The Journal of Immunology

RESEARCH ARTICLE | DECEMBER 01 2004

IFNβ Accelerates Autoimmune Type 1 Diabetes in Nonobese Diabetic Mice and Breaks the Tolerance to β Cells in Nondiabetes-Prone Mice[1]

Aurora Alba; ... et. al

J Immunol (2004) 173 (11): 6667–6675.
https://doi.org/10.4049/jimmunol.173.11.6667

IFNβ Accelerates Autoimmune Type 1 Diabetes in Nonobese Diabetic Mice and Breaks the Tolerance to β Cells in Nondiabetes-Prone Mice[1]

Aurora Alba,* M. Carmen Puertas,* Jorge Carrillo,* Raquel Planas,* Rosa Ampudia,* Xavier Pastor,* Fatima Bosch,† Ricardo Pujol-Borrell,* Joan Verdaguer,* and Marta Vives-Pi[2]*

Genetic and environmental factors are decisive in the etiology of type 1 diabetes. Viruses have been proposed as a triggering environmental event and some evidences have been reported: type I IFNs exist in the pancreata of diabetic patients and transgenic mice expressing these cytokines in β cells develop diabetes. To determine the role of IFNβ in diabetes, we studied transgenic mice expressing human IFNβ in the β cells. Autoimmune features were found: MHC class I islet hyperexpression, T and B cells infiltrating the islets and transfer of the disease by lymphocytes. Moreover, the expression of β_2-microglobulin, preproinsulin, and glucagon in the thymus was not altered by IFNβ, thus suggesting that the disease is caused by a local effect of IFNβ, strong enough to break the peripheral tolerance to β cells. This is the first report of the generation of NOD (a model of spontaneous autoimmune diabetes) and nonobese-resistant (its homologous resistant) transgenic mice expressing a type I IFN in the islets: transgenic NOD and nonobese-resistant mice developed accelerated autoimmune diabetes with a high incidence of the disease. These results indicate that the antiviral cytokine IFNβ breaks peripheral tolerance to β cells, influences the insulitis progression and contributes to autoimmunity in diabetes and nondiabetes- prone mice. *The Journal of Immunology,* **2004, 173: 6667–6675.**

Type 1 diabetes (T1D)[3] is an autoimmune disease of unknown etiology caused by the selective destruction of pancreatic β cells as a consequence of the complex interaction between genetic and environmental factors (1). Genetic susceptibility to the development of the disease has been described. At the present, more than 20 putative diabetes predisposing genes have been identified in the mouse and human genome, but only MHC genes are related to the disease (2–4). Studies on identical twins showed a concordance rate of diabetes lower than 50%, suggesting an important role for the environmental factors, e.g., viral infections (5). Viruses might be involved in the pathogenesis of the disease through exposure to sequestered Ags released by damaged β cells, by altering some mechanisms of peripheral tolerance, by molecular mimicry, or by a direct destruction of the insulin-producing cells (6, 7).

Type I IFNs (IFNα, β, ϵ, κ, λ, and ω) provide the first line of defense against viral infection but their mechanisms of action are only partially understood. Among other effects, type I IFNs increase the expression of MHC class I molecules and induce the activity of NK and CTLs and the production of cytokines. For years, an abnormal expression of type I IFNs has been reported in the sera of patients affected with autoimmune diseases (8). In previous studies, other scientists and we have detected type I IFNs in the pancreata of patients with a recent onset of diabetes (9–11). The expression of type I IFNs, IFNα and IFNκ, by the β cells induces autoimmune diabetes in nondiabetes-prone transgenic mice (12, 13). These mice developed insulitis and hypoinsulinemic diabetes with a cumulative incidence of ~50%. Transgenic mice expressing human IFNβ under the control of rat insulin promoter (RIP-HuIFNβ) were created (14) to avoid the sterility observed in mice expressing high levels of mouse IFNβ (15). HuIFNβ is active in mouse cells although its efficacy is ~1000 times lower than that of mouse IFNβ (14). The transgenic mice in a C57BL6/SJL background showed features of a prediabetic state, with peri-insulitis but without spontaneous diabetes. When these mice were backcrossed to the outbred albino CD-1 mice, they spontaneously developed diabetes with an incidence of 11%. Since type I IFN induction takes place in cells infected mainly by viruses, this model suggests that the initial β cell damage may induce the production of IFNβ by β cells, thus triggering inflammation and cell-mediated autoimmunity.

Because IFNs act as both antiviral factors and inflammatory mediators, the aim of this study was to characterize the immunology of diabetes in the RIP-HuIFNβ transgenic mice and to evaluate the role of IFNβ in animal models with different susceptibility to diabetes: a spontaneous model of diabetes, the NOD mice (16) and a diabetes-free, MHC-matched, homologous strain, the Nonobese-resistant mice (NOR) (17). NOR/LtJ is an insulitis-resistant

*Laboratory of Immunobiology for Research and Diagnostic Applications, Transfusion Center and Tissue Bank, Germans Trias i Pujol University Hospital, Barcelona, Spain; and †Biochemistry Department, Faculty of Veterinary, Autonomous University of Barcelona, Barcelona, Spain

Received for publication March 15, 2004. Accepted for publication September 24, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the Fondo de Investigaciones Sanitarias (Projects 99/1066 and 02/0107 to M.V.-P) and Fundació La Marató de TV3 (Project 99/1810 to M.V.-P.), and Ministry of Science and Technology (Project SAF 2000-050), CICYT to R.P.-B. A.A. was supported by a fellowship from the Instituto de Salud Carlos III (BEFI 01/9065) and J.V. and M.V.-P. are associate researchers supported by Fondo de Investigacion Sanitaria, Spanish Ministry of Health. M.-C.P. was supported by a Comissio Interdepartamental de Recerca I Innovacio Tecnologica Predoctoral Fellowship (2001FI0002) Generalitat de Catalunya, Barcelona, Spain.

[2] Address correspondence and reprint requests to Dr. Marta Vives-Pi, Laboratory of Immunobiology for Research and Diagnostic Applications, Transfusion Centre and Tissue Bank, Germans Trias i Pujol University Hospital, 08916 Badalona, Barcelona, Spain. E-mail address: vivespi@ns.hugtip.scs.es

[3] Abbreviations used in this paper: T1D, type 1 diabetes; RIP, rat insulin promoter; HuIFNβ, human IFNβ; NOR, nonobese resistant; β_2m, β_2-microglobulin; ICA, islet cell Ab.

0022-1767/04/$02.00

and diabetes-free strain where limited regions of the NOD/LtJ genome have been replaced by genome from the C57BLKS/J strain. In this study, we provide evidence that diabetes in RIP-HuIFNβ transgenic mice is autoimmune. The expression of this cytokine in β cells accelerates diabetes in NOD mice and breaks the tolerance to insulin-producing cells in NOR mice. This type I IFN is a functional mediator for the development of autoimmunity to β cells.

Materials and Methods

Mice

Transgenic mice expressing HuIFNβ under the control of the rat insulin I promoter (18) in outbred albino CD-1 background (CD-1 RIP-HuIFNβ) were generated by backcrossing the original C57BL6/SJL RIP-HuIFNβ transgenic mice to CD-1 mice (14). NOD mice, NOR mice, and NOD-SCID mice, unable to produce mature T and B lymphocytes (19), were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were kept in our facility under specific pathogen-free conditions in a 12-h light-dark cycle with free access to a standard diet. The Guidelines for the Use and Care of Laboratory Animals of the Generalitat de Catalunya were followed and animal studies have been approved by the Hospital Germans Trias i Pujol Review Board. To generate inbred NOD RIP-HuIFNβ and NOR RIP-HuIFNβ, the CD-1 RIP-HuIFNβ outbred mice were backcrossed at least six times onto the NOD or NOR strains. The acquisition of the genetic background was controlled by the analysis of microsatellites. NOD RIP-HuIFNβ mice were backcrossed twice onto the NOD-SCID strain to generate NOD-SCID RIP-HuIFNβ. Nontransgenic littermates were used as controls for each strain of mice.

Genotyping

The presence of the transgene was determined by standard PCR using genomic DNA obtained from mice tails and specific primers to HuIFNβ: HuIFNβ sense (5′-TCACCAGGGGAAAACTC-3′) and HuIFNβ antisense (5′-CAGTCACTTAAACAGCATCT-3′). The PCR products were resolved by 2% agarose gel and ethidium bromide staining. The genotyping for the microsatellite markers linked to *Idd* loci (*Idd1–Idd15*) was performed using genomic DNA from the fourth generation of mice from the aforementioned NOD and NOR backcrosses (20, 21), with a set of 11 primers specific for polymorphic loci between NOD/CD-1 and NOR/CD-1: *D6Mit52*, *D3Nds36*, *D2Mit107*, *D4Mit202*, *D1Mit24*, *D5Mit69*, *D11Mit320*, *D7Mit20*, *D7Nds6*, *D14Nds3*, and *D3Nds6*. The chosen markers were evenly spaced along the genome. PCR products were electrophoresed in 4% agarose gel and stained with ethidium bromide. Homozygous mice for Idd NOD or NOR alleles (including Idd1 for H-2g^7) were selected for the following backcross.

Insulin and glycemia assays

Blood glucose levels were measured weekly in CD-1 RIP-HuIFNβ mice and nontransgenic littermates between weeks 4 and 20 using Glucocard strips (Menarini, Barcelona, Spain). Mice with either successive blood glucose levels higher than 200 mg/dl or with a measure higher than 360 mg/dl were considered diabetic ($n = 10$ per condition). Starting at day 21, all CD-1, NOD, and NOR transgenic mice, after N6 backcross onto NOD or NOR background, were monitored daily for glycosuria using Chroma 1 Glucose test strips (Menarini). Insulin levels were determined in both CD-1 RIP-HuIFNβ mice and nontransgenic littermates. Nonfasting insulinemia was determined in sera and pancreatic insulin content was measured in total pancreas homogenized in HCl/ethanol by RIA (Linco Research, St. Charles, MO). The i.p. glucose tolerance test was performed in fasting conditions in control (NOR and NOD-SCID) and transgenic (NOR RIP-HuIFNβ and NOD-SCID RIP-HuIFNβ) adult mice. First, a blood sample was obtained from the tail vein to measure the basal level of glucose. Mice were subsequently given an i.p. injection of 2 mg of glucose per g of body weight. Blood glucose levels were measured at 15, 30, 60, 120, and 210 min after the injection.

Assessment of HuIFNβ in the sera of transgenic mice

The amount of HuIFNβ in the sera of CD-1 RIP-HuIFNβ transgenic mice was determined by ELISA (Fujirebio, Tokyo, Japan). The ELISA test showed no cross-reactivity with murine IFNβ (range of detection, 2.5–200 IU/ml).

Effect of HuIFNβ on the pancreas and the thymus expression profile

Real-time RT-PCR was performed to quantify the specific mRNA for preproinsulins I and II, glucagon and β_2-microglobulin (β_2m). Total RNA was extracted from pancreas, thymus, and liver from CD-1 RIP-HuIFNβ using TRIzol reagent (Invitrogen Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions. RNA was then DNase treated (DNA-free kit; Ambion, Houston, TX). After denaturation, 3 μg of total RNA was reverse transcribed in a 20-μl volume using a Promega Reverse Transcriptase kit (Promega, Southampton, U.K.) for 1 h at 42°C in a solution containing 20 U of RNAsin inhibitor (Promega), 4 μl of 5× reverse transcriptase buffer, 1 mM dNTPs, 5 μM oligo(dT), and 100 U of Moloney murine leukemia virus. Real-time PCR was performed in a Light Cycler (Roche Diagnostic, Mannheim, Germany), using the FastStart DNA Master SYBR Green kit and specific primers (Table I). The results were analyzed

Table I. *List of primers used for the detection of β-actin, preproinsulin I, preproinsulin II, HuIFNβ, β_2m, and glucagon in real-time RT-PCR and list of Abs used on frozen sections and cell suspensions*

Gene	Primer Sense	Primer Antisense	Amplimer Size (bp)
β-Actin	GTTACCAACTGGGACGACA	TGGCCATCTCCTGCTCGAA	459
Preproinsulin I	ACTTCCTACCCCTGCT	CCAAGGTCTGAAGGTCC	218
Preproinsulin II	CCCTGCTGGCCCTGCTCTT	CTGCTCCCGGGCCTCCAC	197
Glucagon	CAGACAGAAGCGCATG	TGGCCCTCCAAGTAAGA	233
HuIFNβ	TCACCAGGGGAAAACTC	CAGTCACTTAAACAGCATCT	238
β_2m	CCGGAGAATGGGAAGC	GTAGACGGTCTTGGGC	240

Ab/hibridoma	Marker	Source
M18/2	Mouse CD18	BD Pharmingen (San Jose, CA)
17A2	Mouse CD3	BD Pharmingen
RM4–5 FITC	Mouse CD4 (L3T4)	BD Pharmingen
53–6.7 PE	Mouse CD8a (Ly-2)	BD Pharmingen
1D3 PE	Mouse CD19	BD Pharmingen
GK1.5/TIB-207	Mouse CD4 (L3T4 Ag)	American Type Culture Collection (ATCC, Manassas, VA)
53–6.72/TIB-105	Mouse CD8 (Lyt-2)	ATCC
RA3–3A1/6.1/TIB-146	Mouse B220 (B cell Ag)	ATCC
F4/80/HB-198	Macrophages	ATCC
N418/HB-224	Mouse CD11c	ATCC
Dx5 PE	Mouse CD49b (NK cell Ag)	BD Pharmingen
N-19	Mouse CD49b (NK cell Ag)	Santa Cruz Biotechnology (Santa Cruz, CA)
M1/42.3.9.8.HLK/TIB-126	H-2 (nonpolymorphic MHC class I)	ATCC
NIMR-4	I-A (nonpolymorphic MHC class II)	Southern Biotechnology Associates
Sheep to human IFNβ	HuIFNβ	BioSource International (Camarillo, CA)
Guinea pig to insulin	Insulin	Biogenesis

using the LDCA software supplied with the machine. A PCR with β-actin-specific primers was performed as control. A standard curve was produced for β-actin, preproinsulins I and II, glucagon, HuIFNβ, and β_2m with serial dilutions of the corresponding quantified cDNAs using a densitometric method (Quantity One, Huntington Station, NY). The real-time PCR was performed with an annealing temperature of 65°C. The results were expressed as the ratio between the level of relevant gene expression and the level of β-actin expression.

Adoptive transfer of diabetes

Adoptive transfer experiments were performed transferring splenocytes or pancreatic-infiltrating cells from NOD RIP-HuIFNβ and NOR RIP-HuIFNβ diabetic mice into adult NOD-SCID mice. NOD-SCID mice were transferred only with splenocytes from NOR nontransgenic littermates because they are insulitis free. As positive control, splenocytes from wild-type NOD diabetic mice were injected in NOD-SCID mice. As negative control, NOD-SCID mice were sham transferred with physiological serum. Splenocytes or pancreatic-infiltrating cells from recent-onset diabetic NOD RIP-HuIFNβ mice were transferred into adult nondiabetic NOD RIP-HuIFNβ mice to assess the acceleration of the disease. The different transfer experiments were performed in a minimum of three recipients per condition ($n = 3–6$). To obtain splenocytes, a mechanically disrupted spleen was incubated in a hypotonic solution to remove erythrocytes. After washing, the cells were counted and injected into the tail vein. Pancreata from recent-onset diabetic animals were digested with collagenase P (Worthington Biochemical, Lakewood, NJ) and total digest was mechanically disrupted. Islet infiltrating lymphocytes were obtained by discontinuous density gradient using a lymphocyte isolation solution (Rafer, Barcelona, Spain). Lymphocytes were recovered from the interphase, washed twice, and transferred as above. In both cases, the cells to transfer were examined for viability by trypan blue exclusion and only the preparations with a viability >80% were transferred. Adult NOD-SCID males were injected i.v. with 2×10^7 splenocytes or 10^6 islet infiltrating cells resuspended in 200 μl of sterile physiological serum. After the transfer, the mice were monitored for assessment of diabetes every 2 days for 15 wk. Pancreata from mice that developed diabetes after the transfer were extracted and frozen for immunohistological analysis.

Autoantibodies

Five-micrometer mouse pancreatic cryosections were air dried and incubated with the sera from control (CD-1, NOD, and NOR nontransgenic littermates) and nondiabetic transgenic animals (CD-1 RIP-HuIFNβ, NOD RIP-HuIFNβ, and NOR RIP-HuIFNβ). The secondary Ab was a FITC-labeled goat anti-mouse Ig (Southern Biotechnology Associates, Birmingham, AL). All sections were blindly evaluated for the presence of autoantibodies to islet cells by two independent observers under an UV microscope and an image analyzer (OpenLab 2.0; Improvision, Coventry, U.K.).

Flow cytometric analysis

Splenocytes from transgenic mice (CD-1 RIP-HuIFNβ, NOD RIP-HuIFNβ, and NOR RIP-HuIFNβ) and nontransgenic littermates were isolated by mechanical disruption, and erythrocytes were lysed with a hypotonic solution. Cell clumps were allowed to sediment and the supernatant was centrifuged at $548 \times g$ for 5 min. Aliquots of 10^5 spleen cells were stained with specific Abs (Table I) conjugated to FITC and PE for 30 min to detect T CD4 and CD8 subsets, respectively. A PE-labeled CD19 Ab was used to stain B cells. The controls included unstained cells (autofluorescence control) and cells stained with an irrelevant isotype-matched control PE/FITC. Dead cells were excluded by propidium iodide staining. The analysis was conducted in a FACScan Cell Analyzer (BD Biosciences, San Jose, CA) and the data were analyzed using CellQuest software (BD Biosciences).

Insulitis development

To determine the degree of islet infiltration, pancreata from transgenic mice in different genetic backgrounds were snap frozen in an isopentane/cold acetone bath and stored at −70°C. Five-micrometer cryostat sections were obtained at five nonoverlapping levels. The sections were stained with H&E. Groups of mice ($n = 6$) were analyzed at different ages (6, 9, and 12 wk), assessing 40–100 islets per animal. Insulitis was scored on a 0–4 scale as described elsewhere (22).

Immunohistological analysis

Consecutive pancreatic cryostat sections (5 μm) from different mice were air dried as described above. To block nonspecific binding, 2% FCS was added to the PBS used to dilute the Abs. The sections were sequentially incubated with 1) Ab to specific marker (Table I), 2) FITC-labeled goat anti-rat IgGs (Southern Biotechnology Associates) or FITC-labeled rabbit anti-sheep FITC (Zymed Laboratories, San Francisco, CA), 3) guinea pig anti-insulin, and 4) tetramethylrhodamine isothiocyanate-labeled goat anti-guinea pig Ab (Biogenesis, Eschwege, Germany). The preparations were assessed with a fluorescence UV microscope and an image analyzer (OpenLab 2.0; Improvision).

Statistical analysis

Statistical analyses to compare independent groups were performed using the *t* test when groups passed normality and showed equal variance tests. When these tests failed, Mann-Whitney *U* test was performed. Differences were considered significant when a value of $p < 0.05$ was reached.

Results

Diabetes is not caused by a dysfunction of β cells in transgenic mice

To discard a reduced capacity to produce insulin by the β cells of transgenic mice, the glycemia, the insulinemia, and the pancreatic insulin content were determined in healthy CD-1 transgenic mice. No significant differences in glycemia were observed between CD-1 RIP-HuIFNβ mice and nontransgenic littermates at weeks 4–20 (Fig. 1*a*), except in animals progressing toward diabetes. The blood glucose concentration was 116.4 ± 6.4 mg/dl (mean ± SEM) in transgenic animals (133.2 ± 2.1, male and 96.7 ± 16, female, respectively) and 106.7 ± 8.7 mg/dl in the control group (120.6 ± 2.4, male and 94.4 ± 1.9, female, respectively). Two weeks before the clinical onset of the disease, diabetic-prone transgenic animals showed progressively increasing blood glucose levels. To rule out the possibility that β cell destruction was caused by the deleterious effects of HuIFNβ, we backcrossed NOD RIP-HuIFNβ mice onto the NOD-SCID strain twice. No significant differences were observed in glycemia between NOD-SCID RIP-HuIFNβ mice (119.4 ± 2.4 mg/dl, mean ± SEM) and nontransgenic littermates (123.9 ± 3.7 mg/dl). In addition, to assess whether the transgene could influence β cell metabolism, we tested the response of transgenic (NOD-SCID and NOR) and control animals to an acute increase of blood glucose levels (Fig. 1*b*). Glycemia of transgenic and control animals reached the highest value 15 min after glucose administration and decreased to basal values at 120 min in all groups. No significant differences were found between transgenic and control mice at any time, demonstrating that insulin production and secretion was unaffected by the transgene.

The pancreatic insulin content was normal in CD-1 RIP-HuIFNβ (156.9 ± 13.7 μg of insulin/g of pancreas, mean ± SEM) when compared with that of the controls (162.2 ± 23.8 μg of insulin/g of pancreas) except in recent-onset diabetic subjects ($p < 0.05$; Fig. 1*c*). Insulinemia was not altered in CD-1 transgenic animals (1.9 ± 0.3 ng/ml) compared with controls (1.3 ± 0.2 ng/ml) and, as expected, diabetic mice showed a significant decrease in the insulin levels in serum (0.3 ± 0.04 ng/ml, $p < 0.05$; Fig. 1*d*). In addition, no significant differences were observed in the levels of preproinsulin or glucagon RNA when compared to transgenic mice and nontransgenic littermates (see below). Apoptotic cells (TUNEL) were not detected in the noninfiltrated islets of healthy mice (data not shown). All of these data suggest that the development of diabetes is not a consequence of β cell dysfunction caused by the effect of the transgene or its product.

HuIFNβ affects the islets but not the thymic environment

HuIFNβ was detected in the sera from CD-1 RIP-HuIFNβ transgenic animals. The amount of this cytokine in sera was 49.2 ± 12.7 IU/ml in transgenic animals, statistically different from 5.6 ±

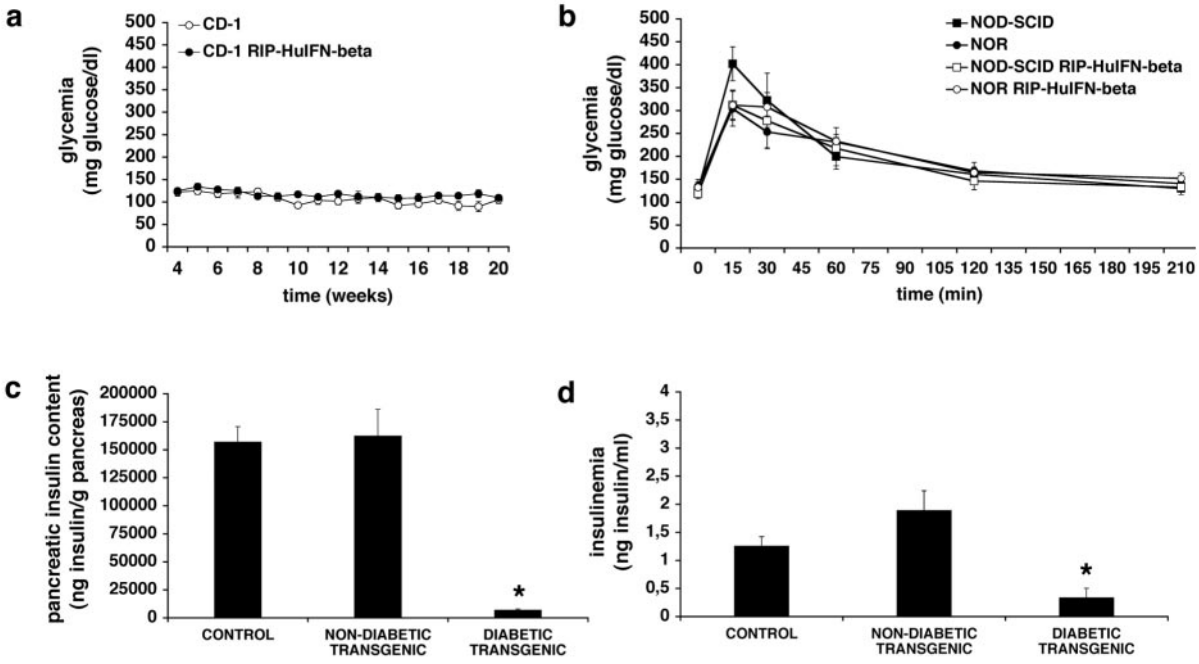

FIGURE 1. RIP-HuIFNβ transgenic mice developed hypoinsulinemic diabetes, but it is not caused by a dysfunction of β cells in transgenic mice. *a,* No significant differences in glycemia (mg/dl) in nonfasting conditions (mean ± SEM) were observed between CD-1 RIP-HuIFNβ mice and nontransgenic littermates. *b,* Intraperitoneal glucose tolerance test was not altered in transgenic mice. Glycemia (mg/dl, mean ± SEM) after i.p. administration of glucose in fasting conditions in control (NOR and NOD-SCID) and transgenic (NOR RIP-HuIFNβ and NOD-SCID RIP-HuIFNβ) adult mice. Glycemia of transgenic and control animals returned to basal levels 120 min after glucose administration. No significant differences were found when compared groups of transgenic and control mice. *c,* Pancreatic insulin content decreased only in diabetic mice. Insulin concentration (ng/g pancreas) in control and transgenic mice did not show significant differences. Diabetic transgenic mice decreased its pancreatic insulin levels ($p < 0.05$) compared with healthy groups. *d,* Insulinemia (ng/ml serum) is not altered by transgenic expression of HuIFNβ. Significant differences in insulin concentration were found between healthy and diabetic animals.

1.6 IU/ml in nontransgenic littermates ($p < 0.01$). These data indicate a systemic distribution of HuIFNβ.

To assess the effects of circulating HuIFNβ on the molecular pattern expression in the thymus (primary lymphoid organ) and in the liver (control tissue), we determined the mRNA levels for preproinsulins I and II, glucagon, and β_2m in CD-1 RIP-HuIFNβ transgenic mice. In addition to the presence of protein (HuIFNβ) in the sera, we confirmed the transcription of HuIFNβ mRNA in the pancreas and the thymus of transgenic mice (Table II). HuIFNβ did not alter the level of expression of preproinsulin I, which was only detected in the pancreas but not in the thymus or in the liver. However, preproinsulin II was detected in the pancreas, thymus, and liver but the differences between transgenic animals and controls were not significant, thus confirming that the transgene did not affect the transcription of insulin. Glucagon was detected in the pancreas and the thymus but not in the liver, and no significant differences were found when comparing transgenic and control animals. The amount of β_2m mRNA was higher in the pancreata of transgenic mice than in the control group ($p < 0.05$), showing an enhanced transcription of MHC class I, due to the effect of HuIFNβ (these data were confirmed by immunofluorescence staining of the islets with Abs to MHC class I). By contrast, no significant differences were found in the levels of β_2m in the thymus of transgenic and control mice. These data confirmed that the islets of Langerhans of transgenic animals have a normal expression profile of insulin I, insulin II, and glucagon and an increased MHC class I hyperexpression caused by the local release of HuIFNβ. Moreover, the expression of β_2m, preproinsulin, and glucagon in the thymus of transgenic mice was unaffected by the expression of the transgene. These results suggests that, in terms of

Table II. *Quantification of the expression of genes related to diabetes in the CD-1 RIP-HuIFNβ mice and controls (CD-1 nontransgenic littermates) by real-time RT-PCR*[a]

	Tissue					
	Thymus		Pancreas		Liver	
Gene Expression	CD-1 RIP-HuIFNβ	CD-1	CD-1 RIP-HuIFNβ	CD-1	CD-1 RIP-HuIFNβ	CD-1
Preproinsulin I	—	—	4.17 ± 1.32	3.81 ± 0.35	—	—
Preproinsulin II	0.1 ± 0.01	0.11 ± 0.008	10.67 ± 1.7	13.79 ± 2.29	0.52 ± 0.06	0.41 ± 0.1
Glucagon	0.01 ± 0.001	0.01 ± 0.002	1.99 ± 0.37	2.25 ± 0.57	—	—
HuIFNβ	0.001 ± 0.0002	—	0.09 ± 0.01	—	—	—
β_2m	1 ± 0.16	0.9 ± 0.12	3.19 ± 0.55*	1.21 ± 0.2*	6.22 ± 0.52	8.04 ± 1.34

[a] Results are expressed as the ratio between the level of expression of the gene of interest and a constitutively expressed gene (β-actin) ± SE. $p < 0.05$.

T cell development, no differences exist between transgenic and wild-type mice.

Genetic background influences the onset and the incidence of diabetes in RIP-HuIFNβ-transgenic mice

The incidence of the disease varies according to the genetic background (Fig. 2). In CD-1 RIP-HuIFNβ mice, 22% of males and 0% of females developed diabetes. The onset of the disease starts after 7 wk of age (Fig. 2*a*). The analysis of the progeny resulting from F6 backcross with NOR strain onward showed not only a spontaneous development of diabetes but also a significant acceleration of the onset of the disease and an increase of the incidence: Fifty-seven percent of male and 22% of female NOR RIP-HuIFNβ mice became diabetic. The onset of diabetes started at 3 wk of age (Fig. 2*b*). Mice resulting from F6 backcross with NOD strain (NOD RIP-HuIFNβ) onward showed a diabetes incidence of 53% in male and 51% in female mice, starting diabetes at 3 wk of age. The incidence of diabetes in NOD nontransgenic littermates was identical to that in wild-type NOD: 27% in males and 66% in females (Fig. 2*c*). As expected, neither CD-1 nor NOR nontransgenic mice developed diabetes during the study. Moreover, NOD-SCID RIP-HuIFNβ mice did not become diabetic.

Most of the NOD and NOR transgenic mice became diabetic just after weaning (3 wk of age), showing a considerable acceleration of the onset of the disease compared with the CD-1 RIP-HuIFNβ mice that started diabetes at 7 wk of age. These data, along with the variation in the incidence of the disease in the three groups of transgenic mice, suggest that the genetic background influences the start point and the percentage of diabetes in mice expressing this transgene.

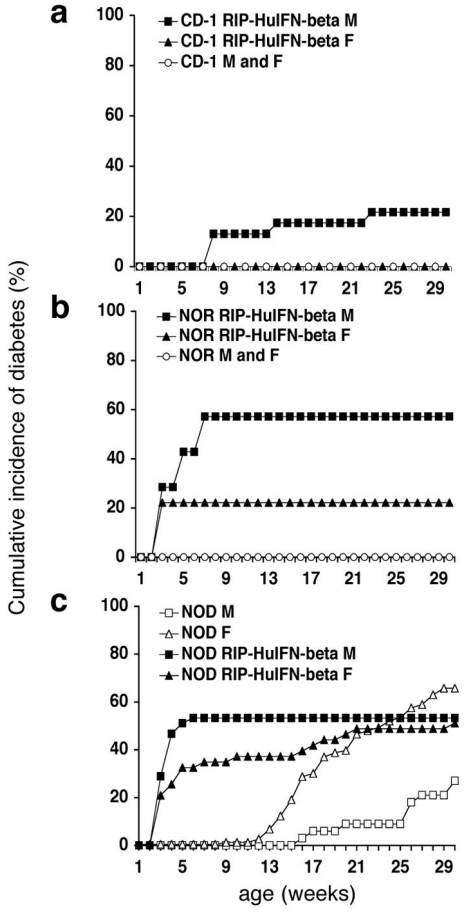

FIGURE 2. The transgenic expression of HuIFNβ in islet β cells contributes to the development of diabetes in nondiabetes-prone strains (CD-1 and NOR) and accelerates the onset of the disease in NOD transgenic mice. Cumulative incidence of diabetes (percentage) in RIP-HuIFNβ transgenic mice in different genetic backgrounds during 30 wk of follow-up ($n > 40$). *a,* Twenty-two percent of CD-1 RIP-HuIFNβ males (M) developed diabetes after 7 wk of age. CD-1 RIP-HuIFNβ females (F) and nontransgenic littermates did not develop the disease. *b,* NOR RIP-HuIFNβ became diabetic after 3 wk of age; the incidence was higher in males (57%) than in females (22%). Nontransgenic littermates did not develop the disease. *c,* NOD RIP-HuIFNβ mice developed early diabetes, after 3 wk of age, when compared with NOD wild-type mice, after 12 wk. The incidence of the disease in transgenic NOD mice was similar in males (53%) and females (61%); as expected, wild-type NOD mice showed a higher incidence of the disease in females (66%) than in males (27%).

The development of diabetes is lymphocyte dependent: evidence of autoimmunity

NOD-SCID RIP-HuIFNβ mice showed no signs of diabetes nor insulitis, thus indicating that the destruction of islet cells in transgenic mice is not caused by the presence of HuIFNβ per se and that it is essential they share immunocompetent T and B cells.

All NOD-SCID mice (four of four) transferred with splenocytes from diabetic nontransgenic NOD mice (positive control), become diabetic after 4–9 wk, as expected (23). All NOD-SCID mice (five of five) transferred with splenocytes or islets infiltrating lymphocytes from diabetic NOD RIP-HuIFNβ mice developed type 1 diabetes after 4–15 wk after transfer. NOR RIP-HuIFNβ mice were also able to transfer diabetes in most (three of four) treated NOD-SCID mice at 8–9 wk, starting 4 wk later than littermates that received cells from diabetic NOD RIP-HuIFNβ. NOD-SCID mice transferred with splenocytes from NOR nontransgenic littermates were insulitis free 3 wk after the injection and at the end of the follow up (15 wk) none of the animals (zero of six) developed diabetes. All NOD RIP-HuIFNβ mice (three of three) transferred with autologous splenocytes or pancreatic-infiltrating lymphocytes from diabetic mice developed diabetes in 3–6 days. As expected, sham-transplanted mice did not develop diabetes nor insulitis. Histological examination of pancreata from transferred diabetic mice showed a high degree of insulitis, confirming that development of the disease is lymphocyte dependent.

Islet cell Abs (ICA) are present in transgenic mice

ICA were detected in mice from all of the study groups, including diabetic and healthy animals: CD-1 RIP-HuIFNβ, NOD RIP-HuIFNβ, NOR RIP-HuIFNβ, and also their nontransgenic littermates. The expression of the transgene did not result in a disappearance of or significant increase of ICA. The ICA-positive staining pattern was weak. Occasionally, ICA were associated with antinuclear autoantibodies in endocrine and exocrine tissues. However, the autoantibodies to islet Ags are not a prerequisite for the subsequent emergence of the disease (24).

Peripheral lymphocyte subsets in transgenic mice

It has been described that type I IFNs have an important effect on the proliferation of different cell types, including lymphocyte subsets (25). To assess the effect of circulating HuIFNβ in lymphocyte subpopulations, we determined the percentages of splenic lymphocyte subsets in transgenic mice. Mice expressing the transgene in different genetic backgrounds (CD-1, NOR, and NOD) had normal percentages (26) of splenic B and T (CD4 and CD8) lymphocyte subsets (Fig. 3).

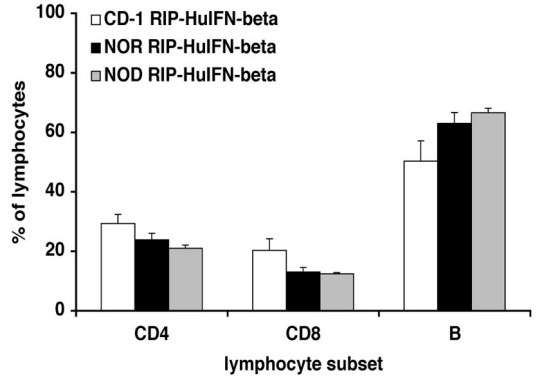

FIGURE 3. Peripheral lymphocyte subsets are not altered in transgenic mice. Histogram of percentages corresponding to splenic lymphocyte subsets: T (CD4 and CD8) and B lymphocytes in CD-1 RIP-HuIFNβ, NOR RIP-HuIFNβ, and NOD RIP-HuIFNβ determined by FACS analysis. No significant differences were found between groups.

Expression of HuIFNβ in islet β cells increases the intensity of insulitis in different genetic backgrounds

The sequential histological examination of the pancreata of nondiabetic RIP-HuIFNβ transgenic mice reveals that the intensity of the insulitis depends on the genetic background. CD-1 RIP-HuIFNβ mice showed normal islets or just weak insulitis at 3 wk of age. Ten percent of the islets of 4-wk-old CD-1 RIP-HuIFNβ mice were weakly infiltrated with mononuclear cells around or inside the islets (peri-insulitis or mild insulitis); this pattern was maintained in adult mice in which 15% of the islets were weakly infiltrated (Table III). The insulitis score (Fig. 4*a*) was very low due to the lack of moderately and severely infiltrated islets and to the absence of infiltrated islets in some subjects. Only at 12 wk of age the intensity of the insulitis was significantly higher than that of controls ($p < 0.05$). As expected, the inflammation was more marked and the insulitis score significantly increased in transgenic mice developing diabetes (Fig. 4*c*).

The pancreata from nondiabetic NOR RIP-HuIFNβ mice showed severe infiltration compared with wild-type NOR mice and nontransgenic littermates ($p < 0.01$). In contrast to the pancreata from CD-1 RIP-HuIFNβ nondiabetic mice, in which most islets were not infiltrated (Table III), NOR transgenic mice showed mononuclear cells infiltrating most islets at 3 wk of age. Adult mice showed 86% of the islets infiltrated (43% moderately or severely). The insulitis score (Fig. 4*b*) was significantly higher ($p < 0.01$) in NOR RIP-HuIFNβ than in CD-1 transgenic mice. Both diabetic NOD and NOR RIP-HuIFNβ mice showed an insulitis score higher than transgenic CD-1 diabetic subjects ($p < 0.05$) and similar between them (Fig. 4*c*). A weak insulitis is enough to cause the disease in the CD-1 transgenic colony, whereas NOD and NOR transgenic strains require massive infiltration.

HuIFNβ in the islets causes MHC class I hyperexpression and recruits lymphomononuclear cells

As expected, HuIFNβ staining was only positive in β cells of transgenic animals. The islets from CD-1 RIP-HuIFNβ healthy mice were predominantly noninfiltrated, peri-infiltrated, or poorly infiltrated. The phenotypic characterization of insulitis (Fig. 5*b*) showed that most mononuclear islet-infiltrating cells were CD4 and CD8 T lymphocytes (ratio 3:1); B cells were only occasionally detected. A few macrophages were also detected surrounding the endocrine islet cells. Dendritic cells were only observed inside the noninfiltrated islets. MHC class I molecules were hyperexpressed in the endocrine cells of all islets, correlating well with molecular data; no MHC class II hyperexpression was observed in islet cells. Diabetic mice showed normal, peri-infiltrated, and mildly infiltrated islets and a stronger infiltration (peri and poorly) than that observed in nondiabetic mice was detected (Fig. 5*a*). The composition of the insulitis in diabetic mice was almost the same as that in healthy animals except for a remarkable increase of B cell clusters in the periphery of the islets.

Most islets from NOR RIP-HuIFNβ nondiabetic mice were moderately or severely infiltrated by CD18-positive cells (leukocytes). As expected, MHC class I, but not MHC class II, was highly hyperexpressed in endocrine islet cells from all of the islets. The insulitis mainly consisted of T and B cells (Fig. 5*a*). We observed B cells, T cells, more CD4 than CD8 cells (ratio 4:1), and few NK cells. B and CD4 cells, but not CD8 cells or NK cells, were distributed in clusters around the islets. These data were confirmed by flow cytometry of pancreatic-infiltrating mononuclear cells (data not shown). Only a few macrophages were observed in the periphery or inside the islets; no dendritic cells were found in the infiltrated islets. The islets from NOR RIP-HuIFNβ diabetic mice (Fig. 5*b*) were almost devoid of β cells and showed a higher intensity of insulitis than healthy mice, but no differences in the composition of the infiltrate were observed. The islets from NOD RIP-HuIFNβ diabetic mice were almost devoid of β cells and showed a high intensity of insulitis, but no differences in the infiltrate composition were observed when compared with NOR RIP-HuIFNβ diabetic mice.

Nontransgenic littermates did not show infiltrating leukocytes or MHC class I hyperexpression (Fig. 5*c*). Histological examination of pancreata from NOD-SCID RIP-HuIFNβ revealed that MHC class I was hyperexpressed in the islets due to the effect of HuIFNβ but, as expected, immunofluorescence staining revealed the lack of insulitis because SCID mice are unable to produce mature T and B cells (Fig. 5*c*).

Transgenic mice in the three analyzed genetic backgrounds showed a HuIFNβ staining pattern that correlates well with the MHC class I hyperexpression in the islets and it is independent of the presence of infiltrating cells.

Discussion

Type I IFNs have been associated with T1D because they have been detected in the islets of diabetic patients (8, 9, 12), thus suggesting that a viral infection and its consequences may be related

Table III. *Percentage of islets of each degree of infiltration in CD-1, CD-1 RIP-HuIFNβ, NOR RIP-HuIFNβ nontransgenic littermates, and NOR RIP-HuIFNβ mice*

Genetic Background	Noninfiltrated	peri-Insulitis	Mild Insulitis	Moderate Insulitis	Severe Insulitis	Insulitis Score
CD-1	85.34	13.62	1.03	0	0	0.14
CD-1 RIP-HuIFNβ	75.51	21.76	2.15	0.34	0.22	0.27
NOR RIP-HuIFNβ (−)	97	3	0	0	0	0.04
NOR RIP-HuIFNβ (+)	14.09	18.79	23.71	26.84	16.55	2.18

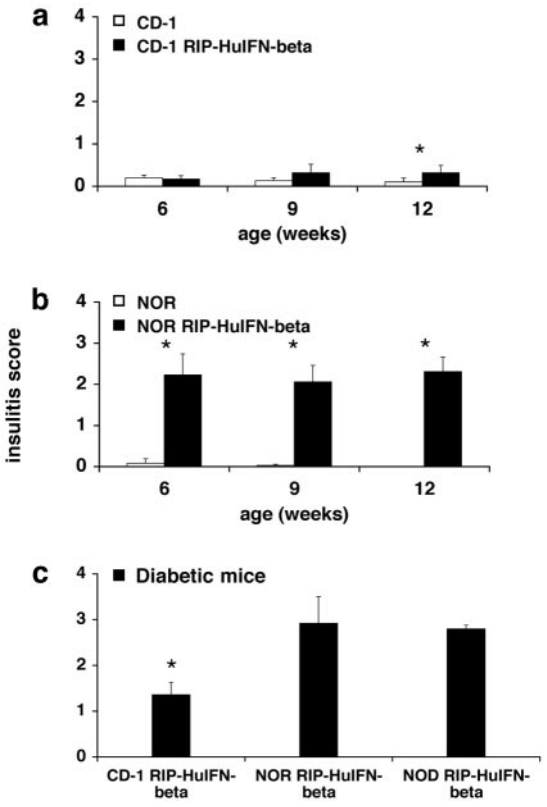

FIGURE 4. Lymphocytic infiltration increases in the islets from mice expressing HuIFNβ in the β cells. *a,* Nondiabetic CD-1 RIP-HuIFNβ colony and nontransgenic littermates at 6, 9, and 12 wk of age. Differences were found at 12 wk of age. *b,* Nondiabetic NOR RIP-HuIFNβ and nontransgenic littermates at 6, 9, and 12 wk of age showed significant differences. *c,* Insulitis score in diabetic CD-1 transgenic mice was lower than that observed in NOR and NOD RIP-HuIFNβ transgenic mice. Insulitis was scored on a 0–4 scale in groups of six animals per condition. Scoring system: 0, no infiltration; 1, some peri-insular infiltration; 2, heavy peri-insular infiltration and <25% of the insular area infiltrated; 3, between 25 and 75% of the insular area infiltrated; and 4, total islet infiltration. The mean score was obtained by division of the sum of all individual islet infiltration scores by the total number of islets analyzed.

to the development of the disease. Furthermore, during the treatment with type I IFNs a small but significant proportion of patients develop autoimmune diabetes (27, 28). Apart from antiviral activities, type I IFNs are also potent immunomodulators: they are involved in the increase of the expression of MHC class I, in the enhancement of T and NK cell cytotoxicity, in the production of proinflammatory cytokines, and other activities (29, 30). Transgenic mice expressing IFNα, β, and κ (12–14) in β cells developed type 1 diabetes, with T and B cells infiltrating the islets. These data suggest a local proinflammatory role for these type I IFNs and their ability to induce T1D.

In this study, we provide evidence that the local expression of HuIFNβ is involved in the development of autoimmune diabetes: 1) the islets from transgenic mice hyperexpress MHC class I molecules, 2) the islets are infiltrated by T and B lymphocytes, 3) the transfer of lymphocytes from a diabetic transgenic animal causes the disease in NOD-SCID recipient mice and accelerates the onset of diabetes in prediabetic recipient mice, and 4) transgenic NOD-SCID RIP-HuIFNβ mice do not develop diabetes. Moreover, systemic HuIFNβ does not alter the expression of analyzed pancreatic hormones or β_2m in the thymus, thus supporting the idea that the disease could be caused by a local effect of IFNβ strong enough to break the peripheral tolerance to β cells rather than by a change in central tolerance. Preliminary results show that HuIFNβ could modulate the expression of adhesion molecules, cytokines, and chemokines in the islets (data not shown). Therefore, we conclude that in this transgenic model, the disease is lymphocyte dependent since transgenic animals lacking functional T and B cells cannot develop diabetes or insulitis, although they increase the expression of MHC class I in the islets.

To study the role of IFNβ in diabetes, we also introduced the RIP-HuIFNβ transgene in different genetic backgrounds with different susceptibility to T1D. We demonstrated that the genetic background not only determines the onset and the severity of the lesions related to the disease but also that these genetic factors cannot protect individuals from T1D by themselves when an inducing factor triggers the autoimmune process.

It has been previously described that a small percentage of N3 generation obtained from the backcross of C57BL6/SJL RIP-HuIFNβ to CD-1 mice spontaneously developed T1D (14). In this study, we showed an increase of the incidence of diabetes in homozygous mice kept under specific pathogen-free conditions. The research in the field of T1D has developed many transgenic mice in nondiabetes-prone strains that were later backcrossed to diabetes-prone NOD mice. This is the first report of the generation of NOD and NOR transgenic mice expressing a type I IFN in the islets. NOD mice expressing HuIFNβ in β cells showed an acceleration of the disease and, unexpectedly, NOR RIP-HuIFNβ mice developed early diabetes with a high incidence. The severity of the insulitis varied according to the genetic background and was correlated with the incidence of diabetes. The three models shared a MHC class I hyperexpression in the islet cells, but whereas diabetic NOD and NOR transgenic mice islets were strongly infiltrated, diabetic CD-1 RIP-HuIFNβ mice were only mildly infiltrated. These results indicated that the genetic background was not strong enough to protect mice from diabetes when an injury triggered an inflammatory response in the islets. In humans, the lack of concordance of diabetes in identical twins indicates that nongenetic factors determine the development of the disease. These factors might be environmental agents but also random factors such as the generation of the immunological repertoire are subject to complex regulation.

The influence of HuIFNβ was always stronger in males than in females. It has been described that male mice have more difficulty maintaining glucose homeostasis under conditions of impaired β cell function and that male islets are more susceptible to an autoimmune damage than are female islets but male islet-specific lymphocytes are less aggressive (31). Results similar to ours have been recently described in nondiabetes-prone transgenic mice expressing IFNκ in β cells (13), where a higher incidence of the disease in males than in females was observed (G. Vassileva, personal communication). Assuming a viral etiology for T1D in humans, females seem to be less susceptible than males to the environmental infectious influences (32).

To date, 14 different viruses have been reported to be associated with the development of autoimmune diabetes in both humans and animal models (5). The most convincing data include an increased frequency of diabetes in patients with enterovirus infection (33), congenital rubella syndrome, measles (34), and the detection of CMV in the lymphocytes of diabetic patients (35). Viral infections result in an enhancement of the MHC class I expression in a number of cell types which normally do not express these molecules (36). This enhancement may occur by a direct interaction between the viral component and the MHC class I gene or indirectly by virus-induced soluble factors, IFN-γ, IFN-$\alpha\beta$, and TNF-α- produced by the infected cells (37). Thus, these viruses induced an

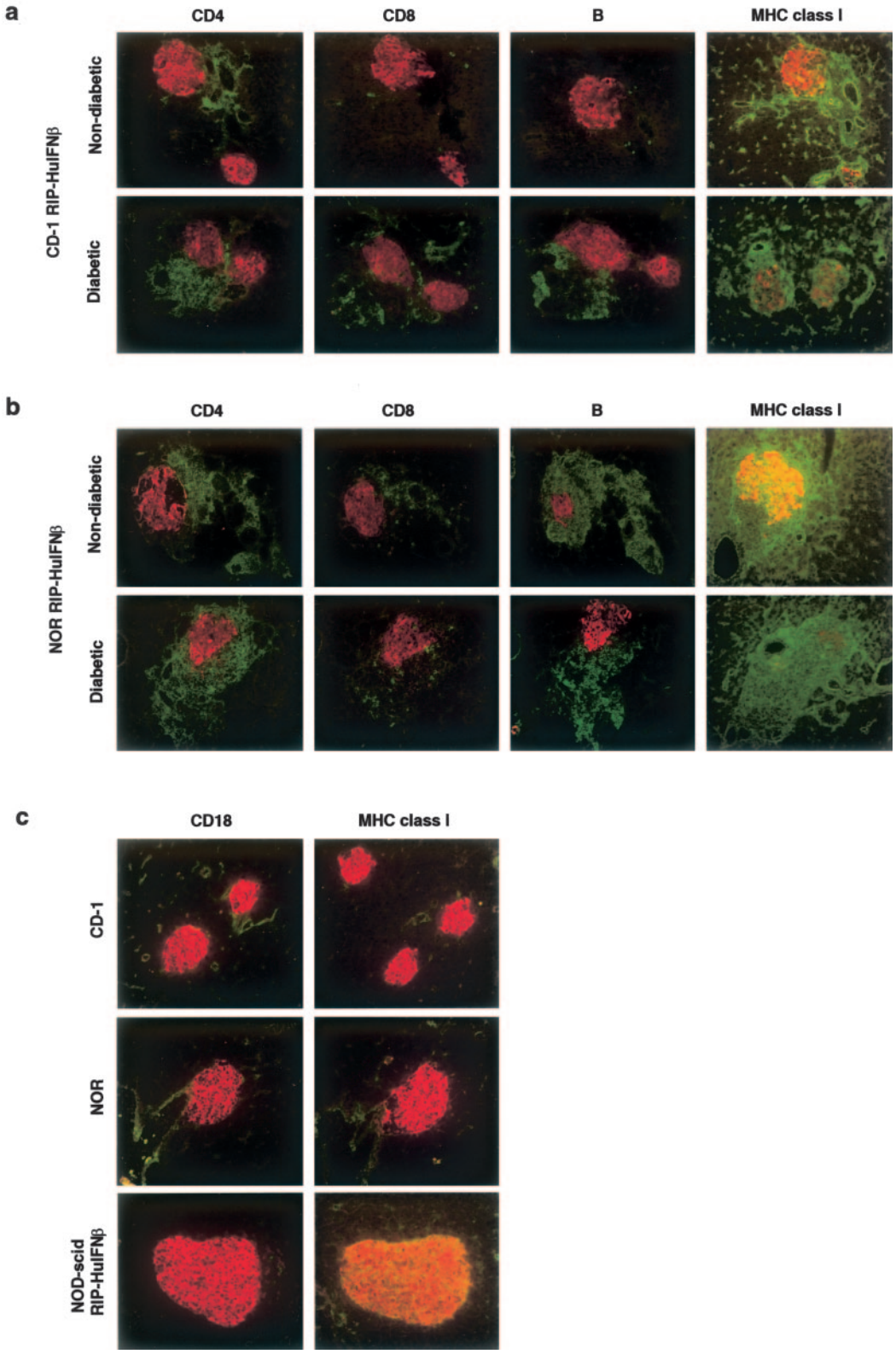

FIGURE 5. HuIFNβ contributes to the recruitment of lymphomononuclear cells to the islets in immunocompetent mice and causes MHC class I hyperexpression in the endocrine cells. Double immunofluorescence staining of 5-μm pancreatic cryostat sections, insulin (in red), and specific markers (in green). MHC class I/insulin overlap shown in orange. Original magnification, ×200. *a,* CD-1 RIP-HuIFNβ transgenic mice, nondiabetic (*upper panels*) and recent-onset diabetic (*lower panels*). *b,* NOR RIP-HuIFNβ transgenic mice, nondiabetic (*upper panels*) and recent-onset diabetic (*lower panels*). *c,* Control animals: CD-1 (*upper*), NOR (*middle*), and NOD-SCID RIP-HuIFNβ (*lower*).

enhanced display of self-peptides in MHC class I, which led to the temporary activation of autoreactive T cells and autoimmunity (38).

Nevertheless, it is very difficult to connect viruses and T1D: it is well known that development of the disease in humans and animal models is reduced in the presence of high amounts of pathogens.

Recently it has been demonstrated that viral infections could influence the progression of insulitis beneficially at the preclinical stage if produced at the correct location, time, and intensity, preventing autoimmune diabetes (39), correlating well with some protocols that prevent diabetes in NOD mice by administration of antiviral cytokines in mice (40, 41). The timing of the infection, the viral strain, the antigenic load, and other unknown factors must be crucial in the protection or predisposition to the disease.

The results presented here, along with previous and future studies, will provide a greater understanding on how the production of type I IFNs in the pancreas affects the recruitment of infiltrating cells and accelerates the onset of diabetes. The genetic background by itself does not protect mice from diabetes when damages, stresses, or dangers affect β cells with intensity strong enough to trigger an inflammatory response. Our data indicate that the antiviral cytokine IFNβ expressed in insulin-producing cells triggers the break of peripheral tolerance to β cells, leading to insulitis and autoimmunity in diabetes and nondiabetes prone mice.

Acknowledgments

We thank L. Sabater, S. Gordillo, and Dr. M. Juan for their assistance with molecular biology, M. A. Fernández for the flow cytometry experiments, and Dr. F. E. Borras for reviewing (Hospital Germans Trias i Pujol). We are grateful to Dr. C. Mora (Hospital Clínic, Barcelona, Spain) for her help with microsatellite technique and to Dr. A. Casellas (Faculty of Veterinary, UAB, Barcelona, Spain).

References

1. Bach, J. F., and L. Chatenoud. 2001. Tolerance to islet autoantigens in type I diabetes. *Annu. Rev. Immunol. 19:131*
2. . Bach, J. F. 1999. The immunology of diabetes: revolution of concepts over a decade. In *The Decade of Autoimmunity,* Y. Shoenfeld, ed. Elsevier, Oxford p. 149.
3. Notkins, A. L. 2002. Immunologic and genetic factors in type 1 diabetes. *J. Biol. Chem. 277:4354*.
4. Todd, J. A., and S. C. Bain. 1992. A practical approach to identification of susceptibility genes for IDDM. *Diabetes 41:1029.*
5. Jun, H. S., and J. W. Yoon. 2003. A new look at viruses in type 1 diabetes. *Diabetes Metab. Res. Rev. 19:8.*
6. Horwitz, M. S., A. Ilic, C. Fine, E. Rodriguez, and N. Sarvetnick. 2002. Presented antigen from damaged pancreatic β cells activates autoreactive T cells in virus-mediated autoimmune diabetes. *J. Clin. Invest. 109:79*.
7. Horwitz, M. S., C. Fine, A. Ilic, and N. Sarvetnick. 2001. Requirements for viral-mediated autoimmune diabetes: β-cell damage and immune infiltration. *J. Autoimmun. 16:211*.
8. Hooks, J. J., H. M. Moutsopoulos, S. A. Geis, N. I. Stahl, J. L. Decker, and A. L. Notkins. 1979. Immune interferon in the circulation of patients with autoimmune disease. *N. Engl. J. Med. 301:5*.
9. Foulis, A. K., M. A. Farquharson, and A. Meager. 1987. Immunoreactive α interferon in insulin secreting β cells in type 1 diabetes mellitus. *Lancet 2:1423*.
10. Somoza, N., F. Vargas, C. Roura-Mir, M. Vives-Pi, M. T. Fernandez-Figueras, A. Ariza, R. Gomis, R. Bragado, M. Marti, D. Jaraquemada, and R. Pujol-Borrell. 1994. Pancreas in recent onset insulin-dependent diabetes mellitus: changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor Vβ usage, and cytokine profile. *J. Immunol. 153:1360*.
11. Huang, X., J. Yuang, A. Goddard, A. Foulis, R. F. James, A. Lernmark, R. Pujol-Borrell, A. Rabinovitch, N. Somoza, and T. A. Stewart. 1995. Interferon expression in the pancreases of patients with type I diabetes. *Diabetes 44:658*.
12. Stewart, T. A., B. Hultgren, X. Huang, S. Pitts-Meek, J. Hully, and N. J. MacLachlan. 1993. Induction of type I diabetes by interferon-α in transgenic mice. *Science 260:1942*.
13. Vassileva, G., S. C. Chen, M. Zeng, S. Abbondanzo, K. Jensen, D. Gorman, B. M. Baroudy, Y. Jiang, N. Murgolo, and S. A. Lira. 2003. Expression of a novel murine type I IFN in the pancreatic islets induces diabetes in mice. *J. Immunol. 170:5748*.
14. Pelegrin, M., D. J. Devedjian, C. Costa, J. Visa, G. Solanes, A. Pujol, G. Asins, A. Valera, and F. Bosch. 1998. Evidence from transgenic mice that interferon-β may be involved in the onset of diabetes mellitus. *J. Biol. Chem. 20:12332*.
15. Iwakura, Y., M. Asano, Y. Nishimune, and Y. Kawade. 1988. Male sterility of transgenic mice carrying exogenous mouse interferon β under the control of metallothionein enhancer-promoter. *EMBO J. 7:3757*.
16. Makino, S., and Y. Tochino. 1978. The spontaneously nonobese diabetic mouse. *Exp. Anim. 27:27.*
17. Prochazca, M., D. V. Serreze, W. N. Frankel, and E. H. Leiter. 1992. NOR/LtJ mice: MHC-matched diabetes-resistant control strain for NOD mice. *Diabetes 41:98*.
18. Dandoy-Dron, F., E. Monthioux, J. Jami, and D. Buchinni. 1991. Regulatory regions of rat insulin I gene necessary for expression in transgenic mice. *Nucleic Acid Res. 19:4925*.
19. Prochazka, M., H. R. Gaskins, L. D. Shultz, and E. H. Leiter. 1992. The nonobese diabetic *scid* mouse: model for spontaneous thymomagenesis associated immunodeficiency. *Proc. Natl. Acad. Sci. USA 89:3290*.
20. Mora, C., F. S. Wong, C. H. Chang, and R. Flavell. 1999. Pancreatic infiltration but not diabetes occurs in the relative absence of MHC class II-restricted CD4 T cells: studies using NOD/CIITA-deficient mice. *J. Immunol. 162:4576*.
21. Benavides, F., E. Glasscock, L. G. Coghlan, M. C. Stern, D. A. Weiss, and C. J. Conti. 2001. PCR-based microsatellite analysis for differentiation and genetic monitoring of nine inbred SENCAR mouse strains. *Lab. Anim. 35:157*.
22. Serreze, D. V., S. A. Fleming, H. D. Chapman, S. D. Richard, E. H. Leiter, and R. M. Tisch. 1998. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J. Immunol. 161:3912.*
23. Rohane, P. W., A. Shimada, D. T. Kim, C. T. Edwards, B. Charlton, L. D. Shultz, and C. G. Fathman. 1995. Islet-infiltrating lymphocytes from prediabetic NOD mice rapidly transfer diabetes to NOD-*scid/scid* mice. *Diabetes 44:550*.
24. Abiru, N., L. Yu, D. Miao, A. K. Maniatis, E. Liu, H. Moriyama, and G. S. Eisenbarth. 2001. Transient insulin autoantibody expression independent of development of diabetes: comparison of NOD and NOR strains. *J. Autoimmun. 17:1*.
25. Kunzi, M. S., and P. P. Rowe. 2001. IFN-α, IFN-β and ω ligands. In *Cytokine Reference*, Vol. 1. S. K. Durum, T. Hirano, J. Vilcek, and N. A. Nicol, eds. Academic, Cornwall, U.K., p. 627.
26. Serreze, D. V., C. M. Post, H. D. Chapman, E. A. Johnson, B. Lu, and P. B. Rothman. 2000. Interferon γ receptor signaling is dispensable in the development of autoimmune type 1 diabetes in NOD mice. *Diabetes 49:2007*.
27. Kado, S., J. Miyamoto, N. Komatsu, Y. Iwaki, H. Ozaki, H. Taguchi, M. Kure, G. Sarashina, T. Watanabe, Y. Katsura, et al. 2000. Type 1 diabetes mellitus caused by treatment with interferon-β. *Intern. Med. 39:146*.
28. Wesche, B., E. Jaeckel, C. Trautwein, H. Wedemeyer, A. Falorni, H. Frank, A. von zur Muhlen, M. P. Manns, and G. Brabant. 2001. Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon α therapy for chronic hepatitis C. *Gut 48:378*.
29. Sinigaglia, F., D. D′Ambrosio, and L. Rogge. 1999. Type I interferons and the Th1/Th2 paradigm. *Dev. Comp. Immunol. 23:657.*
30. Oritani, K., P. W. Kincade, C. Zhang, Y. Tomiyama, and Y. Matsuzawa. 2001. Type I interferons and limitin: a comparison of structures, receptors, and functions. *Cytokine Growth Factor Rev. 12:337*.
31. Ablamunits, V., D. Elias, and I. R. Cohen. 1999. The pathogenicity of islet-infiltrating lymphocytes in the non-obese diabetic (NOD) mouse. *Clin. Exp. Immunol. 115:260*.
32. Roche, E. F., H. Lewy, H. M. Hoey, and Z. Laron. 2003. Differences between males and females in the seasonality of birth and month of clinical onset of disease in children with type 1 diabetes mellitus in Ireland. *J. Pediatr. Endocrinol. Metab. 16:779*.
33. Hyoty, H., and K. W. Taylor. 2002. The role of viruses in human diabetes. *Diabetologia 45:1353.*
34. Lipman, T. H., Y. Chang, and K. M. Murphy. 2002. The epidemiology of type 1 diabetes in children in Philadelphia 1990–1994: evidence of an epidemic. *Diabetes Care 25:1969*.
35. Vives, M., N. Somoza, F. Vargas, and R. Pujol-Borrell. 1995. Overexpression of MHC proteins in pancreatic islets: a link between cytokines, viruses, breach of tolerance and insulin dependent diabetes mellitus. In *Modulation of MHC Antigen Expression and Disease*. E. D. Blair, C. R. Pringle, and D. J. Maudsley, eds. Cambridge Univ. Press, Cambridge, U.K., p. 361.
36. Redwine, J. M., M. J. Buchmeier, and C. F. Evans. 2001. In vivo expression of major histocompatibility complex molecules on oligodendrocytes and neurons during viral infection. *Am. J. Pathol. 159:1219*.
37. Dhib-Jalbut, S. S., and E. P. Cowan. 1993. Direct evidence that interferon-β mediates enhanced HLA-class I expression in measles virus-infected cells. *J. Immunol. 151:6248*
38. Herberts, C. A., J. van Gaans-van den Brink, E. van der Heeft, M. van Wijk, J. Hoekman, A. Jaye, M. C. Poelen, C. J. Boog, P. J. Roholl, H. Whittle, et al. 2003. Autoreactivity against induced or upregulated abundant self-peptides in HLA-A*0201 following measles virus infection. *Hum. Immunol. 64:44*.
39. Christen, U., D. Benke, T. Wolfe, E. Rodrigo, A. Rhode, A. C. Hughes, M. B. A. Oldstone, and M. G. von Herrath. 2004. Cure of prediabetic mice by viral infections involves lymphocyte recruitment along an IP-10 gradient. *J. Clin. Invest. 113:74*.
40. Rabinovitch, A. 1998. An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. *Diabetes Metab. Rev. 14:129*.
41. Hänninen, A., E. Hamilton-Williams, and C. Kurts. 2003. Development of new strategies to prevent type 1 diabetes: the role of animal models. *Ann. Med. 35:546*.