Type 1 diabetes in NOD mice can be prevented through autoantigen vaccination by shifting lymphocyte differentiation toward a T-helper 2 (Th2) response. However, in other models of autoimmunity, this approach may be accompanied by unexpected triggering of Th1-dependent anaphylactic shock. To test the safety of vaccination therapy in the NOD mouse model, we evaluated the effects of immunization with a wide battery of antigens in NOD, BALB/c, and C57BL/6 mice. Surprisingly, a nondiabetogenic antigen, hen egg white lysozyme, induced severe shock exclusively in NOD mice (shock in 11 of 11 mice, lethal in 3 mice). Shock severity was further increased by a more pronounced Th2 setting generated by 1α,25(OH)2D3 administration (17 of 17 mice, lethal in 14 mice, P < 0.0001). Pretreatment with dexamethasone resulted in full rescue, indicating an immune-mediated mechanism. Serum IgE levels and Th1/Th2 cytokine profile analysis showed that the shock phenomenon was paralleled by a Th2 response. mRNA expression of platelet-activating factor receptor (PAF-R) was significantly higher in NOD mice (P < 0.01) and was further increased by 1α,25(OH)2D3. Pretreatment with WEB2086 (PAF-R antagonist) again protected all mice from lethal shock, indicating PAF as an anaphylaxis effector. In conclusion, in NOD mice, vaccination leading to a Th2 immune shift can result in a lethal anaphylactic reaction. Diabetes 52:335–341, 2003

Type 1 diabetes is caused by immune destruction of insulin-producing β-cells in the pancreatic islets of Langerhans (1). In the NOD mouse, a widely used animal model for the study of type 1 diabetes, lymphocytic infiltration into the pancreatic islets (insulitis) is the primary lesion that eventually progresses to overt diabetes. Evidence is accumulating that type 1 diabetes is mediated by T-helper 1 (Th1) cells and that protection or prevention of the disease can be achieved by shifting the immune response toward Th2 activation (2,3).

Several diabetogenic autoantigens have been described, such as GAD, insulin, and heat shock protein, in both animal models and human disease (4,5). Furthermore, epitope spreading and molecular mimicry is enlarging their spectrum during disease evolution (5). The biochemical properties of antigens together with the genetic background of the immune system significantly influence the type (Th1 or Th2) of immune activation (6). Many studies have investigated the effects of administration of diabetes relevant autoantigens or their immunodominant peptides in the NOD mouse model. Most studies convincingly demonstrate a prevention or delayed onset of disease, associated with a shift toward a more Th2-biased response (5,7). Recently, also in type 1 diabetic patients, peptide treatment with specific autoantigens has been shown to prevent or delay the disease and was concluded to be safe (8).

This vaccination-triggered shift from autoreactive Th1 toward Th2 cells has not only been observed in NOD mice (9) but also in experimental autoimmune encephalomyelitis (EAE), another Th1-mediated autoimmune disease (10). Nevertheless, the administration of self-antigen in the EAE model has lead to unforeseen consequences of Th2 activation. Indeed, vaccination with the myelin proteolipid peptide PLPp(139–151) in SJL/J mice under certain conditions, essentially depending on the time point of peptide rechallenge, unexpectedly resulted in lethal anaphylaxis (11). Also in the human situation, a recent phase II trial in which multiple sclerosis (MS) patients received an altered peptide ligand from a myelin basic protein epitope, repeated injections with the peptide induced immediate hypersensitivity reactions in 9% of the subjects (12). These reports suggest that an autovaccine-triggered immune response may produce extremely undesirable allergic reactions. To what extent anaphylaxis depends on the genetic background, the antigen type, or the timing of vaccination is not completely known.

The aim of the present study was to test whether vaccination in NOD mice, animals having an immune system genetically prone to destroy pancreatic islets through Th1-mediated mechanisms, is perfectly safe or whether life-threatening reactions can be induced by manipulating the immune response. Indeed, the NOD mouse strain also displays immune system particularities beyond Th1-dependent autoimmune islet destruction because they seem to be highly susceptible to anaphylactic shock under...
certain specified conditions (13,14). NOD mice might therefore behave differently from other mouse strains in certain situations of immune challenge (13). To investigate whether vaccination could induce lethal shock in NOD mice, we tested a wide panel of antigens. We compared the effects of several diabetes-relevant autoantigens and diabetes-irrelevant peptides or proteins in NOD mice to the effects obtained in two other mouse strains (BALB/c and C57BL/6 mice), in which a Th1-activated immune system is absent. Based on our previous findings, we also tested mice from various strains with an immunomodulatory agent known to favor an autoantigen-specific Th2 shift in NOD mice, namely 1α,25(OH)2D3, and examined its effect on the safety profile of vaccination.

**RESEARCH DESIGN AND METHODS**

**Animals.** NOD mice were originally obtained from Prof. C.Y. Wu in 1989 and further bred in our animal house (Proefdierencentrum, Leuven, Belgium), kept under semi-barrier conditions (15). At the time of the study, diabetes incidence by the age of 200 days in our stock colony was 72% in female mice and 25% in male mice. Nondiabetic mice aged 7–9 weeks of both sexes were used in the experiments. BALB/c and C57BL/6 mice were purchased from Harlan (Horst, the Netherlands). NOD/LtSz-scid and 25% in male mice. Nondiabetic mice aged 7–9 weeks of both sexes were used in the experiments. BALB/c and C57BL/6 mice were purchased from Harlan (Horst, the Netherlands). NOD/LtSz-scid and C57BL/6 mice were purchased from Harlan (Horst, the Netherlands). NOD/LtSz-scid mice were obtained from Prof. Vermylen, University of Leuven, Leuven, Belgium), 500 μg HEL antigen suspended in CFA. All mice were reinjected with the same antigen 3 weeks later in a similar manner.

**Vaccination and intervention regimens.** The 8-week-old NOD, BALB/c, and C57BL/6 mice were immunized by injection of 100 μg antigen (HEL, GAD65, ins-B, hsp65, PLP, OVA, KLH, and TT) emulsified at a 1:1 concentration in complete Freund’s adjuvant (CFA) or incomplete Freund’s adjuvant (Difco Laboratories, Detroit, MI) in the hind footpads. NOD-scid mice were injected with 100 μg HEL antigen suspended in CFA. All mice were reimmunized with the same antigen 3 weeks later in a similar manner.

**Follow-up of mice.** Clinical evolution and survival rate after sensitization with various peptides were monitored in different mouse strains. Shock was characterized by pilo-erection; prostration; erythema of the tail, ears, and footpads; and dyspnea with shallow breathing. Serum for antibody measurement and spleens for quantification of mRNA levels were collected before immunization and again before booster administration.

**Measurement of antibody production.** HEL-specific IgG1, IgG2a, and IgE antibodies were measured in mouse sera by enzyme-linked immunosorbent assay (ELISA). Briefly, for IgG1 and IgG2a ELISA, 96-well microtiter plates were coated overnight with HEL (10 μg/ml). Samples for IgG1 and IgG2a measurement were diluted at 1:3,000 and 1:800, respectively. Bound IgG1 and IgG2a were detected using biotinylated anti-mouse IgG1 and anti-mouse IgG2a, respectively. The plates were further processed with horseradish peroxidase–conjugated streptavidin and tetramethylbenzidine as substrate. To measure HEL-specific IgE antibody, plates were coated with anti-mouse IgE antibody. After incubation with diluted mouse sera (1:20), bound IgE was incubated with HEL. The plates were then processed with peroxidase-conjugated anti-HEL antibody, and tetramethylbenzidine was used as a substrate. Plates were read at 450 nm on a MultiSkan ELISA reader (ThermoLab Systems, Brussels, Belgium).

**Real-time RT-PCR for cytokines, PAF-acetylhydrolase, and PAF-R.** Total IgE was measured by sandwich ELISA (BD Biosciences, Erembodegem, Belgium) according to the manufacturer’s instructions. Serum samples were analyzed in duplicate at a 1:10 dilution.

**Statistical analysis.** Incidence of shock and mortality were compared using the χ2 test. mRNA and antibody levels were compared using the Student’s t test. Differences were considered significant at P < 0.05.

**RESULTS**

**Shock induced by antigen vaccination.** No clinical shock reactions were observed after primary injection for any of the tested autoantigens (GAD65, ins-B, and hsp65) and diabetes-irrelevant antigens (OVA, KLH, TT, and PLP). However, after the booster injection with HEL, all tested NOD mice (11/11) had a clinical picture of shock and three mice (27%) subsequently died (Fig. 1). This shock occurred within the first hour after injection and was characterized by pilo-erection; prostration; erythema of the tail, ears, and footpads; and dyspnea with shallow breathing. In the case of recovery, it occurred within another hour. The severity of these symptoms did not depend on the nature of the adjuvant because similar results were obtained when NOD mice where primed and rechallenged with HEL suspended in incomplete Freund’s adjuvant (shock in five of five mice). No signs of shock were
observed in BALB/c \((n = 9)\) or C57BL/6 \((n = 9)\) mice. Booster injections with all other antigens were also uneventful in all tested mice.

Treatment of NOD mice with Th2 enhancing 1α,25(OH)₂D₃ exacerbated the previous observations of shock after the HEL booster. Whereas all \((n = 17)\) 1α,25(OH)₂D₃-treated NOD mice showed severe clinical signs of shock, 82% never recovered and died \((P < 0.01\) compared with vehicle-treated NOD mice). This enhancement of sensitivity to shock by 1α,25(OH)₂D₃ was also observed in BALB/c mice. Here, in contrast to the symptom-free control BALB/c mice \((n = 9)\), all 1α,25(OH)₂D₃-treated BALB/c mice \((n = 9)\) showed clinical signs of shock, although not life-threatening shock. This exacerbation by treatment with 1α,25(OH)₂D₃ was not observed in C57BL/6 mice \((n = 9)\), which all remained shock free (Fig. 1).

**Th2 shift parallels shock.** Because the clinical picture was compatible with a state of anaphylaxis, rescue treatment with corticosteroids was attempted. As shown in Fig. 2, pretreatment of NOD mice with 50 μg dexamethasone, injected intraperitoneally 48, 24, and 2 h before the HEL booster, rescued all NOD mice—control as well as 1α,25(OH)₂D₃-treated \((n = 8\) for each group). This result suggests involvement of the immune system in the shock phenomenon. To further investigate the mechanism involved, antibody production was measured. Therefore, HEL-specific serum IgG1 and IgG2a and total serum IgE levels of the NOD and C57BL/6 mice, treated with 1α,25(OH)₂D₃ or vehicle, were measured 3 weeks after the first HEL immunization (just before HEL rechallenge).

No significant differences were found for Th1-induced HEL-specific IgG2a antibodies between control NOD and C57BL/6 mice, and no differences were found in the 1α,25(OH)₂D₃-treated groups (Fig. 3A). Levels of HEL-specific IgG1 from NOD mice were very high in all HEL-immunized mice, irrespective of 1α,25(OH)₂D₃ administration. Levels in NOD mice were higher compared with C57BL/6 mice, in vehicle- as well as in 1α,25(OH)₂D₃-treated mice, although no significant difference was reached (Fig. 3B). HEL-specific IgE antibodies were below the detection limit in our ELISA method (data not shown). However, total IgE levels, which were undetectable in the sera of control and CFA-injected NOD and C57BL/6 mice, were elevated in all HEL-immunized mice. Interestingly, total IgE levels were significantly higher in NOD mice than in C57BL/6 controls \((P < 0.01\); Fig. 3C). Moreover, this high IgE level in NOD mice was further increased by treatment with 1α,25(OH)₂D₃ \([P < 0.05\) in 1α,25(OH)₂D₃-treated NOD mice compared with control NOD mice; Fig. 3C], a phenomenon not observed in C57BL/6 mice.
SHOCK INDUCED BY HEL IN NOD MICE

To further investigate the possible involvement of cytokines in this IgE response, cytokine mRNA levels specifically produced by Th1 or Th2 cells were quantified by real-time RT-PCR. In analogy to the serum antibody measurements, mRNA levels were quantified 3 weeks after the first HEL immunization in the spleen of NOD versus C57BL/6 mice, either 1α,25(OH)2D3 or vehicle-treated. IFN-γ mRNA was significantly downregulated by treatment with 1α,25(OH)2D3 in the HEL-immunized NOD mice (P < 0.05, Fig. 4A). Lower values of this Th1 cytokine were also seen in the 1α,25(OH)2D3-treated C57BL/6 mice compared with their nontreated controls, although the difference did not reach significance. However, no difference was observed between the two strains with regard to IFN-γ mRNA (Fig. 4A). In contrast to the downregulated IFN-γ, IL-13 mRNA levels were dramatically upregulated by 1α,25(OH)2D3 in the HEL-immunized NOD mice (P < 0.0001), an effect that was not observed in the C57BL/6 mice. Moreover, for this Th2-specific cytokine, an obvious strain difference was seen between HEL-immunized NOD and C57BL/6 mice (P < 0.05), a difference that was even more pronounced during treatment with 1α,25(OH)2D3 (P < 0.0001, Fig. 4B). No differences in IL-4 or in the macrophage-produced cytokines IL-1, IL-6, IL-12, and TNF-α mRNA levels were observed (data not shown).

Finally, NOD-scid mice lacking T- and B-cells were primed and rechallenged with HEL/CFA. Interestingly, in these mice (n = 6), no symptoms of shock were observed, conclusively demonstrating the key role of T-cells in the observed phenomenon.

**Involvement of platelet activation in shock.** Because the clinical picture of lethal shock was accompanied by hypercoagulation, compatible with anaphylaxis leading to death by disseminated intravascular coagulation, we questioned the involvement of platelet activation in induced shock. We investigated if factors involved in this pathway showed an abnormal expression profile in the NOD mouse or were influenced by 1α,25(OH)2D3. Therefore, mRNA levels for PAF-R and PAF-AH, the enzyme most responsible for degradation of PAF, were quantified by real-time RT-PCR in the spleens of NOD and C57BL/6 mice 3 weeks after the first footpad injection with HEL.

Interestingly, a clear inter-strain difference in the expression of PAF-R mRNA was observed, with 2.5-fold higher levels in NOD mice than in C57BL/6 mice (P < 0.05, Fig. 5A). Additionally, 1α,25(OH)2D3 administration further increased the expression PAF-R mRNA in NOD as well as C57BL/6 mice by 1.5- and 2.5-fold, respectively (P < 0.05, Fig. 5A). These results, showing a regulation of the PAF-R in the analyzed system, are highly indicative for an important role of the PAF pathway in induction and sensitivity for anaphylaxis.

The mRNA expression of PAF-AH in the spleens of HEL-immunized NOD mice was 1.5-fold higher than that in C57BL/6 mice (P < 0.01). 1α,25(OH)2D3 treatment did not influence the PAF-AH mRNA levels. A minor interstrain difference remained after 1α,25(OH)2D3 treatment; however, this difference did not reach significance (Fig. 5B).

To conclusively demonstrate the involvement of the platelet activation pathway in the shock phenomenon, we attempted to rescue the HEL-immunized NOD mice from lethal shock by administration of a PAF-R antagonist. Indeed, mortality was completely abrogated by administration of a single dose of 500 μg of the PAF-R antagonist WEB2086 10 min before the HEL booster in all HEL-immunized NOD mice, irrespective of 1α,25(OH)2D3 treatment (Fig. 6). Only mild nonlethal shock symptoms were observed in 75% of the rescued mice; in each group, shock was completely prevented in two of eight mice, whereas
Efforts have thus been made to induce an anergic state of T-helper cells, which are mainly Th2-dependent (22) and are largely mediated by IgE (22,23). Aggressive allergic reactions to encephalitogenic antigens were also observed when the vaccination approach was tested in human MS (12,24). The beneﬁcial effect of antigenic vaccination in this particular strain with diabetes-relevant autoantigens was also not immediately clear because previous studies suggested that a Th2-dependent differentiation shift toward Th2, driven by IL-12, would be hampered by the allergic side effects.

NOD mice to vaccination was compared with that of two other mouse strains: C57BL/6 and BALB/c mice. To force T-cell differentiation toward a Th1 setting, we submitted groups of mice from the three strains to therapy with 1α,25(OH)2D3. The active form of vitamin D is known as a powerful modulator of the immune system (27). The 1α,25(OH)2D3-induced shift toward Th1 differentiation can even prevent the onset of Th1-mediated autoimmune diseases, such as diabetes in NOD mice (28) or EAE in SJL/J mice (29).

The strain-selective anaphylactic shock induced by HEL in NOD mice. This mouse strain is indeed known to react through Th1-mediated mechanisms (2–4,25) and was moreover described as having a defect in immune modulation by Th1 (31,32). An exacerbation of anaphylaxis caused by the administration of 1α,25(OH)2D3 was also not immediately clear because previous studies suggested that a 1α,25(OH)2D3-dependent differentiation shift toward Th2 takes place exclusively in the presence of diabetes-relevant autoantigens (33). We decided therefore to search for the mechanisms underlying the strain-specific behavior of the NOD immune system.

Complete rescue by therapy with dexamethasone demonstrated undoubtedly that HEL-induced shock in NOD mice depended on immune effectors. Furthermore, the complete absence of symptoms in NOD-scid mice lacking T- and B-cells (34) proved the indispensable role of T-cells in HEL-induced shock. Cytokine modifications caused by 1α,25(OH)2D3 in NOD mice were partially suggestive for a Th1 to Th2 shift, with a decrease in Th1-secreted IFN-γ and a concomitant increase in Th2-specific IL-13. However, no differences in IL-12 and IL-4 levels were found between the 1α,25(OH)2D3-treated and nontreated HEL-sensitized NOD mice. Thus, these changes did not completely overlap the 1α,25(OH)2D3-dependent modifications found when the immune system was activated by autoantigens (35), suggesting antigen-speciﬁc immune modulation by 1α,25(OH)2D3. Comparison of cytokine mRNA spectra between HEL-immunized shock-sensitive NOD mice and shock-resistant C57BL/6 mice showed differences in IL-13 mRNA levels, which were further accentuated by the other six developed milder shock symptoms, from which they all recovered (Fig. 6).

DISCUSSION

Major advances have recently been made in better understanding the pathogenesis of various autoimmune diseases. Data obtained from animal models of human type 1 diabetes and MS suggest that, in these diseases, a pivotal role in immune self-destruction is played by Th1 cells (17). It has been observed that a protective switch toward a Th2 setting, with a decrease in Th1-secreted IFN-γ and a concomitant increase in Th2-specific IL-13, was not unexpected because C57BL/6 mice are known for having a predominantly Th1-driven immune response, though not accompanied by autoimmune phenomena (30).

Although T-cell lymphocyte differentiation is mostly Th2-directed in BALB/c mice (30), HEL vaccination by itself did not evolve to shock. When the immune system was further stressed toward Th2 responses by 1α,25(OH)2D3 administration, all BALB/c mice nevertheless developed a mild reversible form of shock. Unexpectedly, NOD mice, known for their Th1-mediated immune reactivity, also developed shock concomitantly with HEL sensitization. Moreover, shock appeared in NOD mice even without any modulation of the immune system with 1α,25(OH)2D3 and was rather aggressive (even lethal for certain animals). 1α,25(OH)2D3 administration further increased mortality in HEL-sensitized NOD mice.

We were surprised by the strain-selective anaphylactic shock induced by HEL in NOD mice. This mouse strain is indeed known to react through Th1-mediated mechanisms (2–4,25) and was moreover described as having a defect in immune presentation by Th1 (18,19). Moreover, this strategy has been extrapolated to human MS (10) and is about to be adopted in human type 1 diabetes (8,20). However, initial enthusiasm was tempered by the observation that vaccination with self-antigens could elicit anaphylactic shock in the context of a disturbed immune system (11,12). This anaphylactic reaction could be triggered by exactly the same T-cell differentiation shift that provided protection against autoimmunity (21). In contrast to a Th1-mediated autoimmune attack, allergic disease is mainly Th2 dependent (22) and is largely mediated by IgE (22,23). Aggressive allergic reactions to encephalitogenic antigens were also observed when the vaccination approach was tested in human MS (12,24). The benefit of protection against an autoimmune disease could therefore be hampered by the allergic side effects.

NOD mice are animals that have a special immune system, with particularities of both T-cells and antigen-presenting cells (2). Although their most known immune feature is the Th1-driven destruction of pancreatic islets (4), literature data about their Th2 activity are con-

FIG. 6. Effect of rescue treatment with WEB2086 on shock incidence and severity in NOD mice. HEL, HEL-sensitized; HEL+D3, HEL-sensitized, 1α,25(OH)2D3-treated; HEL+D3+WEB, HEL-sensitized, 1α,25(OH)2D3-treated, rescued with WEB2086; HEL+WEB, HEL-sensitized, rescued with WEB2086. □, No signs of shock; ▄, reversible shock; ▄, lethal shock. *P < 0.05 concerning disease incidence. n = 8 mice per group.
1α,25(OH)2D3 administration. Therefore, our data supported that the cytokine has a critical role in the particular behavior of NOD mice toward HEL sensitization. IL-13 is a Th2-specific cytokine closely related to IL-4 (35) and synthesized in both humans and mice (36). IL-13 has been alleged to be importantly involved in Th2-initiated allergic reactions, including anaphylaxis (37). The significant difference in serum IgE levels between the two mouse strains provided evidence of IgE-mediated anaphylactic shock in NOD mice. IgE is an immunoglobulin involved in most allergic reactions (13,38). The parallelism between IL-13 mRNA levels and serum IgE levels assessed in our mice stresses a causal relationship between the two immune parameters. Direct stimulation of IgE synthesis by IL-13 has been actually described (38). Certain NOD mice also displayed higher levels of Th2-dependent IgG1. IgG1 may by itself induce anaphylactic shock in mice (11,13).

PAF is an important mediator of immune, hemostatic, and allergic reactions (39). This bioactive phospholipid can be regarded as a cytokine because of its multiple actions on a wide range of cells (including neutrophils, monocytes, and platelets) and tissues that possess specific membrane receptors (PAF-R) (40). The acute inflammatory effects of PAF are largely due to the stimulation of neutrophilic granulocytes as the first line of defense (40). Exaggerated synthesis of PAF or defective mechanisms of PAF inactivation can however lead to pathologic inflammation, shock, and thrombosis (39). There are data in the literature suggesting significant interference of 1,25(OH)2D3 with PAF actions (41) or metabolism (42). Because 1,25(OH)2D3 had such a powerful impact on shock incidence and severity, we suspected an interference of PAF in HEL-induced shock. PAF-R mRNA levels were significantly higher in NOD mice, pleading in favor of an increased sensitivity to PAF inflammatory actions in this strain. In concordance with other studies (43), 1,25(OH)2D3 therapy caused a further increase in the PAF-R expression, a phenomenon that might partly explain the increase in shock severity. Surprisingly, PAF-AH mRNA levels in HEL-sensitized NOD mice were significantly higher than those in C57BL/6 mice. PAF-AH is the main enzyme involved in PAF degradation. The increase in PAF-AH mRNA levels after HEL sensitization could be the expression of a natural feedback reaction against exaggerated PAF synthesis (44). Contrary to another study (42), 1,25(OH)2D3 therapy did not significantly inhibit PAF-AH mRNA expression in HEL-sensitized NOD mice. However, the minor interstrain difference of PAF-AH levels was no longer significant in the 1,25(OH)2D3-treated groups. The direct proof of PAF involvement in the etiopathogenesis of strain-specific HEL-induced shock was obtained when rescue therapy with a PAF-R antagonist (WEB2086) showed to be efficient in preventing shock or diminishing its severity. The activation of the Th2 arm of the immune attack is probably linked to the activation of the PAF system. IgE, for example, can undeniably cause direct stimulation of PAF synthesis by various cells, such as mastocytes (41). Moreover, a recent article (45) suggested that murine antigen-induced anaphylaxis is primarily mediated by the PAF pathway and not by the classic IgE pathway.

Finally the intriguing question remains why HEL induces anaphylaxis in the NOD mouse model, as opposed to the other antigens tested. An extensive GenBank search did not reveal any peptide homology between HEL and any of the known diabetes-relevant autoantigens, not excluding, however, a possible similarity with an as yet undefined autoantigen.

In conclusion, our data clearly demonstrate the appearance of a severe Th2-biased immune reaction after challenge with a widely used control antigen in a mouse strain known mainly for its genetic propensity toward Th1-mediated autoimmune destruction of β-cells. Strain specificity of this reaction points out that immune systems with a disturbed genetic background may reply unpredictably to an immune challenge, even with “innocent” antigens. Autoimmunity should therefore not be simply viewed as an isolated particularity of the immune response but rather as a general disturbance of the immune system. It is currently accepted that Th1-oriented autoimmunity is involved in the pathogenesis of human type 1 diabetes (1–5). Shifting the activation of the immune system toward the Th2 differentiation pathway has been proven efficient in preventing diabetes in animal models (2,3,5) and might be regarded as an interesting alternative to type 1 diabetes prevention in humans (5,9). The immune system of type 1 diabetic patients may, however, manifest changes beyond the local islet-destructive effect (46). Our data complete the conflicting experience already existing with vaccination strategies in EAE and human MS (11,21,24). Extreme caution is therefore needed when adopting immunomodulating strategies, taking into consideration that a shift in T-helper differentiation might always represent a transfer of risk from a Th1-mediated type IV delayed immune attack to a Th2-mediated anaphylactic reaction, especially in situations with an already-activated Th2 system, such as a coincidental infection or allergy.

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