Anti-GAD Monoclonal Antibody Delays the Onset of Diabetes Mellitus in NOD Mice

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Insulin Dependent Diabetes Mellitus (IDDM type I) is the result of autoimmune destruction of insulin producing pancreatic β-cells by the cellular immune system, specifically, autoreactive T cells. Disease progression is evident by multiple autoantibodies responding to self-antigens in a cascade mechanism, wherein the first self-antigen induces the activation of the immune system, leading to the destruction of β-cells and consequently, exposure of other antigens. Glutamic Acid Decarboxylase (GAD) is recognized in the literature as a primary autoantigen involved in the cascade. We questioned the immunological involvement of this autoantigen in the overall progression of the disease, specifically if antigen recognition by the cellular immune system (T cells) is necessary for organ specific autoimmunity and cellular toxicity. We tested this hypothesis by isolating, purifying and injecting monoclonal antibodies against GAD (anti-GAD Ab; 0.1 mg or 0.3 mg) into non-obese diabetic (NOD) mice on a weekly basis. We suggest that the anti-GAD Ab will bind to the GAD antigen, or perhaps bind to the epitope presented in association with APC-MHC and prevent T cell recognition, thereby delaying disease onset. Our results demonstrate a delay in the onset of diabetes and a decrease in the severity of insulitis in our test animals, when compared to controls. The mechanism of action of the anti-GAD Ab may be associated with a passive protection mechanism, as evidenced by the fact that splenocytes transferred from anti-GAD Ab treated mice did not prevent or delay diabetes in syngeneic irradiated NOD mice. The mechanism of diabetes prevention by administration of anti-GAD antibody could be associated with an interference in recognition of GAD by T cells, and continuing research will be perform to investigate this hypothesis.

KEY WORDS: anti-GAD antibody; insulitis; immune-modulation.

INTRODUCTION

Insulin dependent diabetes mellitus (IDDM) is an autoimmune disease resulting in the selective destruction of pancreatic β-cells. Infiltration of the islets by macrophages and activation of $CD4^+$ T cells possessing specificity for β-cell autoantigens are characteristic of the development of the disease (1). In IDDM and in most autoimmune diseases, the antigen presenting cells (APC)—macrophages, B cells and dendritic cells must first internalize the self-antigens. The processed peptides (antigens) are presented in association with class II MHC molecules on the surface of the APC's to T cell receptors (TCR). The nature of the MHC II molecule-peptide (antigen)-TCR complex and the types of cytokines present at the proximity of cellular involvement will activate and direct the specific T lymphocytes helper cells (Th) subset for directed effector function (2,3). The activation of the Th1 subset leads to a cellular type of response, while activation of the Th2 subset leads to a humoral type of response with production of antibodies. This effector mechanism can result in activation of a cellular response, rather than a humoral response, leading to cell destruction, as observed in IDDM.

In previous research, blocking T cell recognition of the MHC II-processed antigen complexes using anti-CD3 (4), anti-CD4 (5), anti-selectine (6) antibodies, or cytokine administration (7,8) was shown to prevent diabetes in the NOD mouse model. The target molecules in these cases are involved in non-specific immune activation rather than specific immune activation against a specific β-cell antigen. However, interfering with the formation of the specific MHC-processed antigen and TCR complexes for a specific autoantigen may present the most specific type of immune intervention for prevention of autoimmune disease.

Multiple autoantigens have been identified in IDDM (9,10), including glutamic acid decarboxylase (GAD) (11), insulin (12), carboxypeptidase H, and several unspecified islet cell antibodies (13,14). Specifically, GAD is recognized as a major self-antigen involved in the cascade of autoimmunity (15,16) inherent to IDDM in the NOD mouse. Several immunological approaches to prevent IDDM through the administration of GAD have been investigated. Among them, oral or nasal administration of GAD (17,18) or intrathymic injection of GAD (15) prevented diabetes through immune-modulation or the induction of immune tolerance. Immunization with GAD also lead to prevention of diabetes (19,20). In this humoral response model, the production of anti-GAD Ab confirmed that antibodies do not actively participate in β-cell destruction (21–23) and subsequent development of IDDM. Rather, autoreactive B cells may act as antigen presenting cells for the initial T cell activation (24).

Based on the fact that anti-GAD Ab do not play a role in destruction of β-cells, we initiated a process to achieve passive modulation of the immune system of pre-diabetic NOD mice through weekly injections of anti-GAD Ab. This study demonstrates a prevention of IDDM and abatement in the severity of insulitis in NOD mice through weekly injections of anti-GAD Ab. The preventative effect of anti-GAD Ab is specific, in that a significantly greater population of control (polyclonal IgG_1 or normal saline) NOD mice developed IDDM, compared to the test animals.

MATERIALS AND METHODS

Materials

Female NOD mice were purchased at 3–4 weeks of age, male NOD mice were purchased at 6 weeks of age, and Balb/c mice were purchased as retired breeders. The animals were obtained from Jackson Laboratories (Bar Harbor, ME) and housed in the University of Utah Vivarium, an approved AAALAC, USDA facility.

A hybridoma cell line-producing anti-GAD Ab (HB184, IgG_1, anti-GADF-6 that is GAD-65 specific) was obtained from

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ATCC (Rockville, MD). E. coli GAD, expressed and isolated from E. coli-(EC 4.1.1.15), was purchased from Sigma Chemicals, St. Louis MO. Polyclonal mouse IgG_1 was purchased from Zymed, San Francisco, CA and was washed using a concentrator (100,000 MWCO membrane) in PBS and concentrated to 3 mg/ml. Fluorescein Isothiocyanate (FITC)-anti-L3T4 and phycoerythrin-labeled anti-Ly-2 were purchased from Pharmingen, San Diego, CA. IgG isotypes were determined using an isotype kit from Pierce, (Rockford, IL). All other chemicals, reagents and proteins were purchased from Sigma Chemical, St Louis MO. Acrodisk Membranes ware obtained from Gelman Science, Ann Arbor, MI. The Molecular Weight Concentrator was purchased from Amicon, Beverly, MA.

Anti-GAD Antibody Production and Purification

The hybridoma cell line producing anti-GAD Ab (IgG_1, GAD-65 specific) was injected into the peritoneum of Balb/c mice to produce anti-GAD Ab in the ascites fluid. Anti-GAD Ab was extracted and purified after precipitation with saturated ammonium sulfate, followed by anion exchange chromatography and protein A-Sepharose chromatography, as described by Gottlieb *et al.* (25).

The purified fractions were pooled, dialyzed, and filtered with 0.2 μm sterile Acrodisk membranes, diluted to a concentration of 3 mg/ml in phosphate buffered saline (PBS), and stored at −20°C. The purified fractions were characterized by electrophoresis (Phastgel 8/25, Pharmacia) under native and denaturing conditions, and with a homogeneous gel in denaturing and reducing conditions.

Anti-GAD Ab Binding Activity

E. coli GAD, expressed and isolated from E. coli (EC 4.1.1.15) was used in ELISA studies to determine the purity of anti-GAD Ab obtained from anti-GAD Ab producing hybridoma cells, the ascites, and purified fractions obtained from chromatography. The cross-reactivity between the E. coli GAD and recombinant mouse GAD 65 was previously demonstrated (26).

E. coli GAD was dissolved in a coating buffer (Na_2CO_3 0.1 M, $NaHCO_3$ 0.1M, pH = 9.5), and the solution (0.5 mg/mL) was transferred into wells of microplates (50 μl/well) and incubated for 1 hour at 37°C. The wells were washed with 200 μl of PBS, 0.02% Tween 20, pH = 7.3, (wash buffer) and then exposed to 250 μl of PBS, 0.02% Tween 20, 1% bovine serum albumin (blocking buffer) for 1 hour at 37°C. After incubation, the blocking buffer was removed and the microplates dried. Duplicate dilution (50 μl) of samples containing anti-GAD Ab were incubated for 2 hours at 37°C, followed by 3 washes with washing buffer (blocking buffer without BSA). Rabbit anti-mouse IgG conjugated to alkaline phosphatase, at a concentration of 4 μg/ml in blocking buffer, was added to all wells and incubated for 2 hours at 37°C, followed by 3 washes with washing buffer. The wells were then incubated with p-nitrophenol phosphate chromogen at 1 mg/mL in 1M diethylethanolamine buffer for 40 min at room temperature. The optical density at 405 nm was then determined using a microplate autoreader EL311 (Bio-Tek Instruments). These values were compared to standard curves (known anti-GAD concentrations) to extrapolate anti-GAD concentrations.

Treatment

Female non-obese diabetic (NOD) mice were used in all therapeutic evaluation. Animals receiving weekly interperitoneal (IP) injection of anti-GAD Ab were treated under different protocols to define an optimum age of initial treatment or optimum dosage. Groups received weekly IP injections of 0.3 mg of anti-GAD Ab, beginning at either 7 weeks of age (n = 13) or 12 weeks of age (n = 6), until they reached 32 weeks of age. This dosage was selected on the basis of previous studies using antibodies targeted to tumor cells. The doses and time schedules were optimized to insure high concentrations of antibody at the target site as a function of time (27,28). Another group received 0.1 mg/week/mouse (n = 13) beginning at 7 weeks of age until they reached 32 weeks of age.

To determine if the effect of anti-GAD Ab on the delay of IDDM was an immunization process, a group of female NOD mice (n = 4) received one IP injection of 0.3 mg anti-GAD Ab at 7 weeks of age, and another IP injection of 0.3 mg anti-GAD Ab at 12 weeks of age.

Control groups consisted of female NOD mice, maintained similar to the test group, and received weekly IP injections of normal saline (n = 10). Another group of animals were injected IP weekly with 0.3 mg of polyclonal mouse IgG_1 beginning at 7 weeks of age (n = 6).

Evaluation of Hyperglycemia

Weekly blood glucose levels were obtained by collecting blood samples from the tail vein and analyzing glucose levels with an Accu-Check Advantage glucometer (Boehringer Mannheim Corporation). Hyperglycemia and IDDM was characterized by weight loss and blood glucose levels >250 mg/dl that persistent for two consecutive weeks.

Evaluation of Insulitis

The development of diabetes is directly correlated to the extent of insulitis (inflammation and infiltration by lymphocytes) present in the β-cells. Therefore, a measure of the efficacy of anti-GAD Ab treatment is to compare insulitis in both the test and control groups. The severity of insulitis was determined by physically counting stained macrophage and lymphocyte infiltrated β-cells. At determined times, either at the onset of hyperglycemia or at the end of treatment for the test group (~32 weeks), the animals were sacrificed (Metofane inhalation and cervical dislocation) and the pancreas were removed. Each pancreas was fixed with 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The severity of insulitis was determined by 2 independent researchers using light microscopy. The scoring system of Charlton *et al.* (29) was used to assess the extent of insulitis: (1) normal islet; (2) mononuclear infiltration in less than 25% of the islet; (3) 25 to 50% of the islets infiltrated; (4) over 50% infiltrated; (5) extensive intra-islet infiltration with obvious β-cell damage. The mean score for each pancreas was calculated by dividing the total score by the number of islets scored.

FACS Analysis

Splenocytes from control animals and from anti-GAD Ab treated mice were prepared. Immunofuorescent analysis was

conducted using commercially available monoclonal antibodies such as fluorescein isothiocyanate (FITC)-anti-L3T4 and phycoerythrin-labeled anti-Ly-2. The monoclonal antibodies (2 μl) were added to 1×10^6 splenocytes and incubated for 30 min at 4°C in PBS containing 1% FCS and 0.1% sodium azide. Cells were washed twice in the same buffer and then analyzed using a Fluorescent Antibody Cell Sorter (Becton Dickinson, Sunnyvale, CA).

Transfer of Diabetes

To assess the effect of anti-GAD Ab treatment on the activation of T suppressor cells, two groups of mice were treated with either 0.1 mg or 0.3 mg of anti-GAD until 25 weeks of age, sacrificed and their spleen cells prepared for disease transfer experiments as follows:

7–8 week-old NOD mice were irradiated (750 rad) and then separated into three groups.

- Group 1 mice were injected IV with either 10^7 (n = 6) or 2×10^7 (n = 4) spleen cells collected from diabetic mice prepared aseptically in 200 μl HBSS.
- Group 2 mice were injected IV with a mixture of 10^7 splenocytes from diabetic mice and 10^7 splenocytes from 0.3 mg anti-GAD Ab treated mice ~24 weeks of age (n = 6), or with a mixture of 2×10^7 splenocytes from diabetic mice and 2×10^7 splenocytes from 0.3 mg anti-GAD treated mice (n = 6).
- Group 3 mice were injected IV only with splenocytes from anti-GAD Ab treated mice (2×10^7 splenocytes from 0.1 mg anti-GAD treated mice (n = 6) or with 10^7 splenocytes from 0.3 mg anti-GAD Ab treated mice (n = 6)).

Onset of diabetes was monitored by testing the blood glucose level three times a week and was confirmed by two consecutive measures of glucose level >250 mg/dl.

Statistics

The statistical significance for all evaluations was determined using ANOVA.

RESULTS

Administration of anti-GAD Antibodies Specifically Prevents Diabetes in NOD Mice

Female NOD mice were injected IP once a week with either 0.1 or 0.3 mg of anti-GAD Ab, beginning at 7 weeks of age, and blood glucose levels measured. As shown in Fig. 1, ~83% of the control group developed hyperglycermia between 15 and 20 weeks of age. However, only ~30% ($p < 0.001$) of the group treated with anti-GAD Ab displayed symptoms of hyperglycemia after 32 weeks of age. Furthermore, no statistical difference ($p = 0.12$) was determined between the 0.1 mg and 0.3 mg anti-GAD treatment groups.

Another group of NOD mice was injected weekly with 0.3 mg of polyclonal mouse IgG_1 beginning at 7 weeks of age. As shown in Fig. 2, no significant difference in the incidence of hyperglycemia was observed at 32 weeks of age between the two groups. The median age of hyperglycemic onset of IgG_1 injected mice is 20 weeks, and both the IgG_1 treated group and the control group developed a high incidence of hyperglycemia (>75%) by 32 weeks of age which is statistically different from the anti-GAD Ab treated groups ($p < 0.01$).

Two groups of mice were injected beginning at 12 weeks of age with 0.3 mg of anti-GAD Ab. As shown in Fig. 3, the incidence of IDDM development was not statistically different from the control group. The median age of onset for the development of diabetes in this treatment group was 17 weeks, similar for the control group.

Another group of 4 mice received only 2 injections of 0.3 mg of anti-GAD Ab at 7 and 12 weeks of age to determine if

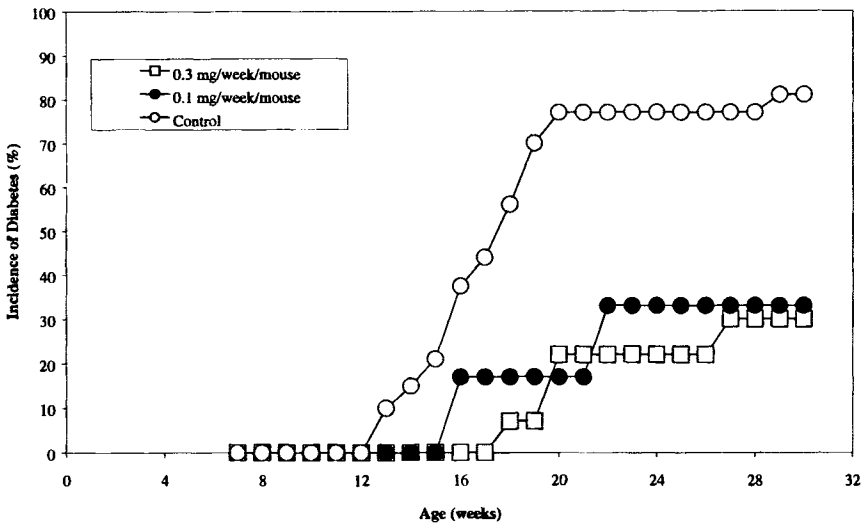

Fig. 1. The effect of administering 0.1 mg/mouse/week (closed circle) or 0.3 mg/mouse/week (open square) of anti-GAD Ab to prediabetic NOD mice at 7 weeks of age is compared to control (open circle—administration of 0.9% saline). There is no statistical difference in the incidence of type I diabetes between mice receiving 0.1 mg and those receiving 0.3 mg of anti-GAD Ab ($p = 0.12$). There is a statistical difference in the delay of diabetes onset between the test groups and the control group ($p < 0.001$).

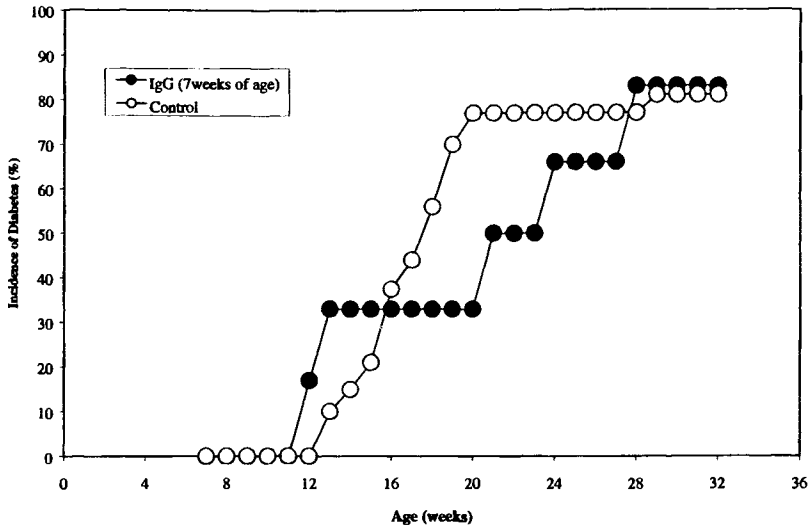

Fig. 2. The effect of administering polyclonal IgG_1 (closed circle) to NOD prediabetic mice at 7 weeks of age is compared to a control group (open circle—administration of 0.9% saline). There is no statistical difference in the development of type I diabetes between the mice receiving polyclonal IgG_1 or 0.9% saline ($p = 0.12$) at 30 weeks of age.

prevention is an immunization effect. In this group, 25% of the mice became diabetic at 20 weeks of age (data not shown). The remainder of the treatment group became hyperglycemic by 32 weeks of age, suggesting that the efficiency of the treatment is transient and that continuous administration of anti-GAD Ab is required to prevent diabetes in the NOD mouse model.

Anti-GAD Antibody Administration Lessens the Severity of Insulitis

Pancreatic islets were examined under a light microscope to correlate the effect of anti-GAD Ab treatment with the development of insulitis. The severity of Scale 5 infiltration for the control group was ~90% while ~50–60% of the islets from

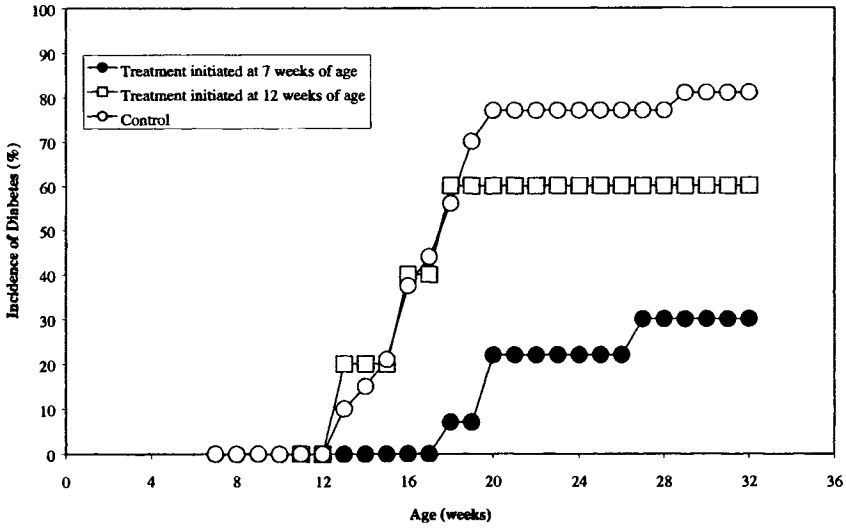

Fig. 3. The effect of age of initial treatment on the incidence of IDDM. NOD prediabetic mice (n = 6) were administered 0.3 mg/mouse/week beginning at 7 weeks of age (closed circle), or NOD mice received 0.3 mg/mouse/week beginning at 12 weeks of age (open square). Control groups (open circle) received 0.9% saline at the same age and for the duration of therapy. There is a statistical difference in the incidence of type I diabetes between mice treated with 0.3 mg of anti-GAD Ab at 7 weeks and those treated at 12 weeks of age ($p < 0.05$).

both 0.1 or 0.3 mg of anti-GAD Ab treated mice received a score of 5, as shown in Fig. 4. As can be seen, there is only a slight difference in the extent of insulitis between the two anti-GAD Ab treatment groups, indicating that both treatments protected β-cells from T cell infiltrates when compared to controls. It should be noted that control animals succumbed to IDDM before 32 weeks of age, and most of these islet cells had significant infiltrates (Score 5) at a much earlier age.

The Effect of Anti-GAD Antibody Administration on the Activation of Regulatory Cells

Two groups of NOD mice were treated with either 0.1 mg/week/mouse (n = 5), or 0.3 mg/week/mouse (n = 5) beginning between 7 and 8 weeks of age. At 25 weeks of age, 3 non diabetic mice treated with 0.1 mg anti-GAD Ab/week/mouse, and 4 non diabetic mice treated with 0.3 mg anti-GAD Ab/week/mouse were sacrificed and the splenocytes were isolated. The cells were transferred into non-diabetic mice to determine if splenocytes from anti-GAD Ab treated mice could actively inhibit the adoptive transfer of diabetes. The splenocytes from mice treated with anti-GAD Ab-treated mice (0.1 mg/week/mouse or 0.3 mg/week/mouse) were co-transferred with splenocytes from diabetic mice. No difference in the incidence of diabetes was observed between the different groups (Fig. 5 a & b). These results were confirmed by demonstrating the lack of T suppressor cells in treated mice (0.1 or 0.3 mg) which is independent of cell number (20 million Fig. 5 a, versus 10 million Fig. 5b). Furthermore, splenocytes from anti-GAD Ab treated mice alone, contained autoimmune lymphocytes that were able to induce diabetes in syngeneic irradiated NOD mice. In addition, no difference in the different T cells subset was observed between the anti-GAD treated mice and control group (Table 1).

DISCUSSION

This study demonstrated that administering high doses of anti-GAD Ab delayed the onset of diabetes in a NOD mouse model. The treatment group was compared to control groups that received either a saline solution or an equivalent quantity of polyclonal non-specific mouse IgG_1. To define the importance of the anti-GAD Ab and GAD auto-antigenicity in the cascade of events that lead to the destruction of the β-cells, we initiated treatment on NOD mice with anti-GAD Ab at various ages. The incidence of diabetes decreased as a function of age of initial treatment, since treatment begun at 7 weeks of age was more efficacious that treatment that was initiated

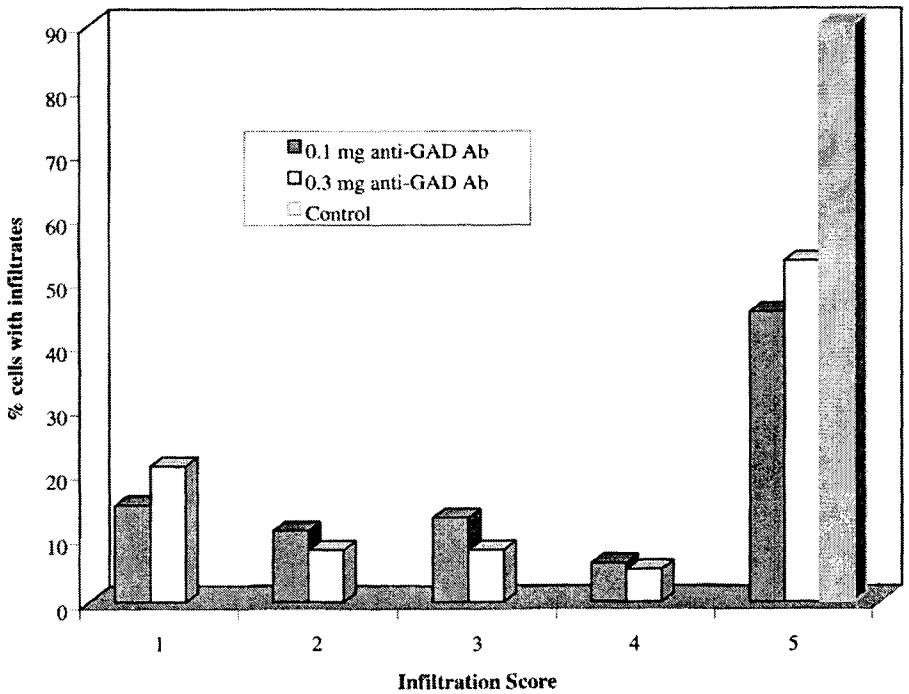

Fig. 4. The severity of the insulitis was evaluated in NOD mice injected with 0.3 mg anti-GAD Ab (n = 5) (white bar), 0.1 mg anti-GAD (n = 4) (dark gray bar). Surviving animals receiving anti-GAD Ab were sacrificed at 32 weeks of age and the pancreas extracted. Control animals (light gray bar) succumbed to the disease and pancreata were analyzed postmortem at ~18 weeks of age. The sectioned pancreata were stained with eosin and hematoxylin, and examined for lymphocyte infiltration. The severity of insulitis in individual cells was assessed by two independent researchers using the following scoring system: (1) Normal islet; (2) Mononuclear infiltration less than 25% of the islet cells cross-sectioned; (3) Mononuclear infiltration in 25 to 50% of the islet cells cross-sectioned; (4) Mononuclear infiltration in greater than 50% of the islet cells cross sectioned; (5) Extensive intra-islet infiltration with obvious ß-cell damage. The mean score for each pancreas was calculated by dividing the total score by the number of islets scored.

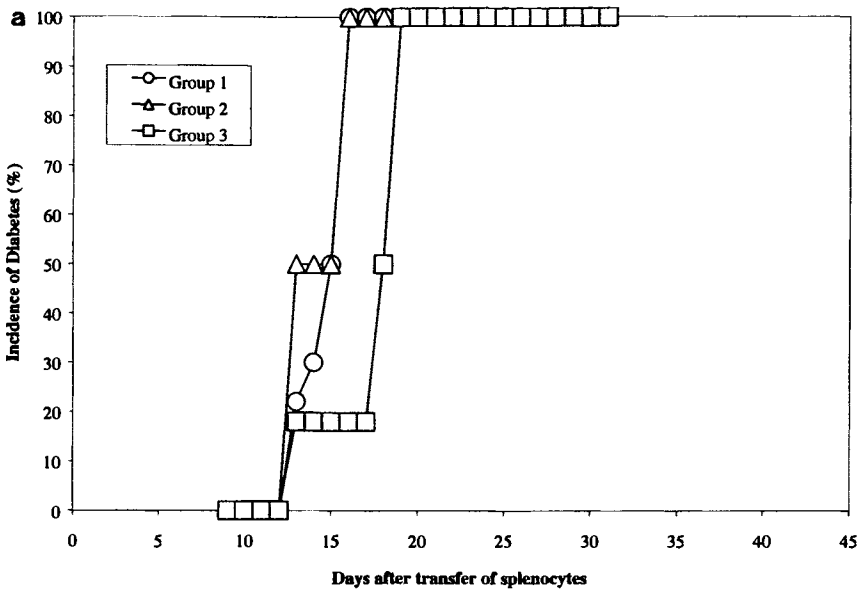

Fig. 5a. Effect of prophylactic therapy with anti-GAD Ab on the adoptive transfer of diabetes into 7–8 week old irradiated male NOD mice. Four male NOD mice were injected with 2×10^7 of diabetic splenocytes (Group 1), 6 male NOD mice were injected with 2×10^7 diabetic splenocytes and 2×10^7 cells from 0.1 mg/week/mouse of anti-GAD Ab treated mice (Group 2), and 6 male NOD mice were injected with 2×10^7 cells from 0.1 mg/week/mouse of anti-GAD treated mice (Group 3).

at 12 weeks of age. This demonstrates that up to 7 weeks, GAD autoantigenicity is very important in the activation of autoimmunity, since anti-GAD Ab injections are able to alter the immune responses. However, after a certain age, the importance of GAD antigenicity becomes secondary in the reactions that lead to the destruction of the β-cells. These results corroborate the hypothesis of a cascade of antigenicities as described by Tisch *et al.* (15), where GAD is the first autoantigen involved in the autoimmune activation in the NOD mouse model. Furthermore, the monoclonal anti-GAD (GAD-6) used binds specifically to the COOH region of the GAD. This region has been shown by Kaufman *et al.* (16) to be involved in the autoimmune response in the NOD mouse beginning at three week of age. At seven weeks of age, the autoimmune response has spread to other parts of the GAD molecules, but an epitope is still involved with the COOH terminus. Given the importance of the

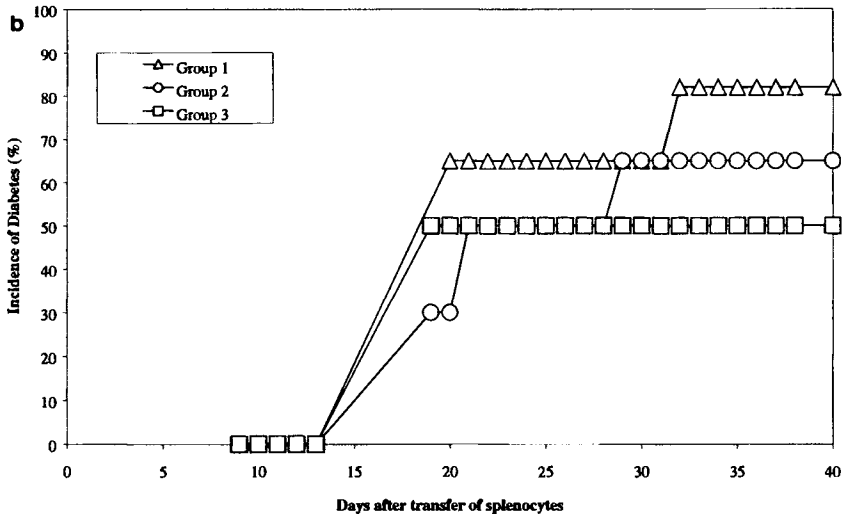

Fig. 5b. Effect of prophylactic therapy with anti-GAD Ab on the adoptive transfer of diabetes into 7–8 weeks old irradiated male NOD mice. Six male NOD mice were injected with 10^7 cells of diabetic splenocytes (Group 1), 6 male NOD mice were injected with 10^7 cells of diabetic splenocytes and 10^7 cells from 0.3 mg/week/mouse of anti-GAD Ab treated mice (Group 2), and 6 male NOD mice were injected with 10^7 cells from 0.3 mg/week/mouse of anti-GAD treated mice (Group 3).

Table I. Analysis of T Cells Subsets by FACS Analysis of Splenocyte of Anti-GAD Treated and Control Nontreated NOD Mice

Treatment	T cell subset (% of total lymphocytes)	
	$CD4^+$	$CD8^+$
Anti-GAD (0.3 mg/week)	31.45 ± 0.97	14.18 ± 1.53
Control	32.58 ± 1.33	12.12 ± 0.24

COOH region in the autoimmune response, it may be possible to augment the passive abatement of the immune response by the co-administration of the anti-GAD (GAD-6) other antibody directed against other epitopes (peptide 247–266) as described by Dr. Kaufman (16).

The injection of anti-GAD does not completely protect the islets from insulitis, as observed with other methods for prevention of diabetes. A significant reduction in insulitis was observed by tolerance induction through intrathymic injection of mouse recombinant GAD-65 to young NOD mice (16). Other types of immune prevention however, do not confer this cellular protection, as observed by injection of Freund's adjuvant (30) or by the injection or oral administration of insulin (31,32). From these observations, it can be concluded that insulitis by itself is not sufficient for the development of clinical disease, and that differences may exist in the T cells subsets of insulitis between destructive and non destructive lymphocytes infiltration.

The function of T cells to promote diabetes, presumably involving GAD recognition, could be inhibited for a long period of time by regular administration of anti-GAD Ab. However, as shown by the transfer of disease experiments, the T cells do not develop tolerance, but remained in the host in an inactive state. No activation of suppressor cells was observed in the irradiated mice co-injected with diabetogenic splenocytes and anti-GAD treated mice splenocytes. In addition, T cells treated with anti-GAD were able to induce the destruction of the β cells of the injected recipients at the same rate and in the same proportion as diabetogenic splenocytes. Only the rate of development of diabetes was shown to be a function of the number of cells injected per mouse. This dormant state was reactivated when such T cells were subsequently transplanted into irradiated recipients. It seems likely that GAD autoantigenicity can be masked by binding to anti-GAD Ab which may lead to a non activation of the T cells, and the prevention of diabetes. In this approach anti-GAD could alter the processing of GAD by the APC. Other groups have shown that by modification of (antigen) amino acid sequence, it is possible to lower the affinity of the processed antigen for a specific MHC II molecule and change the T lymphocyte differentiation from a Th1 to a Th2 type of response (33,34). Following the same concept, monoclonal antibodies specific for a particular antigen were shown to bind and prevent efficient digestion, processing and presentation of the antigen by the APC to primed T cells (35). In this report, the anti-GAD Ab was shown to delay the onset of diabetes, presumable through binding to the specific GAD antigen or the processed antigen (APC) and modulate the T lymphocyte cell response. Since the antibody mediated cytotoxic response has been shown to be unable to induce diabetes in an animal model (21), and no complement lytic activity exists in the NOD mouse (36), the anti-GAD could not be directly involved in destruction of β-cells. Based on these preliminary results, it is probable that anti-GAD Ab ~ GAD complexes could alter the immune system toward a non-destructive type of immune response, however, further studies need to be performed to confirm this hypothesis.

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