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Decreased Prevalence of Lymphatic Filariasis Among Subjects with Type-1 Diabetes

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Abstract. Several animal studies have shown a protective effect of helminth infections against type-1 diabetes mellitus (T1DM). However, epidemiologic studies demonstrating this protective relationship with T1DM are largely lacking, although an inverse correlation between the prevalence of lymphatic filariasis (LF) and prevalence of allergies and autoimmunity has been shown. A cross-sectional study was undertaken in southern India to assess the baseline prevalence of seropositivity of LF among persons with T1DM (n = 200) and normal glucose tolerant (NGT) persons (n = 562). The prevalence of LF was 0% among persons with T1DM and 2.6% among NGT persons ($P = 0.026$). The percentage of persons who were positive for filarial antigen-specific IgG4 (but not antigen-specific IgG) was also significantly lower in persons with T1DM (2%) compared with NGT persons (28%) ($P < 0.001$). Thus, there appears to be a striking inverse relationship between the prevalence of LF and T1DM in southern India.

INTRODUCTION

Insulin-dependent (type-1) diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by the progressive loss of insulin-producing beta cells.[1] Although the mechanism responsible for the pancreatic loss is not completely understood, self-reactive T cells (and to a lesser extent, B cells) probably play a significant role in beta cell destruction.[2–4] The prevalence of T1DM and other autoimmune diseases has increased dramatically over the past few decades.[5–7] Although genetic factors may play a role in the susceptibility to T1DM,[8] the dramatic worldwide increase in T1DM prevalence is likely the result of environmental factors.[9] One environmental change that may be responsible for the recent increase in autoimmune diseases is the decrease in chronic parasitic infections in developed countries (hygiene hypothesis).[10]

Experimentally, a number of helminthic parasites, including the trematode *Schistosoma mansoni*[11] and the nematodes *Litomosoides sigmodontis, Heligmosomoides polygyrus,* and *Trichinella spiralis*, prevent the onset or suppress the severity of T1DM in non-obese diabetic mice.[12,13] Several studies have found that persons infected with chronic parasitic worm infections have lower rates of autoimmune diseases than persons without these infections.[14,15] Currently, trials are under way using *Trichuris suum* and *Necator americanus* infection for the treatment of T1DM, multiple sclerosis, inflammatory bowel disease, asthma, and food allergies.[16]

However, large-scale epidemiologic studies examining the association of helminth infections and the prevalence of autoimmunity are lacking. Thus, there is a need to conduct such studies, mainly in developing countries that are at a transition state with increasing incidence of noncommunicable diseases and decreasing incidence of infectious diseases largely caused by globalization/urbanization.[17,18] We previously showed reduced prevalence of lymphatic filariasis (LF) among persons with type-2 diabetic (T2DM).[19] In the present study, we examined the prevalence of LF among persons with T1DM.

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MATERIALS AND METHODS

Study participants and sample-size calculation. Patients with T1DM (n = 200) were recruited from Dr. Mohan's Diabetic Specialties Center, a tertiary center for diabetes in Chennai (formerly Madras) in southern India. For each participant, a medical history was obtained, a physical examination was undertaken, and fasting plasma glucose, serum lipids, and glycated hemoglobin (HbA1C) estimations were obtained. Type-1 diabetes mellitus was diagnosed by the presence of lean body weight in combination with hyperglycemia, ketonuria, and absence of insulin release as shown by C-peptide assay.[20] Persons who had fasting and stimulated C-peptide values < 0.3 pg/mL were diagnosed with T1DM.[20] Persons with T1DM who had serum glutamic acid decarboxylase (GAD)–specific autoantibody levels ≥ 10 IU/mL were classified as GAD positive.[20] Healthy control participants (n = 562) were recruited from the Prevention Awareness Counseling and Evaluation project.[21] All the healthy controls had serum C-peptide values > 0.3 pg/mL and were GAD autoantibody negative. Institutional ethical committee approval was obtained from the Madras Diabetes Research Foundation Ethics Committee (Ref. No. MDRF-EC/SOC/2009/05), and written informed consent was obtained from all the study participants. Initially, 200 normal glucose tolerant (NGT) persons and 100 persons with T1DM were screened for LF. Of 200 NGT persons, 6 (3%) were LF+, and all 100 (0%) persons with T1DM were LF–. On the basis of these preliminary results, with a confidence interval of 95%, an estimated P value < 0.05, and a power of 80%, the sample size estimates were 500 subjects for NGT persons and 200 subjects for persons with T1DM.

Anthropometric and biochemical parameters. Anthropometric measurements including height, weight, and waist circumference were obtained by using standardized techniques. The body mass index was calculated as the weight in kilograms divided by the square of height in meters. Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method), high-density lipoprotein cholesterol (direct method polyethylene glycol-pretreated enzymes), and creatinine (Jaffe's method) were measured using a Hitachi-912

autoanalyzer (Hitachi, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation for biochemical assays ranged between 3.1% and 5.6%. Levels of HbA1c were estimated by high-pressure liquid chromatography using a variant apparatus (Bio-Rad, Hercules, CA). The intra-assay and inter-assay coefficient of variation of HbA1c was < 5%.

Detection of Bancroftian LF. To quantify the filarial antigen levels and prevalence, serum samples were analyzed by using the *Wuchereria bancrofti* Og4C3 antigen-capture enzyme-linked immunosorbent assay (ELISA) (TropBio, Townsville, Queensland, Australia) according to the manufacturer's instructions. The TropBio values were log transformed before statistical analysis.

Determination of antibody titer to LF. The serum antibody (IgG and IgG4) levels against *Brugia malayi* antigen was determined by ELISA as described.[22] IgG titers > 14 and IgG4 titers > 100 were considered positive.

Assessment of socioeconomic status. Monthly income and job designation were recorded to assess the socioeconomic status of the study participants. The study participants were categorized into three socioeconomic groups. The basis for these assignments can be found in Supplementary Table 1.

Statistical analysis. All statistical analyses were performed by using SigmaPlot software version 15.0.0 (SPSS, Inc., Chicago, IL). Differences in prevalence of filarial infections among different groups were compared by using the chi-square test. Antibody titer was analyzed by using the Mann-Whitney *U* test. Clinical and biochemical characteristics of study participants were compared by using one-way analysis. $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics including clinical and biochemical features of the study populations are shown in Table 1. There was no significant difference between the 562 NGT persons and the 200 persons with T1DM for age, sex distribution, body mass index, systolic and diastolic blood pressure, serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels.

TABLE 1
Biochemical and anthropometric characteristics of study participants*

Parameter	NGT (n = 562)	T1DM (n = 200)
Age (years)	35 ± 12	28 ± 16
Onset of diabetes (years)	NA	18 ± 13
Sex (M/F)	203/359	95/105
Body mass index (kg/m^2)	23 ± 5	21 ± 5
Systolic blood pressure (mm Hg)	117 ± 20	115 ± 15
Diastolic blood pressure (mm Hg)	73 ± 12	73 ± 7
Fasting plasma glucose† (mg/dL)	84 ± 9	190 ± 102‡
HbA1C† (%)	5 ± 1	9 ± 2‡
Serum cholesterol (mg/dL)	175 ± 36	159 ± 34
Serum triglyceride (mg/dL)	110 ± 60	84 ± 34
Serum high-density cholesterol (mg/dL)	43 ± 10	49 ± 13
Serum low-density cholesterol (mg/dL)	110 ± 31	91 ± 31
C-peptide fasting (pmol/mL)	NA	0.3 ± 0.2
C-peptide stimulated (pmol/mL)	NA	0.5 ± 0.4
GAD positivity	0%	63%

*Values are mean ± SD unless otherwise indicated. NGT = normal glucose tolerance; T1DM = type-1 diabetes mellitus; NA = not available; HbA1C = glycated hemoglobin; GAD = serum glutamic acid decarboxylase.
†The fasting plasma glucose and HbA1C levels were compared by one-way analysis of variance.
‡$P < 0.01$.

The prevalence of LF among the control and T1DM groups was determined by quantitative TropBio ELISA (> 128 U/mL) (Figure 1A). The prevalence of LF was significantly different ($P = 0.026$) between NGT persons (15 of 562, 2.6%) and those

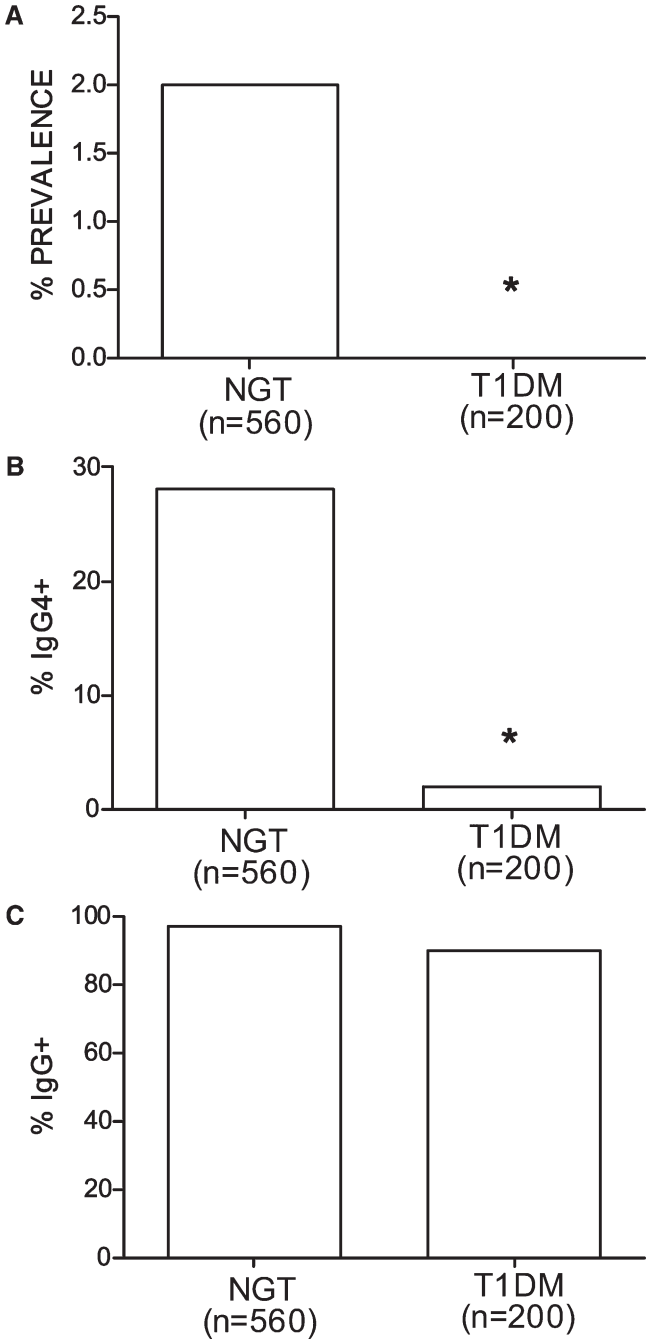

FIGURE 1. Reduced prevalence of lymphatic filariasis (LF) among persons with type-1 diabetes (T1DM), Chennai, India. **A**, Percentage of LF-positive persons in those with normal glucose tolerance (NGT) (n = 560) and in those with T1DM (n = 200) was determined by enyzyme-linked immunosorbent assay (ELISA) (TropBio Pty. Ltd., Townsville, Queensland, Australia). Statistical difference in prevalence was determined by chi-square analysis. $P < 0.05$ was considered significant. **B**, Percentage of filarial antigen–specific IgG4-positive persons in NGT and T1DM groups. **C**, Percentage of filarial antigen-specific IgG-positive persons in NGT and T1DM groups. Serum levels of filaria-specific IgG and IgG4 levels were quantified by ELISA. Statistical difference in the prevalence was determined by chi-square analysis. $P < 0.05$ was considered significant.

with T1DM (0 of 200, 0%). Not surprisingly, the percentage of persons with measurable filaria-specific IgG4 was significantly lower in the T1DM group (2%) than in the NGT group (14%) ($P < 0.001$) (Figure 1B). In contrast, the percentage of persons who had measurable antifilarial IgG, which is believed to be an indicator of exposure rather than infection,[22] was not statistically different between the two groups (92% in NGT versus 90% in T1DM) (Figure 1C).

Because the socioeconomic status of the population under study could be a potential confounding factor in interpreting the data, we performed a socioeconomic stratification of the study population. As shown in Supplementary Table 1, there were no significant differences in the socioeconomic breakdown of NGT persons and those with T1DM, indicating that socioeconomic differences are unlikely to be a confounding factor for the differences in the prevalence of LF.

DISCUSSION

The hypothesis that parasitic helminths may protect against development of allergic and autoimmune processes has spurred an interest in examining the protective effects of helminths and their immunomodulatory products in animal models. In addition, clinical trials in humans examining effects of helminth products on disease severity in autoimmune diseases have shown promising results, as well as showing the safety of these helminth-derived immunomodulators for use as therapeutic agents. Although there is evidence to show that helminth infection can prevent T1DM in mice,[11,13,23–25] there are no reports to support this hypothesis in humans. Epidemiologic studies carried out in southern India have shown reduced prevalence of LF after mass drug administration.[26] Whether this reduced prevalence is a contributing factor for the increasing incidence of T1DM and T2DM in this region (www.diabetes atlas.org/ content/diabetes-and-impaired-glucose-tolerance) is currently not known. We have recently shown reduced prevalence of LF among persons with T2DM and have also provided evidence for the possible involvement of filarial-mediated immune modulation in conferring protection against T2DM.[19]

Reduced prevalence of LF was associated with reduced levels of filaria-specific IgG4 among persons with T1DM. Because *B. malayi* antigen–specific IgG4 is also an indicator of current infection,[22] the fact that IgG4 levels are lower among persons with T1DM is probably a secondary confirmation of the prevalence data assessed by circulating filarial antigen. Our findings thus support the animal experiments that have shown that infection with the filarial parasite was enough to confer protection against T1DM.[23] It is unlikely that the decreased prevalence of LF among persons with diabetes was caused by LF-mediated mortality because LF is a chronic, nonlethal disease. More studies are clearly needed on the prevalence of diabetes (T1DM and T2DM) and other infectious diseases to have a better understanding of the interplay of infection/inflammation and diabetes.[27]

Differences in exposure to *W. bancrofti* is a potential confounding factor for the differences in the prevalence of LF. Because filarial antigen-specific IgG has been used as a marker for estimating exposure to filarial infection,[22] we examined the levels of antigen-specific IgG and showed that IgG levels were remarkably similar between the two groups. In addition, differences in socioeconomic status, another potential confounding factor,[28] was also shown to be noncontributory in the context of the present study (Supplementary Table 1) because no differences were seen in socioeconomic status between the control and T1DM groups.

One limitation of this study is that, being cross-sectional, it cannot provide a direct causal link between the decreased prevalence of LF and T1DM. Nevertheless, the study highlights the importance of studying the complex interactions between a chronic infectious disease (LF) associated with immune modulation and an autoimmune disorder (T1DM). It is surprising that T1DM (an autoimmune disorder) and T2DM (a metabolic disorder), despite completely different underlying mechanisms for glucose intolerance, are modulated by LF infection. However, T1DM and T2DM are characterized by the presence of an intense inflammatory milieu, and filarial infections are known inducers of regulatory networks including regulatory T cells,[29,30] tolerogenic dendritic cells,[31] alternatively activated macrophages,[32] and anti-inflammatory cytokines.[33] Thus, it is not surprising that filarial infections exert such a profound effect on the prevalence of diabetes mellitus of autoimmune and metabolic origin.

Elucidation of the mechanism by which filarial infections can impart protection against diabetes mellitus could not only lead to identification of filarial products with therapeutic potential but also to defining key molecular events in induction of anti-inflammatory response that might prove subsequently beneficial in defining new therapeutic targets and avenues. Finally, the decreasing incidence of LF (caused by mass eradication programs) could have an impact on the incidence of T1DM in the future and obviously is an important area for future study.

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Note: Supplemental table is available at www.ajtmh.org.

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