Rapid Publications

Evidence for Initial Involvement of Macrophage in Development of Insulitis in NOD Mice

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Macrophages have been shown to be the major population of infiltrated immunocytes at the early stage of insulitis in diabetes-prone BB rats. This study was undertaken to investigate the role of macrophages in the development of insulitis in nonobese diabetic (NOD) mice. Administration of cyclophosphamide to NOD mice resulted in a significant increase in the incidence of overt diabetes and severity of insulitis compared with that in untreated NOD mice. Intraperitoneal injections of silica completely prevented the development of diabetes and insulitis in both cyclophosphamide-treated and untreated animals. Because silica is selectively toxic to macrophages, the results suggest that macrophages play an important role in the initiation of insulitis in NOD mice. *Diabetes* 37:989–91, 1988

Evidence indicates that many patients with insulin-dependent diabetes mellitus (IDDM) may result from an autoimmune process directed against pancreatic β-cells (1). Animal models for IDDM, such as BB rats and nonobese diabetic (NOD) mice, support the autoimmune hypothesis. However, the mechanism underlying the autoimmunity remains unknown (1). Various effector systems, e.g., T-lymphocytes, natural killer (NK) cells, macrophages, and/or humoral mediators, have been implicated as the possible effectors of immune responses.

Recent studies from our laboratory and others revealed that most infiltrated immunocytes at the early stage of insulitis in BB rats are macrophages (2,3). These findings suggested that macrophages may be involved in the initiation of insulitis. We investigated whether macrophages have an initial permissive role in the immune response against pancreatic β-cells in NOD mice.

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MATERIALS AND METHODS

Our NOD mouse colony was produced from a breeding stock obtained from Clea Japan (Tokyo). These animals were maintained on regular rat chow and tap water ad libitum at the University of Calgary. Beginning at ~8 wk of age, their urinary glucose and ketone levels were determined twice weekly with Diastix and Ketostix reagent slips (Miles, Ontario, Canada). Individual mice were classified as diabetic on the basis of positive glycosuria. The overall cumulative incidence of diabetes among the colony was ~15% in males and 50% in females at 24 wk of age.

A total of 32 male animals from 10 litters, divided into four groups (n = 8/group), were used for this study. We injected silica (Steinkohlen-Bergbau-Verein, Essen, FRG), at a dose of 200 mg · kg^{-1} body wt · wk^{-1} i.p., into one group of animals from 4 to 10 wk of age. The second group of animals received silica, as described above, and subsequently cyclophosphamide (Horner, Montreal, Canada), at a dose of 150 mg/kg body wt twice per 3-day interval, at age 10 wk. The third group received only cyclophosphamide, administered at a dose identical to the second group. The fourth group of animals received no chemicals and were used as controls.

Two weeks after the second injection of cyclophosphamide, the mice were killed by suffocation with carbon dioxide, and their pancreases were fixed in 6% formalin. Paraffin-embedded sections were stained by hematoxylin and eosin and were examined. Severity of insulitis in each islet was evaluated and classified according to the following system of grades: 0, intact islet; 1, the area of mononuclear cell infiltration within an islet is <25%; 2, 25–50%; 3, >50%; and 4, end stage of insulitis, which is characterized by small retracted islets with or without some residual infiltrates. For statistical analysis of the obtained data, χ^2-test was performed (4).

TABLE 1
Effect of cyclophosphamide and silica on incidence of diabetes and insulitis

Group	*n*	Animals with diabetes (*n*)	Animals with insulitis (*n*)	Grade of insulitis lesions (%)				
				0	1	2	3	4
1	8	0	0	100	0	0	0	0
2	8	0	0	100	0	0	0	0
3	8	5	8	0	0	16	48	36
4	8	0	8	32	25	19	24	0

Group 1, injections of silica only; group 2, injections of silica and cyclophosphamide; group 3, injections of cyclophosphamide only; group 4, no treatment. Grades: 0, normal islet; 1, <25% of area with mononuclear cell infiltration within an islet; 2, 25–50%; 3, >50%; 4, small retracted islet with few mononuclear infiltrates.

RESULTS

Administration of cyclophosphamide in NOD male mice resulted in a significant increase in the incidence of overt diabetes and severity of insulitis (Table 1). Five of 8 cyclophosphamide-only male NOD mice developed diabetes within 2 wk of the second injection of the drug, whereas none of the control mice became diabetic. About 84% of the islets from cyclophosphamide-only animals showed advanced insulitis lesions in the islets (grades 3 and 4; Fig. 1*A*), whereas only 24% of the islets from the control group showed similar lesions ($P < .005$). None of the silica-injected mice developed diabetes, and examination of the islets from these animals showed no insulitis (Table 1; Fig. 1*B*). Therefore, silica treatment completely protected the mice from the development of insulitis and diabetes.

DISCUSSION

We show in this study that administration of silica completely prevents the development of insulitis and diabetes in cyclophosphamide-treated NOD mice. Cyclophosphamide significantly increases the incidence of diabetes in NOD mice (5), either by inhibiting suppressor T-lymphocytes or by activating cytotoxic T-lymphocytes (6). Because silica is selectively toxic to macrophages (7), our data suggest that macrophages have a permissive role in the development of insulitis in NOD mice.

Our data are consistent with earlier observations obtained from BB rats (8). The initial role of macrophages in the development of immune responses against pancreatic β-cells

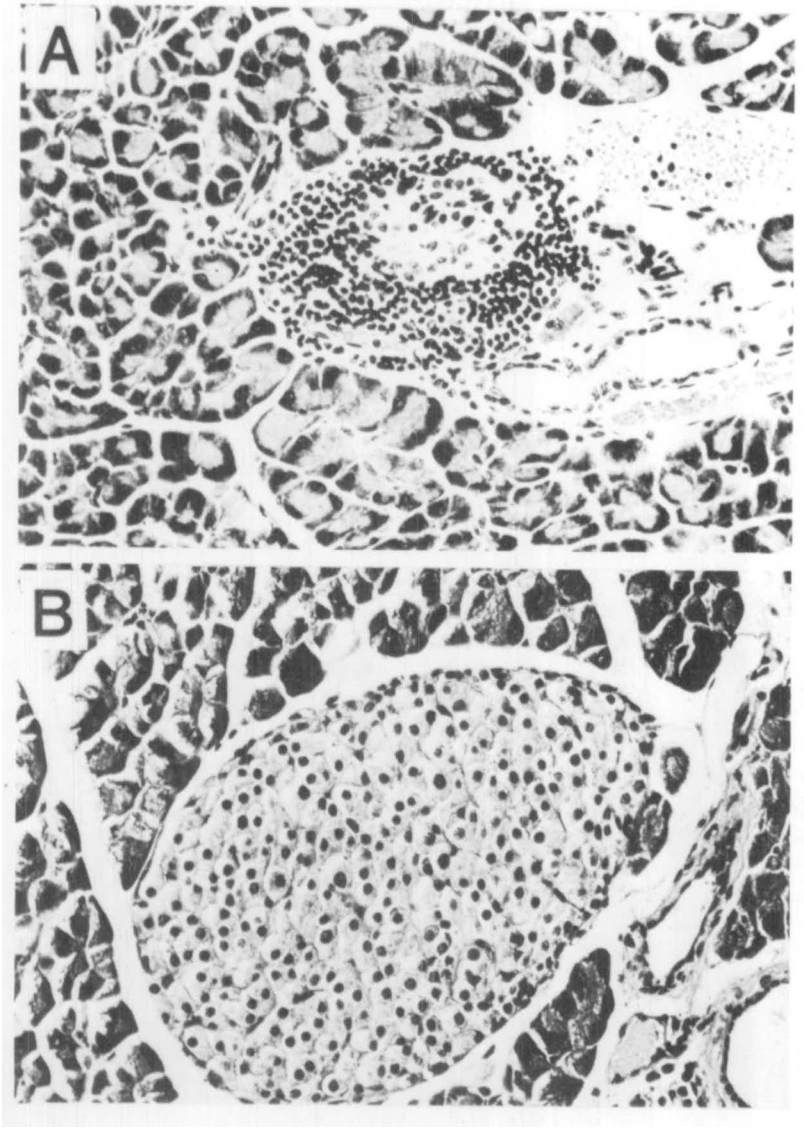

FIG. 1. Pancreatic islet of nonobese diabetic mouse treated with cyclophosphamide (*A*) and with silica and cyclophosphamide (*B*). ×300.

has been further emphasized by recent studies (2,3). Most of the infiltrated immunocytes at the early stage of insulitis in BB rats were shown to be macrophages. Because the cytotoxic effect of activated macrophages is generally assumed not to be specific for any given antigen, it was assumed that an initial change in target β-cells, which can attract macrophages, might precede their immune destruction.

Macrophages play a central role in the immune response against immunologically active molecules (9). The key roles of macrophages include the presentation of processed antigen to helper T-lymphocytes in the context of MHC class II molecules present on the surface of macrophages. Recent observations emphasized that L3T4$^+$ helper T-lymphocytes play a role in the pathogenesis of diabetes in NOD mice (10,11). Administration of anti-L3T4 monoclonal antibody to NOD mice prevented the development of diabetes and the rejection of islet allografts. On the basis of information from the cited studies and our study, we suggest that the presentation of β-cell–specific autoantigen by macrophages and the recognition of the antigen by helper T-lymphocytes might be the initial steps in the development of insulitis.

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