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Nasal Administration Of Glutamate Decarboxylase (GAD65) Peptides Induces Th2 Responses and Prevents Murine Insulin-dependent Diabetes

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Summary

We previously demonstrated that a spontaneous Th1 response against glutamate decarboxylase (GAD65) arises in NOD mice at four weeks in age and subsequently T cell autoimmunity spreads both intramolecularly and intermolecularly. Induction of passive tolerance to GAD65, through the inactivation of reactive T cells before the onset of autoimmunity, prevented determinant spreading and the development of insulin-dependent diabetes mellitus (IDDM). Here, we examined whether an alternative strategy, designed to induce active tolerance via the engagement of Th2 immune responses to GAD65, before the spontaneous onset of autoimmunity, could inhibit the cascade of Th1 responses that lead to IDDM. We observed that a single intranasal administration of GAD65 peptides to 2–3-wk-old NOD mice induced high levels of IgG_1 antibodies to GAD65. GAD65 peptide treated mice displayed greatly reduced IFNγ responses and increased IL-5 responses to GAD65, confirming the diversion of the spontaneous GAD65 Th1 response toward a Th2 phenotype. Consistent with the induction of an active tolerance mechanism, splenic $CD4^+$ (but not $CD8^+$) T cells from GAD65 peptide-treated mice, inhibited the adoptive transfer of IDDM to NOD-*scid/scid* mice. This active mechanism not only inhibited the development of proliferative T cell responses to GAD65, it also limited the expansion of autoreactive T cell responses to other β cell antigens (i.e., determinant spreading). Finally, GAD65 peptide treatment reduced insulitis and long-term IDDM incidence. Collectively, these data suggest that the nasal administration of GAD65 peptides induces a Th2 cell response that inhibits the spontaneous development of autoreactive Th1 responses and the progression of β cell autoimmunity in NOD mice.

We previously demonstrated in non-obese diabetic (NOD)[1] mice that autoreactive Th1 cell responses develop spontaneously and gradually spread both intramolecularly and intermolecularly, creating a cascade of responses that ultimately destroys the insulin-secreting β cells and results in insulin-dependent diabetes mellitus (IDDM) (1). Autoantigen based therapies offer the opportunity to prevent, or inhibit, this amplificatory cascade through mechanisms that are minimally invasive. There are two basic autoantigen-based immunotherapeutic strategies. The first strategy, passive tolerance, aims to inactivate, or prevent the priming of autoreactive T cells by presenting antigen without appropriate costimulatory signals, or by interfering with the formation of MHC/TCR complexes through peptide antagonists or monoclonal antibodies (reviewed in references 2 and 3). Therapies based on passive tolerance are highly specific, but require detailed knowledge of the target antigens, the effector TCR or the peptide/MHC complex they recognize. Therefore, passive tolerance has almost exclusively been used to prevent experimentally induced autoimmune disease, in which the initiating autoantigen is defined.

We demonstrated the efficacy of passive tolerance to prevent spontaneous β cell autoimmunity in NOD mice by identifying GAD65 as the earliest known T cell target antigen and demonstrating that inactivation of GAD65-

[1] *Abbreviations used in this paper:* GAD, glutamate decarboxylase; hsp, heat shock protein; IDDM, insulin-dependent diabetes mellitus; NOD, non-obese diabetic.

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reactive T cells (by the intravenous administration of GAD65), before the loss of self-tolerance, prevented the development of β cell autoimmunity (1). The GAD65 treated mice did not have detectable levels of GAD65 autoantibodies and their splenic T cells failed to protect recipients in adoptive transfer experiments, consistent with the induction of passive tolerance (J. Tian and D.L. Kaufman, unpublished observations).

The second immunotherapeutic strategy involves administering autoantigens in modes that induce protective immune responses, creating active forms of self-tolerance (3–8). This approach does not require knowledge of the initial target antigen and its protective effect may not be confined only to the administered autoantigen, as the activated immune responses may downregulate effector T cells through "bystander" suppression (4). A recently favored mechanism for active tolerance involves the induction of Th2 cells that protect a target tissue via the local release of cytokines that downregulate Th1 cells (9–12). However, evidence demonstrating the protective effects of Th2 cells in autoimmune disease has been largely confined to experimentally induced diseases (4). In the case of IDDM, Th2 cells have in fact been suggested to be both nonprotective and nondiabetogenic in adoptive transfer experiments (13).

We reasoned that if inactivation of Th1 responses to GAD65 before the onset of autoimmunity prevents β cell autoimmunity in NOD mice, the early priming of a Th2 response to GAD65 could also interfere with diabetogenesis—either by diverting the pool of GAD65-reactive precursor T cells from developing toward a Th1 phenotype, or by downregulating effector T cells in the target organ via bystander suppression. It is not known however, what effect the induction of Th2 responses to an autoantigen, before the spontaneous development of pathogenic Th1 responses to that autoantigen, would have on determinant spreading and disease progression. Moreover, it is unclear what modes of antigen administration would induce Th2 responses, whereas antigens are easily delivered to mucosal surfaces, this treatment has been recently reported to induce a variety of T cell responses (i.e., peripheral deletion, anergy, and activation of $CD4^+$ and $CD8^+$ cells, references 4, 6, 14–16). Here, we show in NOD mice, that a single nasal administration of GAD65 peptides, before the onset of autoimmunity, induces a Th2 cell response that actively diverts/downregulates the development of autoreactive Th1 responses and inhibits autoimmune disease progression.

Materials and Methods

Mice. NOD mice (Taconic Farms Inc., Germantown, NY) were bred under SPF conditions. Only female NOD mice were used in this study. Insulitis begins at 4 wk and the average age of disease onset in females is at 22 wk. Approximately 80% of the female mice display IDDM by 30 wk in age. IDDM increases to ~90% by 40 wk in age and remains stable thereafter.

Antigens. We previously characterized in NOD mice the development of T cell autoreactivity to a panel of overlapping GAD65 peptides that span the molecule (1). We administered a mixture of the synthetic peptides that contain the earliest GAD65 T cell target determinants (peptides 17, 34, 35, and 36, references 1 and 17), or a control GAD65 peptide that does not contain a T cell target determinant (peptide 11 in references 1 and 17). These peptides, as well as the hsp65 immunodominant peptide (18) were prepared as previously described (17). To avoid confusion between the experimental GAD65 peptides (peptides 17, 34, 35, 36) that contain T cell determinants and the control GAD65 peptide (peptide 11) which does not, we refer to peptide 11 as the "control" peptide. Mouse GAD65 (19) and control *Escherichia coli* β-galactosidase, used in T cell proliferation assays, were purified as previously described (1). Human GAD65, used in ELISA assays for autoantibodies, was provided by Synectics Biotechnology (Stockholm, Sweden). Bovine insulin B chain was from Sigma Chemical Co. (St. Louis, MO). Hen egg white lysozyme peptide (HEL_{11-25}), which contains a dominant determinant, was provided by Eli Sercarz.

Treatment. Female NOD mice at 2–3 wk were given a single intranasal dose of a mixture of four GAD65 peptides (peptides 17, 34, 35, 36, 50 μg of each in 50 μl PBS). Control NOD mice received a single dose of the control peptide (200 μg in 50 μl PBS). Mice received peptides while under light ether anesthesia using a thin drawn-out plastic tube (inserted 5–10 mm intranasally) attached to a Hamilton syringe. At 12 wk, some mice were killed to characterize splenic T cell responses and insulitis. The remaining mice were followed up to 1 yr to determine the effect of treatment on long-term IDDM frequency. Urine glucose levels were monitored weekly for diabetes by Tes-tape. After observing abnormal glucose in the urine, blood glucose levels were monitored twice weekly. Two consecutive blood glucose levels >13 μmol/L were considered as IDDM onset.

GAD65 Autoantibody Assays. GAD65 (10 μg/ml) was bound to 96-well plates (Nunc) in 0.1 M $NaHCO_3$, pH 8.5, at 4°C overnight. The wells were rinsed with PBS and then blocked with 3% BSA in PBS for 1 h. Mouse sera was added (0.1 ml of a 1/500 dilution) and incubated 1 h at 37°C. Following washing, bound Ig was characterized using affinity-purified HRP-coupled goat anti–mouse IgG+A+M (H+L) (Pierce Chem.), or HRP-coupled goat anti–mouse isotype-specific antibodies for IgG_1, IgG_{2a}, IgM (Southern Biotech Associates) and 2,2′-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co.). Sera from untreated BALB/c and AKR mice were used as negative controls.

Cytokine Assays. Spleen cells were plated at 8×10^6 cells/ml in 24-well microtiter plates as previously described (1). GAD65 or β-galactosidase were present at 100 μg/ml. After 48 h, culture supernatants were analyzed by lymphokine-specific ELISA. For the IFNγ ELISA, mAb R4-6A2 was used as the capturing agent and XMG 1.2-biotin (PharMingen, San Diego, CA) was used in conjunction with SAV-AP and PNPP for the detection of bound lymphokine. For IL-5 detection, mAb TRFK5 and TRFK4-biotin (PharMingen) were used. The concentration of lymphokines was determined by comparison with a standard curve of recombinant murine IFNγ or IL-5 (PharMingen).

Adoptive Transfer of Diabetes. Ten million splenic mononuclear cells from untreated-diabetic mice were mixed with an equal number of splenic mononuclear cells from (18–20 wk old) experimental or control peptide treated mice, and the mixture injected i.v. into 10-wk-old NOD-*scid/scid* mice (20). A positive control group received 1×10^7 splenic mononuclear cells obtained from only untreated diabetic mice.

To examine the T cell subset that conferred protection from the adoptive transfer of disease, $CD4^+$ and $CD8^+$ splenic T cell populations were isolated from 5-wk-old control and GAD65

peptide–treated mice (3 wk after treatment) using T cell and $CD4^+$ and $CD8^+$ enrichment columns (R&D Systems, Minneapolis, MN). Three-color FACS® analysis using PE-anti-CD3, Cy-chrome-anti-$CD4^+$, and FITC-anti-$CD8^+$ antibodies (PharMingen) showed that the negatively selected $CD4^+$ subset contained less than 1% $CD8^+$ T cells, and that the $CD8^+$ subset contained less than 3% $CD4^+$ T cells. 5×10^6 purified T cell subsets from control or GAD65 peptide treated mice were mixed with 10^7 splenic mononuclear cells from diabetic NOD mice and injected i.v. into NOD-*scid/scid* mice.

T Cell Proliferation Assays. At 12 wk of age the mice were killed and their spleen cells were tested directly in vitro for proliferative responses to GAD65, heat shock protein (hsp65), β-galactosidase and the insulin B chain, as previously described (1).

Histopathology. At 12 wk of age, pancreata from control and experimental NOD mice (at least five from each group) were examined for insulitis as described previously (1). The severity of mononuclear cell infiltration was defined histologically (0 = no lymphocytic infiltration; 1 = <25%; 2 = 25–50%; 3 = 50–75%; 4 = >75%, reference 21).

Results

Nasal Administration of GAD65 Determinants Induces GAD65-specific Autoantibodies of the IgG_1 Isotype. Experimental and control NOD mice were given a single intranasal dose of peptides at 2–3 wk and humoral responses to GAD65 were tested at 12 wk. We observed that NOD mice treated with the control peptide had low levels of GAD65 autoantibodies (Fig. 1), similar to that observed in unmanipulated age-matched NOD mice, and only slightly greater than that found in BALB/c and AKR mice (data not shown). However, high levels of GAD65 autoantibodies were observed in most of the GAD65 peptide–treated NOD mice, indicating that the treatment triggered a T cell–dependent anti-GAD65 B cell response. To identify the type of T cell that provided help for GAD65 autoantibody production, we characterized the isotype of the anti-GAD65 immunoglobins. The GAD65 autoantibodies in the GAD65 peptide–treated group were almost exclusively of the IgG_1 subclass (Fig. 1), which is indicative of Th2 help (22, 23). In contrast, IgG_{2a} GAD65 autoantibodies, which require Th1 help, were at very low levels in both the experimental and control groups. These data suggest that nasal administration of GAD65 peptides activates a GAD65-specific Th2 response.

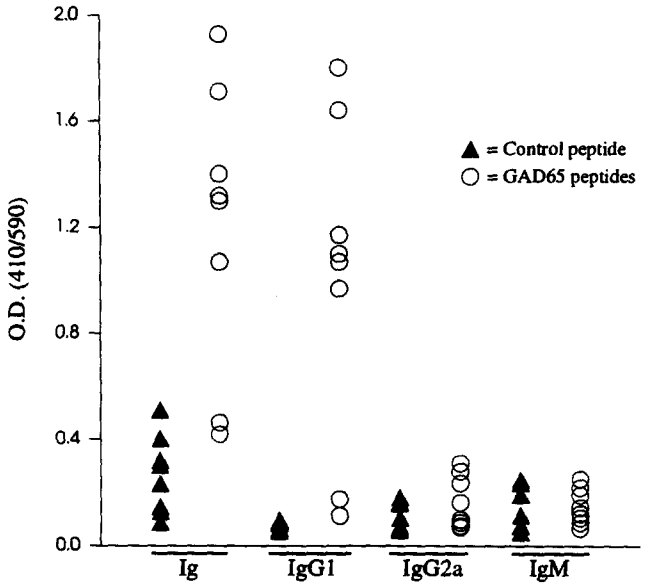

Figure 1. The nasal administration of GAD65 determinants induces GAD65-specific autoantibodies of the IgG_1 isotype. Female NOD mice at 2–3 wk were given a single intranasal dose of a control peptide, or GAD65 peptides that contain early T cell target determinants. At 12 wk, sera from control (▲) and GAD65 peptide (○) treated mice were tested for anti-GAD65 antibodies. The isotype of the GAD65 antibodies was characterized using an ELISA assay and isotype-specific antibodies as described in Materials and Methods. Unmanipulated NOD mice and control peptide–treated mice had low levels of GAD65 autoantibodies, that were only slightly greater than the background levels we observed in sera from BALB/c and AKR mice (data not shown). There was a linear relationship between the OD and the titer of the antibody (data not shown). The data are represented as the mean absorbance values of samples from individual mice. The background OD ranged from 0.05–0.07 in two separate experiments.

T Cells from GAD65 Peptide Treated Mice Display Decreased IFNγ and Increased IL-5 Production in Response to GAD65. We previously demonstrated that unmanipulated NOD mice at 12 wk have a clonally expanded, $CD4^+$ L-selectin$^-$, IFNγ secreting population of GAD65-specific T cells, (i.e., a Th1 response to GAD65, reference 1). Consistent with our previous findings, we observed that T cells from 12 wk old control peptide-treated NOD mice secreted high levels of IFNγ in response to GAD65 challenge in vitro (Fig. 2). In contrast, T cells from GAD65 peptide–treated NOD mice displayed greatly reduced IFNγ responses. Further-

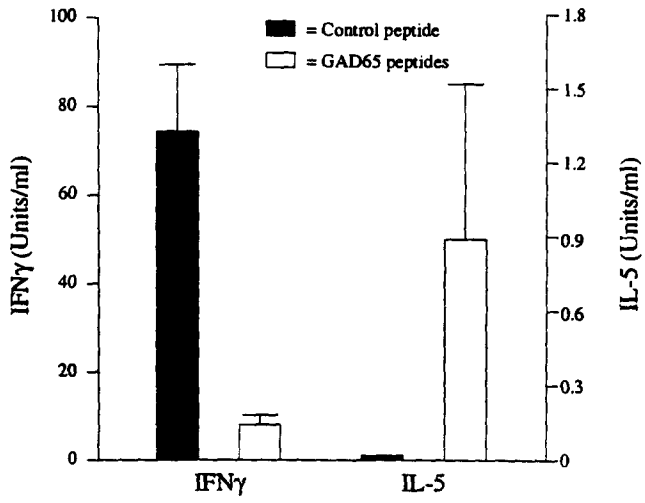

Figure 2. T cells from GAD65 peptide-treated mice display decreased IFNγ and increased IL-5 production in response to GAD65. Splenic T cells from 12-wk-old NOD mice that had received a single nasal administration at 2–3 wk of experimental (*open bar*) or control GAD65 peptides (*black bar*) were tested for their pattern of cytokine responses to GAD65. The IFNγ responses in the control group were similar to that found in the unmanipulated NOD mice of our previous study (1). IFNγ or IL-5 were not detected in cultures with control β-galactosidase or medium alone (data not shown). Values shown are mean ± SEM of triplicate assays in two separate experiments.

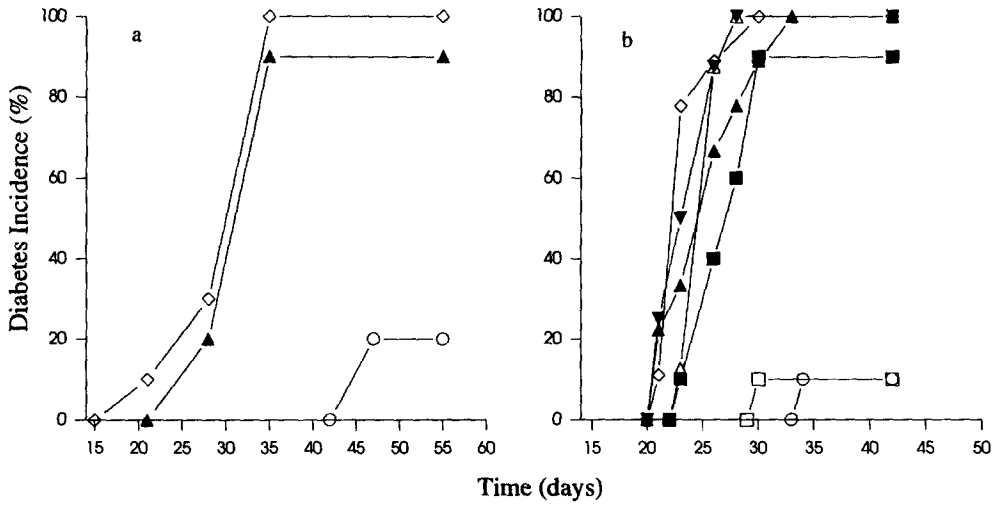

Figure 3. Adoptive cotransfer of $CD4^+$ splenic T cells from GAD65 peptide–treated mice protects recipient NOD-*scid/scid* mice from IDDM. (a) At 18–20 wk, splenic mononuclear cells from mice treated with control (▲) or GAD65 (○) peptides were cotransferred with T cells from diabetic NOD mice to NOD-*scid/scid* mice. A positive control group received cells only from untreated diabetic NOD mice (◇) (n = 10 for all groups). (b) Unfractionated splenic T cells (▲), $CD4^+$ (■) and $CD8^+$ (▼) T cells from control peptide-treated mice, or unfractionated splenic T cells (○), $CD4^+$ (□) and $CD8^+$ (△) T cells from GAD65 peptide-treated mice, were mixed with mononuclear cells from unmanipulated diabetic NOD mice and cotransferred into NOD-*scid/scid* mice. Positive control, unfractionated T cells from only untreated diabetic mice (◇). (n = 8–10 for all groups).

more, while IL-5 secretion was almost undetectable in the control group, clear IL-5 responses were detectable in some mice of the experimental group, confirming the activation of Th2 responses.

Adoptive Cotransfer of Splenic T Cells from GAD65 Peptide–treated Mice Protects Recipient NOD-scid/scid Mice from IDDM. We next examined whether T cells from GAD65 peptide–treated NOD mice could actively inhibit the adoptive transfer of diabetes. Splenic mononuclear cells from mice treated with GAD65 or control peptides were cotransferred with T cells from diabetic NOD mice to NOD-*scid/scid* mice. All of the positive control mice that received cells from unmanipulated diabetic NOD mice, and 90% of the mice that received a mixture of cells from unmanipulated diabetic and control peptide-treated NOD mice, became diabetic within 5 wk after transfer (Fig. 3 *a*). In contrast, only 20% (n = 10) of the NOD-*scid/scid* mice that received a mixture of cells from GAD65 determinant treated mice and unmanipulated diabetic mice developed IDDM ($P < 0.01$). Those recipients that did develop IDDM showed a delayed onset of disease relative to the control groups.

To examine the T cell subset that inhibited disease development, splenic $CD4^+$ and $CD8^+$ T cell populations were isolated from the spleens of control or GAD65 peptide–treated mice and cotransferred with mononuclear cells from diabetic NOD mice into NOD-*scid/scid* mice. Mice that received $CD4^+$ or $CD8^+$ T cells from control peptide–treated mice progressed to IDDM within 4 wk (Fig. 3 *b*). In contrast, the $CD4^+$, but not the $CD8^+$, T cell population of the GAD65 peptide–treated mice conferred protection from the adoptive transfer of disease, at a level similar to that of unfractionated T cells from these mice. These

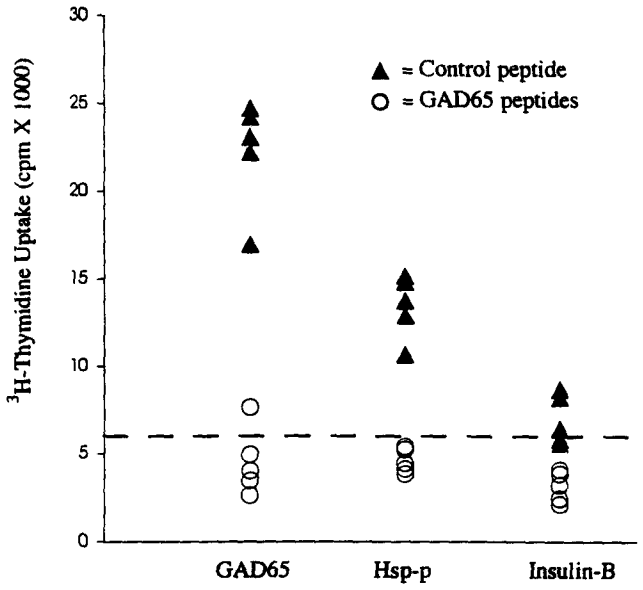

Figure 4. The nasal administration of GAD65 peptides inhibits determinant spreading of T cell autoimmunity. Splenic T cells from 12-wk-old control (▲) or GAD65 peptide-treated (○) mice were tested for proliferative responses to GAD65, the immunodominant hsp65 peptide and the insulin B chain. Background for medium alone ranged from 1,200–2,500 cpm. Positive control Con A (1 μg/ml)–stimulated T cell proliferation ranged from 38,000–43,000 cpm. The dashed line indicates the level considered to be a significant response (threefold above the mean background level). Mice from both groups were tested simultaneously in two separate experiments. None of the antigens induced proliferation of splenic T cells from BALB/c mice (data not shown).

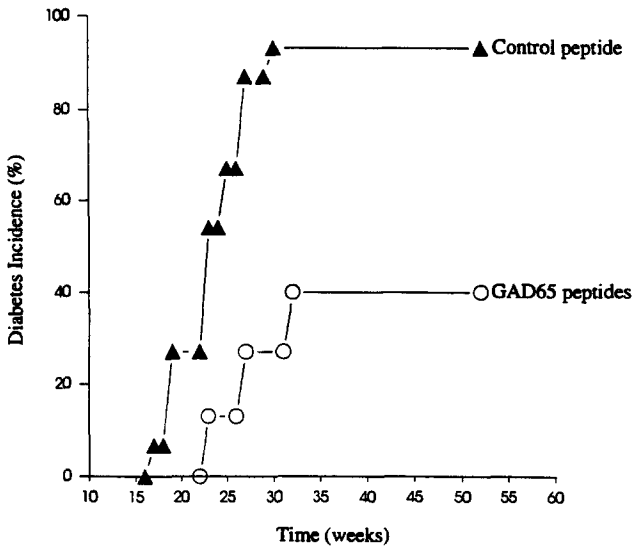

Figure 5. The nasal administration of GAD65 peptides inhibits IDDM. Female NOD mice at 2–3 wk were given a single intranasal dose of control (▲) or GAD65 peptides (○) and were followed up to 52 wk to determine the effect of treatment on long-term IDDM incidence as described in Materials and Methods. (n = 15 for each group).

data demonstrate that the nasal administration of GAD65 peptides can induce potent $CD4^+$ regulatory cells that suppress the adoptive transfer of IDDM.

The Nasal Administration of GAD65 Peptides Inhibits Spreading of T Cell Autoimmunity. Splenic T cells from 12-wk-old experimental and control mice were tested for their proliferative responses to β cell autoantigens. T cells from control mice displayed strong proliferative responses to GAD65 and hsp65 and some of the mice displayed T cell responses to insulin B chain (Fig. 4). The levels of these proliferative responses were similar to those we observed in untreated NOD mice (1). In contrast, nearly all of the NOD mice treated with peptides containing GAD65 target determinants failed to display significant proliferative responses to GAD65 (Fig. 4). Furthermore, none of the mice in the experimental group displayed significant proliferative responses to hsp65 or the B chain of insulin. Thus, the nasal administration of GAD65 determinants inhibited the intermolecular spreading of the autoreactive Th1 responses that naturally occur in NOD mice.

The Nasal Administration of Key GAD65 Peptides Reduces Insulitis and IDDM Incidence. Examination of the pancreata from 12-wk-old control mice revealed that all islets had infiltrating lymphocytes at levels essentially identical to those we observed in unmanipulated NOD mice (1). In contrast, several of the pancreata from the GAD65 peptide–treated group were virtually free of insulitis. The overall insulitis score for pancreata from mice in the experimental group (0.9 ± 0.4) was less than half that of pancreata from the control group (1.9 ± 0.7).

We next examined the effect of GAD65 peptide administration on the long-term frequency of IDDM in NOD mice. 90% of the mice receiving the control peptide were diabetic by 35 wk (Fig. 5), matching the time course of IDDM incidence observed in unmanipulated female NOD mice. In contrast, 60% of the GAD65 peptide–treated mice remained disease free at 52 wk (P <0.03), and those that did become diabetic displayed a delayed onset of disease. Thus, the single nasal administration of GAD65 determinants early in life significantly delayed, or prevented, IDDM onset.

Interestingly, high levels of anti-GAD65 IgG_1 autoantibodies were detected in the sera of all 52-wk-old GAD65 peptide treated mice that had remained disease free. However, GAD65 autoantibody levels greatly declined just before disease onset in the GAD65-treated mice which developed IDDM (data not shown).

Discussion

The NOD mouse has been suggested to be deficient in Th2 development (24) and the spontaneous autoimmune response to β cell antigens in NOD mice appears to be highly Th1 biased (1), factors which may predispose these mice to IDDM susceptibility. We hypothesized that if Th2 responses could be induced to β cell autoantigens at an early age, it might inhibit the development of pathogenic Th1 responses and autoimmune disease progression. As GAD65 is a key initial target antigen in NOD mice, we examined whether it is possible to administer GAD65 in a mode that would induce GAD65-specific Th2 responses and shift the development of a Th1 biased anti-GAD65 response in NOD mice.

We observed that a single intranasal administration of GAD65 peptides induced high levels of IgG_1 anti-GAD65 antibodies and greatly reduced IFNγ while increasing IL-5 responses to GAD65, apparently shifting the normal Th1/Th2 balance toward a Th2 response. Consistent with the induction of an active tolerance mechanism, splenic T cells from GAD65 peptide–treated mice inhibited the adoptive transfer of disease to NOD-*scid*/*scid* mice. This protection was conferred by the $CD4^+$, and not the $CD8^+$, T cell subset. This active mechanism inhibited the development of proliferative T cell responses to GAD65 as well as other β cell antigens and reduced insulitis and long-term IDDM incidence. While GAD65 autoantibodies remained high in the GAD65 peptide–treated mice that did not develop disease, GAD65 autoantibody levels greatly declined in the GAD65-treated mice which developed IDDM, consistent with Th2 regulation of IDDM development. Collectively, these data suggest that the nasal administration of GAD65 peptides interferes with the development of the diabetogenic process through the induction of a Th2 response (active tolerance), rather than the clonal inactivation of GAD65-reactive cells (passive tolerance).

The mechanism by which the induction of Th2 responses interferes with disease progression may involve one, or several, of the following: (*a*) the induction of Th2 responses before the spontaneous priming of Th1 responses may have driven a limited number of uncommitted, or not fully differentiated, Th0 cells toward a Th2 lineage; (*b*) the

first generation of Th2 cells may have migrated to the target organ where their local secretion of cytokines may have downregulated antigen presentation in the islets (25, 26), thereby limiting the amplificatory cascade of Th1 responses; (*c*) alternatively, the local release of cytokines by Th2 cells may have upregulated islet antigen presentation, which in the absence of appropriate costimulatory factors, caused the antigen-specific T cells that engaged these determinants to be anergized or deleted (27), or; (*d*) the upregulation of islet antigen presentation may have caused T cell priming that was Th2 biased due to the IL-4 environment that was established by the first wave of Th2 cells, creating an amplificatory cascade of Th2 responses; (*e*) The GAD-specific Th2 response may have locally downregulated Th1 cells through the secretion of cytokines such as IL-10 and IL-4 (bystander suppression, references 4, 10, 11, 22).

Th2 cells are generally thought to exert protective functions in experimental models of autoimmune disease (3, 4, 9, 15) and administration of IL-4 and IL-10 are anti-inflammatory (24, 28). However, two recent reports question whether Th2 cells exert anti-inflammatory functions. Katz et al. showed that Th2 cells fail to mitigate β cell destruction when coinjected with Th1 cells in adoptive transfer experiments (13). Our conflicting findings may be due to differences in the nature and frequency of the autoantigen-reactive T cells used in our experiments. Katz et al. simultaneously injected clonal populations of preactivated β cell–specific Th1 and Th2 cells. Under these conditions it is likely that Th2 cell secreted cytokines could not exert regulatory functions before Th1 cells engaged target determinants. We injected a mixed population of unactivated spleen cells, in which the frequency of GAD65-reactive T cells was ~1 in 10^4–10^5. Our protocol may not have flooded the target organ with an autoreactive Th1 component, thus providing an opportunity for Th2 cells to exert protective functions. These experiments also targeted different β cell antigens, whereas our protocol induced GAD65-specific Th2 cells, the T cell populations used by Katz et al. have an unknown specificity.

Apparently also contradicting the protective Th2 cell hypothesis, is the finding that IDDM is accelerated in transgenic mice expressing IL-10 in their β cells (29). However, IL-10 may have complex functions. For example, IL-10 is a growth factor for CTL and facilitates their priming (30, 31). Since $CD8^+$ cells are involved in the pathogenesis of IDDM in NOD mice (32, 33), the IL-10 produced in the transgenic islets may have promoted an anti–β cell $CD8^+$ response and accelerated disease progression. It is also possible that other cytokines such as IL-4 and IL-13 mediate the anti-inflammatory functions of Th2 cells. Thus, these two reports do not disprove a protective role for Th2 cells. To the contrary, our data substantiate the contention that Th2 cells can serve a protective role.

In conclusion, we have shown that a single nasal administration of peptides containing T cell target determinants can induce antigen-specific Th2 responses and inhibit the spontaneous cascade of events that lead to IDDM. Thus the inhalation of autoantigen peptides may provide an easily administered, minimally invasive, immunotherapy to prevent spontaneous autoimmune disease.

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