent toxicity of IL-12 and the inability to target IL-12 to antigen-specific T-cell populations may limit its therapeutic potential.

Additionally, these results may have relevance to chronic hepatitis B virus (HBV) infection. Recent serologic studies comparing patients with acute self-limited HBV infection and symptomatic chronic patients revealed significantly greater antibody responses in the chronic patients (19) even though cellular immune responses appear to be significantly greater in acute patients (20). Further, approximately 50% of asymptomatic chronic HBV-infected patients demonstrate significant antibody responses in the absence of T-cell-mediated liver disease (21). We have proposed that the predominance of HBeAg-specific Th_2-type cells in chronic HBV infection may explain these observations (19, 21). In this event, IL-12 treatment in chronic HBV patients may be useful to shift the balance of HBeAg-specific Th cells from Th_2 to Th_1 predominance. Because IL-12 appears more effective when present at the time of T-cell priming, a combination strategy including IL-12 plus therapeutic vaccination of established HBV carriers may be more beneficial than IL-12 alone. Inclusion of IL-12 as a vaccine adjuvant has been proposed to induce protective cellular immunity against leishmaniasis (22).

Last, this study highlights this unique transgenic model of autoimmunity in which autoantibody production, autoantigen clearance from the serum, and the phenotype of the self-reactive Th cells can all be determined within 2–3 weeks with a simple blood sample. This model should be useful to screen a variety of immunomodulatory drugs or therapies.

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