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Promotion of spontaneous diabetes in non-obese diabetes-prone mice by cyclophosphamide

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Summary. Cyclophosphamide promoted the onset of overt diabetes in non-obese diabetes-prone mice of both sexes. Two injections of this agent at 2 weeks apart were necessary to obtain a constant high incidence, although even a single injection was effective in some animals. Clinical symptoms of the cyclophosphamide-induced diabetes were similar to those of the naturally occurring type. The same schedule of cyclophosphamide treatment failed to induce diabetes in non-diabetic mouse strains, such as DS/Shi, Jcl: ICR or in non-obese non-diabetes-prone mice, which suggests that the promotion of diabetes by cyclophosphamide in non-obese diabetes-prone mice is not due to the simple pharmacological action of this agent but to some immunopharmacological action.

Key words: Non-obese diabetes-prone mouse, cyclophosphamide.

The non-obese diabetes-prone (NOD) mouse, which develops spontaneous diabetes of the non-obese type, is one of two sister strains established during the course of breeding of cataractous mice from the Jcl: ICR mouse [1, 2]. The occurrence of overt diabetes in this strain is sex-related [3]. In the female mice, the disease is observed first at 13 weeks of age and thereafter the number of mice with diabetes increases, the cumulative incidence reaching approximately 75% at 30 weeks of age. On the other hand, diabetes occurs in a few males only, the cumulative incidence being approximately 10% at 30 weeks from birth. However, histological examination has revealed that insulitis occurs in almost all the mice of both sexes after 5 weeks of age and is followed by islet atrophy [3]. These histological changes are very similar to those observed in human juvenile-onset diabetes mellitus and spontaneous diabetes in the BB rat [4]. To elucidate the pathogenic mechanism in NOD mice, we started a series of experiments from the immunological standpoint, because recent information has suggested that humoral and/or cell-mediated immunities to pancreatic islets are involved in the production of insulin-dependent diabetes in man [5–9] and animals [4, 10, 11]. In the first attempt, we examined the effect of cyclophosphamide (CY) on the production of diabetes in NOD mice. This agent is known to enhance some autoimmune diseases, such as experimental allergic encephalomyelitis [12] and streptozotocin-induced diabetes in the mouse [13] probably by impairing suppressor cells.

Materials and methods

Animals

NOD mice of both sexes at differing ages were used. The non-diabetic strains used were DS/Shi (inbred, female, aged 8 weeks), Jcl:ICR (closed colony, female, aged 8 weeks, purchased from Clea Japan Inc., Tokyo, Japan) and non-obese non-diabetes-prone (inbred, both sexes, aged 5 weeks) mice. All but the Jcl: ICR mice were bred in our laboratory. The non-obese non-diabetes-prone mouse is a sister strain isolated during breeding of the cataractous mouse [1]. All the mice were kept under conventional conditions at a constant temperature (22–25 °C), and fed on commercial diet CA-1 (Clea Japan Inc., Tokyo, Japan) and tap water ad libitum.

Cyclophosphamide treatment

Cyclophosphamide (CY, 150 mg/kg, Asta, Frankfurt, FRG) was injected intraperitoneally once or twice with a 2-week interval. As the vehicle control, NaCl (0.154 mol/l) was injected in place of CY. Numbers and ages of CY- or saline-treated mice are shown in Table 1.

Examination of overt diabetes

The onset of overt diabetes was examined by testing the urinary glucose with Tes-Tape (Eli-Lilly, Indianapolis, Illinois, USA). The animals showing strongly positive coloration (> grade + + +) were recorded as diabetic mice. Plasma and urinary glucose concentrations of some randomly selected Tes-Tape-positive and negative mice were quantitatively determined by the glucose oxidase method using a commercial kit (Blood Sugar-GOD-Perid-Test, Boehringer-Mannheim-Yamanouchi, Tokyo, Japan) [3].

Table 1. Effect of intraperitoneal injection of cyclophosphamide (CY) on the production of overt diabetes in non-obese diabetes-prone and three other strains of mouse

Strain	Injection	Age at the first injection (weeks)	Sex	Number of diabetic mice	
				One week after second injection of CY	Two weeks after second injection of CY[a]
Non-obese diabetes-prone	CY (150 mg/kg)	3	F	0/20	2/20 (10)
			M	0/17	1/17 (6)
		5	F	3/11 (27)[b]	5/11 (46)
			M	5/19 (26)	8/19 (42)
		8–9	F	10/22 (46)	14/22 (64)
			M	14/22 (64)	16/22 (73)
		10	F	5/18 (28)	12/18 (67)
			M	8/20 (40)	14/20 (70)
		15–20	M	20/40 (50)	26/40 (65)
		>20	M	10/30 (33)	13/30 (43)
	Physiological saline (0.2 ml)	5	F	0/18	0/18
			M	0/20	0/20
		8	M	0/ 6	0/ 6
DS/Shi	CY (150 mg/kg)	8	F	0/10	0/10
Jcl:ICR	CY (150 mg/kg)	8	F	0/10	0/10
Non-obese non-diabetes-prone	CY (150 mg/kg)	5	F	0/11	0/11
			M	0/10	0/10

[a] Injections given 2 weeks apart. [b] Corresponding percentage given in parentheses

Results

Overt diabetes was induced in a number of NOD mice injected twice with CY (Table 1). The promotion of pathogenesis was striking in both sexes. The disease was induced in many young females aged 5–9 weeks that otherwise would not have developed spontaneous diabtes so early. Male mice also displayed an incidence comparable with that in females, despite the fact that males rarely develop diabetes spontaneously. The effect of CY did not differ with age, except for the incidence being low in very young mice (aged 3 weeks) in which insulitis was not yet observed. Even a single injection of CY was effective, but the incidence varied with the experiment. To obtain a high incidence more constantly, re-injection at a 2-week interval was necessary. In this case, cumulative incidence attained the maximal level between 1 and 2 weeks after the second injection.

In the control mice treated with physiological saline solution, no promotion of diabetes was observed.

Determination of plasma and urinary glucose concentrations confirmed the above results using Tes-Tape. Plasma glucose levels of randomly selected age-matched (8–9 weeks) diabetic and control mice of either sex were 36.6 ± 5.9 mmol/l (mean $\pm$ SD, $n = 6$) and 9.1 ± 1.9 mmol/l ($n = 4$) respectively 2 weeks after the second injection of CY or saline solution. Urinary glucose levels of these diabetic and control mice determined at the same time were 590.8 ± 35.3 mmol/l ($n = 6$) and 4.1 ± 0.3 mmol/l ($n = 4$) respectively. In addition, consumption of food and water and the urine volume increased greatly in the diseased animals. Loss of body weight and wasting were also pronounced. The mice weighed 25 to 30 g before the onset of diabetes and less than 20 g at the time of death.

In contrast with NOD mice, the three normal strains of mouse, i.e., DS/Shi, Jcl:ICR and non-obese non-diabetes-prone mouse, did not become diabetic after the same CY treatment (Table 1).

Discussion

The present experiment demonstrates that CY treatment promotes the onset of diabetes in NOD mice. Because the same treatment failed to induce the disease in three other strains, promotion of the pathogenesis is unlikely to be due simply to the pharmacological action of this agent. Induction of overt diabetes by CY seems to require some pathological background unique to both sexes of NOD strain aged more than 5 weeks; e.g., insulitis. CY may potentiate the immunological damage of pancreatic islets in this strain, although the development of anti-islet autoimmunity is somewhat controversial at present. Support for this speculation comes from the well-documented information that CY can enhance the immune response to various immunogens including proteinous antigens [14–16] and foreign red blood cells

[17, 18] by depleting suppressor T lymphocytes or their precursors. More closely related findings show that CY converts resistant mouse strains into susceptible ones to some autoimmune diseases, such as experimental allergic encephalomyelitis [12] and streptozotocin-induced diabetes [13], thus offering more support for our speculation.

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