Erratum

Cerkovic-Cvrlje M, Gerling IC, Muir A, Atkinson MA, Elliott JF, Leiter EH: Retardation or acceleration of diabetes in NOD/Lt mice mediated by intrathymic administration of candidate β-cell antigens. *Diabetes* 46:1975–1982, 1997

Because of a typesetting error, the Greek symbol "μ" appeared as an "m" eight times in the article mentioned above. The correct paragraphs are printed below, with the corrected units shown in bold.

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RT-PCR semiquantification of cytokine mRNA. Total RNA was extracted from islets isolated by collagenase digestion (3) or from whole pancreases of 8-week-old NOD/Lt females intrathymically treated with islet cells, GAD65, insulin A and B chain, and PBS at age 4 weeks, using the same single-step RNA extraction method as described previously (23). Isolated islets (100–150 per pancreas) were handpicked under a dissecting microscope and pooled from 5–6 mice per each treatment group. RNA samples were treated with DNase (DNase I, Amplification Grade; Gibco BRL, Gaithersburg, MD). Approximately 0.5 **μg** of DNase-treated RNA from each sample was reverse transcribed to cDNA (60 min at 42° C) in a 30-**μl** reaction containing 50 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 10 mmol/l $MgCl_2$, 0.5 mmol/l spermidine, 10 mmol/l DTT, 1 mmol/l of each dNTP, 40 U RNAsin (Promega, Madison,WI), 600 ng random hexamers (Pharmacia, Uppsala, Sweden), and 30 U AMV-reverse transcriptase (Promega, Madison, WI). The resulting cDNA was amplified by radiolabeled PCR using separate pairs of oligonucleotide primers for β-actin, IFN-γ, IL-4 (33), IL-10 (30) and the constant region of the T-cell receptor β-chain (TCR-Cβ) (31). PCR reactions (30 μl) contained 5 **μl** cDNA, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mmol/l $MgCl_2$, 0.1 mmol/l of each dNTPs, 50 pmol of the appropriate 5' and 3' primers, 2.5 U Taq DNA polymerase (Promega, Madison, WI) and 0.3 **μCi** (α-^{32}P) dCTP. Reactions were loaded in a Perkin-Elmer Thermal Cycler 9600 (Perkin-Elmer/Cetus, Norwalk, CT, heat-treated at 94° C for 3 min, and then subjected to 23 (β-actin) or 30–35 (IFN-γ, IL-4, IL-10, and TCR) amplification cycles. The number of cycles chosen was empirically determined for each primer set to ensure that amplification of all specific cDNA products was exponential. Each cycle consisted of denaturation at 94° C for 20 s, primer annealing at 55° C for 20 s, and extension at 72° C for 60 s. After amplification, 5 **μl** of the PCR products were electrophoresed in a 1.7% polyacrylamide gel for 45 min at 300 V. The IFN-γ, IL-4, IL-10 and β-actin PCR products were 220, 217, 479, and 348 bp, respectively. Gels were dried at 80° C for 40 min, and the radiolabeled PCR products were semiquantified using the phosphor-imaging system (Fujix BAS 2000; Fuji, Tokyo, Japan). The intensity of each radiolabeled signal was expressed in arbitrary phosphor stimulated luminescence (PSL) units. To correct for variations in the amount of RNA used, and more importantly, the variability in the efficiency of reverse transcription, the radiolabeled signal for each cytokine PCR product was normalized to the β-actin PCR signal of the same sample. To obtain information about cytokine expression in islets resulting from a different level of T-cell infiltration, the cytokine/β-actin PSL ratio was normalized to the TCR/β-actin PSL ratio.

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DISCUSSION

The present study confirmed previous reports demonstrating protection/retardation of IDDM in NOD mice (3–5) and BB rats (35) by intrathymic treatment with whole islets or islet cells. Furthermore, our finding that the extent of IDDM resistance elicited by intrathymic injection of 32 **μg** of GAD65 was equivalent to that obtained by intrathymic injection of a cell suspension from 200 islets confirmed previous reports of the IDDM-suppressive effect of GAD65 treatments in the prediabetic state (7,11). Moreover, we showed for the first time that intrathymic injection of 200 **μg** of insulin B chain could retard IDDM development as well. In contrast to the high levels of insulin transcripts and product in collagenase-isolated and handpicked islets from young, prediabetic NOD/Lt mice, we observed very low levels of GAD mRNA transcripts and enzymatic activity (M.C.-C., E.H.L., unpublished observations). Given the high ratio of insulin to GAD protein in NOD islets, the level of GAD65 administered intrathymically (32 **μg** total per two lobes) represented a huge excess from the level expected in a preparation of cells from 200 islets and also in comparison with the concentration of insulin in these cells. Therefore, multiple antigens found in NOD islets at low abundance (as exemplified by GAD) amplify the protective effects of the higher concentrations of insulin present in islet cell preparations given intrathymically to NOD females.