

## Homeostasis as Regulated by Activated Macrophage. V. Suppression of Diabetes Mellitus in Non-obese Diabetic Mice by LPSw (a Lipopolysaccharide from Wheat Flour)

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A lipopolysaccharide from wheat flour (LPSw) which was isolated and characterized is shown to exert definitely a suppressive effect on incidence of type 1 diabetes in mice. Therapeutic effect on type 2 diabetes in patients was also obtained, though only from preliminary results. The percutaneous route of administration is most favorable. The important role that precursor tumor necrosis factor, free or cell bound, may play in this mechanism is discussed.

**Keywords** diabetes; non-obese diabetic mice; lipopolysaccharide; macrophage; homeostasis

### Introduction

We earlier described "ontogenic inflammation" which regulates the homeostasis of ontogenesis by endogenous production of tumor necrosis factor (TNF).<sup>1)</sup> Reproduction of ontogenic inflammation in adults, especially in patients of intractable diseases, may restore their homeostasis from a distorted or deviated state.<sup>2)</sup>

In the first report<sup>2)</sup> of this series of papers we described a new lipopolysaccharide (called LPSw) which was isolated and purified from wheat flour. LPSw can activate macrophage up to the primed state for endogenous production of TNF to induce ontogenic inflammation when administered percutaneously or orally.

The present report describes the effect of LPSw on diabetes, by showing its suppression of incidence of type 1 diabetes in non-obese diabetic (NOD) mice. Preliminary accounts of this treatment on clinical trials are also reported. The results are quite promising and the innovative results in related TNF production are discussed.

### Materials and Methods

**Preparation of LPSw (Wheat Flour LPS) and LPSw-H (Partially Purified LPSw)** The method of LPS purification from wheat flour was described.<sup>2)</sup> Briefly, low molecular sized substances (<5 kilodaltons (kDa)) were removed from a water extract of wheat flour by ultrafiltration. The remaining fraction, called LPSw-H, contained 0.01—0.1% of LPSw as estimated by Limulus test.<sup>3)</sup> LPSw was further purified to 95% by trichloroacetic acid (TCA)-extraction of LPSw-H, gel filtration, enzyme digestion and then ion-exchange chromatography.

**Experimental Animals** NOD female mice, age 4 weeks (19—23 g), were purchased from Clea Japan, Inc. (Tokyo, Japan). This strain was used as a model for type 1 (insulin-dependent) diabetes.<sup>4)</sup>

**Occurrence of Diabetes** In NOD mice, lymphocytic infiltration into pancreatic islets, or insulinitis, appears at approximately 4 to 5 weeks of age and causes an autoimmune destruction of insulin-secreting cells. At about 28 weeks of age, from 70 to 80% of the females develop diabetes.<sup>5)</sup>

**TNF** Recombinant human TNF ( $2 \times 10^6$  U/mg protein) was prepared and purified to more than 95% homogeneity in our laboratory. Details were described in a previous paper.<sup>6)</sup>

**Preliminary Clinical Trial** A preliminary clinical trial was made with 4 cases of type 2 non-insulin dependent diabetes. Two cases were treated with LPSw-H alone and 2 others with LPSw-H in combination with insulin or an oral antidiabetic. We report the effect of treatment by LPSw-H alone in type 2 diabetes.

**Diagnosis of Diabetes** The diagnosis of diabetes was made by the onset of glycosuria which was examined by glycosuria-test paper called "Tes-tape" developed by Shionogi Pharm. Co. (Osaka, Japan).

### Results

**Suppressive Effect of LPSw as Compared with TNF via the Intravenous Route** Satoh *et al.*<sup>7)</sup> have reported that TNF

can suppress the occurrence of type 1 diabetes in NOD mice when administered intravenously and intraperitoneally. We administered LPSw intravenously to compare its effect with that of TNF. The result is shown in Fig. 1. Three  $\mu\text{g}/\text{mouse}$  of LPSw and 5  $\mu\text{g}/\text{mouse}$  of TNF was given intravenously once a week. Definite suppression is observed in both cases.

We also examined effect of LPSw-H by the oral route. LPSw-H was dissolved in distilled water to a concentration of 0.1  $\mu\text{g}$  LPSw/ml (by calculation) and the solution was orally administered to mice *ad libitum*. The incidence of type 1 diabetes showed a similar pattern as the control, suggesting that LPSw-H was not effective against diabetes when ingested freely at this concentration.

**Suppressive Effect of LPSw on Incidence of Diabetes of NOD Mice via the Intradermal Route** LPSw has shown marked therapeutic effect even when used percutaneously or orally against some intractable diseases.<sup>8)</sup> We previously reported also that LPSw shows a similar effect by intravenous or intradermal administration.<sup>2)</sup> We did a similar experiment examining the dosage and response *via* the intradermal route. The result is shown in Fig. 2. Incidence of diabetes was delayed by about 9 weeks and survival rate was more than 80%. A ten-fold greater dosage, 10  $\mu\text{g}/\text{mouse}/\text{week}$  of LPSw *via* the intradermal route gives

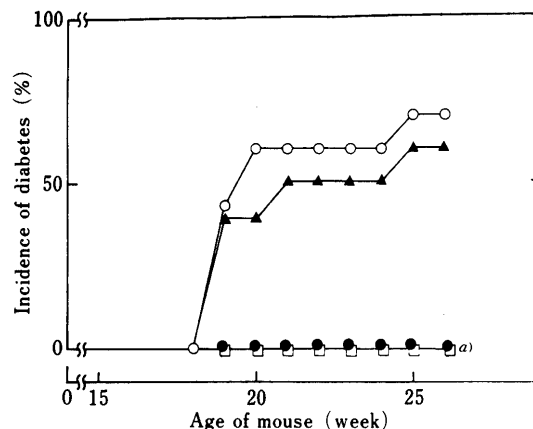


Fig. 1. Suppressive Effect of LPSw and TNF on Incidence of Type 1 Diabetes in NOD Mice

Ten NOD mice were used in each group and treated with samples from 6 to 12 weeks of age. (○), control (non-treated); (●), LPSw *i.v.* (3  $\mu\text{g}/\text{mouse}/\text{week}$ ); (▲), LPSw-H in drinking water *ad libitum* (LPSw, 0.1  $\mu\text{g}/\text{ml}$ ); (□), TNF *i.v.* (5  $\mu\text{g}/\text{mouse}/\text{week}$ ). a) Significant difference ( $p < 0.05$ ) from the control group by Student's *t*-test.

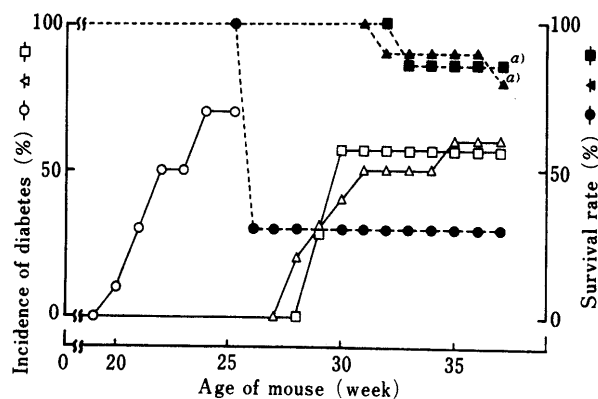


Fig. 2. Suppressive Effect of LPSw on Incidence of Type 1 Diabetes in NOD Mice

Ten NOD mice were used in each group and treated with LPSw from 6 to 12 weeks of age. (○, ●), control (non-treated); (△, ▲), LPSw i.d. (10 µg/mouse/week); (□, ■), LPSw i.v. (1 µg/mouse/week). Open symbols indicated incidence of diabetes and closed symbols indicated survival rate. a) Significant difference ( $p < 0.01$ ) from the control group by Student's *t*-test.

TABLE I

Case 1 (female, 57 years)		Before a meal	1 h after a meal	2 h after a meal
Before administration	Blood glucose <sup>a)</sup> level (mg/dl)	99	214	216
	Glycosuria <sup>b)</sup>	—	—	+++
1 month after administration	Blood glucose level (mg/dl)	92	194	182
	Glycosuria	—	—	—
Normal blood glucose level (mg/dl)		60—110	<160	<120
Normal glycosuria		—	—	—

a) Blood glucose level was measured by glucose-oxidase method. b) Glycosuria was examined by glycosuria-test paper. Glycosuria score roughly indicated concentration of glucose in urine. (—), <0.1%; (+), 0.1%; (++) , 0.25%; (+++) , 0.5%; (++++), >2%.

TABLE II

Case 2 (male, 56 years)	Dietotherapy			1 month after administration
	Dec. 1985	May 1988	Jul. 1989	Aug. 1990
Before a meal				
Blood glucose <sup>a)</sup> level (mg/dl)	183	223	192	139
Glycosuria <sup>b)</sup>	+	+++	+++	—

a) Blood glucose level was measured by glucose-oxidase method. b) Glycosuria was examined by glycosuria-test paper. Glycosuria score roughly indicated concentration of glucose in urine. (—), <0.1%; (+), 0.1%; (++) , 0.25%; (+++) , 0.5%; (++++), >2%.

the same pattern as given by 1 µg/mouse/week of LPSw *via* the intravenous route.

**Preliminary Clinical Trial on Patients of Diabetes** Since LPS is non-toxic when given orally or percutaneously, we applied LPSw-H to type 2 (non-insulin dependent diabetes). One ml of a liniment containing LPSw-H (estimated at 1 µg of LPSw/50% (w/v) glycerol) was applied percutaneously once a day to the medial femoral region.

Table I shows the change in case 1. Before the administration the blood glucose levels before a meal, and 1 and 2 h after a meal were 99, 214, and 216 mg/dl, respectively. Glycosuria 2 h after a meal was 3 plus. After

one month of administration of LPSw-H the blood glucose levels before a meal, and 1 and 2 h after a meal lowered to 92, 194, and 182 mg/dl, respectively and glycosuria was completely negative. Normal levels are also shown in Table I.

In case 2 in Table II, the blood glucose level before a meal was 183 mg/dl. The patient had been treated with dietary regulation, but glycosuria had deteriorated to 3 plus. After one month of the treatment with LPSw-H the blood glucose level declined to 139 mg/dl, glycosuria was negative and the symptoms disappeared.

Similar results were obtained in two other cases, though data are not shown.

## Discussion

The results obtained now show quite definitely that LPSw suppresses incidence of diabetes type 1 in NOD mice, when administered intravenously or intradermally. As previously reported, LPSw at this dose can activate macrophage to prime endogenous production of TNF.<sup>2)</sup> Macrophage at this stage contains large quantity of precursor TNF around the surface of cells.<sup>9)</sup> Since the present experiment showed positive results with the primed macrophage, that is to say LPSw can reproduced "ontogenic inflammation"<sup>1)</sup> in adults, agents considered to prime macrophage are presumed to be available in this therapy. In fact, OK-432 (a streptococcal preparation), BCG (bacillus Calmette-Guérin) even TNF, all of which are shown to be a good primer,<sup>10)</sup> have been reported to be applicable in this therapy.<sup>7,11)</sup> Thus, we consider that the primed stage for endogenous production of macrophage is an essential requisite of this therapy. Furthermore, precursor TNF may be the key molecule which drives the whole phenomenon.

Among all agents now known to suppress diabetes, LPSw may be the best, since it can be administered percutaneously without harm, while others are active when given parenterally and have some degree of harm.

In experimental animal type 1 diabetes has been shown to be suppressed, whereas in clinical trials patients of type 2 might have actually been cured by LPSw, though it is preliminary result. These two types are completely different from each other in their mechanisms of incidence. We do not yet know the reason for this difference. It is considered, however, that the precursor TNF, free or cell-bound, might work to regulate the homeostasis. Trials to suppress the incidence of type 2 in experimental animals are now under way.

As shown previously,<sup>8)</sup> LPS of smaller molecular size can do the same work at LPSw. Therefore, LPS in general, regardless of its origin, whether from bacteria or plant, is expected to be useful for therapy by the oral or percutaneous route, so long as its molecular size is smaller than 5 kDa.

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