Original Article

Transient Upregulation of Indoleamine 2,3-Dioxygenase in Dendritic Cells by Human Chorionic Gonadotropin Downregulates Autoimmune Diabetes

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OBJECTIVE—Pregnancy induces a state of immunological tolerance that aims at suppressing immune responses against the fetus and has been linked to temporal remission of preexisting autoimmune disorders. To understand the mechanisms of this reversible immune regulation, we investigated the role of a key pregnancy hormone, human chorionic gonadotropin (hCG), in immune tolerance against autoimmune type 1 diabetes in nonobese diabetic (NOD) mice.

RESEARCH DESIGN AND METHODS—We injected hCG into cytokine gene–deficient NOD mice and evaluated the effects of hCG administration on T-cells and dendritic cells (DCs).

RESULTS—We show that administration of hCG to NOD mice inhibits both the activation of diabetogenic $CD4^+$ and $CD8^+$ T-cells, in vitro and in vivo, and the progression of type 1 diabetes by upregulating the expression of indoleamine 2,3-dioxygenase (IDO) in DCs. IDO upregulation is transient and declined shortly after hCG withdrawal. DC depletion restores the diabetetogenic activity of splenic T-cells from hCG-treated mice, and inhibition of IDO activity by 1-methyl-tryptophan abrogates the hCG-induced T-cell suppression and resistance to type 1 diabetes.

CONCLUSIONS—We propose that hCG-induced upregulation of IDO in DCs plays a major role in pregnancy-associated resistance to autoimmunity. ***Diabetes*** **56:1686–1693, 2007**

Type 1 diabetes is a T-cell–mediated autoimmune disorder, resulting from the selective destruction of the insulin-producing β-cells of the pancreas by islet antigen–specific $CD4^+$ and $CD8^+$ T-cells (1). The onset of hyperglycemia in NOD mice is preceded by a protracted preclinical period of islet inflammation. This preclinical period is associated with the activation, recruitment, and local accumulation of diabetogenic T-cells. These processes are driven by islet antigen–loaded professional antigen-presenting cells (APCs), consisting primarily of dendritic cells (DCs) in pancreatic lymph nodes (PLN) (2,3). Dysregulated DC functions, such as defective γ-interferon (IFN-γ)-dependent induction of indoleamine 2,3-dioxygenase (IDO) (4), might contribute to the autoimmune proclivity of this strain.

IDO is a rate-limiting enzyme for tryptophan degradation via the kynurenine pathway. The depletion of local tryptophan by IDO-expressing DCs triggers proliferation arrest and anergy in T-cells (5), whereas tryptophan metabolites, such as kynurenine and picolinic acid, induce apoptosis of activated T-cells (6). IDO upregulation has been shown to protect allogeneic engrafts from rejection (7) and to inhibit experimental asthma (8), presumably because of the suppressive effects of IDO on T-cell–mediated immune responses. In addition, administration of a tryptophan catabolite inhibited the relapse of experimental autoimmune encephalomyelitis (EAE) (9). By contrast, IDO inhibition exacerbated EAE and autoimmune colitis (10,11). Thus, IDO expression in DCs clearly has the potential to regulate autoimmune responses. IDO expression in DCs is often a cytokine-dependent (including type I and II interferons) process that functions as a feedback regulatory mechanism to downregulate inflammatory responses (6).

The placenta transiently expresses IDO, largely in an IFN-γ–independent manner (12). IDO expression by the placenta likely constitutes an important component of maternal tolerance, since inhibition of IDO activity in pregnant mice promoted the rejection of the allogeneic fetus (13,14). However, IDO expression in the placenta is unlikely to be programmed genetically in the fetus because cells of both maternal and fetal origins express IDO (12, 15–17). In addition, increased numbers of IDO-expressing cells were found in both the decidua and the circulation in pregnant women (18), suggesting that an as yet unknown pregnancy-associated factor induces a systemic, albeit transient, expression of IDO.

As a major pregnancy hormone, chorionic gonadotropin is produced by the embryo and placenta, and the concentration of chorionic gonadotropin in the maternal circulation increases exponentially during early pregnancy. In human gestation, the peak concentration reaches >100 IU/ml from <1 IU/ml in a few weeks (19). Chorionic gonadotropin has been found to induce multiple genes in

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1-MT, 1-methyl-tryptophan; 3,4-DAA, *N*-(3,4,-dimethoxycinnamoyl) anthralic acid; APC, antigen-presenting cell; CFSE, carboxyfluorescein diacetate succinimidyl ester; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; hCG, human chorionic gonadotropin; IDO, indoleamine 2,3-dioxygenase; IFN-γ, γ-interferon; IL, interleukin; MHC, major histocompatibility complex.

different tissues and cells to facilitate embryo implantation and fetal development (20), and the receptor for hCG (human chorionic gonadotropin) (LH/hCG receptor) is expressed in almost every tissue and cell (21). Interestingly, administration of hCG to NOD mice inhibited the development of type 1 diabetes (22), suggesting a role of hCG in immune regulation. In the present study, we investigated the potential mechanisms through which hCG may inhibit diabetogenesis. We show that hCG induces the expression of IDO in DCs in an IFN-γ–independent manner and that IDO-expressing DCs inhibit the expansion of islet-specific autoreactive T-cells and the onset of type 1 diabetes in NOD mice. hCG induction of IDO is transient, and autoreactive T-cells from hCG-treated NOD mice regain their diabetogenic potential in the absence of IDO-expressing DCs.

RESEARCH DESIGN AND METHODS

NOD, NOD.scid, NOD$^{tcr\alpha-/-}$ mice, and IL-10– and IFN-γ–deficient NOD mice (NOD$^{Il10-/-}$) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Stat6-deficient NOD mice were produced by backcrossing a mutant *Stat*6 gene from the B6 onto the NOD background for 11 generations. The deficient *Il*10 (*Interleukin*-10) gene was then crossed from interleukin (IL)-10 deficient NOD mice into Stat6-deficient NOD to develop Stat6/IL-10 double-deficient NOD mice. NOD-BDC2.5 transgenic mice were provided by Dr. J.D. Katz (University of Cincinnati, OH). NOD-NY8.3 transgenic mice were as described previously (23). All mice were maintained in a specific pathogen-free facility at University of Calgary, according to the Institutional Animal Care and Use Committee guidelines.

Recombinant hCG and 1-methyl-DL-tryptophan (1-MT) were purchased from Sigma-Aldrich Canada (Oakville, ON). Slow-releasing placebo and 1-MT pellets were obtained from Innovative Research of America (Sarasota, FL). All antibodies were purchased from BD Pharmingen Canada (Mississauga, ON).

hCG administration. To inhibit type 1 diabetes, hCG was injected intraperitoneally into 9-week-old female mice daily. Mice were injected with 200 IU/day for the first week and with 100 IU/day for the following 4 weeks. Mice in the control groups were injected with PBS. To inhibit IDO activity, slow-releasing 1-MT (10 mg/day) or placebo pellets were implanted under the dorsal skin of recipient mice. The onset of type 1 diabetes was monitored twice per week by measuring glucose levels in both blood and urine. Mice were considered diabetic once blood glucose was >12 mmol/l.

RT-PCR and Western blot. Total RNA was extracted using TRIzol reagent (Invitrogen, CA); 2 μg of RNA from each sample was used for reverse transcription, and PCR was conducted as previously described (13). Sequences of the PCR primers for IDO mRNA were: 5′AGCTTCGAGAAGTT GAAAAGC3′ and 5′ATTCCCAGAAGG-ACATCAAGACTC3′; for β-actin mRNA: 5′GTTACCAACTGGGACGACA3′ and 5′TGGCCATCTC-CTGCTCGAA3′; and for FasL mRNA: 5′ACACCTATGGAATTGTCCTGC3′ and 5′GACCAG-AGAGA GCTCAGATAC3′.

For Western blots, purified splenic CD11c$^+$ cells were cultured in granulocyte/macrophage colony-stimulating factor–containing (5 ng/ml) RPMI medium free of serum in the presence or absence of IFN-γ (1,000 IU/ml, Peprotech, NJ) or recombinant hCG at various concentrations for 24 h. Cells were collected, lysed, and subjected to 10% PAGE gel and immunobloting using an anti-IDO monoclonal antibody (Chemicon International, CA). The membranes were stripped and reblotted with an anti-Stat6 monoclonal antibody (Biotech, Santa Cruz, CA) to estimate the amount of protein in each sample.

Cell preparation and T-cell activation. Splenic DCs, T-cells, and CD4$^+$ and CD8$^+$ T-cells were isolated with antibody-conjugated microbeads (Miltenyi Biotec, Auburn, CA). Peptides P1040–51 and NRP-A7 were used as ligands for in vitro activation of BDC2.5-CD4$^+$ and 8.3-CD8$^+$ T-cells, respectively (23, 24). Splenocytes (2.5×10^6/ml) were cultured for 3 days with various concentrations of immobilized anti-CD3 antibody, and CD4$^+$ or CD8$^+$ T-cells (0.5×10^6/ml) were cultured with DCs (0.1×10^6/ml) for 3 days in the presence of various concentrations of ligands. [H^3]-thymidine incorporation was used to measure T-cell proliferation, and cytokine production was measured using a DuoSet kit (R&D System, Minneapolis, MN). 1-MT was first resolved in 0.5% Polysorbate-20 (Kirin Brewery, Japan) and then diluted into various concentrations in culture medium.

Reconstitution of NOD$^{tcr\alpha-/-}$ and NOD.scid mice. To reconstitute NOD$^{tcr\alpha-/-}$ mice, T-cells purified from control and hCG-injected NOD mice (200 IU/day for 1 week and 100 IU/day for an additional 4 weeks) were transferred into NOD$^{tcr\alpha-/-}$ mice aged 6–7 weeks [$10 \times (10^6$/recipient)]. The onset of type 1 diabetes was monitored twice a week. To reconstitute NOD.scid mice, splenocytes from control and hCG-injected (200 IU/day for 1 week and 100 IU/day for an additional 4 weeks) NOD mice were prepared and transferred into 7-week-old NOD.scid mice [$20 \times (10^6$/recipient)]. In some recipient mice, DC-depleted splenocytes from hCG-injected donor mice were transferred. The onset of type 1 diabetes was monitored twice a week by measuring blood glucose concentration as described above.

Statistical analysis. The results of the in vivo studies were analyzed with a Kaplan-Meier log-rank test, and a Welch *t* test was used for in vitro data.

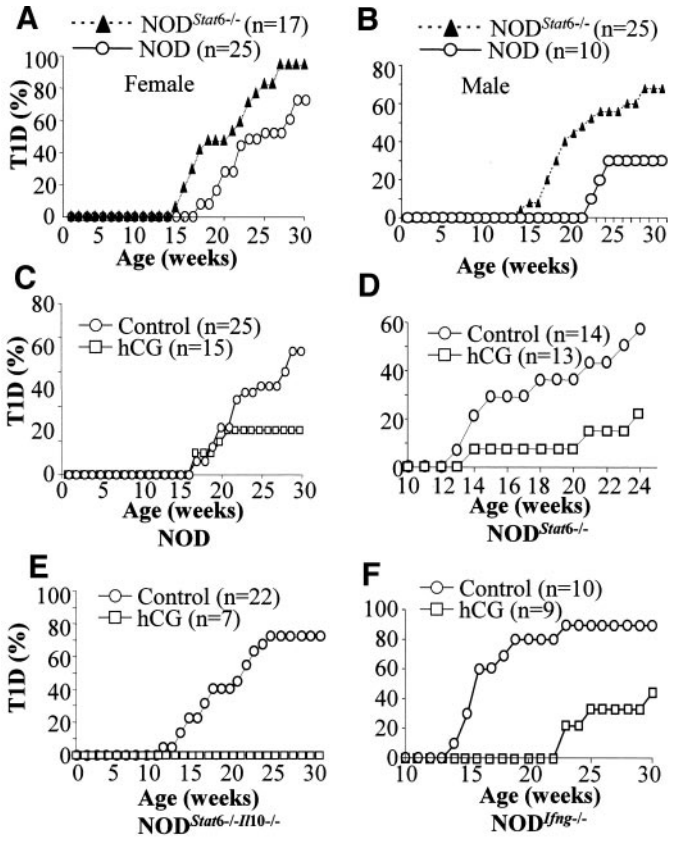

FIG. 1. Reduced type 1 diabetes incidence in hCG-treated NOD and cytokine gene–deficient NOD mice. *A* and *B*: Incidence of type 1 diabetes in female and male NOD and NOD$^{Stat6-/-}$ mice. *C–F*: Incidence of type 1 diabetes in control and hCG-injected female NOD mice (*C*), NOD$^{Stat6-/-}$ mice (*D*), NOD$^{Stat6-/-Il10-/-}$ mice (*E*), and NOD$^{Ifng-/-}$ mice (*F*). The differences in type 1 diabetes incidence between the groups of control and hCG-injected female NOD mice were statistically significant ($P < 0.05$).

RESULTS

Inhibition of type 1 diabetes in cytokine gene–deficient NOD mice by hCG administration. Previous work has suggested that administration of hCG protects NOD mice from type 1 diabetes by promoting the development of Th2-type CD4$^+$ T-cells (22). To determine the role of cytokines in this process, we intraperitoneally injected hCG into NOD and Stat6-deficient NOD mice. Stat6 is a signal transducer shared by two key Th2 cytokines, IL-4 and Il-13; thus, signals from IL-4 and IL-13 are both abolished in *Stat*6-gene deficient NOD (NOD$^{Stat6-/-}$) mice. Unlike IL-4 deficient NOD mice, which develop type 1 diabetes with a frequency and age at onset similar to NOD mice (25), both female and male NOD$^{Stat6-/-}$ mice exhibited a slightly accelerated form of type 1 diabetes (Fig. 1*A* and *B*), suggesting a modest protective effect of IL-4 and/or IL-13 against type 1 diabetes development. Injection of

hCG for 5 weeks (starting at 9 weeks of age) delayed and reduced the incidence of type 1 diabetes both in female NOD and NOD$^{Stat6-/-}$ mice (Fig. 1*C* and *D*). We also evaluated NOD$^{Stat6-/-Il10-/-}$ mice, which develop type 1 diabetes essentially like NOD$^{Stat6-/-}$ mice (data not shown). Again, hCG administration inhibited type 1 diabetes development in NOD$^{Stat6-/-Il10-/-}$ mice (Fig. 1*E*). Interestingly, hCG treatment also protected IFN-γ–deficient NOD (NOD$^{Ifng-/-}$) mice from type 1 diabetes development (Fig. 1*F*). These results indicate that neither Th1 nor Th2 cytokines plays a critical role in hCG-induced resistance to type 1 diabetes.

hCG administration inhibits T-cell responsiveness in vivo, but not in vitro. To ascertain whether hCG administration had any effect on T-cell function, we isolated CD4$^+$ T-cells from young NOD mice and activated these cells using immobilized anti-CD3/CD28 antibodies in the presence or absence of hCG (100 IU/ml) and in the absence of APCs. We found that the purified T-cells proliferated similarly and produced IFN-γ and IL-4 at similar levels in both the presence and absence of hCG (Fig. 2*A*), indicating that hCG has no direct effect on T-cell responsiveness. Similar results were obtained from CD4$^+$ T-cells purified from control and hCG-injected (200 IU/day for 5 days) NOD mice (data not shown).

To study the potential effects of hCG on APC function, we examined the expression levels of major histocompatibility complex (MHC) and costimulatory molecules on splenic CD11c$^+$ DCs isolated from control and hCG-injected NOD mice. In addition, purified splenic CD11c$^+$ DCs from NOD mice were cultured for 2 days in granulocyte/macrophage colony stimulating factor–containing medium in the presence or absence of hCG. No differences in the expression levels of MHC class I, MHC class II, CD40, CD80, or CD86 were noted (Fig. 2*B* and data not shown).

Although T-cells and DCs from hCG-treated mice were apparently normal, we found that total splenocytes from hCG-injected NOD mice (2 days, 100 IU/day) proliferated significantly less in response to immoblized anti-CD3 antibody than did those isolated from control NOD mice. The effects of hCG treatment on splenocyte responsiveness were identical in age-matched female and male NOD mice, suggesting that immune cells from both sexes were equally affected by hCG (Fig. 2*C*). We noted a strong upregulation of IDO gene expression in splenocytes from hCG-treated mice compared with splenocytes from control mice, which expressed barely detectable levels of IDO mRNA (Fig. 2*D*). The reduced T-cell responsiveness was more pronounced in NOD mice that had been treated with hCG for 5 days (Fig. 2*E*). The addition of an IDO-specific inhibitor, 1-MT, into the cultures did not affect the proliferative responsiveness of splenocytes from control NOD mice but did significantly increase the responsiveness of splenocytes from hCG-treated NOD mice (Fig. 2*F*). Because we could not document any obvious direct effects of hCG on purified T-cells, we reasoned that the reduced proliferation of anti-CD3–stimulated splenocytes from hCG-treated mice might be mediated by the upregulation of IDO.

IDO-expressing DCs in hCG-treated mice suppress T-cell responsiveness. To examine the effects of hCG on antigen-specific T-cell proliferation in vivo, we isolated CD4$^+$ T-cells from NOD-BDC2.5 transgenic mice, which preferentially proliferate in the pancreatic lymph nodes (PLN), but not in other lymph nodes on adoptive transfer into syngeneic hosts (3). Purified BDC2.5 CD4$^+$ T-cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and then transfused into NOD mice treated with hCG (100 IU/day) or PBS for 5 days. PLNs were removed from the recipient NOD mice 6 days after the transfer to analyze the extent of CFSE dilution in the transfused T-cells and the expression of IDO. The T-cells proliferated vigorously in the PLNs of PBS-treated NOD mice but proliferated only weakly in the PLNs of hCG-treated mice (Fig. 3*A*). High levels of IDO mRNA were detected in the PLNs of hCG-treated mice (Fig. 3*B*). To determine whether the reduced proliferation of BDC2.5 T-cells in the host PLNs was mediated by hCG-induced upregulation of IDO in vivo, slow-releasing 1-MT (10 mg/day) or placebo pellets were implanted under the dorsal skin of another cohort of NOD mice. Both groups of mice were transfused with CFSE-labeled BDC2.5 T-cells and then injected with hCG (100 IU/day) or PBS for 5 days. CFSE$^+$ T-cells proliferated similarly in the PLNs of PBS-treated control mice and hCG-treated mice implanted with 1-MT–containing pellets, indicating that implantation of 1-MT pellets abolished the effect of hCG. By contrast, far fewer BDC2.5 T-cells proliferated in the PLN of hCG-treated mice that had received a placebo implant (Fig. 3*C*). On average, hCG administration inhibited the proliferation of BDC2.5 T-cells by more than 40% in the mice that received placebo pellets but by less than 10% in mice implanted with 1-MT pellets (Fig. 3*D*).

The above data suggested that inhibition of antigen-specific T-cell proliferation by hCG treatment might be mediated by IDO-expressing DCs (6). To investigate this, we isolated splenic CD11c$^+$ DCs from control and hCG-treated NOD mice and used them as APCs to activate BDC2.5 T-cells in the presence of a peptide ligand, p1040–51 (24). DCs from both types of mice were able to present the antigen to T-cells; however, BDC2.5 T-cell proliferation was much lower with DCs from hCG-treated mice than with those isolated from control mice. As expected, the addition of 1-MT significantly increased T-cell proliferation (Fig. 3*E*).

We also isolated CD11c$^+$ splenic DCs from NOD mice treated with hCG (200 IU/day for 5 days) and implanted with slow-releasing 1-MT or placebo pellets and used these DCs as APCs to present peptide antigens NRP-A7 to CD8$^+$ T-cells from 8.3-NOD mice (23). The proliferation of 8.3-CD8$^+$ T-cells was significantly lower in cultures using DCs from hCG-placebo treated NOD mice than in cultures using DCs from hCG-1-MT treated mice (Fig. 3*F*). To further assess the IDO activity expressed by DCs, NOD and 8.3 T-cells were activated by immobilized anti-CD3 or peptide antigen NRP-A7 in the presence of DCs from control or hCG-treated mice, with/without excess tryptophan. DCs from hCG-treated mice significantly reduced proliferation of the activated T-cells, while the addition of excess tryptophan restored the reduced proliferation (Fig. 3*G*). Together, these results indicate that the immunesuppressive effects of hCG-treatment on T-cell proliferation are mediated by IDO-expressing DCs.

The induction of IDO by hCG in DCs is transient. We next investigated the influence of hCG on IDO expression in DCs. Splenic CD11c$^+$ DCs were isolated from 8-week-old NOD mice and cultured in the presence or absence of various concentrations of recombinant hCG to determine whether hCG directly induced IDO expression in DCs. Splenic CD11c$^+$ DCs were also cultured with IFN-γ, a strong inducer of IDO gene expression (6). Indeed, both IFN-γ (1,000 IU/ml) and hCG (200 and 400 IU/ml) upregu-

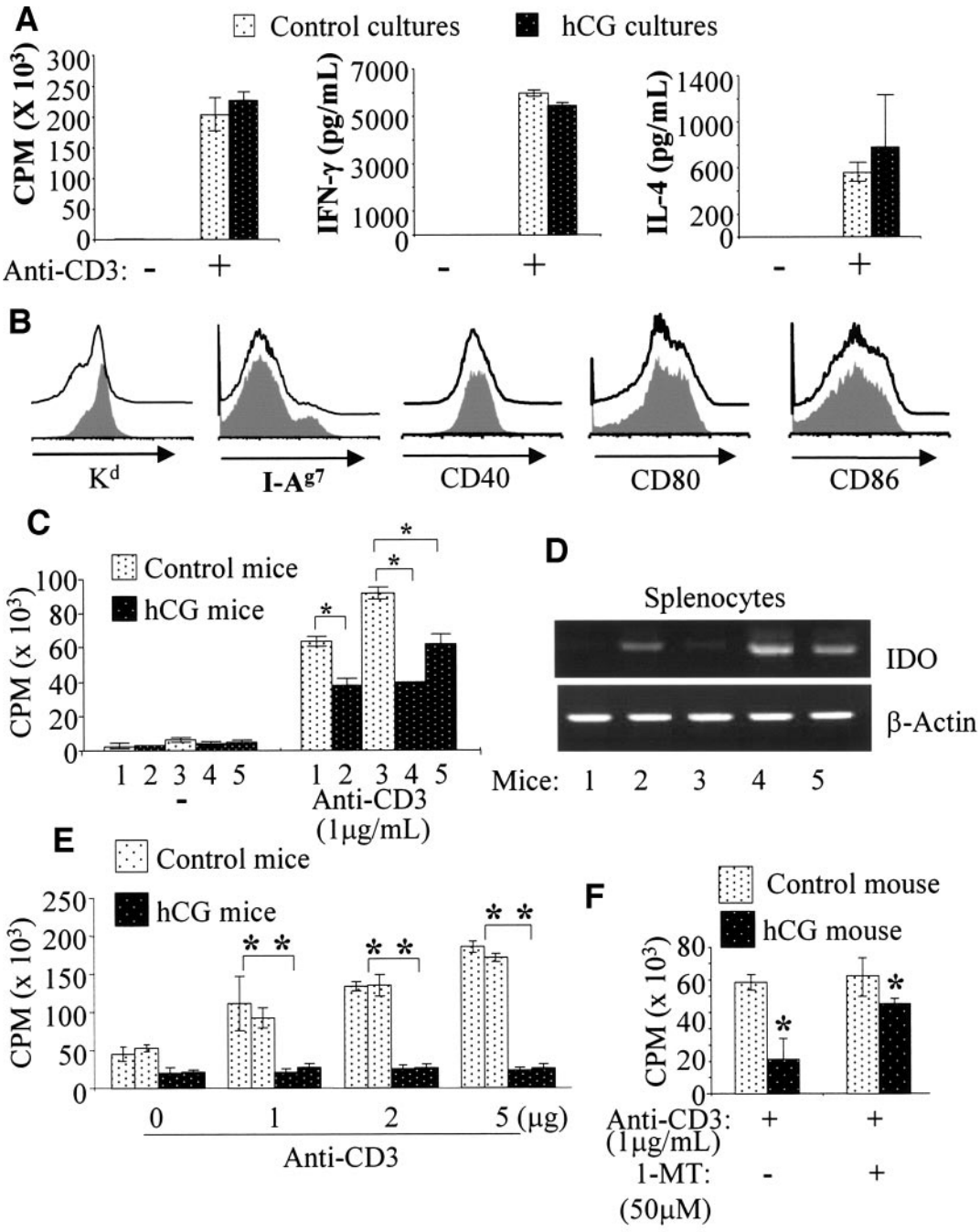

FIG. 2. Suppressed T-cell activation in splenocytes from hCG-treated NOD mice. *A*: Proliferation and cytokine production of CD4$^+$ T-cells activated by immobilized anti-CD3 antibody (5 μg/ml) and anti-CD28 antibody (2 μg/ml) in the absence or presence of hCG (200 IU/ml) for 3 days. *B*: The expression of MHC and co-stimulatory molecules on splenic CD11c$^+$ DCs from control (shade) and hCG-injected (200 IU/day for 5 days; lines) NOD mice. Representative results from two independent experiments are shown. *C*: Proliferation of splenocytes from control and hCG-treated (100 IU/day for 2 days) female and male NOD mice (*$P < 0.05$). *Columns 1* and *2* were female and 3–5 were male NOD mice. *D*: RT-PCR profile of splenocytes from control and hCG-injected NOD mice as described in (*C*). *E*: Proliferation of splenocytes from control NOD mice and hCG-injected (200 IU/day for 5 days) NOD mice (columns represented individual mice; **$P < 0.01$). Splenocytes were stimulated by anti-CD3 antibody at the indicated concentrations for 72 h. *F*: Proliferation of splenocytes from control and hCG-injected mice in the presence or absence of 1-MT. Representative results from at least three independent experiments are shown.

lated IDO mRNA and protein expression in DCs (Fig. 4*A* and *B*). Notably, the hCG-induced upregulation of IDO also occurred in CD11c$^+$ DCs from NOD$^{Ifng-/-}$ mice, indicating that this upregulation was independent of IFN-γ (Fig. 4*C*).

We then isolated CD11c$^+$ DCs from NOD mice at different time points after a single hCG or mock injection and found that DCs also expressed IDO in vivo within 24 h of hCG treatment (Fig. 4*D*). The induction of IDO was hCG dose dependent and, curiously, disappeared at very high doses of hCG (>400 U/mouse) (Fig. 4*E*). The expression of two other genes, *FasL* and β-*actin*, in DCs from hCG-injected mice was similar to that seen in PBS-treated animals (Fig. 4*D* and *E*), despite the fact that hCG has been reported to enhance *FasL* expression in decidual cells during early pregnancy (26). Importantly, IDO induction in DCs by hCG was transient, reverting to background levels within 48 h of hCG withdrawal (Fig. 4*D*); DCs from hCG-injected B6 mice also expressed IDO within 48 h (Fig.

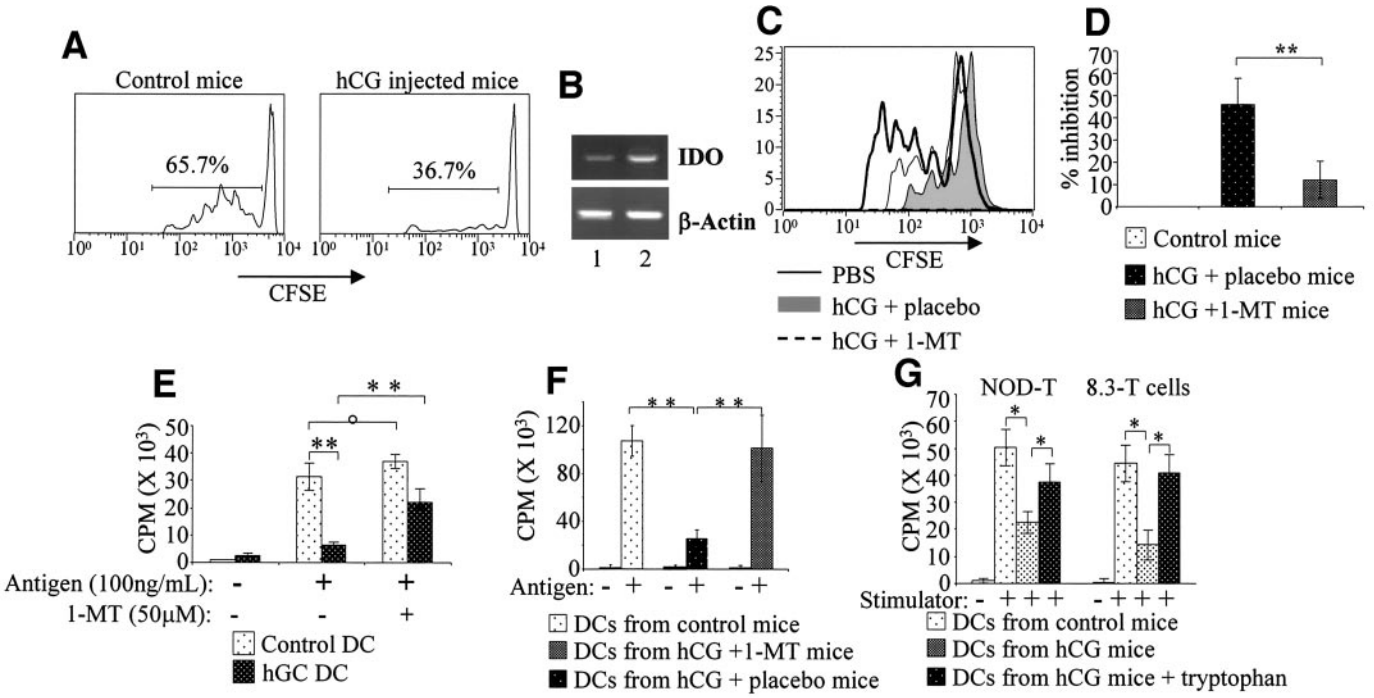

FIG. 3. DC-mediated suppression of antigenic responses of islet-specific T-cells in hCG-treated NOD mice. *A*: Representative profiles of CFSE-labeled CD4$^+$ BDC2.5 T-cells in PLN from control and hCG-injected NOD mice (4–5 mice/group). *B*: RT-PCR profile of PLN cells from control (*lane 1*) and hCG-injected (*lane 2*) mice. *C*: Representative proliferation profiles of BDC2.5 T-cells in control or hCG-injected recipient NOD mice implanted with 1-MT or placebo pellets (five to six mice per group). *D*: Inhibitory rates of in vivo proliferation of BDC2.5 T-cells in different groups of recipient NOD mice ($P < 0.01$). *E*: Proliferation of BDC2.5 T-cells that were cultured with splenic CD11c$^+$ DCs from control and hCG-injected (100 IU/day for 2 days) NOD mice in the presence or absence of peptide ligand (p1040–51). The IDO inhibitor 1-MT was added into cultures as indicated (**$P < 0.01$; °$P > 0.1$). *F*: Proliferation of 8.3 T-cells cultured in the presence or absence of peptide ligand (NRP-A7) presented by splenic CD11c$^+$ DCs from control and hCG-injected (100 IU/day for 5 days) NOD mice that carried slow-releasing 1-MT (10 mg/day) or placebo pellets (**$P < 0.01$). *G*: Proliferation of NOD T-cells and 8.3 T-cells activated by immobilized anti-CD3 (2 μg/ml), or peptide NRP-A7 (100 ng/ml for 8.3 T-cells), in the presence of DCs from control or hCG-treated mice. Excess tryptophan (10 μmol/l) was added into the cultures as indicated (*$P < 0.03$).**

4*F*), indicating that the hCG-inducted transient expression of IDO in DCs is not specific for NOD mice.

The antidiabetogenic effect of hCG treatment is reversible. To test whether hCG administration irreversibly reduced the pathogenic potential of autoreactive T-cells, we isolated T-cells from control and hCG-treated NOD mice and transfused these T-cells into TCR (T-cell receptor)α–gene-deficient NOD (NOD$^{tcr\alpha-/-}$) mice, which are T-cell deficient and, hence, are type 1 diabetes–free. Reconstitution of NOD$^{tcr\alpha-/-}$ mice with purified T-cells from NOD mice induced type 1 diabetes within 20 weeks. T-cells from NOD mice that had been treated with hCG for 5 weeks also induced type 1 diabetes in NOD$^{tcr\alpha-/-}$ mice and did so even more rapidly than did T-cells from control NOD mice (Fig. 5*A*). Thus, hCG treatment did not eliminate islet-specific autoreactive T-cells from the peripheral T-cell repertoire, and these T-cells retained their diabetogenic potential. These results also suggested that hCG administration did not enhance the development of regulatory subsets of T-cells and that diabetogenic T-cells released from a suppressive environment in hCG-treated NOD mice expanded more aggressively in NOD$^{tcr\alpha-/-}$ recipients. We then transferred splenocytes from control and hCG-treated female NOD mice into NOD.scid recipients to assess a potential role of splenic DCs in type 1 diabetes inhibition by hCG. All mice that received splenocytes from control donors developed type 1 diabetes within 10 weeks of transfer. In contrast, recipients of splenocytes from hCG-treated NOD mice developed delayed type 1 diabetes with a slightly reduced incidence (Fig. 5*B*). Depletion of DCs from splenocytes of hCG-treated NOD mice before cell transfer rapidly induced type 1 diabetes in the NOD.scid recipient mice (Fig. 5*B*), supporting the assertion that DCs mediated, though transiently, the diabetes resistance afforded by hCG treatment.

Last, we used slow-releasing 1-MT pellets (10 mg/day for 35 days) to inhibit IDO activity in NOD mice throughout the entire period of hCG administration. As expected, type 1 diabetes development in hCG-treated NOD mice carrying placebo pellets was delayed and reduced in frequency. In contrast, type 1 diabetes incidence was restored to almost normal levels in hCG-treated NOD mice carrying 1-MT pellets (Fig. 5*C*), providing direct evidence that IDO induction was responsible for the hCG-induced resistance to type 1 diabetes.

DISCUSSION

In the present study, we demonstrate that hCG upregulates IDO in DCs and that this accounts for hCG's ability to suppress autoimmune diabetes in NOD mice. In the presence of 1-MT, an IDO inhibitor, hCG could no longer do so, suggesting a direct link between the hormone and the regulator. IDO expression in the islet cells has been shown to be able to prolong the survival of these cells when they were transplanted into diabetic NOD mice (27). It is likely that IDO expression in islet cells protects these cells from local inflammatory responses. However, because of the critical role of DCs in initiation and maintaining cascades responses of autoimmunity in type 1 diabetes, IDO expres-

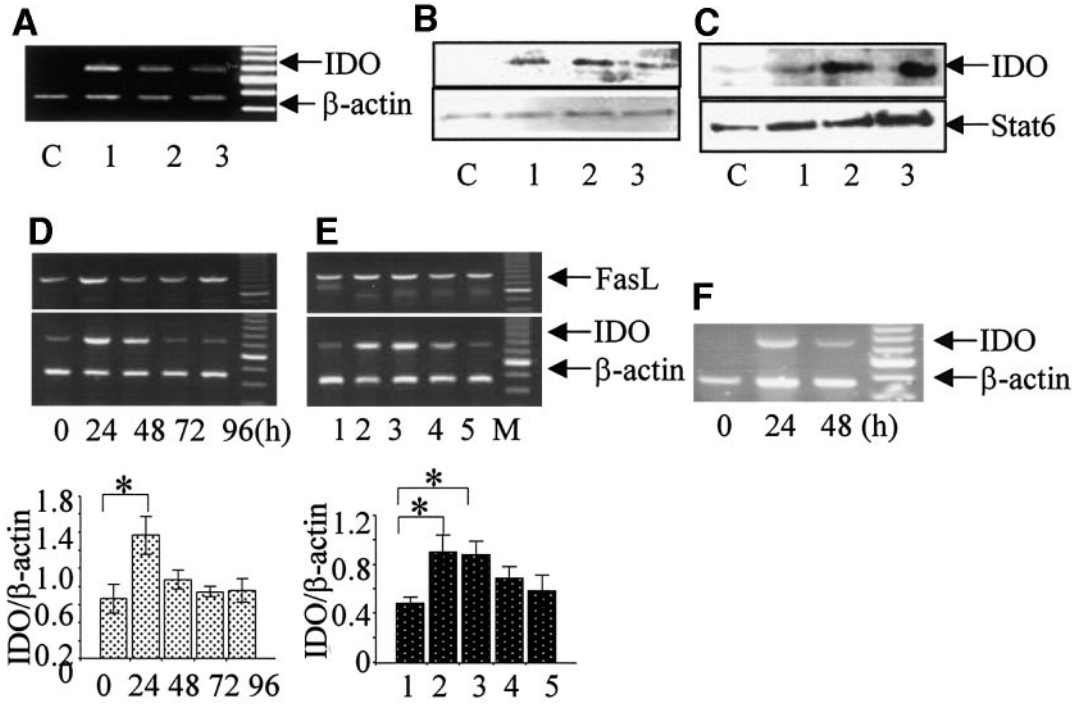

FIG. 4. IFN-γ–independent induction of IDO in DCs by hCG. *A*: IDO and β-actin gene expression in splenic CD11c$^+$ DCs from NOD mice cultured with IFN-γ (1,000 IU/ml; *lane 1*) or recombinant hCG (200 and 400 IU/ml; *lanes 2* and *3*, respectively) for 24 h, as well as from control DCs (*lane C*). *B*: Immunoblotting profile of IDO and Stat6 protein expression in CD11c$^+$ DCs that were cultured with/without IFN-γ (*lane 1*) or recombinant hCG (200 and 400 IU/ml; *lanes 2* and *3*, respectively) for 24 h, as well as in control DCs (*lane C*). *C*: Immunoblotting profile of IDO and Stat6 protein expression in CD11c$^+$ DCs from IFN-γ–deficient NOD mice 24 h after cultures with IFN-γ (*lane 1*) or hCG (200 and 400 IU/ml; *lanes 2* and *3*, respectively). *D*: IDO gene expression in splenic CD11c$^+$ DCs 24, 48, 72, and 96 h after hCG injection. Representative results, and densitometry analysis of the ratio between IDO/β-actin gene expression at different time points (*$P < 0.05$), from three independent experiments are shown. *E*: RT-PCR profile of IDO gene expression in DCs from NOD mice 24 h after injection of PBS (*lane 1*), 100 IU (*lane 2*), 200 IU (*lane 3*), 400 IU (*lane 4*) or 1,000 IU (*lane 5*) of hCG. Representative results, and densitometry analysis of the ratio between IDO/β-actin gene expression with different hCG dosages (*$P < 0.05$), from two similar experiments are shown. *F*: IDO gene expression in splenic CD11c$^+$ DCs from B6 mice 0, 24, and 48 h (200 IU) after hCG injection.

sion in DCs may be more effective for type 1 diabetes prevention. Treatment with the tryptophan catabolites *N*-(3,4-dimethoxycinnamoyl) anthralic acid (also generated by IDO) suppressed T-cell activation and Th1 cytokine production and inhibited EAE in mice (9). Although the APC function of DCs was also suppressed by high doses of 3,4-DAA, the inhibition of EAE was associated with the generation of IL-10–producing, central nervous system antigen-specific T-cells (9), suggesting a role for regulatory T-cells (Tregs) or certain cytokines in 3,4-DAA–mediated suppression of autoimmunity. In the present study, we did not find evidence supporting a role for Tregs or Th1/Th2 cytokines in the antidiabetogenic effect of hCG as suggested by previous studies (9,22). Efficient protection of Stat6-, IL-10–, and IFN-γ–deficient NOD mice from onset of type 1 diabetes by hCG treatment suggests that neither Th1- nor Th2-type cytokines are required for this process. Furthermore, T-cells from hCG-treated NOD mice could rapidly transfer type 1 diabetes into immunocompromised NOD hosts if the DCs were removed from the inoculum, suggesting a Treg-independent role for DCs as mediators of immunosuppression. Because the hCG concentrations used in this study were within the physiological range, the treatment might not result in a large amount of tryptophan catabolites, which itself can lead to sustained suppression of T-cell and APC functions. However, our results indicate that even a mild and temporary upregulation of functional IDO in DCs could efficiently

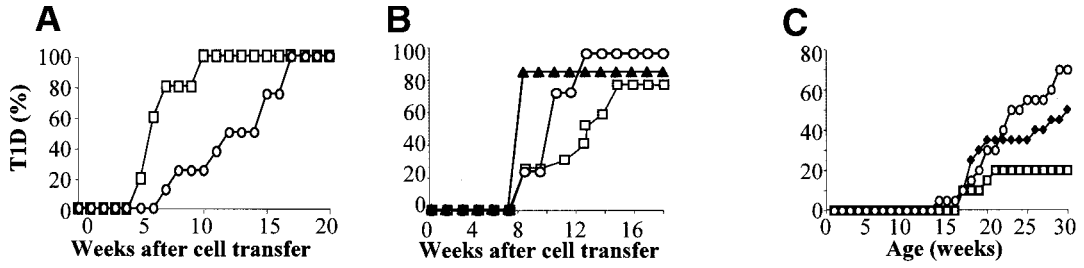

FIG. 5. Reversible effects of hCG treatment on type 1 diabetes pathogenesis. *A*: Type 1 diabetes development in the NOD$^{tcr\alpha-/-}$ mice reconstituted with T-cells purified from control (○, $n = 8$) and hCG-injected (□, $n = 5$) NOD mice. *B*: Type 1 diabetes development in NOD.scid mice that received splenocytes from control (○, $n = 9$) and hCG-injected (□, $n = 9$) NOD mice. ▲, DCs depleted hCG splenocytes ($n = 8$). *C*: Type 1 diabetes development in hCG-injected NOD mice carrying slow-releasing pellets of the IDO inhibitor, 1-MT (♦, $n = 20$), or placebo (□, $n = 20$). The differences in type 1 diabetes incidence between control (○) and placebo groups, or between 1-MT and placebo groups, were statistically significant ($P < 0.05$).

downregulate autoimmune responses and halt diabetogenesis.

IFN-γ induces IDO expression in $CD8^+$ DC subsets, whereas type I interferon induced IDO in $CD19^+$ DCs (6). Whether hCG has different effects on subsets of DCs remains to be defined; our results clearly show that hCG induces IDO expression though an IFN-γ–independent pathway. Because expression of IDO in the placenta during pregnancy is largely IFN-γ independent (12), our results raise the possibility that hCG is responsible, at least in part, for inducing the expression of IDO in both the placenta and maternal DCs. Maternal tolerance is a transient and reversible condition caused by several poorly defined mechanisms (28). For example, pregnancy has been associated with increased numbers of Tregs in the decidua and circulation, suggesting that this T-cell subset contributes to maternal tolerance (29). However, factors that drive the expansion and decline in the number of these Treg cells during and after gestation remain unknown. Although a dilution of fetal antigens on delivery presumably contributes to the downregulation of fetal antigen-specific immune regulation in the mother, other mechanisms must be at play to explain the restoration of systemic immune competence that occurs in the postpartum period. Because hCG disappears from the circulation shortly after delivery (30), we propose that the short-lived, systemic induction of IDO in DCs by hCG described herein accounts for the transient nature of maternal tolerance. In addition to fetal antigen-specific maternal tolerance, several types of autoimmune disorders, primarily T-cell–dependent autoimmune disorders such as rheumatoid arthritis (31) and multiple sclerosis (32), often undergo remission during pregnancy only to relapse in the 1st trimester postpartum. This pregnancy-associated resistance to autoimmunity appears to occur systemically in a nonantigen-specific fashion, consistent with the effects of the upregulation of IDO in DCs demonstrated herein. We thus further propose that hCG affords the mother protection from autoimmunity.

The physiological relevance of IDO induction by hCG in both the placenta and periphery remains to be examined in humans. However, consistent with the systemic immunosuppressive effects of hCG shown in this study, hCG therapy has been shown to cause a transient immunosuppressive state in patients (33). In addition to the placenta, several different types of cancer cells express hCG, and appearance of anti-hCG antibodies upon vaccination was associated with improved survival (34). Furthermore, IDO-expressing DCs that suppress antitumor responses have been found in lymph nodes draining to certain types of tumors in mice (35). It is tempting to speculate, in light of the studies presented here, that hCG expression helps certain tumors evade immune surveillance through upregulation of IDO in DCs.

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