Dapsone Decreases the Cumulative Incidence of Diabetes in Non-Obese Diabetic Female Mice (44137)

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Abstract. **Dapsone (4,4′-diaminodiphenyl sulfone) has a large clinical experience due to its antimicrobial effects against *Mycobacterium leprae,* the causative agent of leprosy, and is used clinically where inflammation mediated by neutrophils is perceived to play a role. We administered dapsone in two concentrations (0.001% and 0.0001% w/w of diet) to 30 female non-obese diabetic (NOD) mice to explore the effect of dapsone on the development of IDDM following either a 1-week pulse or 20 weeks of continuous oral dapsone administration. Those mice receiving either the high or low doses of dapsone in the continuous group had a significantly reduced cumulative percentage of onset of IDDM. One of the seven mice given 0.0001% dapsone became diabetic (age 25 weeks), while none of the eight high dose (0.001%) mice developed the disease. Histological examination of pancreatic sections revealed islet infiltration in all groups of animals. The pulse and continuous experiments showed no statistically significant difference in the frequency or severity of lymphocytic infiltration. Dapsone administration did not inhibit growth, and growth rates were greater in those animals receiving the higher dapsone dose compared with the lower dose comparable to controls.**

We studied whether dapsone influenced murine lymphocyte function in addition to the published effects of the drug on neutrophils. At doses approximating those achieved *in vivo* (0.4 and 2 μg/ml), dapsone was found to inhibit murine splenocyte IL-2 and IL-4 secretion in response to concanavalin A. In view of the wide clinical experience with dapsone, randomized trials of the drug in new onset diabetes may be warranted.

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Dapsone (4,4′-diaminodiphenyl sulfone) is a compound that has a large clinical experience due to its proven antimicrobial effects against *Mycobacterium leprae,* the causative agent of leprosy (1). It is also increasingly used in a number of clinical situations where inflammation mediated by neutrophils may play an ancillary role (2, 3). For example, the treatment of asthma is undergoing significant change, with an emphasis on anti-inflammatory therapy that has utilized *inter alia* the properties of dapsone (4).

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Booth and co-workers (5) demonstrated that the anti-inflammatory influence of dapsone may involve suppression of neutrophil chemotaxis to selected attractants. Dapsone may suppress migration of neutrophils to extravascular sites through inhibition of adherence functions required for neutrophil recruitment. Dapsone did not affect unstimulated neutrophil adherence but, when present with a stimulus, produced dose-related inhibitory effects on adherence. Fifty percent inhibitory doses were approximately 150 μg/ml dapsone. The observed ability of dapsone to inhibit neutrophil chemotaxis under agarose to FMLP (N-formyl-methionyl-leucyl-phenylalanine, a synthetic neutrophil chemoattractant) and interleukin-8 (IL-8) may be explained by an interference with integrin-mediated adherence required for motility in this assay system.

The non-obese diabetic mouse (NOD) is a generally accepted model of autoimmune diabetes mellitus. The nature of the destruction of insulin secreting β cells of the pancreas is not completely understood, although T cells are generally conceded to play a role (6). The role of inflam-

matory cytokines in islet cell destruction is an area of active investigation. An inhibitory effect or lack thereof by dapsone on the cumulative incidence of diabetes mellitus in the NOD mouse would have mechanistic as well as therapeutic implications. Therefore, we administered dapsone in two doses to female NOD mice and compared the cumulative incidence of diabetes and pancreatic histology with control animals following either a 1-week pulse or 20 weeks of oral drug administration.

Research Design and Methods

Mice. Female NOD mice, aged 8 weeks, were raised in the animal facility of Sansum Medical Research Foundation. All mice were maintained at 23°C in a relative humidity of 50% and under a normal light:dark cycle. All animals were administered untreated tap water and either treated or untreated Purina Mouse Chow (5015) *ad libitum.* Littermates were randomly allotted to one of five groups. Five animals received 7 days of low-dose dapsone (0.0001%); five animals received 7 days of high-dose dapsone (0.001%); seven animals received continuous administration of the low dapsone dose; and eight animals received continuous administration of the higher dapsone dose. Six animals served as controls and received no dapsone. Mice were weighed at 2-week intervals throughout the study. The NOD strain has been described elsewhere (7). The NOD mouse colony (NOD/Sansum) originated from five breeding pairs obtained from Clea, Japan, and has been maintained through brother/sister in breeding for over 40 generations. Female mice start developing overt diabetes at 12 weeks, and the cumulative incidence of diabetes reaches 60%–65% by 34 weeks of age. Less than 8% of male mice become diabetic by 30 weeks of age. All protocols were approved by appropriate institutional review. The Smrf laboratory code (for Sansum Medical Research Foundation) has been assigned to the NOD and NON colonies of the Sansum Medical Research Foundation by the National Research Council Institute of Laboratory Animal Resources.

Dapsone (4,4′-Diaminodiphenyl Sulfone) Preparation. Two solutions were prepared: one containing 7.19 g dapsone (0.0001%; Jacobus, Princeton, NJ); and one containing 71.90 g dapsone (0.001%) in 1 liter of distilled, deionized water. The solutions were stored at room temperature. Dapsone was first dissolved in 3 ml DMSO (Sigma Chemical Co., St. Louis, MO) to aid dissolution before being diluted to final concentrations. Pellets were then soaked in the solution, dried, and given to the animals.

Dapsone Administration. As part of the continuous administration experiment, seven mice were given dapsone as 0.0001% (w/w) of their diet. Mean food consumption was 11 g food/2 days, and 0.0011 g dapsone was given every 2 days. The second group of eight mice were given 0.001% or 0.011 g dapsone every 2 days. The protocol follows that used for testing of efficacy against *M. leprae* (8, 9). Administration of the drug to the two continuous dose groups continued until diabetes appeared or until the mice reached 30 weeks of age. The mice that were given 7 days of dapsone were followed until they reached 30 weeks of age or until the onset of diabetes as was done for the continuous dosing experiment.

Onset of Diabetes. Urine glucose levels were measured at 2-week intervals. The onset of IDDM was defined by urine glucose levels >2%, confirmed by a blood glucose level >200 mg/dl. Blood was obtained from the retro-orbital sinus following light Aerrane anesthesia and analyzed for glucose (YSI, Yellow Springs, OH) and glycated hemoglobin (Primus CLC330, Kansas City, MO).

Histology. After the onset of diabetes as defined above or at 30 weeks of age (whichever occurred first), the animals were bled retro-orbitally for final blood glucose and glycated hemoglobin measurements, and sacrificed for histological examination of the pancreas. Hematoxylin and eosin–stained pancreatic sections were scored by two readers, who assigned numerical values to each organ based upon the following scale: 0 = no infiltrate; 1 = perivascular only; 2 = periislet at pole <50%; 3 = periislet >50%; 4 = insulitis <50%; and 5 = insulitis >50%. A minimum of eight sections per organ were scored by two observers unaware of the treatment status of the mice as described previously (10, 11).

Effect of Dapsone on IL-2 and IL-4 Production in Murine Splenocytes *in Vitro*. To determine whether cytokine secretion might be altered by dapsone, IL-2 and IL-4 secretion in response to concanavalin A was evaluated as described previously (12). Splenocytes were harvested from the spleens of male C57/BL6 mice, aged 12 weeks. Cells were cultured in triplicate in complete growth medium and 10% fetal bovine serum (FBS) and were stimulated with concanavalin A (ConA; 4 μg/ml). Dapsone was added as a solution in phosphate-buffered saline (PBS) to wells at 0.4 and 2 μg/ml to approximate the concentration range documented after *in vivo* administration (13, 14). Cells were cultured for 48 hr until harvested for IL-2 and IL-4 determinations. Cell culture supernatants were collected and analyzed in triplicate for IL-2 and IL-4 concentration using a standard sandwich enzyme-linked immunosorbent assay (ELISA; Endogen, Boston, MA).

Statistics. Statistical significance among groups was determined by unpaired *t* test (Statview 4.01; Abacus Concepts, Berkeley, CA).

Results

Cumulative Incidence of Diabetes. The occurrence of diabetes in each experimental group is shown in Figure 1. Both of the 7-day-dose groups of animals had 100% occurrence of diabetes by Week 20 of the experiment (27 weeks of age). The control group animals had 67% (four of six animals) occurrence of diabetes by Week 20. The continuous-dosage animals, in contrast, experienced significantly reduced occurrence of diabetes: 14% of the low-dose group (one out of seven animals) developed IDDM; and none of the eight high-dose animals developed the disease

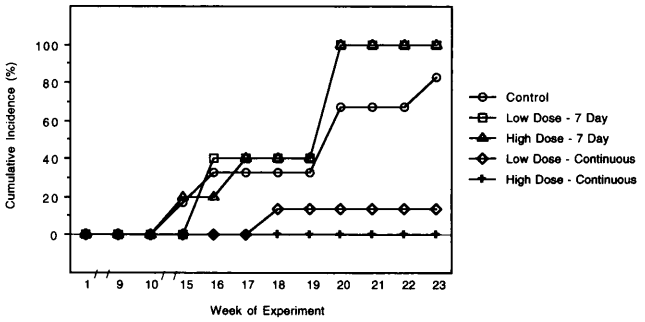

Figure 1. Cumulative incidence of diabetes in the various treatment groups. The mice were 30 weeks of age at Week 23 of the experiment.

($P < 0.001$) when compared with control animals. Comparison of continuous versus pulse dapsone treatment at both doses was also highly significant ($P < 0.0001$).

Histology. Histological examination of pancreatic sections revealed no statistically significant differences in islet infiltration among the groups of animals (Table I). In neither the 7-day nor the continuous experiments did dapsone prevent the occurrence of lymphocytic infiltration. There was no statistically significant difference in the frequency or severity of lymphocytic infiltration among the treatment groups. All deaths were due to diabetes, but some animals could not be processed for histology. Low histology scores in diabetic animals were often associated with few islets per pancreas, thus complicating comparisons between individual animals.

Effects of Dapsone on IL-2 and IL-4 Production in Splenocytes. Dapsone was found to have a dose-dependent effect on IL-2 production in stimulated splenocytes (Fig. 2). The same effect was noted in a similar experiment where culture supernatants collected after 24 hr were studied (data not shown). In the IL-4 experiments, both low and high doses of dapsone resulted in a similar diminution in secretion (Fig. 3) that, when comparing control samples to those given dapsone, was statistically significant ($P = 0.01$).

Table I. Pancreas Histology following Dapsone Treatment

Treatment group	Week of study	Histology score
Control		
1	30	4.6
2	30	0.1
3	30	5.0
4	30	5.0
5	30	4.4
6	20	—[a]
7-Day dose dapsone 0.0001%		
1	30	0.0
2	30	0.0
3	30	3.8
4	22	—[a]
5	23	—[a]
7-Day dose dapsone 0.001%		
1	30	0.0
2	30	1.7
3	30	5.0
4	30	3.1
5	22	—[a]
Continuous dapsone 0.0001%		
1	30	0.0
2	30	4.3
3	30	1.4
4	30	0.1
5	30	0.1
6	30	4.8
7	30	5.0
Continuous dapsone 0.001%		
1	30	4.6
2	30	4.6
3	30	0.0
4	30	0.0
5	30	4.3
6	30	0.7
7	30	4.5
8	30	0.9

[a] Deceased from hyperglycemia; histology not available.

Discussion

While used primarily to treat leprosy, dapsone has anti-inflammatory properties that have been exploited in the treatment of a variety of diseases, including dermatitis herpetiformis and asthma (3, 4). As noted by Lang and coworkers (15), dapsone appears to give greatest benefits to the treatment of diseases characterized by neutrophil infiltration. While the process is not completely understood, dapsone likely is metabolized within neutrophils and monocytes by myeloperoxidase (16–18). The reactive metabolites (for example, hydroxylamine derivatives) have been shown to inhibit hypochlorous acid production by myeloperoxidase (19). Neutrophils have not been implicated directly in the course of insulin-dependent diabetes mellitus (IDDM). However, the suggested scavenger activity of dapsone for reactive oxygen species may be important in prolonging the time to onset of disease seen in our experiments reported here (15).

An unknown mechanism is involved in the recruitment of lymphocytes to the pancreatic β cells, which is a hallmark of the destruction of β cell mass in IDDM (20). Dapsone did not prevent lymphocytic infiltration of the perivasculature or islets of the animals in our study.

We observed that, despite lymphocytic infiltration of the islets, those animals given continuous doses of dapsone were not as vulnerable to the development of IDDM. This finding is consistent with the idea that some of the deleterious effects of the lymphocytes themselves may be ameliorated by dapsone. To test the effect of dapsone on cytokine production, we incubated murine splenocytes in complete growth medium with dapsone doses calculated to approximate the range of concentrations obtained *in vivo* after oral administration of the drug (13, 14). After both 24 and 48 hr of incubation, both the IL-2 and IL-4 concentrations in the supernatants were found to be diminished for

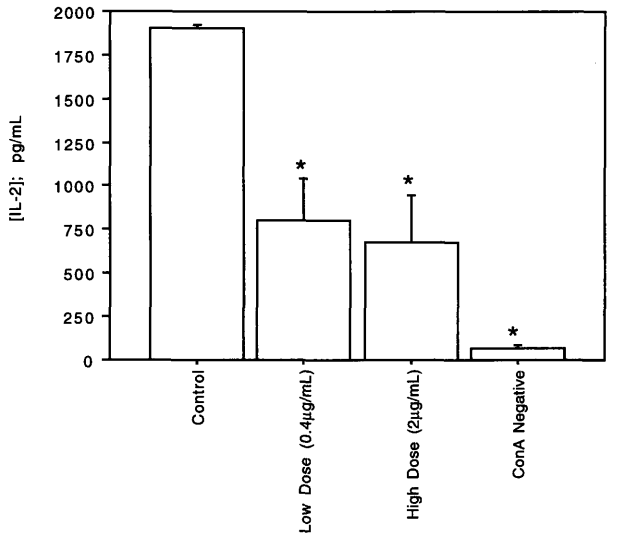

Figure 2. Concentration of IL-2 in splenocyte supernatants determined by sandwich ELISA assay following ConA stimulation as a function of dapsone incubation concentration. *A significance (*P*) level of <0.01 when compared with the control value.

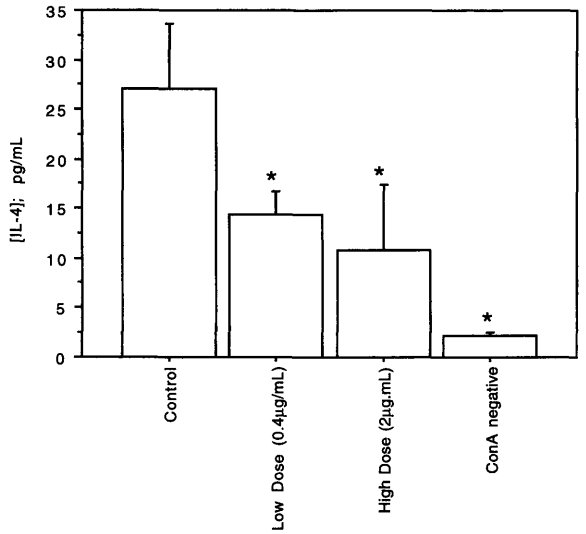

Figure 3. IL-4 secretion in murine splenocyte supernatants determined by sandwich ELISA assay following ConA stimulation as a function of dapsone incubation concentration. *A significance (*P*) level of <0.01 when compared with the control value.

both the low and the high concentrations of dapsone used. The diminution in IL-2 and IL-4 suggests that lymphocyte activity may be diminished by the presence of dapsone, thereby reducing the β-cell damage in those animals. Therefore, β-cell function may be spared once immunomodulation has occurred despite lymphocyte infiltration. The fact that both IL-2 and IL-4 were diminished by the presence of dapsone in concentrations less than those reported for neutrophil modulatory effects (5) suggests that both the Th1 (IL-2–secreting cells associated with cell-associated immunity) and Th2 (IL-4–secreting cells associated in part with suppressor activity) arms of the cellular immune response are affected by dapsone administration. The extent of involvement of Th1 and Th2 requires further study.

In conclusion, the incidence of IDDM in NOD mice treated with continuous dapsone administration was reduced compared with control animals, despite lymphocytic infiltration of pancreatic islets. Dapsone was found to reduce IL-2 and IL-4 secretion *in vitro*. These results support the hypothesis that the destruction of β cells in IDDM may involve multiple destructive pathways acting in concert. Therefore, the use of several drugs that interrupt different pathways of the destructive process may be useful in ameliorating the progressive β-cell destruction seen in IDDM. Dapsone has been available for many years, and as an immunomodulating agent for diabetes might be considered an "orphan drug" (21). In view of the wide clinical experience with the compound, clinical trials incorporating dapsone in persons at risk for or with new onset IDDM (where insulitis is invariably already present) may be warranted if the drug can be shown to spare β-cell destruction in animal models at the onset of diabetes.

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