Interleukin-13 Prevents Autoimmune Diabetes in NOD Mice

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Interleukin (IL)-13 is a cytokine primarily produced by the T-helper (Th)-2 subset of lymphocytes that possesses powerful anti-inflammatory properties. Here, we have evaluated the impact of IL-13 treatment on development of type 1 diabetes in diabetes-prone nonobese diabetic (NOD) mice. Prolonged treatment with recombinant human IL-13 (hIL-13) markedly diminished the incidence of spontaneous type 1 diabetes in the mice. Female NOD mice treated from age 5–16 weeks with hIL-13 also showed significantly milder insulitis than control mice. The preventive action of hIL-13 was associated with a slight but significant change from a type 1 to a type 2 cytokine response. Accordingly, splenic lymphoid cells (SLC) from hIL-13–treated mice secreted less interferon (IFN)-γ upon ex vivo stimulation with Concanavalin A than controls, and anti-CD3 monoclonal antibody–induced activation of T-cells in vivo resulted in lower blood levels of IFN-γ and tumor necrosis factor-α and augmented blood levels of IL-4 in NOD mice pretreated with hIL-13. hIL-13 treatment also increased the blood levels of IgE and inhibited the transfer of type 1 diabetes by spleen cells from a diabetic donor to irradiated recipients. Taken together, these data add hIL-13 to the list of cytokines capable of downregulating immunoinflammatory diabetogenic pathways in NOD mice, and further support the concept that IL-4–related anti-inflammatory cytokines might have a role in the prevention of type 1 diabetes. *Diabetes* 48:1522–1528, 1999

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ConA, Concanavalin A; ELISA, enzyme-linked immunosorbent assay; hIL-13, human recombinant interleukin-13; IFN, interferon; IL, interleukin; IS, insulitis score; mAb, monoclonal antibody; NK, natural killer; PBS, phosphate-buffered saline; PBTN, phosphate-buffered saline containing 1% bovine serum albumin, 0.05% Tween-20, and 0.02% sodium azide; PT, phosphate-buffered saline and 0.05% Tween; SLC, splenic lymphoid cells; TNF, tumor necrosis factor.

Cytokines are (glyco)proteins centrally implicated in the growth, maturation, and functional activities of immune cells. At least two major subsets of cytokines exist, type 1 and type 2 cytokines, often with antagonistic functions. While type 1 cytokines such as interferon (IFN)-γ, interleukin (IL)-2, IL-12, and tumor necrosis factor (TNF)-α stimulate cell-mediated immune responses and isotype switching to IgG2a and IgG3, type 2 anti-inflammatory cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) induce a functional state of macrophages and T-cells that is not cytotoxic and favor humoral immune responses and production of IgG1, IgG2b, and IgE (1). Because of their capacity to stimulate cell-mediated immune effectors such as macrophages, T-cells, and natural killer (NK) cells, type 1 cytokines are thought to play a part in the pathogenesis of organ-specific autoimmune diseases such as multiple sclerosis and type 1 diabetes, while type 2 cytokines may contribute to the development of allergic reactions and humoral autoantibody-mediated autoimmune diseases such as Graves' disease, rheumatoid arthritis, and systemic lupus erythematosus (2–4).

A tightly regulated balance exists between type 1 and type 2 cytokines, and it has been hypothesized that alterations of this balance leading to dysregulated production of type 1 proinflammatory cytokines may be implicated in the clinical course, and perhaps even the appearance, of organ-specific autoimmune pathologies such as multiple sclerosis, autoimmune thyroiditis, and type 1 diabetes (2–4).

Type 1 diabetes is a chronic immunoinflammatory disease, probably autoimmune in nature, due to the destruction of the insulin-producing β-cells of Langerhans from autoreactive mononuclear cells that specifically infiltrate the islets (insulitis) before and shortly after disease development (5). Recent studies indicate that type 1 diabetes is a prototypical type 1 cytokine-mediated immunoinflammatory disease. IL-1, IL-12, IFN-γ, and TNF-α impair β-cell function, immunogenicity, and viability in vitro and might recruit and activate further autoreactive effectors in vivo, amplifying β-cell destruction (4,6). The essential pathogenetic role of endogenous IL-1, IL-12, IFN-γ, and TNF-α in autoimmune diabetogenesis in vivo is emphasized by the preventive efficacy observed in nonobese diabetic (NOD) mouse and/or BB rat models by immunotherapeutical approaches aimed at blocking their actions (7–16).

Increasing evidence suggests that the propensity to mount an upregulated type 1 cytokine response in human and rodent type 1 diabetes may depend on, or be favored by, a defective production of type 2 cytokines (2). Lymphocytes

from both newly diagnosed type 1 diabetic patients and NOD mice produce more IFN-γ and less IL-4 in vitro (2,17–19), the latter depending on a diminished population of CD1 restricted T-cells (20–22). Moreover, blood levels of type 1, but not type 2, cytokines are elevated at the onset of type 1 diabetes in human and BB rat diabetes (15,23,24). A polarized type 1 cytokine profile with high levels of IL-1, IL-2, TNF-α, IFN-γ, and IL-12 and low levels of IL-4 is also observed during the advanced destructive phase of the insulitis process in female NOD mice with high diabetes incidence and/or in DP-BB rats (25–29). In contrast, a type 2 cytokine profile with high levels of IL-4 and low levels of IFN-γ occurs in the insulitis lesions of both male NOD mice with low diabetes incidence and female NOD mice protected against type 1 diabetes by administration of complete Freund's adjuvant (25,26). Finally, exogenously administered IL-4 (17,30) and IL-10 (13) and pancreatically expressed IL-4 prevent type 1 diabetes (31), and the effects of IL-4 are associated with a deviation of the cytokine production toward a type 2 phenotype in female NOD mice (30).

Other data suggest that the type 1/type 2 cytokine hypothesis in type 1 diabetes may be oversimplistic. In fact, although Trembleu et al. (32) have demonstrated that IL-12, which promotes IFN-γ production and type 1 T-cell generation, accelerates disease development in NOD mice, O'Hara et al. (33) have found that a different treatment regimen with IL-12 actually inhibits disease development in these mice. In addition, exogenously administered IFN-γ does not influence type 1 diabetes development in NOD mice (34) and, paradoxically, protects DP-BB rats from disease development (35). In a similar manner, the onset of diabetes may be prevented in NOD mice and BB rats by systemically administered type 1 cytokines such as IL-1, TNF-α, and IL-2 (2,34,36), and the diabetogenic process is accelerated in NOD mice by transgenic expression of IL-10 in the islets (37). It was recently demonstrated that IL-4 deficiency neither accelerates the tempo nor the cumulative incidence of type 1 diabetes in NOD mice (38) and that type 2 T-cells induce acute pancreatitis and diabetes in immune-compromised NOD mice (39). Finally, Huang et al. (40) did not find IL-2 and TNF-α expression in pancreases of recently diagnosed type 1 diabetic patients, and only found IFN-γ transcripts in one out of four pancreases. IFN-α was the only cytokine consistently found in that study (40).

These complex, and often paradoxical, effects of exogenously administered cytokines on the course of the disease in NOD mice prompted us to evaluate the effect of treatment with the anti-inflammatory cytokine recombinant human IL-13 (hIL-13) (41) on the development of spontaneous and adoptively transferred type 1 diabetes in NOD mice. The data show a marked and sustained antidiabetogenic effect of hIL-13 associated with profound modifications of the cytokine secretory capacity of the mice.

RESEARCH DESIGN AND METHODS

hIL-13. hIL-13 was produced as described elsewhere (42). The biological efficacy of hIL-13 in mice has been demonstrated (43). Control mice were treated with a similar volume of phosphate-buffered saline (PBS). The endotoxin concentration of hIL-13 was between 23 and 38 pg/mg protein.

NOD mice. Euglycemic female NOD mice purchased from Charles River (Calco, Italy) were housed at the animal facilities of the Institute of Microbiology of the University of Catania. These mice were used to test the effects of hIL-13 administration on the development of spontaneous diabetes. Male NOD mice, obtained from a breeding nucleus maintained at the CRC (Northwick Park, U.K.) and originally provided by Dr. E. Leiter, were housed at the animal facilities of the Department of Pathology of the University of Cambridge and were used to test the effects of hIL-13 on adoptively transferred diabetes. In both animal facilities, the mice were kept under standard laboratory conditions (nonspecific pathogen free), with ad libitum food and water.

Ex vivo cytokine secretion. Individual spleens from either hIL-13– or PBS-treated mice were passed through a sterile sieve and suspended in Hanks' balanced salt solution (Sigma Chimica, Milan, Italy). Splenic lymphoid cells (SLC) obtained and cultured for 48 h at 37°C with 5% CO_2, as described elsewhere (14), were either unstimulated or stimulated with Concanavalin A (ConA; Sigma). At the end of the culture period, the cells were centrifuged, and the supernatant collected was aliquoted at –20°C until cytokine measurement.

In vivo cytokine release. Female NOD mice were treated with either hIL-13 or PBS 1 h before the intraperitoneal injection of 10 μg of hamster anti-mouse CD3 monoclonal antibody (mAb) (clone 145-2C11; Pharmingen, San Diego, CA) that reacts with the 25-kDa ε chain of the T-cell receptor–associated CD3 complex, which is expressed on thymocytes and mature T-cells.

TNF-α, IFN-γ, and IL-4 quantitation. These cytokines were measured using mouse-specific enzyme-linked immunosorbent assay (ELISA) purchased from Endogen (Boston, MA). The limits of sensitivity of the assays were 10 pg/ml for IL-4 and TNF-α and 1 pg/ml for IFN-γ. Intra- and interassay coefficients of variation were within 12 and 19%, respectively. To calculate mean values, samples with undetectable cytokine concentrations were assigned the lower limit of sensitivity of the assay as a theoretical value.

Mouse IgE quantitation. Total serum IgE levels were measured by solid-phase ELISA (44). Plates were coated overnight at 4°C with a rat anti-mouse IgE mAb (clone R35-72; Pharmingen) diluted 2 μg/ml in carbonate buffer. After five washes with PT (PBS and 0.05% Tween), the plates were blocked with PBTN (PBS containing 1% bovine serum albumin, 0.05% Tween-20, and 0.02% sodium azide) for 30 min. Sera, diluted 1/100 in PBS with 1% bovine serum albumin and 0.02% sodium azide, were added to wells and incubated at room temperature for 2 h. Serum samples were washed six times with PT, and the secondary antibody, biotinylated rat anti-mouse IgE (clone R35-92; Pharmingen), at a concentration of 2 μg/ml in PBTN was added to the wells and incubated at room temperature for 45 min. After six washes with PT, streptavidin-AP (Pharmingen) diluted 1/2,000 in PBTN was added to each well and allowed to stand at room temperature for 45 min. After several washes with PT, p-nitrophenylphosphate buffer (Sigma) was added to each well. Absorbance values were measured at 405 nm after 2 h.

Histological examination of pancreatic islets. The degree of mononuclear cell infiltration was graded in a blind fashion as described elsewhere (14): 0, no infiltrate; 1, periductular infiltrate; 2, peri-islet infiltrate; 3, intraislet infiltrate; 4, intraislet infiltrate associated with β-cell destruction. At least 15 islets were counted for each mouse. A mean score for each pancreas was calculated by dividing the total score by the numbers of islets examined. Insulitis scores (ISs) are expressed as mean values ± SD.

RESULTS

Early prophylaxis with hIL-13 prevents insulitis and diabetes. To evaluate the effects of hIL-13 on the early diabetogenic pathways of the NOD mice and because virtually all the mice are free from insulitis until 5 weeks of age (45,46), a first experiment was conducted in which NOD mice were treated with hIL-13 from the 5th until the 16th week of age.

By the age of 16 weeks, none of the mice treated with hIL-13 had developed type 1 diabetes, while 29% (7 of 24) of the control mice had type 1 diabetes (Fig. 1). Moreover, the histological analysis performed at the end of the study of pancreases from euglycemic animals revealed a milder insulitis in the treated mice (n = 18) compared with the PBS-treated control mice (n = 17). This was also reflected by the significantly lower IS of the treated animals: 0.4 ± 0.3 vs. 2.2 ± 1; $P < 0.0001$, analysis of variance (ANOVA). This effect is unlikely to be secondary to an unspecific effect of the human protein because NOD mice treated with 10 μg recombinant human IL-6 (biologically active in mice) on alternate days from the 5th to the 16th week of age had cumulative diabetes incidence and insulitis similar to those of PBS-treated controls (data not shown). It is also known that exogenously administered recombinant human IFN-α does not influence type 1 diabetes development in NOD mice (47).

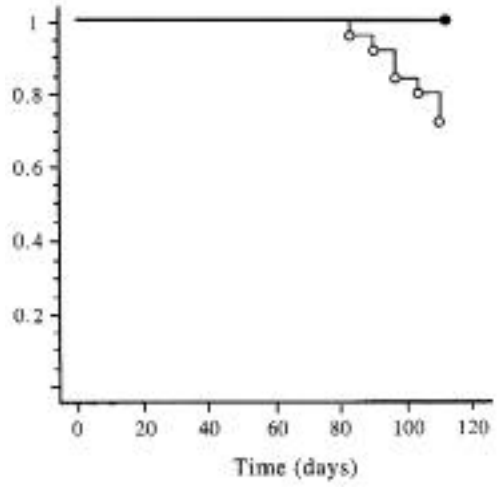

FIG. 1. Cumulative time free of type 1 diabetes. Euglycemic female NOD mice were treated from the 5th until the 16th week of age either with hIL-13 (●, 10 µg/mouse i.p. three times weekly on alternate days, *n* = 18) or with PBS (○, *n* = 24). The mice were examined for diabetes development once a week. Diabetes was diagnosed in the presence of two consecutive days of glycosuria followed by fasting glycemia >11.8 mmol/l. *P* = 0.0163 vs. PBS-treated controls by log-rank testing (Mantel-Cox). The results of three independent experiments are shown.

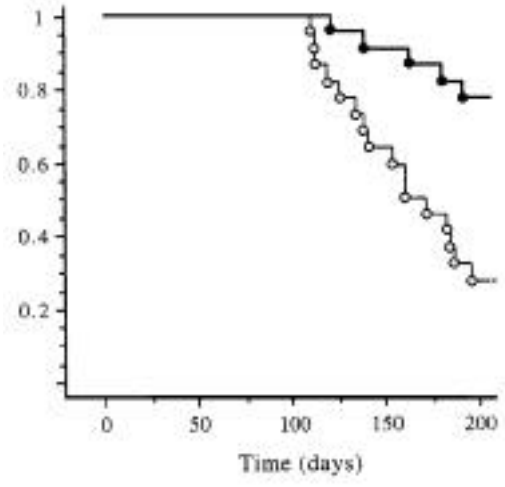

FIG. 2. Cumulative time free of type 1 diabetes: the effects of late prophylaxis with hIL-13 on spontaneous type 1 diabetes in NOD mice. Euglycemic 14-week-old female NOD mice were treated three times weekly on alternate days with either 10 µg hIL-13 (●, *n* = 22) or PBS (○, *n* = 22) until the age of 28 weeks. Diabetes was diagnosed in the presence of two consecutive days of glycosuria followed by fasting glycemia >11.8 mmol/l. *P* = 0.0006 vs. PBS-treated controls by log-rank testing (Mantel-Cox). The results of three independent experiments are shown.

Late prophylaxis with hIL-13 confers long-lasting protection against type 1 diabetes and blocks progression of insulitis in NOD mice. Another set of experiments was conducted to ascertain whether hIL-13 was also effective in ameliorating clinical and histological signs of type 1 diabetes in NOD mice when administered late during the prediabetic period. At age 14 weeks, most female NOD mice show signs of actively ongoing insulitis (45,46). From this age to 28 weeks, 10 µg hIL-13 per mouse, in a final volume of 100 µl, or PBS was administered intraperitoneally three times weekly on alternate days. As shown in Fig. 2, most of the mice treated with PBS became diabetic during the study period, and the incidence of the disease was significantly diminished by hIL-13 treatment. Moreover, type 1 diabetes has presently occurred in none of 7 hIL-13–treated mice that were euglycemic at the end of the study, 50 weeks after treatment withdrawal, thus demonstrating that the antidiabetogenic action of hIL-13 may be long-lasting.

To evaluate whether late hIL-13 treatment also affected the progression of insulitis, some of the mice were sacrificed at the onset of type 1 diabetes or at the end of the study period. A massive intraislet inflammatory infiltration of the pancreatic islets of Langerhans associated with β-cell destruction was found in virtually all PBS-treated control mice, regardless of whether they were diabetic (IS = 3.37 ± 0.44, n = 16) or euglycemic (IS = 3.2 ± 0.51, n = 6). Severe insulitis was also seen in the hIL-13–treated mice that developed diabetes (IS = 3.32 ± 0.54, n = 5). In contrast, those hIL-13–treated NOD mice that remained euglycemic at the end of the study (n = 10) had milder insulitis, mostly perisletitis rarely associated with β-cell destruction (IS = 2.22 ± 0.6). This was significantly lower than that observed in both healthy (P = 0.005) and diabetic (P < 0.0001) PBS-treated mice (ANOVA). Hence, institution of hIL-13, even after the insulitis process has started, delays progression to the destructive type of insulitis.

hIL-13 prevents type 1 diabetes and diminishes insulitis adoptively transferred by spleen cells. Autoimmune diabetes can be adoptively transferred to euglycemic syngeneic recipients by intravenous injection of spleen cells from acutely diabetic animals; the model is T-cell dependent, and type 1 diabetes occurs within 3–8 weeks after cell transfer (46). hIL-13, 30 µg/mouse, was administered intraperitoneally starting 1 day before the injection of spleen cells. Control mice were treated under the same experimental conditions with PBS. As shown in Fig. 3, type 1 diabetes occurred in the majority of PBS-treated NOD mice within 4 weeks of cell transfer, but the incidence of the disease was markedly diminished by hIL-13. In agreement with the clinical data, the histological analysis of pancreases isolated 28 days after transfer of diabetic spleen cells from mice in the second experiment revealed significantly lower ISs in the 10 hIL-13–treated mice than those in the 11 PBS-treated controls: 2.76 ± 0.85 vs. 3.68 ± 0.3 (P = 0.003, ANOVA). However, when diabetic and euglycemic mice from the two groups were examined separately, comparable ISs were found in the diabetic mice treated with either PBS (IS = 3.8 ± 0.02, n = 8) or hIL-13 (IS = 3.6 ± 0.34, n = 4). Insulitis was significantly milder in the hIL-13–treated healthy euglycemic mice (IS = 2.2 ± 0.51, n = 6) compared with both the diabetic and euglycemic controls (IS = 3.37 ± 0.26, n = 3), P < 0.0001 and 0.008 by ANOVA, respectively.

As in the spontaneously occurring diabetes in the NOD mouse, hIL-13 prophylaxis also seemed to not simply delay the onset of diabetes in the transfer model. In fact, in experiment 1, the mice were observed for late development of diabetes up to 2 weeks after hIL-13 (or PBS) treatment withdrawal, and during this period of time, the incidence of the disease increased from 80 to 100% in control mice but remained stable in hIL-13–treated mice (Fig. 3). hIL-13 administered under the same experimental conditions at the lower dose of 10 µg/mouse was not effective (data not shown).

hIL-13 downregulates ex vivo secretion of IFN-γ. To evaluate the impact of prolonged hIL-13 treatment on the ex vivo secretory capacity of NOD mice, SLC were isolated when the animals were killed at the age of 16 weeks and were cul-

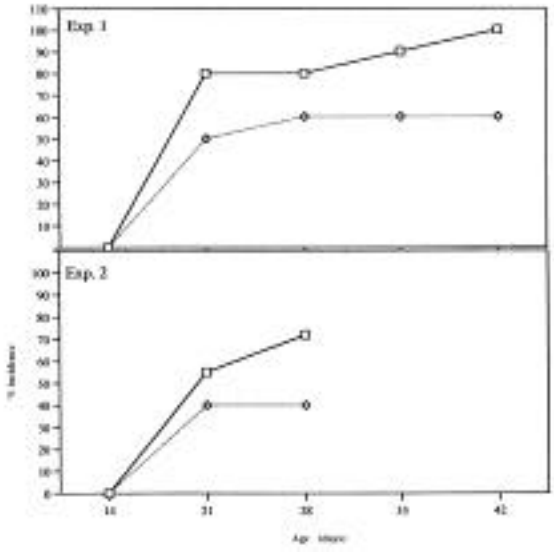

FIG. 3. Reduction of adoptively transferred type 1 diabetes in NOD mice by hIL-13. Six-week-old euglycemic male NOD mice were irradiated with 650 rads before intravenous injection with 2×10^7 spleen cells from acutely diabetic female NOD mice, as described elsewhere (67). Mice were treated with either hIL-13 (◇, 30 µg/mouse three times weekly on alternate days starting 1 day before transfer) or PBS (□). The mice were tested for diabetes development once a week by glycemia measurement. Diabetes was diagnosed in the presence of nonfasting glycemia >11.8 mmol/l. For statistical analysis, the results of the two experiments were pooled. Experiment (Exp.) 1 contained 10 mice in both the experimental and control groups. In Exp. 2, there were 10 mice treated with hIL-13 and 11 treated with PBS. The cumulative incidence of type 1 diabetes in the mice treated with hIL-13 (10 of 20, 50%) was significantly ($P = 0.034$ by χ^2) lower than that of PBS-treated control mice (18 of 21, 85.7%).

tured with and without ConA. As shown in Fig. 4, SLC from hIL-13–treated mice secreted less IFN-γ than SLC from controls.

hIL-13 diminishes IFN-γ and TNF-α and augments IL-4 blood levels after anti-CD3 treatment. $CD3^+$ T-cells play a pivotal role in NOD mouse type 1 diabetes (46), and in vivo injection with anti-CD3 mAb induces T-cells to release several cytokines, such as IL-2, IL-4, IL-6, TNF-α, and IFN-γ (48), which appear with different kinetics in the circulation. As shown in Fig. 5, pretreatment of female NOD mice with 30 µg hIL-13, the dose previously found to prevent development of adoptively transferred type 1 diabetes, modified the blood levels of IFN-γ, IL-4, and TNF-α in anti-CD3–injected mice toward a polarized type 2 cytokine profile. In fact, relative to PBS-treated mice injected with anti-CD3 mAb, the blood levels of hIL-13–treated mice contained less IFN-γ and TNF-α and more IL-4.

hIL-13 increases IgE levels. Because marked increase in IgE serum levels has been observed in hIL-13 transgenic mice (49), it was important to determine whether increased IgE blood levels had occurred in response to hIL-13 treatment. As shown in Fig. 6, the sera of NOD mice treated with the largest dose of hIL-13 in the adoptive transfer model contained significantly larger amounts of IgE than control mice.

Lack of systemic toxicity of hIL-13 treatment. hIL-13 was well tolerated, judging from the behavior and general appearance of the mice. There were also no significant differences in body weights between hIL-13– and PBS-treated control mice throughout the study period. Moreover, azotemia and transaminases in the male mice treated with the largest dose of hIL-13 (30 µg/mouse three times weekly for up to 28 days) were similar to those observed in PBS-treated controls.

DISCUSSION

hIL-13 is a cytokine produced primarily by $CD4^+$ type 2 T-cells but also by type 0 T-cells, $CD8^+$ T-cells, and $CD45RA^+$ T-cells and in low levels by type 1 T-cells (41). Although hIL-13 is

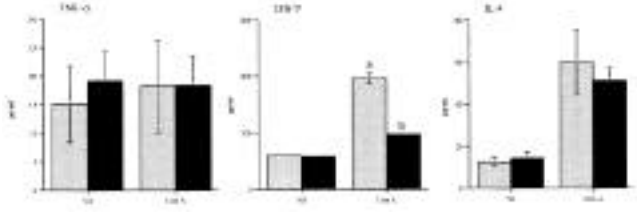

FIG. 4. Ex vivo effect of hIL-13 treatment on ConA-induced secretion of TNF-α, IFN-γ, and IL-4. Individual spleens were obtained from 16-week-old female euglycemic NOD mice treated from the age of 5 weeks either with hIL-13 (■, 10 µg/mouse i.p. three times weekly on alternate days, $n = 10$) or PBS (▨, $n = 10$). Spleen cells were cultured in complete medium at 37°C, 5% CO_2 in the presence or absence of 2 µg/ml ConA. After 48 h, culture supernatants were collected by centrifugation and stored at –20°C until cytokine measurements. Results (picograms per milliliter) are expressed as mean values ± SD. Where SD bars are not visible, they are <5%. $P < 0.0001$ (ANOVA) for *b* vs. *a*. The results of two independent experiments are shown.

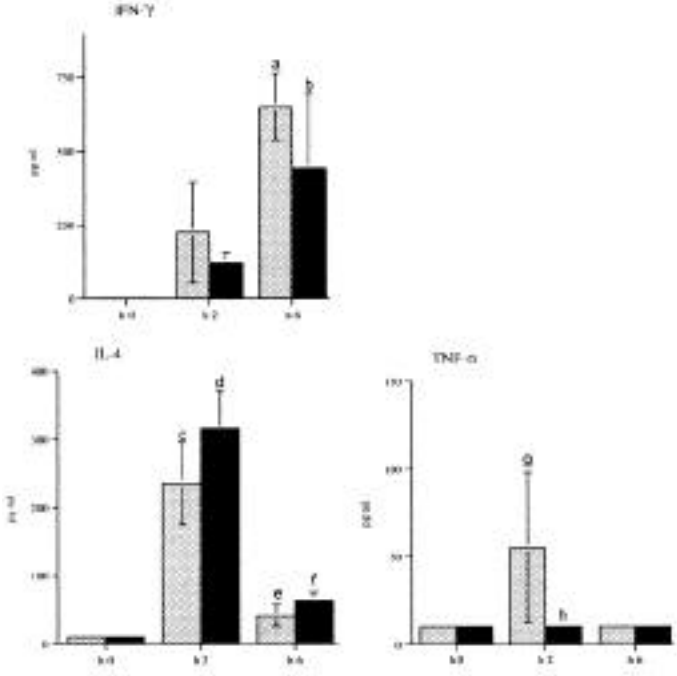

FIG. 5. Modulation by hIL-13 of anti-CD3–induced appearance blood levels of IFN-γ, IL-4, and TNF-α. Euglycemic 13-week-old female NOD mice were treated either with hIL-13 (■, 30 µg/mouse, $n = 10$) or with PBS (▨, $n = 10$) 1 h before intraperitoneal injection with 10 µg of anti-mouse CD3 mAb. Blood samples were collected from 10 mice for each group killed at $t = 0$, just before injection with the anti-CD3 mAb, and 2 and 6 h thereafter. Blood samples were allowed to clot at room temperature, and serum was immediately separated by centrifugation at 1,000*g* and stored at –20°C until measurement of TNF-α, IFN-γ, and IL-4. Results (picograms per milliliter) are expressed as mean values ± SD. Where SD bars are not visible, they are <5%. Statistical analysis was performed by ANOVA. Each group is compared with PBS-treated mice. *b* vs. *a*, $P = 0.029$; *d* vs. *c* = 0.008; *e* vs. *f*, $P = 0.003$; *g* vs. *h*, $P = 0.004$. The results of two independent experiments are shown.

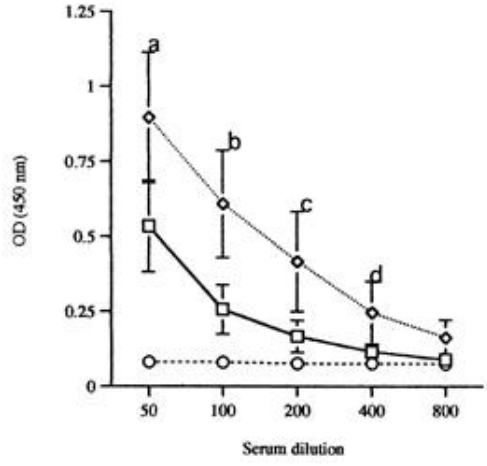

FIG. 6. hIL-13 treatment increases IgE blood levels in NOD mice. Six-week-old male NOD mice that were used for the adoptive transfer model of type 1 diabetes were treated either with hIL-13 (30 µg/mouse, n = 10) or PBS (n = 11) starting 1 day before intravenous injection with 2×10^7 spleen cells from donor diabetic female NOD mice. Treatment was continued three times weekly on alternate days for four consecutive weeks. At the end of the study, serum samples were obtained from either group, and the total levels of IgE were measured by solid-phase ELISA. Results (means ± SD) are shown as optical density (OD) values. Statistical analysis was performed by ANOVA. Each group is compared with PBS-treated control mice: *a*, $P = 0.024$; *b*, $P > 0.0001$; *c*, $P = 0.001$; *d*, $P = 0.004$; *e*, $P = 0.005$. □, control; ◇, hIL-13; ○, background.

closely related to IL-4 and shares the receptor α-chain, it is not a typical type 2 cytokine. Moreover, unlike IL-4, hIL-13 acts only on macrophages and B-cells, but not on T-cells, and the two cytokines are also distinguished by different patterns of production from T-cells (50). Accordingly, hIL-13 plays a distinct role in type 2 T-cell–mediated immune responses (51).

hIL-13 possesses powerful immunodownregulatory properties, at least in part related to its capacity to inhibit the production of inflammatory cytokines, such as IL-1β, IL-12, TNF-α, and IFN-γ, through suppression of nuclear factor (NF)-KB and preservation of inhibitory protein KB (IKB)-α under various in vitro and in vivo conditions (52–54). These properties might be related to the beneficial effects of hIL-13 in immunoinflammatory conditions such as septic shock (43,53,54), type II collagen–induced arthritis (55), experimental allergic encephalomyelitis (56), and lung-induced injury in rodents (57).

The antidiabetogenic action of hIL-13 observed in the present study was associated with modifications of the cytokine secretory capacity of the mice. Ex vivo studies with SLC from hIL-13–treated mice, but not from controls, showed a significantly diminished ConA-induced IFN-γ secretion without any effect on TNF-α and IL-4 upon ex vivo conditions. And activation with anti-CD3 mAb resulted in reduced levels of IFN-γ and TNF-α and elevated levels of IL-4. The reason(s) for this discrepancy is unknown. The ex vivo conditions, e.g., washing of cells and their maintenance in medium with mitogens for 2 days without exogenously added hIL-13, may have minimized the bioavailability of hIL-13 in the cultured cells. However, the release of TNF-α from mononuclear cells is more rapid than that of IFN-γ and IL-4. This is well illustrated in the in vivo study with anti-CD3 mAb in which, unlike IFN-γ and IL-4, TNF-α was found in the blood at 2 h, but not at 6 h, after challenge. Therefore, an early release of TNF-α may also have been suppressed by hIL-13, an effect that is no longer seen at 48 h, when the TNF-α levels are back at nonstimulated levels. Nonetheless, the diminished production/secretion of IFN-γ and TNF-α, along with the simultaneous increase of IL-4 in the hIL-13–treated NOD mice, accords with a polarization toward a type 2 cytokine profile that may play a role in mediating the beneficial effects of hIL-13 (2). This accords with the impaired development of T-helper (Th)-2 cells in hIL-13–deficient mice (58).

The ability of hIL-13 to inhibit the action/production of the diabetogenic cytokine IL-1β (53) by suppressing its production and upregulating that of IL-1 receptor antagonist (59) might also explain its ability to prevent type 1 diabetes. In a similar manner, inhibiting macrophage production of nitric oxide (60), which is involved in NOD mouse type 1 diabetes (2), and counteracting IL-1β–induced suppression of glucose metabolism in rodent β-cells in vitro (61) might be additional protective mechanisms. It has also been very recently demonstrated that hIL-13 suppresses TNF-induced apoptosis (62), one possible mode of β-cell death in type 1 diabetes (63).

Because IL-4 and hIL-13 share several immunological properties (41), and exogenously administered IL-4 prevents NOD mouse type 1 diabetes (17,30), the antidiabetogenic effects of hIL-13 were not unexpected. However, in agreement with the distinct roles of IL-4 and hIL-13 in modulation of type 2 T-cell responses (51), several differences emerge regarding the underlying immunomodulatory effects of the two cytokines. Although IL-4 treatment markedly augmented both spontaneous and anti-CD3–induced secretion of IL-4 from splenic T-cells (30), exogenously administered hIL-13 augmented IL-4 blood levels upon in vivo challenge with anti-CD3 mAb, but failed to do so in either unstimulated or ex vivo–stimulated SLC; in addition, IL-4, but not hIL-13, treatment also downregulated the spontaneous secretion of IFN-γ from spleen cells (30). Taken together, these data suggest that the immunomodulatory effects of prolonged hIL-13 treatment are less pronounced than those induced by IL-4. This is likely to be associated with the fact that IL-4 acts directly on T-cells, whereas the effect of hIL-13 is secondary to its action on macrophages. hIL-13 would then downregulate type 1 T-cells by inhibiting macrophage production of IL-12 (53,60) and augmenting production of IL-6 (54,64), which favors type 2 T-cell generation through IL-4 production (65). Along this line of reasoning, because mouse B lymphocytes are not responsive to hIL-13, the increased titers of IgE in the blood of hIL-13–treated mice also fits in with indirect operational mechanisms that are induced by hIL-13 in upregulating IgE secretion. These may not necessarily, however, be IL-4 mediated, since recent reports by Emson et al. (49) have demonstrated IL-4–independent induction of IgE in hIL-13 transgenic mice that express up to 100-fold higher IgE blood levels than their littermate controls. Moreover, when comparing the immunomodulatory effects of IL-4 with those of hIL-13 in NOD mice, it should also be noticed that hIL-13 was used in our study, compared with the mouse IL-4 used by others (17,30). hIL-13 is up to 100-fold less effective than murine IL-13 in mouse (66), and its prolonged application might induce formation of neutralizing antibodies capable of further reducing the bioactivity of the cytokine. Thus, it can be expected that mouse IL-13 exerts even more powerful antidiabetogenic and immunomodulatory effects than hIL-13 in NOD mice.

The effects of mouse IL-4 and hIL-13 on the clinical course of the disease were similar with regard to both the degree of protection and the long-lasting duration with protection in excess of 10 months after treatment withdrawal (17,30).

The capacity of hIL-13 to arrest the progression of insulitis when treatment was started in animals with manifest insulitis is also of interest. The effect may again be secondary to hIL-13–induced intraislet inhibition of type 1 cytokines implicated in the progression toward destructive type 1 insulitis (2). It should be noticed, however, that while late prophylactic treatment of NOD mice with hIL-13, or its administration to irradiated NOD mice injected with already committed diabetogenic T-cells, prevented histological and clinical signs of type 1 diabetes, the early prophylactic treatment with hIL-13 ameliorated both parameters to a greater extent. It seems possible, therefore, that mechanisms besides modulation of intrainsular type 1 cytokine production may have been involved in the antidiabetogenic action in early hIL-13 prophylaxis. For example, hIL-13 could influence the homing process of leukocytes into pancreatic islets and/or modify early events in the generation of autoreactive T-cells in the periphery or the thymus.

If NOD mouse prediabetes resembles the clinical and histoimmunological features of human prediabetes, hIL-13 could be considered for prevention, and perhaps even treatment, of human type 1 diabetes. The sustained antidiabetogenic action of hIL-13 in NOD mice might even envisage a relatively short-term treatment with hIL-13 to be capable of conferring long-lasting protection from disease development. Although hIL-13 was well tolerated and no clinical or biochemical signs of liver and kidney toxicity were observed, the use of hIL-13 in the clinical setting must be carefully weighted against possible side effects in humans. In this regard, the observed increase in blood IgE levels induced by prolonged treatment could constitute a risk for development of allergic reactions, especially in individuals with atopic/allergic diseases.

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