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Effects of BCG, Lymphotoxin and Bee Venom on Insulitis and Development of IDDM in Non-obese Diabetic Mice

To investigate whether BCG, lymphtoxin (LT) or bee venom (BV) can prevent insulitis and development of diabetes in non-obese diabetic (NOD) mice, we measured the degree of insulitis and incidence of diabetes in 24 ICR and 96 female NOD mice. NOD mice were randomly assigned to control, BCG-, LT-, and BV-treated groups. The BCG was given once at 6 weeks of age, and LT was given in 3 weekly doses from the age of 4 to 10 weeks. The BV was injected in 2 weekly doses from the age of 4 to 10 weeks. Diabetes started in control group at 18 weeks of age, in BCG group at 24 weeks of age, and in LT- or BV-treated group at 23 weeks of age. Cumulative incidences of diabetes at 25 weeks of age in control, BCG-, LT-, and BV-treated NOD mice are 58, 17, 25, and 21%, respectively. Incidence and severity of insulitis were reduced by BCG, LT and BV treatment. In conclusion, these results suggest that BCG, LT or BV treatment in NOD mice at early age inhibit insulitis, onset and cumulative incidence of diabetes.

Key Words: *Diabetes mellitus, insulin-dependent; Autoimmunity; Cytokines*

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INTRODUCTION

Type 1 diabetes both in humans and in animals models is a multifactorial disease resulting from destruction of islet B-cells that leads to an absence of intrinsic insulin secretion (1). The destruction of islet B-cells may be caused by autoimmune mechanisms, viral infection (2, 3), or chemicals (4), each in a setting of some genetic predisposition (5). Dietary and other environmental factors are also important to the development of diabetes (6, 7). The NOD mice, which show many features of human type I diabetes, are extensively used to evaluate the role of immunomodulators in the pathogenesis of autoimmune diabetes. The development of diabetes in this model appears to be controlled by a finely tuned immunoregulatory balance between autoaggressive T cells and regulatory immune phenomena (8).

Recently, many reports suggest various immune interventions have prevented development of autoimmune diabetes in NOD mice: e.g., lymphocyte vaccination (9), TNF-alpha (10, 11), OK-432 (12), immunosuppressive drug (13, 14), *bacille* Calmette-Guerin (BCG) (15, 16), and lymphtoxin (LT) (17, 18). Bee Venom (BV) has many powerful immunomodulators; eg. apamin, mellitin, phospholipase, and so on, but its effect on the development of diabetes has not been studied yet.

In this study, we investigated the effects of BCG, LT and BV on insulitis and development of diabetes in NOD mice, and compared their ability to suppress autoimmune diabetes.

MATERIALS AND METHODS

Animals

NOD mice, purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A.) and maintained in specific pathogen-free rack of the Animal Care Unit of Yeungnam University College of Medicine, were used as a model for IDDM, and ICR mice from KIST (Taejon, Korea) were used for non-diabetic control mice (19). The animals were fed with chow diet. Only female animals were used for the experiments.

Groups

NOD mice were subdivided into control (n=24), BCG- (n=24), LT- (n=24) and BV- (n=24) treated groups.

Immunomodulators and administration

BCG (Pasteur Merieux, Lyon, France) in a dose of 100 μL was injected subcutaneously in back and intraperitoneally at 5 weeks of age. LT (Sigma, MO, U.S.A.) in a dose of 200 U was injected intraperitoneally three times per week from 4 to 10 weeks of age. BV (Sigma, MO, U.S.A.) in a dose of 0.5 mg/kg was injected into hindlimb muscle twice per week from 4 to 10 weeks of age.

Assessment of diabetes and insulitis

Insulitis was assessed by histology at 19-25 weeks of age. Pancreatic tissue was prepared for histology by fixing in 10% neutral buffered formalin and then embedding in paraffin. The fixed blocks were sectioned and stained by hematoxylin and eosin. The slides were examined by light microscopy. Severity of insulitis in each islet was evaluated and classified according to the following system of grades (12): Grade 0 refers to an intact islet; grade 1 indicates that the area of mononuclear cell infiltration surrounding or within an islet is <25%; grade 2, 25-50%, grade 3, >50%.

To assess diabetes, mice were monitored twice a week for urine glucose from 15 weeks of age. Diabetes was diagnosed when mice were glycosuric for at least two consecutive times (1).

Sacrifice of animals

Diabetic mice were sacrificed 1 week after development of diabetes, and non-diabetic mice were sacrificed at 25 weeks of age, following 7 hr of fasting. Anesthesia was carried out with pentothal sodium (40 mg/kg), and blood was drawn from inferior vena cava. Then, mice were sacrificed by blood loss, and then pancreatic tissue was excised.

Measurement of blood chemicals

Plasma triglycerides and total cholesterol were measured by enzymatic methods.

Statistical analysis

Statistical significances were determined by χ^2 with Yates correction and analysis of variance (ANOVA).

RESULTS

Diabetes was developed between 18-25 weeks of age in control, between 24-25 weeks of age in BCG and between 23-25 weeks of age in LT- or BV-treated NOD mice (Fig. 1). Cumulative incidences of insulitis from specimens by 25 weeks of age in control, BCG-, LT- or BV-treated NOD mice were 68.7, 51.7, 57.1, and 57.2%, respectively compared with ICR mice (Table 1, Fig. 2) and insulitis was developed in all NOD mice. Cumulative incidences of diabetes by 25 weeks of age in control, BCG-, LT- or BV-treated NOD mice were

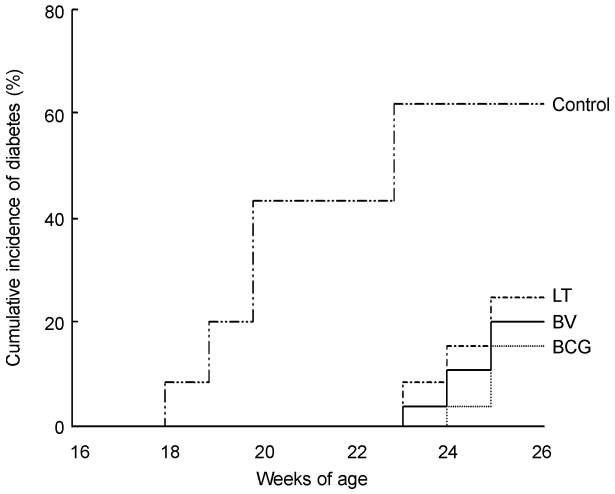

Fig. 1. Effect of BCG, LT, or BV on onset of diabetes mellitus in female NOD mice. NOD, non-obese diabetic; LT, lymphtoxin; BV, bee venom.

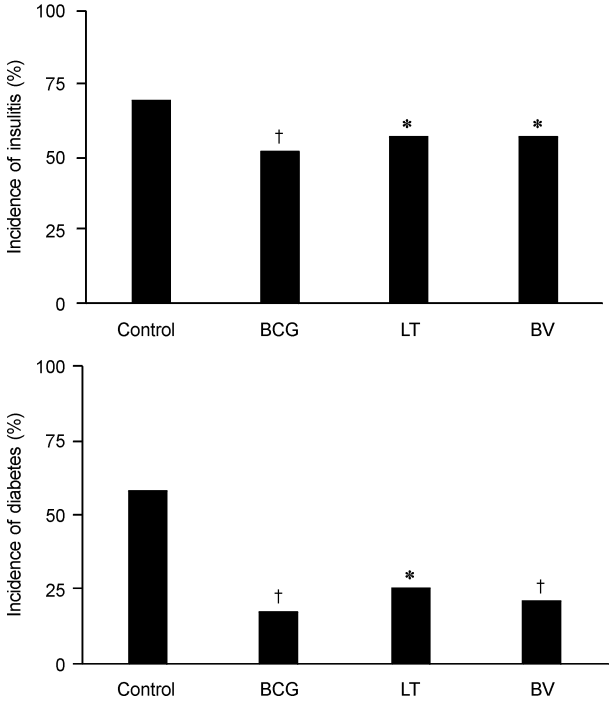

Fig. 2. Effects of BCG, LT, or BV on cumulative incidences of insulitis and diabetes mellitus in female NOD mice. NOD, non-obese diabetic; LT, lymphtoxin; BV, bee venom. *$p<0.01$ or †$p<0.001$ vs control NOD mice by χ^2 test.

Table 1. Effects of BCG, LT, or BV on development and severity of insulitis in female NOD mice

Group	N	Number of islets counted	Grade of insulitis lesions (%)				*p*
			0	1	2	3	
ICR	12	50	100				
NOD							
Control	24	186	31	31	23	15	
Diabetic	14	102	20	31	27	22	
Non-diabetic	10	84	47	31	17	5	
BCG	24	144	49	24	23	4	0.001
Diabetic	4	40	30	40	25	5	
Non-diabetic	20	104	52	21	23	4	
LT	22	148	43	34	16	7	0.01
Diabetic	6	56	24	36	24	16	
Non-diabetic	16	92	50	33	13	4	
BV	24	147	43	31	20	8	0.01
Diabetic	5	45	27	34	23	16	
Non-diabetic	19	102	47	30	19	4	

NOD, non-obese diabetic; LT, lymphtoxin; BV, bee venom; N indicates number of cases
Severity of insulitis is expressed as follows; grade 0 refers to an intact islet; grade 1 indicates that the area of mononuclear cell infiltration surrounding or within an islet is <25%; grade 2, 25-50%, grade 3, >50%
P values are versus control NOD mice by χ^2 test

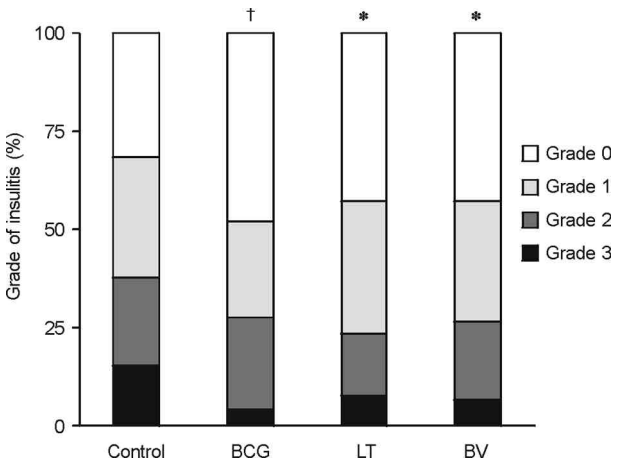

Fig. 3. Effects of BCG, LT, or BV on severity of insulitis in female NOD mice. NOD, non-obese diabetic; LT, lymphtoxin; BV, bee venom. Severity of insulitis is expressed as follows; grade 0 refers to an intact islet; grade 1 indicates that the area of mononuclear cell infiltration surrounding or within an islet is <25%; grade 2, 25-50%; grade 3, >50%. *$p<0.01$ or †$p<0.001$ vs control NOD mice by χ^2 test.

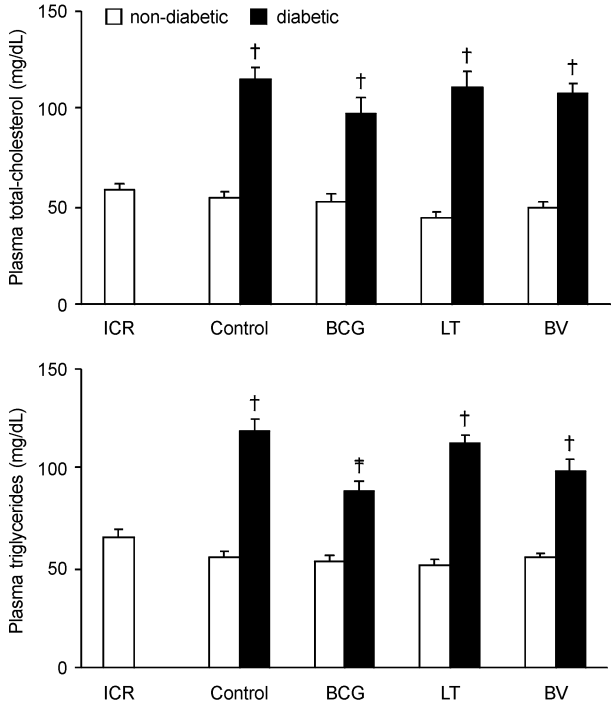

Fig. 4. Plasma total-cholesterol and triglyceride concentrations in diabetic and non-diabetic NOD mice. NOD, non-obese diabetic; LT, lymphtoxin; BV, bee venom. Values are mean±SE for 4-24 rats. ‡$p<0.05$ vs ICR, non-diabetic or diabetic control, LT or BV treated NOD mice, †$p<0.001$ vs ICR or non-diabetic NOD mice by ANOVA.

58.3, 16.7, 25.0, and 20.8%, respectively compared with ICR mice (Fig. 2). Severity of insulitis was exacerbated by development of diabetes and reduced by BCG, LT, or BV treatment (Fig. 3).

Blood glucose concentration was increased markedly by development of diabetes compared to non-diabetic mice in all groups (data not shown). Plasma insulin concentration was lowered in NOD compared to ICR mice, and decreased by development of diabetes compared to non-diabetic NOD mice (data not shown). Plasma triglycerides and total cholesterol levels were increased markedly in diabetic mice than in non-diabetic mice (Fig. 4).

DISCUSSION

This study was carried out to investigate the effects of various immunomodulators on development of insulitis and diabetes in NOD mice.

Various experimental immunotherapies have successfully prevented IDDM in animal models (9-18, 20), and some of these therapies have been used in humans (21, 22). Recently, Harada et al. (15) reported that a single BCG injection suppressed development of insulitis and diabetes in NOD mice, and Gearon et al. (9) insisted lymphocyte vaccination protects prediabetic NOD mice from developing diabetes mellitus.

In this experiment, cumulative incidence of diabetes at 25 weeks of age in control NOD mice was 58.3% compared to ~80% of other reports. The lower incidence of diabetes in this experiment may be due to environmental differences, but further study is needed. By single injection of BCG, incidence of diabetes was lowered to 16.7% compared to 58.3% of control, and insulitis was inhibited. In previous studies, LT production of peripheral blood mononuclear cells was lower in IDDM subjects than in controls, and an early age at onset was correlated with low LT-alpha production (23). It is known that LT inhibits development of diabetes in BB rats (24) and in NOD mice (17, 18). In our experiment, LT suppressed insulitis and overt diabetes. The result was different from Seino et al. (17), who reported LT did not inhibit insulitis significantly at 8 and 12 weeks of age either. It is suspected that the discrepancy is due to the determination of insulitis at a different age. BV inhibited insulitis and development of diabetes in NOD mice. Suppressive ability of BV was intermediate between BCG and LT treatment. No apparent side effects by BV injection during the experimental period were found.

The results imply immunomodulators play a role in treatment of insulitis and diabetes of NOD mice, although precise immune and metabolic mechanisms remain to be elucidated.

In conclusion, our results suggest that BCG, LT or BV treatment suppress insulitis and inhibit development of diabetes in NOD mice.

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