Unaltered Diabetes Presentation in NOD Mice Lacking the Vitamin D Receptor

Conny Gysemans,[1] Evelyne van Etten,[1] Lutgart Overbergh,[1] Annapaula Giulietti,[1] Guy Eelen,[1] Mark Waer,[2] Annemieke Verstuyf,[1] Roger Bouillon,[1] and Chantal Mathieu[1]

OBJECTIVE—Vitamin D deficiency increases risk for type 1 diabetes in genetically predisposed individuals, while high doses of 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] prevent insulitis and diabetes in NOD mice.

RESEARCH DESIGN AND METHODS—Since 1,25$(OH)_2D_3$ regulates gene transcription through the vitamin D receptor (VDR), we investigated the role of VDR in diabetes development by creating NOD mice without functional VDR.

RESULTS—$VDR^{-/-}$ NOD mice are rachitic and have lower numbers of putative regulator cells [TCR-$\alpha/\beta^+CD4^-CD8^-$ (natural killer T-cells) and $CD4^+CD25^+$ T-cells [in central and peripheral immune organs compared with $VDR^{+/+}$ NOD littermates. Lipopolysaccharide-stimulated $VDR^{-/-}$ NOD macrophages expressed lower interleukin (IL)-1, IL-6, and CC chemokine ligand 2 mRNA, correlating with less nuclear translocation of p65 nuclear factor-κB compared with $VDR^{+/+}$ NOD macrophages. Thymic and lymph node dendritic cells from $VDR^{-/-}$ NOD mice displayed an even less mature $CD11c^+CD86^+$ phenotype than $VDR^{+/+}$ NOD mice. Despite this immune phenotype linked to diabetes in NOD mice, $VDR^{-/-}$ NOD mice developed insulitis and diabetes at the same rate and incidence as $VDR^{+/+}$ NOD littermates.

CONCLUSIONS—Despite aggravating known immune abnormalities in NOD mice, disruption of VDR does not alter disease presentation in NOD mice in contrast to the more aggressive diabetes presentation in vitamin D–deficient NOD mice. ***Diabetes*** **57:269–275, 2008**

Environmental factors influence the presentation of type 1 diabetes in humans and in animal models. Like many autoimmune diseases, type 1 diabetes is less frequent in regions close to the equator (1). An obvious candidate to explain this difference is sunlight exposure and resulting vitamin D status.

Experimental, clinical and epidemiological data show that maintenance of an optimal vitamin D status is fundamental for proper calcium absorption and bone mineralization and is also critical for avoidance of a variety of diseases, such as tuberculosis (2), but vitamin D insufficiency and rickets are reemerging (3). A Finnish study revealed that children with suspected rickets during the first year of life have an increased frequency of type 1 diabetes compared with children without vitamin D deficiency (4). In parallel, we and others demonstrated that vitamin D–deficient NOD mice have a more aggressive disease (5,6), as reflected by earlier onset and doubled disease incidence, whereas high doses of active vitamin D, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], prevent insulitis and diabetes in NOD mice (7,8).

Immune changes induced by 1,25$(OH)_2D_3$ include modulation of phenotype and function of dendritic cells, restoration of regulatory T cells, and improved elimination of autoimmune effector cells (9). 1,25$(OH)_2D_3$ acts via the vitamin D receptor (VDR), which belongs to the steroid hormone receptor superfamily. As VDR is expressed in β-cells and in most immune cells, it is not surprising that the 1,25$(OH)_2D_3$-VDR complex modulates insulin secretion, cell differentiation, and innate and adaptive immune functions. Moreover, associations between some VDR polymorphisms and type 1 diabetes have been described in several populations (10,11). In view of these data, we investigated the role of the VDR gene in autoimmune diabetes development by generating and studying a congenic stock of NOD mice with a disruption of the VDR gene.

RESEARCH DESIGN AND METHODS

The original $Vdr^{tm1Ska}/Vdr^{tm1Ska}$ ($VDR^{-/-}$) mice were produced by Dr. S. Kato (University of Tokyo, Tokyo, Japan) and were kept on C57BL/6 × CBA background (12). After backcrossing male $VDR^{-/+}$ heterozygotes to female NOD mice (N10 and N14), $VDR^{-/+}$ NOD mice were intercrossed to produce experimental animals of three genotypes. Genotypes of mice from N2 to N14 generation were determined by testing tail DNA with microsatellite markers linked to 15 insulin-dependent diabetes loci (*Idd*1–*Idd*15), as previously described (13).

All experiments were performed on age- and sex-matched $VDR^{-/-}$ and $VDR^{+/+}$ NOD littermates from intercrosses of N10 and N14 generations. Complete methods, including a description of the procedures for all in vitro immune phenotyping, assessment of diabetes, pancreatic histopathology with insulitis scoring and insulin content determination, calcium and bone parameters, RNA isolation, and quantitative RT-PCR analysis are provided in an online appendix (available at http://dx.doi.org/10.2337/db07-1095).

Data were analyzed using NCSS 2000 statistical software (Kaysville, UT). Results are expressed as means ± SEM, and differences are considered significant when $P \leq 0.05$. Differences between groups were evaluated by ANOVA and post hoc unpaired Student's t test. For comparing the in vivo insulitis and diabetes incidence, Kaplan-Meier survival curves, the log-rank test, and the χ^2 test were used.

From the [1]Laboratory of Experimental Medicine and Endocrinology, Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium; and the [2]Laboratory of Experimental Transplantation, Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium.

Address correspondence and reprint requests to Chantal Mathieu, MD, PhD, LEGENDO, Campus Gasthuisberg O&N1, Katholieke Universiteit Leuven, Herestraat 49, 3000 Leuven, Belgium. E-mail: chantal.mathieu@med.kuleuven.be.

Received for publication 6 August 2007 and accepted in revised form 16 October 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 24 October 2007. DOI: 10.2337/db07-1095.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-1095.

AUC, area under the curve; 1,25-dihydroxyvitamin D_3, 1,25$(OH)_2D_3$; CCL2, CC chemokine ligand 2; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; VDR, vitamin D receptor.

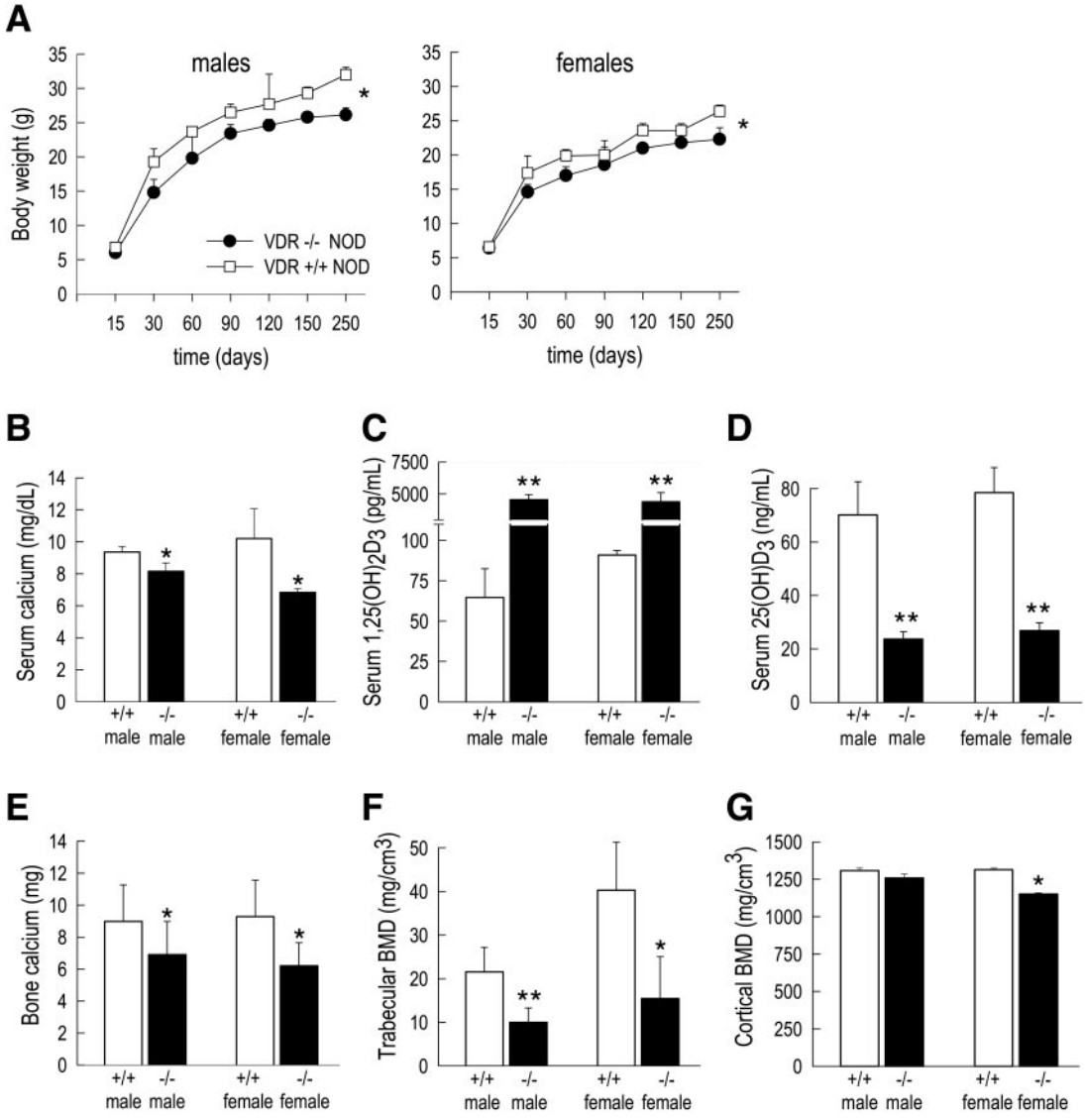


FIG. 1. Phenotype of VDR$^{-/-}$ and VDR$^{+/+}$ NOD mice. *A*: Body weight from VDR$^{-/-}$ and VDR$^{+/+}$ NOD littermates was evaluated by weekly intervals over a period of 250 days. Data are expressed as means ± SEM and are based on at least 10 animals at each time point. Note that both male and female VDR$^{-/-}$ NOD mice gained weight less rapidly after weaning than VDR$^{+/+}$ NOD mice (male, 14.8 ± 1.0 vs. 19.3 ± 1.9 g, respectively, at 30 days of age, $P \leq 0.001$; female, 14.6 ± 1.1 vs. 17.4 ± 2.5 g at 30 days of age, $P \leq 0.005$). *B*: Serum calcium. *C*: Serum 1,25(OH)$_2$D$_3$. *D*: Serum 25(OH)D$_3$. *E*: Bone calcium. *F*: Trabecular bone mineral density (BMD). *G*: Cortical bone mineral density. Results are means ± SEM of determinations in 3–8 mice of the same genotype and sex. Hypocalcemia and bone loss with extremely high 1,25(OH)$_2$D$_3$ levels, coincident with decreased 25(OH)D$_3$ concentrations, were detected in VDR$^{-/-}$ NOD mice compared with that in VDR$^{+/+}$ NOD mice.

RESULTS

Vitamin D–resistant phenotype of VDR$^{-/-}$ NOD mice. VDR$^{-/-}$ NOD mice exhibited alopecia and growth retardation. As expected, VDR was undetectable, whereas CYP27B1 was overinduced and CYP24A1 was inhibited in VDR$^{-/-}$ NOD kidneys (online appendix Fig. 1*A–C*). Also, calbindin-D$_{9k}$ was reduced in VDR$^{-/-}$ NOD kidneys (online appendix Fig. 1*D*). VDR$^{-/-}$ NOD mice showed anticipated abnormalities in mineral homeostasis including hypocalcemia (Fig. 1*B*). VDR$^{-/-}$ NOD mice displayed abnormally high levels of 1,25(OH)$_2$D$_3$ and low 25(OH)D$_3$ (Fig. 1*C–D*). These metabolic imbalances resulted in typical skeletal defects, including hypocalcemia (Fig. 1*E*), decreased bone mineral density (Fig. 1*F–G*), widened growth plates with hypomineralization, less trabeculae, and thicker osteoid seams (data not shown).

Effect of VDR deficiency on T-lymphocytes in NOD mice. VDR$^{-/-}$ NOD mice had less immature CD4$^-$CD8$^-$ T-cells expressing the T-cell receptor-α/β in the periphery and fewer regulatory CD4$^+$CD25$^+$ T-cells in spleen and mesenteric lymph nodes and, to a lesser extent, in thymus (Table 1). VDR$^{-/-}$ NOD splenocytes displayed equal responses to anti-CD3 compared with VDR$^{+/+}$ NOD splenocytes (male, 14 ± 3 vs. 14 ± 1 stimulation index [SI], respectively, P = NS; female, 17 ± 5 vs. 16 ± 3 SI, P = NS). Percentage of activation-induced cell death was not altered in VDR$^{-/-}$ vs. VDR$^{+/+}$ NOD mice (male, 32 ± 5 vs. 34 ± 1%, respectively, P = NS; female, 37 ± 6 vs. 39 ± 5%, P = NS).

Effect of VDR deficiency on gene expression and nuclear factor-κB activation of NOD macrophages after lipopolysaccharide stimulation. Disruption of VDR in NOD mice did not induce significant changes in

TABLE 1
Flow-cytometric analysis of cell surface proteins expressed by immune cells prepared from thymus, spleen, and mesenteric lymph nodes of $VDR^{-/-}$ and $VDR^{+/+}$ NOD mice

	Thymus				Spleen				Lymph nodes			
	$VDR^{+/+}$ NOD male	$VDR^{+/+}$ NOD female	$VDR^{-/-}$ NOD male	$VDR^{-/-}$ NOD female	$VDR^{+/+}$ NOD male	$VDR^{+/+}$ NOD female	$VDR^{-/-}$ NOD male	$VDR^{-/-}$ NOD female	$VDR^{+/+}$ NOD male	$VDR^{+/+}$ NOD female	$VDR^{-/-}$ NOD male	$VDR^{-/-}$ NOD female
$CD4^-CD8^-$	7.6 ± 0.3	4.6 ± 0.2	10.5 ± 2.2	7.4 ± 0.8	32.1 ± 8.7	29.1 ± 1.4	24.5 ± 0.2†	23.1 ± 0.6†	7.2 ± 3.2	3.4 ± 0.6	7.5 ± 2.9	3.9 ± 1.1
$CD4^-CD8^-$ (TCR-α/β^+)	0.09 ± 0.02	0.10 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.40 ± 0.03	0.39 ± 0.03	0.03 ± 0.02‡	0.07 ± 0.01†	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
$CD4^+CD8^-$	24.6 ± 1.1	20.6 ± 1.9	19.1 ± 8.0	18.3 ± 3.5	44.1 ± 1.1	47.6 ± 1.5	48.3 ± 3.4	48.0 ± 0.2	65.1 ± 0.9	68.3 ± 1.6	63.7 ± 1.1	61.6 ± 6.7
$CD4^-CD8^+$	13.5 ± 3.2	9.6 ± 0.1	13.9 ± 2.4	10.4 ± 1.3	21.3 ± 2.0	19.8 ± 1.6	26.7 ± 0.4	23.6 ± 0.4	22.4 ± 8.5	26.2 ± 0.7	25.6 ± 8.4	32.6 ± 4.2
$CD4^+CD8^+$	49.3 ± 4.2	62.2 ± 2.1	55.0 ± 10.4	63.9 ± 5.6	3.7 ± 0.4	4.7 ± 0.6	4.7 ± 0.2	4.7 ± 0.2	5.3 ± 0.1	2.1 ± 0.3	3.2 ± 1.4	1.9 ± 1.4
$CD4^+CD25^+$	4.8 ± 0.6	4.2 ± 0.6	2.8 ± 0.7†	2.8 ± 0.2	11.5 ± 1.0	12.0 ± 1.1	3.5 ± 2.0†	5.1 ± 0.1†	28.5 ± 11.9	28.8 ± 2.1	11.4 ± 2.6†	19.1 ± 1.4†
$CD4^+CD62L^+$	11.6 ± 4.4	9.4 ± 1.7	10.4 ± 1.5	10.4 ± 0.8	24.3 ± 3.7	26.9 ± 3.4	20.9 ± 2.6	26.2 ± 2.6	15.7 ± 3.1	16.9 ± 3.3	20.6 ± 7.2	21.2 ± 12.6
$B220^+$	3.3 ± 1.1	1.3 ± 0.4	1.9 ± 0.6	1.6 ± 0.5	21.4 ± 1.8	22.0 ± 1.2	24.1 ± 0.4	27.8 ± 7.2	26.4 ± 7.9	19.6 ± 5.1	26.0 ± 1.3	15.7 ± 1.6
$CD11b^+$	0.6 ± 0.2	0.3 ± 0.1	0.6 ± 0.0	0.6 ± 0.2	3.6 ± 1.1	4.1 ± 0.7	3.5 ± 0.1	3.8 ± 0.4	2.8 ± 0.2	1.3 ± 0.1	3.3 ± 0.9	1.0 ± 0.4
$CD11c^+$	1.2 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.4	2.6 ± 0.1	3.1 ± 0.4	2.9 ± 0.6	2.4 ± 0.2	1.6 ± 1.0	0.6 ± 0.0	1.6 ± 0.5	0.8 ± 0.2

Data are means ± SEM. A total of 4 mice were examined for each experimental group and sex. All tests were performed on 100-day-old mice. Compared with $VDR^{+/+}$ NOD mice, †$P \leq 0.05$ and ‡$P \leq 0.01$. TCR, T-cell receptor.

number (Table 1) or function (chemotaxis, phagocytosis, and respiratory burst capacity) of $CD11b^+$ myeloid cells (data not shown). As expected, VDR was undetectable in $VDR^{-/-}$ NOD macrophages, while lipopolysaccharide (LPS) upregulated VDR in $VDR^{+/+}$ NOD macrophages (Fig. 2*A*). Comparably low levels of CYP27B1 were present in macrophages of both types of NOD mice (Fig. 2*B*). Basal expression of interleukin (IL)-1, IL-6, and CC chemokine ligand 2 (CCL2) was lower in $VDR^{-/-}$ NOD macrophages, while toll-like receptor 4 ligation amplified IL-1, IL-6, and CCL2 expression in $VDR^{-/-}$ NOD macrophages (though the effect was less pronounced than in $VDR^{+/+}$ NOD macrophages) (Fig. 2*C–E*). In contrast to $VDR^{+/+}$ NOD macrophages, no induction of CAMP (cathelicidan antimicrobial peptide) transcripts upon LPS stimulation was observed in $VDR^{-/-}$ NOD macrophages (Fig. 2*F*).

In unstimulated macrophages from $VDR^{-/-}$ and $VDR^{+/+}$ NOD mice, p65 nuclear factor-κB (NF-κB) was mainly located in cytoplasm. In response to LPS, translocation of p65 to the nucleus was significantly less pronounced in $VDR^{-/-}$ NOD macrophages (Fig. 2*G*).

Effect of VDR deficiency on dendritic cell phenotype. Ablation of VDR in NOD mice did not alter numbers of $CD11c^+$ dendritic cells in central and peripheral immune organs (Table 1). Nevertheless, thymic and lymph node dendritic cells from $VDR^{-/-}$ NOD mice showed defective differentiation into mature dendritic cells as indicated by an even lower CD86 expression (online appendix Fig. 2*A*).

Different results were observed in dendritic cells obtained in vitro from bone marrow cell cultures. Although percentage of $CD11c^+$ dendritic cells was similar for $VDR^{-/-}$ and $VDR^{+/+}$ NOD mice (data not shown), expression of major histocompatibility complex II and CD86 molecules was higher on bone marrow–derived dendritic cells from $VDR^{-/-}$ NOD mice relative to $VDR^{+/+}$ NOD mice (online appendix Fig. 2*B*).

Effect of VDR deficiency on insulitis and diabetes incidence of NOD mice. $VDR^{-/-}$ NOD mice had normal islet architecture and developed insulitis with similar severity as $VDR^{+/+}$ NOD littermates (Fig. 3*A*). In N10 generation, diabetes development by the age of 250 days was similar between $VDR^{-/-}$ and $VDR^{+/+}$ NOD littermates in final outcome (male, 50 vs. 50%, respectively, P = NS; female, 67 vs. 80%, P = NS) and in disease onset (male, 87 ± 2 vs. 113 ± 38 days, P = NS; female, 129 ± 30 vs. 111 ± 24 days, P = NS). In N14 generation, diabetes incidence in $VDR^{-/-}$ NOD males and females was 30 and 69%, respectively, while 38% of the $VDR^{+/+}$ NOD males and 70% of the $VDR^{+/+}$ NOD females became diabetic by 250 days (NS) (Fig. 3*B*). Timing of disease onset was similar in $VDR^{-/-}$ and $VDR^{+/+}$ NOD littermates (male, 163 ± 7 vs. 166 ± 10 days, respectively, P = NS; female, 142 ± 18 vs. 139 ± 13 days, P = NS).

Glucose tolerance tests in $VDR^{-/-}$ and $VDR^{+/+}$ NOD mice were identical as indicated by areas under the curve (AUC) (male, 29,928 ± 5,807 vs. 21,998 ± 2,123 AUC, respectively, P = NS; female, 23,409 ± 711 vs. 21147 ± 2,973 AUC, P = NS).

DISCUSSION

Vitamin D deficiency in early life leads to a doubling in diabetes incidence in later life in NOD mice (5) and is associated with a higher risk for type 1 diabetes in humans (4). To date, only one receptor for the active form of vitamin D has been identified; it was therefore surprising

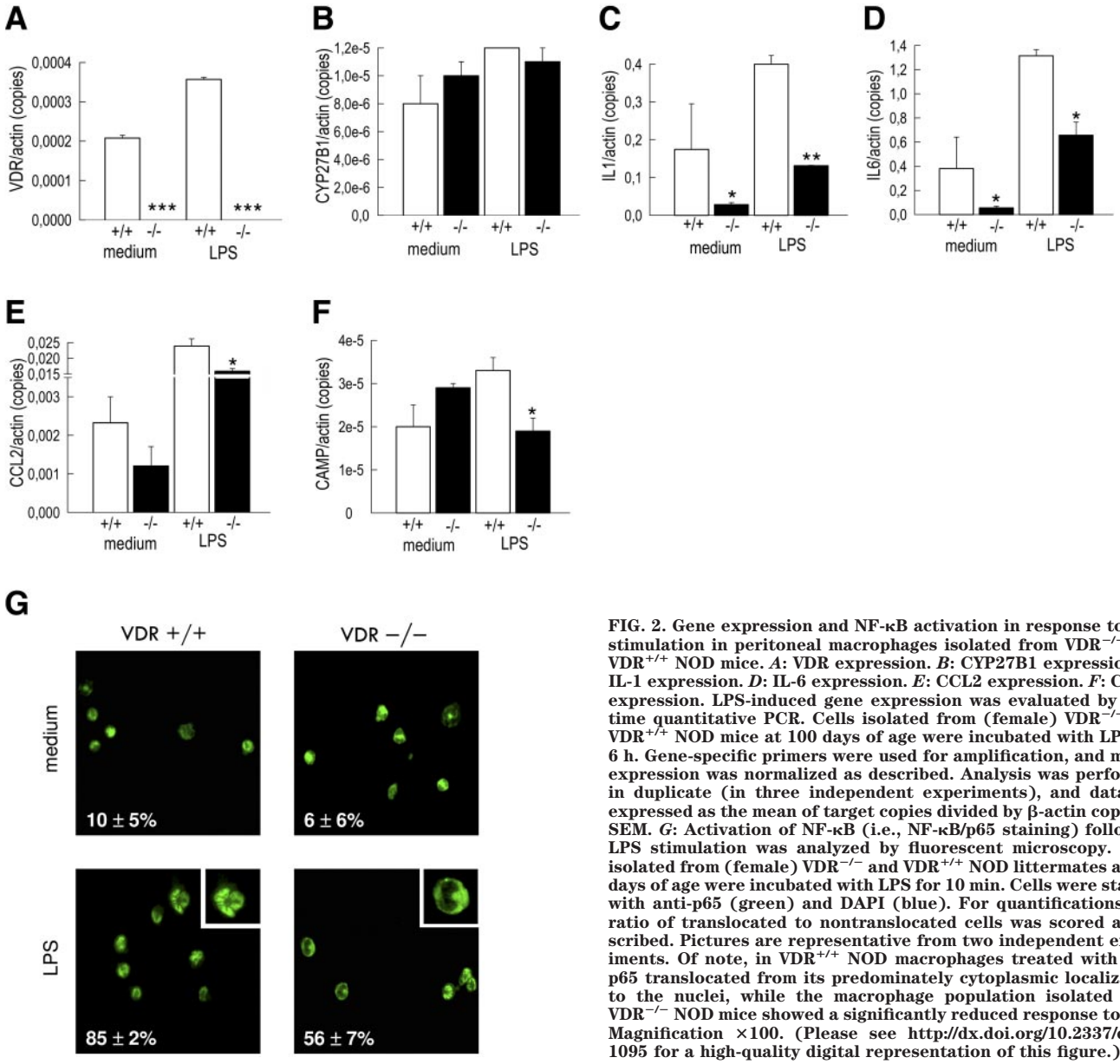


FIG. 2. Gene expression and NF-κB activation in response to LPS stimulation in peritoneal macrophages isolated from VDR$^{-/-}$ and VDR$^{+/+}$ NOD mice. *A*: VDR expression. *B*: CYP27B1 expression. *C*: IL-1 expression. *D*: IL-6 expression. *E*: CCL2 expression. *F*: CAMP expression. LPS-induced gene expression was evaluated by real-time quantitative PCR. Cells isolated from (female) VDR$^{-/-}$ and VDR$^{+/+}$ NOD mice at 100 days of age were incubated with LPS for 6 h. Gene-specific primers were used for amplification, and mRNA expression was normalized as described. Analysis was performed in duplicate (in three independent experiments), and data are expressed as the mean of target copies divided by β-actin copies ± SEM. *G*: Activation of NF-κB (i.e., NF-κB/p65 staining) following LPS stimulation was analyzed by fluorescent microscopy. Cells isolated from (female) VDR$^{-/-}$ and VDR$^{+/+}$ NOD littermates at 100 days of age were incubated with LPS for 10 min. Cells were stained with anti-p65 (green) and DAPI (blue). For quantifications, the ratio of translocated to nontranslocated cells was scored as described. Pictures are representative from two independent experiments. Of note, in VDR$^{+/+}$ NOD macrophages treated with LPS, p65 translocated from its predominately cytoplasmic localization to the nuclei, while the macrophage population isolated from VDR$^{-/-}$ NOD mice showed a significantly reduced response to LPS. Magnification ×100. (Please see http://dx.doi.org/10.2337/db07-1095 for a high-quality digital representation of this figure.)

to find that introduction of the VDR$^{-/-}$ phenotype onto the NOD background did not alter insulitis or diabetes presentation in NOD mice. These findings are in contrast to the more severe and accelerated diabetes in vitamin D–deficient NOD mice (5).

This discrepancy in disease phenotype between absence of ligand and absence of receptor is also described for other members of the nuclear receptor superfamily, e.g., the thyroid hormone receptor-α/β. The hypothyroid state results in severe abnormalities in cerebral cortical, cerebellar, and ear development, whereas normal central nervous system structure and function, apart from deafness caused by an inner ear defect, are observed in thyroid hormone receptor-β$^{-/-}$ mice (14). These data emphasize that nuclear receptors play independent roles even in the absence of an agonistic ligand.

This difference between loss of ligand and loss of receptor is not homogenous for different tissues. For the VDR, this discrepancy has already been reported for hair growth (15). VDR$^{-/-}$ mice and humans with VDR mutations develop alopecia, whereas vitamin D–deficient mice or humans have normal hair cycles.

Also, in the immune system, differences between loss of ligand and loss of receptor are found. Vitamin D–deficient animals and humans have an increased sensitivity to mycobacteria (2), probably related to deficient macrophage function, whereas macrophage function was normal in VDR$^{-/-}$ NOD mice. On the other hand, aberrances in dendritic cell phenotype were observed here and previously in VDR$^{-/-}$ mice (16), whereas dendritic cells from vitamin D–deficient mice do not present abnormalities (E. van Etten, unpublished observations). These changes in dendritic cells may contribute to the changes in T-lymphocyte subsets observed in VDR$^{-/-}$ NOD mice but not in vitamin D–deficient mice.

Absence of ligand and absence of a nuclear receptor also affect disease presentation differently. Experimental autoimmune encephalomyelitis incidence was de-

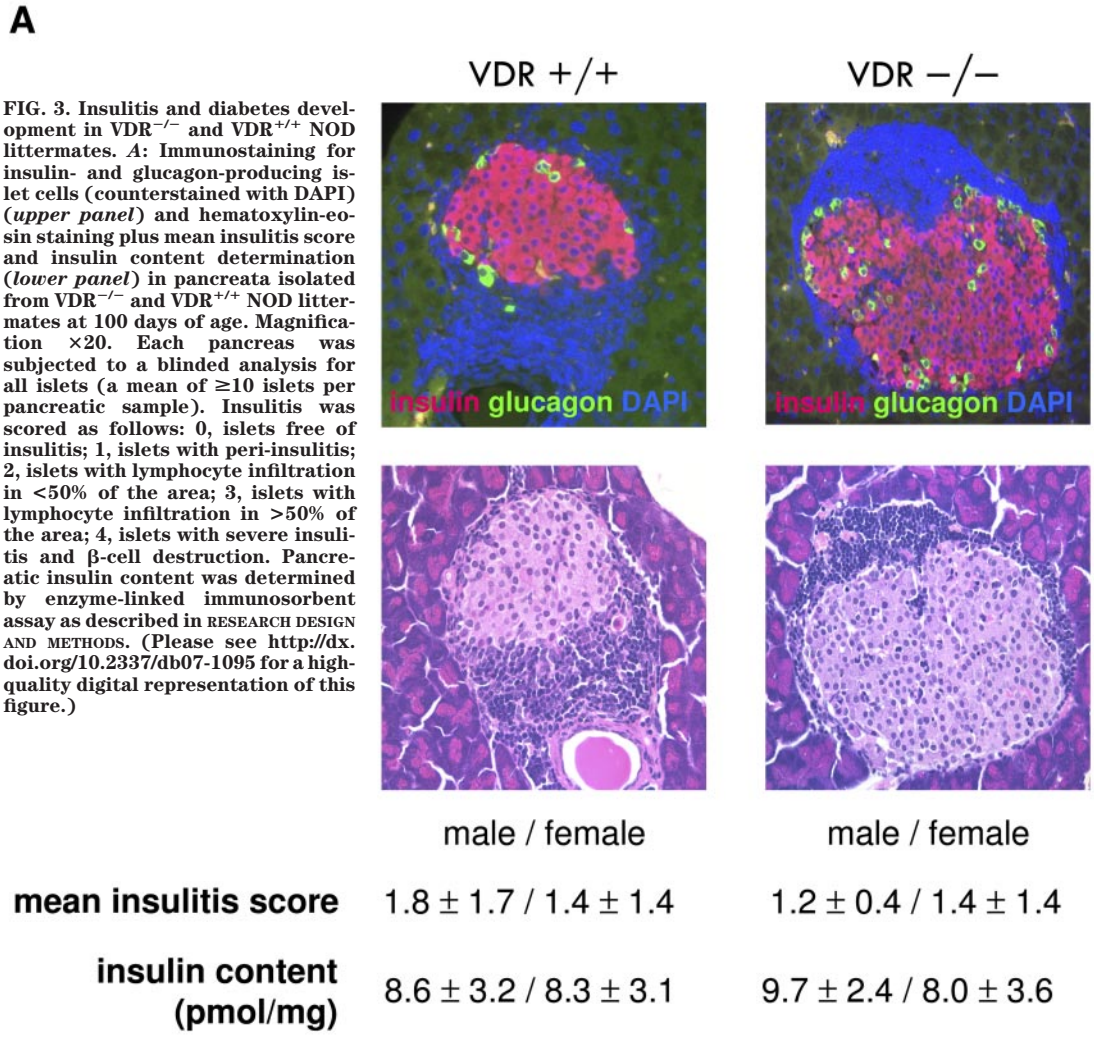
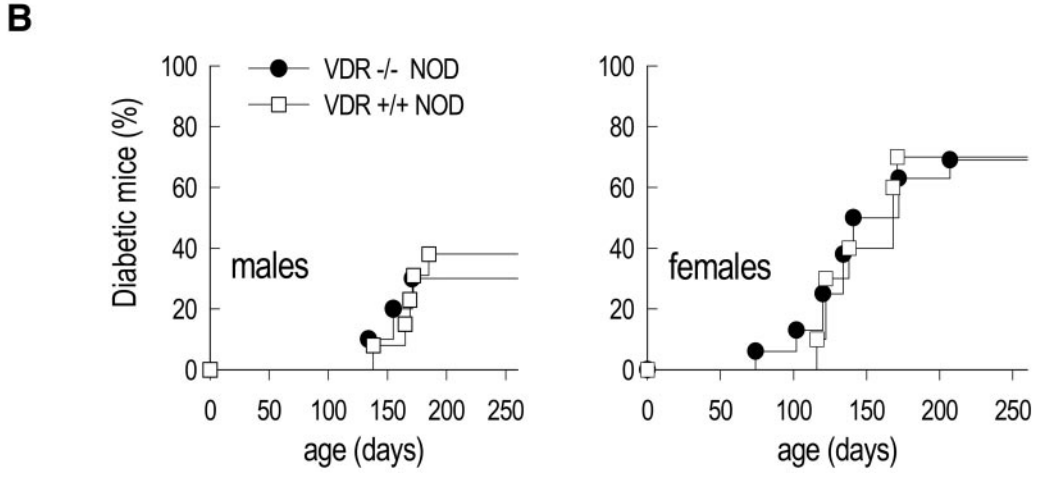


FIG. 3. Insulitis and diabetes development in VDR$^{-/-}$ and VDR$^{+/+}$ NOD littermates. *A*: Immunostaining for insulin- and glucagon-producing islet cells (counterstained with DAPI) (*upper panel*) and hematoxylin-eosin staining plus mean insulitis score and insulin content determination (*lower panel*) in pancreata isolated from VDR$^{-/-}$ and VDR$^{+/+}$ NOD littermates at 100 days of age. Magnification ×20. Each pancreas was subjected to a blinded analysis for all islets (a mean of ≥10 islets per pancreatic sample). Insulitis was scored as follows: 0, islets free of insulitis; 1, islets with peri-insulitis; 2, islets with lymphocyte infiltration in <50% of the area; 3, islets with lymphocyte infiltration in >50% of the area; 4, islets with severe insulitis and β-cell destruction. Pancreatic insulin content was determined by enzyme-linked immunosorbent assay as described in RESEARCH DESIGN AND METHODS. (Please see http://dx.doi.org/10.2337/db07-1095 for a high-quality digital representation of this figure.)



***B*: Cohorts of VDR$^{-/-}$ and VDR$^{+/+}$ NOD mice (N14 backcross mice were used to generate these plots) were monitored for the development of diabetes. Mice with blood glucose levels >250 mg/dl were scored as having diabetes. VDR$^{-/-}$ NOD males reached an incidence of 30% against 38% in VDR$^{+/+}$ NOD males (P = NS). Females presented the highest incidence, reaching 69% in VDR$^{-/-}$ NOD mice, which was fully comparable with that of VDR$^{+/+}$ NOD females, with 70% (P = NS).**

creased in $VDR^{-/-}$ mice (17), whereas vitamin D deficiency induced acceleration of disease (18). In contrast, in models of inflammatory bowel disease, more severe colitis was present in the absence of either vitamin D or its receptor (19). A clear explanation for these diverse disease phenotypes is lacking, but involvement of different immune cells in respective target organs that are affected in a different way by ligand or receptor loss may be responsible.

A remarkable finding here was that $VDR^{-/-}$ NOD mice had severe immune defects considered crucial for diabetes development in NOD mice, whereas $VDR^{-/-}$ NOD mice did not display an enhanced susceptibility to disease. For instance, less T-cell receptor-α/β^+ $CD4^-CD8^-$ T-cells and regulatory $CD4^+CD25^+$ T-cells were present in $VDR^{-/-}$ NOD immune organs, postulated to contribute to an inability to maintain peripheral tolerance and, hence, diabetes development in NOD mice (20,21). Moreover, inactivation of VDR in NOD mice resulted in disturbed cytokine and chemokine profile with extremely low IL-1, IL-6, and CCL2 transcripts, implicated in aberrant migratory capacity, possibly trapping the macrophages in the pancreas and leading to nonspecific damage of β-cells (22). Moreover, thymus and lymph nodes of $VDR^{-/-}$ NOD mice contained even fewer mature $CD11c^+$ dendritic cells than $VDR^{+/+}$ NOD mice, as reflected by low levels of CD86, proposed to contribute to a defective regulation of diabetogenic T-cells by preventing the full activation of T-cells (23). Taken together, it is clear that inactivation of VDR intensifies several identified defects in both innate and adaptive immune system of NOD mice—without, however, direct translation in an aggravated disease onset and incidence.

In conclusion, in contrast to vitamin D deficiency, disruption of its receptor, VDR, does not influence the onset, severity, or incidence of insulitis and diabetes in NOD mice of either sex despite the induction of an array of defects in innate and adaptive immune system. Considering this discrepancy in disease phenotype between the ligand-deficient and the receptor-deficient situation, it would be wrong to conclude that no role for vitamin D and its active form, $1,25(OH)_2D_3$, exists in the prevention of type 1 diabetes or other autoimmune diseases. The link between vitamin D deficiency and a higher prevalence of autoimmune diabetes in genetically high-risk individuals, mice or humans, has clearly been proven. Our present data point toward the interesting observation that nuclear receptors have a function of their own and that having an unliganded receptor present results in a different situation from that of having no receptor present.

ACKNOWLEDGMENTS

This work was supported by the Belgium Program on Interuniversity Poles of Attraction initiated by the Belgian State (IAP P5/17 and P6/40), the Katholieke Universiteit Leuven (Geconcerteerde Onderzoeksacties 2004/10), the Flemish Research Foundation (Fonds Voor Wetenschappelijk Onderzoek [FWO], Vlaanderen G.0084.02, G.0233.04, G.0552.06, and G.0649.08), and the European Union Project SAVEBETA (contract no. 036903 in FP6 of the European Community). C.M. has a clinical research fellowship from FWO, and C.G. and G.E. have a postdoctoral fellowship from FWO. E.V.E. has a postdoctoral fellowship from the Juvenile Diabetes Research Foundation (3-2006-33).

We thank Wim Cockx, Jos Depovere, Julianna Hagenbrock, Molly Holte-Nelson, Ivo Jans, Jos Laureys, Suzanne Marcelis, Dirk Valckx, Erik Van Herck, and Riet van Looveren for excellent technical assistance. We thank Dr. Edward Leiter (The Jackson Laboratory, Bar Harbor, ME) for his expert guidance in the backcrossing and for critical reading of the manuscript.

REFERENCES

1. Beran D, Yudkin JS: Diabetes care in sub-Saharan Africa. *Lancet* 368:1689–1695, 2006
2. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL: Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773, 2006
3. Holick MF: Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 116:2062–2072, 2006
4. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM: Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 358:1500–1503, 2001
5. Giulietti A, Gysemans C, Stoffels K, van Etten E, Decallonne B, Overbergh L, Bouillon R, Mathieu C: Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. *Diabetologia* 47:451–462, 2004
6. Zella JB, McCary LC, DeLuca HF: Oral administration of 1,25-dihydroxyvitamin D_3 completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch Biochem Biophys* 417:77–80, 2003
7. Mathieu C, Laureys J, Sobis H, Vandeputte M, Wer M, Bouillon R: 1,25-Dihydroxyvitamin D_3 prevents insulitis in NOD mice. *Diabetes* 41:1491–1495, 1992
8. Mathieu C, Wer M, Laureys J, Rutgeerts O, Bouillon R: Prevention of autoimmune diabetes in NOD mice by 1,25-dihydroxyvitamin D_3. *Diabetologia* 37:552–558, 1994
9. Mathieu,C, Adorini,L: The coming of age of 1,25-dihydroxyvitamin D_3 analogs as immunomodulatory agents. *Trends Mol Med* 8:174–179, 2002
10. Chang TJ, Lei HH, Yeh JI, Chiu KC, Lee KC, Chen MC, Tai TY, Chuang LM: Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. *Clin Endocrinol (Oxf)* 52:575–580, 2000
11. Motohashi Y, Yamada S, Yanagawa T, Maruyama T, Suzuki R, Niino M, Fukazawa T, Kasuga A, Hirose H, Matsubara K, Shimada A, Saruta T: Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. *J Clin Endocrinol Metab* 88:3137–3140, 2003
12. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, Masushige S, Fukamizu A, Matsumoto T, Kato S: Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet* 16:391–396, 1997
13. Serreze DV, Chapman HD, Varnum DS, Hanson MS, Reifsnyder PC, Richard SD, Fleming SA, Leiter EH, Shultz LD: B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu null mice. *J Exp Med* 184:2049–2053, 1996
14. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN, Wondisford FE: An unliganded thyroid hormone receptor causes severe neurological dysfunction. *Proc Natl Acad Sci USA* 98:3998–4003, 2001
15. Skorija K, Cox M, Sisk JM, Dowd DR, Macdonald PN, Thompson CC, Demay MB: Ligand-independent actions of the vitamin D receptor maintain hair follicle homeostasis. *Mol Endocrinol* 19:855–862, 2005
16. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R: Dendritic cell modulation by 1α,25-dihydroxyvitamin D_3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. *Proc Natl Acad Sci USA* 98:6800–6805, 2001
17. Meehan TF, DeLuca HF: The vitamin D receptor is necessary for 1α,25-dihydroxyvitamin D_3 to suppress experimental autoimmune encephalomyelitis in mice. *Arch Biochem Biophys* 408:200–204, 2002
18. Cantorna MT, Hayes CE, DeLuca HF: 1,25-Dihydroxyvitamin D_3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA* 93:7861–7864, 1996
19. Froicu M, Cantorna MT: Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC Immunol* 8:5, 2007

20. Godfrey DI, Kinder SJ, Silvera P, Baxter AG: Flow cytometric study of T cell development in NOD mice reveals a deficiency in TCR-α/β+CD4-CD8- thymocytes. *J Autoimmun* 10:279–285, 1997
21. Alard P, Manirarora JN, Parnell SA, Hudkins JL, Clark SL, Kosiewicz MM: Deficiency in NOD antigen-presenting cell function may be responsible for suboptimal $CD4^+CD25^+$ T-cell–mediated regulation and type 1 diabetes development in NOD mice. *Diabetes* 55:2098–2105, 2006
22. Serreze DV, Gedeke JW, Leiter EH: Hematopoietic stem-cell defects underlying abnormal macrophage development and maturation in NOD/Lt mice: defective regulation of cytokine receptors and protein kinase C. *Proc Natl Acad Sci USA* 90:9625–9629, 1993
23. Dahlen E, Hedlund G, Dawe K: Low CD86 expression in the non-obese diabetic mouse results in the impairment of both T cell activation and CTLA-4 up-regulation. *J Immunol* 164:2444–2456, 2000