Insulin in Oral Immune "Tolerance": A One-Amino Acid Change in the B Chain Makes the Difference

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Insulin in Oral Immune “Tolerance”: A One-Amino Acid Change in the B Chain Makes the Difference

Dirk Homann, Thomas Dyrberg, Jacob Petersen, Michael B. A. Oldstone, and Matthias G. von Herrath

Oral administration of self-Ags can dampen or prevent autoimmune processes by induction of bystander suppression. Based on encouraging results from experiments in nonobese diabetic (NOD) mice, clinical trials have been initiated in type 1 diabetes using human insulin as an oral Ag. However, neither the precise antigenic requirements nor the mechanism of bystander suppression are currently understood in detail. Here we report that 1) a 1-aa difference in position 30 of the insulin B chain abrogated the ability of insulin to confer protection in both NOD as well as a virus-induced transgenic mouse model for type 1 diabetes. In the latter model transgenic mice express the nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV) under the control of the rat insulin promoter (RIP) in the pancreatic β cells and develop diabetes only following LCMV infection; and 2) protection could be transferred with insulin B chain-restimulated but not LCMV-restimulated splenocytes from RIP-NP transgenic mice, demonstrating that the mechanism of diabetes prevention in the RIP-NP model is mediated by insulin B chain-specific, IL-4-producing regulatory cells acting as bystander suppressors. The Journal of Immunology, 1999, 163: 1833–1838.

Type 1 diabetes is considered a T cell-mediated autoimmune disease that results from specific destruction of insulin-producing β cells residing in the pancreatic islets of Langerhans. Pathogenetically, MHC genes, aberrant immune responses, and possibly viruses have been implicated (1); however, the precise nature of self-Ags involved in the initiation of the autoimmune process remains largely unknown. Currently, no cure or effective prevention is available, and despite treatment with insulin, long term complications are frequent. Oral administration of self (islet) Ags has been shown to reduce diabetes in various animal models (2, 3), and trials using human insulin orally or intranasally have been initiated. However, the precise mechanism(s) and antigenic requirements are not completely understood, and no certain predictions about the efficacy of an orally administered Ag are available. These issues need further exploration, especially since human trials using oral self-Ags are ongoing or planned and are the focus of this article.

For this study we used two different models for type 1 diabetes. Diabetes in the nonobese diabetic (NOD) mouse is genetically predetermined, is linked to the MHC class II complex (IAβ), and develops within 3–9 mo in most females (4). RIP-NP transgenic mice develop autoimmune diabetes upon infection with lymphocytic choriomeningitis virus (LCMV) (5, 6). In this model the immune response against the virus also targets β cells expressing the nucleoprotein (NP) of LCMV as a transgene. In both models mononuclear cell infiltration of islets (requiring islet Ag or viral NP-specific CD8+ CTL as well CD4+ T cells) eventually leads to destruction of β cells, resulting in cessation of insulin production and hyperglycemia.

Successful prevention of autoimmune diabetes has been reported by oral insulin administration in NOD and RIP-NP mice (7, 8). A likely mechanism for oral tolerance has been termed bystander suppression (2, 3) and relies on the induction of regulatory cells in the gut specific for the orally administered self Ag. During the prediabetic phase (preceding spontaneous onset in NOD mice and following virus infection in RIP-NP mice), initial β cell destruction leads to release of various self Ags, including insulin, which can be presented by resident APCs. After migration to the diseased organ, regulatory cells could be activated, and by secretion of immunosuppressive cytokines (IL-4, IL-10, and TGF-β) (2, 3) lead to suppression of the ongoing autoimmune response to an unrelated self Ag, e.g., the viral NP expressed as a transgene in the islets of the RIP-NP mice. Evidence suggests that immune regulation and bystander suppression occur with intermediate oral Ag doses via the induction of insulin B chain-specific T cells, whereas deletion of Ag-reactive lymphocytes is detected at high dosages (2, 9–10). Therefore, the term “oral tolerance” may, but not necessarily does, signify the deletion of specific lymphocytes (2, 3).

In this report we have delineated several crucial aspects of oral Ag therapy for the prevention of type 1 diabetes. Protection occurs via bystander suppression, thus circumventing the need for identification of the initiating autoantigen(s). However, the fed Ag has to be specific for the target cell under destruction (pancreatic β cells). Hormonal activity of insulin, potentially causing a β cell rest, is not required. Most importantly, minute differences in Ag composition, such as a 1-aa change in the immunogenic insulin B chain, can abrogate the protective effect.
Materials and Methods

Animals

Female NOD mice were obtained from Bommice (Ry, Denmark) at 4 wk of age and were housed in a specific pathogen-free environment. The average incidence of diabetes in untreated mice housed under identical conditions during the study period was 60–75% at 40 wk of age. The transgenic RIP-NP 25-3 H-2d line used in this study expressed the NP of LCMV under control of the rat insulin promoter (RIP) in the pancreatic β cells as well as in the thymus, but not in any other tissues (5, 6). BALB/c non-transgenic H-2d mice were used for the evaluation of metabolic activity after feeding of oral Ags and for assessment of CTL precursor frequencies. The virus used was LCMV Armstrong (ARM) strain (clone 53b). Four to twenty-one-week-old RIP-NP 25–3 mice were inoculated i.p. with $1 \times 10^3$ PFU LCMV ARM in a volume of 0.2 ml to initiate diabetes.

Analysis of blood glucose

NOD mice were screened for diabetes twice a week from 10 wk of age by testing for glucosuria. Subsequently, diabetes was defined by two consecutive blood glucose analyses (Accucheck III, Boehringer Mannheim, Indianapolis, IN) with values $>300$ mg/dl. Blood samples from RIP-NP mice were analyzed biweekly accordingly.

Oral Ags

The human and analogue insulin as well as glucagon were recombinant proteins; porcine insulin was purified from pancreatic glands, all from Novo Nordisk (Bagsvaerd, Denmark). All proteins were obtained as dry crystals from the very last stage of the purification process, immediately before formulation into the injectable product used for patients. Porcine, human, and murine (1+2) insulin B chains were also provided by Novo Nordisk. All insulins were solubilized in acid buffer, the pH was adjusted, and the solution was stored at $-20^\circ$ until used. Oral Ag was administered via a blunt-ended curved feeding tube inserted into the esophagus/stomach. In a total of three sequential studies NOD mice were fed buffer and human or porcine insulins from 5 wk of age at doses of 1 mg twice a week. From 10 wk of age the mice were given insulin only once a week. In two of the studies the animals were followed until 45 wk of age, and in the third they were followed until 30 wk of age. There was no difference in the diabetes incidence relative to treatment in the three studies, so the combined data are presented. RIP-NP mice were fed biweekly with 0.5 ml of an aqueous solution containing 2 mg/ml Ag. Feeding was started 1 wk before infection with LCMV and was discontinued after 8 wk. Control groups received saline or BSA at a concentration of 2 mg/ml.

Cytotoxicity assays

LCMV-specific CTL activity in spleens harvested 7 days after inoculation with $10^7$ LCMV ARM i.p. was assessed in a standard 4- to 5-h $^{51}$Cr release assay in LCMV-infected and uninfected, MHC-matched (BALB/c17 [H-2b]) and mismatched (MC57 [H-2d]) target cells (11). For determination of LCMV-specific CTL precursor frequencies (pCTL) 7 days after infection, spleen cells from immunized mice were serial diluted and cultured in 96-well flat-bottom plates (12 wells/dilution; highest dilution, 16,000 cells/well) with LCMV-infected and irradiated (2,000 rad) macrophages as well as irradiated spleen feeder cells. After 8 days, cells from each well were split and tested on LCMV infected and uninfected BALB/c17 targets in a 4- to 5-h $^{51}$Cr release assay. The pCTL frequencies were assessed by plotting the fraction of negative cultures on a semilogarithmic scale against the number of splenocytes per culture; pCTL frequencies are defined by the slope of the linear regression among at least three separate data points.

Adoptive transfers

Splenocytes from diabetic or protected porcine insulin-fed RIP-NP mice were cultured for 3 days in the presence of 100 μg/ml porcine insulin B chain or $10^{-5}$ M of the immunodominant, MHC class I-restricted, LCMV NP (aa 118–126). Supernatants were analyzed for IFN-γ and IL-4 as described previously (11), and 5 $\times 10^6$ cells were transferred i.p. into non-irradiated, prediabetic (day 5 after LCMV) RIP-NP recipients.

Results

A single amino acid difference in the insulin B chain abrogates its protective effect to induce “oral tolerance”

To define the precise sequence (structural) requirements for oral Ags, we investigated several different pancreatic hormones (Table I and Figs. 1 and 2). Oral administration of porcine insulin significantly reduced the development of diabetes in both NOD (Fig. 1) and RIP-NP (Fig. 2) mice. In marked contrast to an earlier report using NOD mice (12), oral treatment with human insulin, which differs by a single amino acid in position 30 of the B chain from porcine insulin (Table I), did not result in protection from diabetes in either NOD or RIP-NP mice. Protection was associated with peri-itis in the absence of MHC class I up-regulation, while diabetic mice showed profound islet infiltration by CD8 and CD4 T cells as well as up-regulation of MHC class I and II (data not shown) (7).

Hormonal activity of insulin is not required for oral tolerance induction

We further explored whether hormonal activity was required for “oral tolerance” induction. Prophylactic insulin treatment of individuals at risk for type 1 diabetes has been explored as a means to prevent or delay the onset of disease (13, 14), and the protective effect in this situation has been hypothesized to be mediated through induction of a “β cell rest”, making β cells less sensitive.

Table I. Insulin sequence alignment

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$^a$ Sequence alignment of porcine, human, analog, and mouse insulins. Differences between experimental insulins affect the carboxy-terminal end of the B-chain. Human insulin differs from porcine insulin by one amino acid (Ala B25→Thr). Metabolically inactive analog insulin has been engineered by substitution of one amino acid in human insulin (Phe B30→Asn). In addition, analog insulin preparation did not contain zinc required for insulin homodimer and homo hexamer formation. All three insulins differ by several amino acids (3–5) from mouse insulin 1 or II.
to immune destruction. We find that oral administration of porcine and human insulin to fasted mice resulted in acute, but transient, blood glucose reduction (Fig. 3). Statistically significant alterations in blood glucose only occurred 10 min after feeding, and blood glucose returned to normal levels in all mice after they were left to feed freely. Although these observations could suggest a metabolic component in oral tolerance and thus lend support to the β cell rest hypothesis, both porcine and human insulin had similar metabolic effects, but only porcine insulin was protective. Therefore, β cell rest is not the mechanism by which oral insulin can prevent diabetes.

Target cell specificity is required for effective oral tolerogens

It has been hypothesized that organ specificity of the oral Ag may be sufficient for successful tolerance induction. More specifically, if “oral tolerance” is to occur via bystander suppression as outlined above, target cell specificity should be a necessary condition. In human type 1 diabetes, as in our two animal models, β cell destruction is specific, and glucagon-producing α cells in the immediate vicinity remain largely untouched. To study whether other islet-derived Ags derived from non-β islet cells can induce oral tolerance we fed RIP-NP mice recombinant glucagon. This treatment did not influence the development of diabetes, indicating that bystander suppression does not occur if the orally administered Ag is not derived from the target cell (Fig. 2). However, the lack of immunogenicity or the absence of a glucagon-specific T cell repertoire could also account for the failure to induce protection.

Oral Ag treatment does not affect the systemic immune response

We have previously shown that protection from diabetes after oral administration of porcine insulin in RIP-NP mice is associated with abrogation of virus (transgene)-specific CTL activity in the pancreas, but not in the spleen. As expected, none of the orally administered Ags used in the present study affected the systemic generation of LCMV-specific CTL as determined by CTL activity, lytic units, and precursor frequencies (Table II). Thus, protection does not occur via systemic deletion of NP (self)-specific CTL. As expected (7), pancreatic NP-specific CTL activity was only observed in diabetic RIP-NP, not protected mice (data not shown).

Protection from type 1 diabetes occurs via bystander suppression

We have previously demonstrated, by comparison of cytokine profiles in protected and diabetic RIP-NP mice, that bystander suppression is the likely mechanism for protection after oral insulin administration (7). We now show that protection can be adoptively transferred. Although ex vivo transfers of splenocytes from protected mice did not abrogate the development of diabetes (data not shown), short term in vitro stimulation with insulin B chain, but not the MHC class I-restricted immunodominant LCMV NP (aa

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**FIGURE 1.** Cumulative incidences of diabetes in oral Ag-treated NOD mice. Biweekly administration of 1 mg of oral Ag was started at 5 wk of age (NOD). Protection from diabetes was observed only in porcine insulin-treated mice. PBS treatment, n = 90; porcine insulin treatment, n = 79; human insulin (B30A→T) treatment, n = 52 (combined results from three different experiments, data corrected for death).

**FIGURE 2.** Cumulative incidences of diabetes in oral Ag-treated RIP-NP mice. Biweekly administration of 1 mg oral Ag was started 1 wk before i.p. immunization with $1 \times 10^5$ PFU LCMV ARM and was continued until 8 wk after immunization. Sixty-five percent of porcine insulin-treated mice were protected from diabetes, while no protection was observed in buffer/BSA-treated mice (diabetes incidence, 94%), human insulin-treated mice (92%), analogue insulin (B30A→T, B25F→N)-treated mice (91%), or glucagon-treated mice (89%). No new cases of diabetes were recorded 6 wk after infection.
Discussion

“Oral tolerance” induction provides an attractive experimental concept for the prevention of autoimmune diseases. Although bystander suppression as well as deletion and anergy of autoreactive cells have been proposed as potential mechanisms, a better understanding of the characteristics of “oral tolerogens” is also needed. The emphasis of the present study lies on an analysis of antigenic frequencies of B chain-specific cells in the spleen, which also may account for the failure of unstimulated cells to confer protection after transfer. Nevertheless, a 3-day stimulation of splenocytes derived from protected, but not diabetic, donors proved sufficient to activate and probably expand the regulatory cell population.

FIGURE 3. Effect of orally administered Ags on blood glucose levels in fasted mice. Orally administered, intrinsically active Ag partially reaches the bloodstream in its intact form, as determined by the effect on blood glucose levels compatible with respective hormonal activity. Five or six mice per group were fasted for 4 h before administration of 1 mg of Ag and during the time of the study. Blood glucose levels were determined 10, 20, 40, 60, and 120 min after feeding. Statistically significant alteration of blood glucose levels with \( p < 0.035 \) (by paired \( t \) test) were observed 10 min after feeding in all groups except the BSA-treated control group.

118–126), led to significant production of IL-4 and diabetes protection after subsequent adoptive transfer into prediabetic RIP-NP mice (Table III). Transfer of cells cultured in the absence of Ag also failed to prevent diabetes (data not shown). It is of interest to note that splenic CTL activity was not affected by the presence of regulatory cells (Table II), presumably due to low precursor frequencies of B chain-specific cells in the spleen, which also may account for the failure of unstimulated cells to confer protection after transfer. Nevertheless, a 3-day stimulation of splenocytes derived from protected, but not diabetic, donors proved sufficient to activate and probably expand the regulatory cell population.

Discussion

“Oral tolerance” induction provides an attractive experimental concept for the prevention of autoimmune diseases. Although bystander suppression as well as deletion and anergy of autoreactive cells have been proposed as potential mechanisms, a better understanding of the characteristics of “oral tolerogens” is also needed. The emphasis of the present study lies on an analysis of antigenic requirements for oral Ag treatment of autoimmune diabetes as well as bystander suppression as the potential mechanism for diabetes protection. By using oral Ags that differ from the initiating autoantigens in two models for type 1 diabetes (unknown in NOD mice, viral transgene in RIP-NP mice), we assessed the efficacy of various islet cell proteins to confer protection from spontaneous (NOD) and virus-induced (RIP-NP) diabetes. Our data clearly demonstrate that a single amino acid change (B30A→T) can abrogate the protective effect after oral administration. Previous studies using porcine, equine, bovine, and ovine insulin, which differ from porcine insulin by one, two, and three amino acids, respectively, in the A, but not the B, chain, have also been shown to confer diabetes protection in NOD mice after oral (porcine, equine) \( (8, 12, 15) \) or i.v. (bovine, ovine) \( (16) \) administration. Further, s.c. administration of the metabolically inactive B chain \( (17) \) or B9-23 \( (18) \), but not of the A chain \( (17) \), in IFA was also protective in NOD mice. Although tolerance induction after parenteral insulin administration is likely to have a mechanism different from that of mucosal tolerance induction, the oral B chain administration has also been associated with a shift from Th1 to Th2 cytokines and diabetes protection in the NOD cotransfer model \( (10) \).

Furthermore, porcine, but neither human nor mouse, B chains were protective in RIP-NP mice (M. von Herrath, unpublished observations). The single amino acid difference in the present study did not affect the insulin epitope B9-23 \( (18) \), indicating that flanking sequences might affect processing of the B chain before presentation by the NOD MHC \( (D^K^I^A^B) \) as discussed below.

The idea that small structural differences in the primary sequence can produce dramatic differences in the clinical outcome is supported by data from a different autoimmune model. Studies in experimental autoimmune encephalitis demonstrated a preventive effect after a single amino acid change in the immunogenic myelin basic protein peptide \( (19) \). Furthermore, experimental autoimmune encephalitis protection induced in Lewis rats with guinea pig \( (GP) \) MBP68–88 or rat MBP68–88 was observed after feeding of GP68–88, but not rat MBP68–88, which differs from GP68–88 by one amino acid \( (S^e^r^6^8^→^T^h^r^) \) \( (20) \). It should be noted, however, that these studies used related peptides for both tolerance and autoimmune disease induction, while the present study used Ags (insulin) different from the initiating autoantigens. Our present findings stand in contrast to those from studies in NOD mice demonstrating a protective effect of human insulin after oral \( (12) \) or aerosol \( (21) \) administration in NOD mice. Other studies using oral human insulin treatment of NOD \( (22, 23) \) mice, while not having investigated the prevention of spontaneous onset diabetes, have reported the generation of regulatory cells that, when cotransferred with diabetogenic cells, protected against autoimmune diabetes. In our NOD colony, cotransfers were much more effective using porcine than human insulin-induced regulatory cells (T. Dyrberg, unpublished observations). These discrepancies may be related to differences in colonies, feeding protocols, or preparation of the Ag, but a clear explanation remains elusive, since a direct comparison based on experimental evidence is and will be difficult to achieve. However, given these considerations, probably very subtle differences can greatly influence the outcome of oral Ag therapy, a fact that should be taken into account for human applications.

Our observation of a temporary decrease in blood glucose was observed only in mice fasted before insulin administration. Furthermore, significant blood glucose reductions were found only 10 min after feeding. Later measurements were not significantly different from initial blood glucose values and are in agreement with other reports that have not found such effects on blood glucose \( (8, 12) \). More importantly, however, standard techniques for oral Ag administration in mice may result in uptake of unprocessed Ag. Because both porcine and human insulin had an intrinsic effect after feeding, but only porcine insulin protected against diabetes,
10 6 cells were transferred intraperitoneally into nonirradiated, prediabetic (day 5 after LCMV) RIP-NP recipients. Recipients were monitored biweekly for determination of blood glucose. Such as glutamate decarboxylase have supported the notion that the adoptive transfer of regulatory lymphocytes prevents type 1 diabetes in RIP-NP transgenic mice. A Th2-like cytokine profile and prevent type 1 diabetes when induced by oral insulin (insulin B chain) makes the RIP-NP model the only diabetes model to date in which bystander suppression as the mechanism for oral tolerance induced by feeding of intermediate dosages of Ag. The clear distinction between the specificity of autoaggressive CTL (viral NP expressed as transgene in β cells) and IL-4-producing regulatory cells induced by oral insulin (insulin B chain) makes the RIP-NP model the only diabetes model to date in which bystander suppression could be unequivocally demonstrated. The reasons for incomplete protection after oral porcine insulin are not clear, but may lie in the generation of regulatory cells at different precursor frequencies.

Further analysis of antigenic requirements indicates that tolerizing Ags need to be target cell specific. Oral administration of glucagon, produced by pancreatic α cells that are not subject to autoimmune destruction in type 1 diabetes, is not protective. Here, lack of local glucagon peptide presentation may have precluded activation of glucagon-specific regulatory cells. Furthermore, it cannot be excluded that such regulatory cells were not generated by glucagon feeding. Nonetheless, studies using other β cell Ags, such as glutamate dehydrogenase have support the notion that the orally (25) or nasally (26) administered Ag has to be derived from the target cell under immunological attack.

Transfer of protection by B chain-stimulated, but not NP-stimulated, splenocytes derived from protected mice establishes bystander suppression as the mechanism for oral tolerance induced by feeding of intermediate dosages of Ag. The clear distinction between the specificity of autoaggressive CTL (viral NP expressed as transgene in β cells) and IL-4-producing regulatory cells induced by oral insulin (insulin B chain) makes the RIP-NP model the only diabetes model to date in which bystander suppression could be unequivocally demonstrated. The reasons for incomplete protection after oral porcine insulin are not clear, but may lie in the generation of regulatory cells at different precursor frequencies. We have recently generated insulin-specific cell lines that exhibit a Th2-like cytokine profile and prevent type 1 diabetes when infused into prediabetic RIP-NP mice and thus allow this hypothesis to be tested (D. Homann, unpublished observations).

In summary, our study points to the importance of oral Ag selection for treatment of human autoimmune disease. Protection via

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### Table II. Oral Ag treatment does not affect generation of anti-LCMV (“self”) CTL

<table>
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<th>Splenocytes after LCMV 7 days</th>
<th>E:T</th>
<th>H-2(^a) uninfected</th>
<th>H-2(^a) LCMV</th>
<th>H-2(^b) LCMV</th>
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<td>0</td>
<td>67 ± 2</td>
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<td>1.30</td>
<td>1/1425 ± 53</td>
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<td>40 ± 10</td>
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<td>12.5:1</td>
<td>0</td>
<td>25 ± 9</td>
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<td></td>
<td>6.25:1</td>
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<td>20 ± 4</td>
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<td>Porcine insulin</td>
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<td>65 ± 5</td>
<td>2 ± 1</td>
<td>1.28</td>
<td>1/1217 ± 174</td>
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<td>17 ± 3</td>
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</tbody>
</table>

* BALB/c (H-2\(^a\)) mice were treated orally with porcine, human, and analog insulin or glucagon as described. All mice were infected with 1 × 10\(^5\) PFU LCMV, and 7 days later spleens were removed to test for CTL activity and determine LCMV-specific precursor frequencies (pCTL). Target cells were syngeneic (H-2\(^a\)) or allogeneic (H-2\(^b\)) fibroblasts infected with LCMV (MOI = 1) or uninfected. All samples were run in triplicate, displayed are mean ± 1 SE. Lytic units were defined as the reciprocal of the number of >10\(^5\) effector cells required for 25% lysis of 10\(^4\) targets determined by correlating four different E:T ratios with the respective \(^{3}\text{H}\)Cr release values. Precursor frequencies are shown as CTL precursors per number of splenocytes.

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### Table III. Adoptive transfer of regulatory lymphocytes prevents type 1 diabetes in RIP-NP transgenic mice

<table>
<thead>
<tr>
<th>Donor Splenocytes</th>
<th>Stimulation with</th>
<th>Cytokine Production (ng/100 µl)</th>
<th>Adoptive Transfer Recipient</th>
<th>Type 1 Diabetes Incidence After Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protected RIP-NP mouse</td>
<td>Porcine B-chain</td>
<td>IL-4: 0.25 ± 0.1, IFN-γ: 0.21 ± 0.1</td>
<td>RIP-NP mouse day 5 after LCMV</td>
<td>50% (3/6)</td>
</tr>
<tr>
<td>Protected RIP-NP mouse</td>
<td>LCMV NP(_{118–126})</td>
<td>IL-4: &lt;0.05, IFN-γ: 1.1 ± 0.32</td>
<td>RIP-NP mouse day 5 after LCMV</td>
<td>100% (4/4)</td>
</tr>
<tr>
<td>Diabetic RIP-NP mouse</td>
<td>Porcine B-chain</td>
<td>IL-4: &lt;0.05, IFN-γ: 0.3 ± 0.11</td>
<td>RIP-NP mouse day 5 after LCMV</td>
<td>83% (1/6)</td>
</tr>
<tr>
<td>Diabetic RIP-NP mouse</td>
<td>LCMV NP(_{118–126})</td>
<td>IL-4: &lt;0.05, IFN-γ: 0.9 ± 0.20</td>
<td>RIP-NP mouse day 5 after LCMV</td>
<td>100% (4/4)</td>
</tr>
</tbody>
</table>

* Donor splenocytes were derived from protected and diabetic mice treated with porcine insulin. After a 3-day stimulation with porcine B-chain or the immunodominant MHC class I-restricted LCMV NP-peptide (aa 118–126), cytokine production (IL-4 and IFN-γ) was determined in culture supernatants as described in Materials and Methods and 5 × 10\(^6\) cells were transferred intraperitoneally into nonirradiated, prediabetic (day 5 after LCMV) RIP-NP recipients. Recipients were monitored biweekly for determination of blood glucose.
bystander suppression obviates the need for autoantigen identification. However, while hormonal function is not related to protection, the Ag chosen probably has to be specific for the target cell under attack. Minute differences in Ag structure/sequence can have significant biological implications for the induction of “oral tolerance”. Thus, an appreciation of the full therapeutic potential of orally administered Ags for treating or preventing human autoimmune diseases depends on a better understanding of the underlying structural requirements as well as a defined testing system that can predict their efficacy. In vitro testing of MHC class II-restricted binding of insulin B chains does not appear to be a suitable criterion to predict protective capacity of oral Ags (unpublished observations). Parameters critical for induction of regulatory cells thus probably include Ag processing and specific T cell repertoires.

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References