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SIGNIFICANT INVERSE RELATIONSHIP BETWEEN ANTIBODIES AGAINST *Entamoeba histolytica, Ascaris lumbricoides, Toxocara spp* AND THE PRESENCE OF TYPE 1 DIABETES MELLITUS

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ABSTRACT
Autoimmunity is a very heterogeneous disorder with diverse clinical phenotypes, among them T1 DM affects particularly children in certain ethnic groups, there are several explanations for the increasing incidence in prevalence and incidence of T1DM worldwide, epidemiological studies show variability in certain geographical regions and socioeconomically status, besides ethnical and genetic influences, the presence of parasitic infections seems to be at play; thus, those individuals expose to parasites are protected for developing autoimmunity probably due to the expression of FoxP3, which would act as a protective factor by reducing the expression of pro-inflammatory cytokines in the pancreatic B cells protecting for the development of T1DM. Here we analyzed the association of the presence of parasites and antibodies to *Ascaris lumbricoides*, *Toxocara spp* and *Entamoeba histolytica* with HLA-DR genotypes in 26 patients with T1DM who attended to the Children's Diabetes Clinic (CANDI) at the Hospital Infantil de Mexico; and 33 of their siblings. Intestinal parasites were identified by coproparasitoscopic, IgG antibodies against the parasites *Entamoeba histolytica, Ascaris lumbricoides and Toxocara spp*. Of the 59 stool samples and sera (26 T1DM and 33 siblings), *Entamoeba coli and Endolimax nana* in 13.6% of siblings of children with T1DM was identified by CPS. Antibodies against *Entamoeba histolytica* were detected in 18.6%, *Toxocara spp* was 37.3% and *Ascaris lumbricoides* was 35.6% of siblings of children with T1DM; This difference was statistically significant ($p < 0.05$, OR: 0.45,). The HLA-DR3 and HLA-DR4 alleles were increased in the patient group compared to controls ($p = 0.0002$, OR: 3.5, CI 95% 2.2-5.2). We also found a statistically significant relationship between the presence of antibodies against the three parasites analyzed, *Entamoeba histolytica*, *Ascaris lumbricoides* and *Toxocara spp* and the absence of T1DM manifestations in the 33 siblings of the patients. This would suggest the possible role of parasites in the resistance to the development of autoimmunity.

KEYWORDS: Type 1 Diabetes mellitus, *Entamoeba histolytica, Ascaris lumbricoides, Toxocara spp.*

INTRODUCTION
Type 1 Diabetes Mellitus (T1DM) incidence is increasing worldwide and seems to coincide with a decrease in helminth infection.[1] T1DM is characterized by self-destruction of pancreatic beta cells mediated by pro-inflammatory cytokines derived from CD4+ T cells activation, this results in profound decrease in insulin production. In addition to the strong genetic influence, the recently increase in T1DM in Mexico suggest a significant role of environment as well. Several epidemiological studies have shown that T1DM is particularly infrequent in Mexicans; one of the many possible explanations could be the historical high prevalence of parasite infections which could serve as a protective factor.[2] One hypothesis is the induction of regulatory T cell (Treg) driven by parasites burden along with the increase expression of P3 transcription factor (FoxP3).[3] Regulatory T cells are central in maintaining tolerance and immune homeostasis within the mucosal tissue, thus Treg dysfunction would drive immune response toward autoimmune diseases. Cross sectional studies in Mexicans both in adults and children describe

a wide variety of parasites, (up to 53% of 49.1% prevalence respectively) being the protozoan *Entamoeba histolytica* and the helminths *Ascaris lumbricoides* and *Toxocara spp* the most frequently described pathogens.

MATERIALS AND METHODS

Here we report the study of parasitosis in 26 Mexican children suffering from T1DM (according to American Diabetes Association criteria)[4] and the relationship with the HLA-DR genotypes and compared them with those present in 33 of their siblings; protocol was approved by the Institutional Research, ethics and biosafety committee at "Hospital Infantil de México Dr. Federico Gómez" in Mexico City. Samples of stool and serum from all individuals were collected. Patients with T1DM had less than 3 months of diagnosis and siblings were similar in all demographic features. Coproparasitoscopic analysis were done in three consecutive days after which serum anti-IgG titles against *Entamoeba histolytica, Ascaris lumbricoides* and *Toxocara spp* were tested using an ELISA technique. To carry out this technique, total extract of each parasite was used at a protein concentration of 1µg/mL, 1:500 dilution of the first antibody and 1:10 000 anti-human IgG conjugated to peroxidase. For the typing HLA-DR genome DNA was obtained for peripheral venous blood leucocytes by the "Modified salting out" technique.[5] For HLA-DR genotyping we used a low resolution technique after PCR amplification. Statistical analysis included multivariated logistic regression using the PASW statistical package version 18.0.

RESULTS AND DISCUSSION

Antibodies against *Ascaris lumbricoides* were detected in 23.7% of the patients compared to 35.6% of siblings, antibodies against *Toxocara spp* in 28.8% in children with T1DM compared to 37.3% and finally antibodies against *Entamoeba histolytica* in 15.3% of the patients versus 18.6% in siblings. *Entamoeba coli* and *Endolimax nana* were identified and in 8.5% of the patients and in 13.6% of the siblings. The results were statistically significant (p: <0.05, OR 0.45, 95% CI). Frequencies of HLA-DR3 and HLA-DR4 alleles were significantly increased in patients and siblings as compared to healthy ethnically matched Mexican Mestizo individuals (p = 0.0002, OR 3.5, 95% CI: 2.2- 5.2); this would suggest that parasite infections have a role in resistance to the development of autoimmunity. This is supported by reports describing a lower risk for developing T1DM in children exposed to infections during their first years of life.[6] Initial observations of the immunomodulation of parasites in autoimmune diseases arose when it was recognized that children with helminthiasis had fewer allergic diseases than uninfected children, however sensitivity to environmental allergens increased when antiparasitic treatment was given.[7] Furthermore, experimental animal models have shown that in NOD (non-obese diabetic) mice the highest rate of diabetes occurs if mice are in sterile housing conditions[8], but if they are infected with the adult worm or the eggs from the *Schistosoma mansoni* or with *Heligmosomoides polygirus* or *Trichinella spiralis* the development of diabetes is significantly retarded.[9] Additional studies from our group in seven children affected by inflammatory bowel disease, who were given eggs of *Trichuris suis* nematode, (which infects the pig but not the man), obtained remission in six of them, without any collateral complications.[10] We concluded that there is a significant inverse relationship between the presence of antibodies against *Entamoeba histolytica, Ascaris lumbricoides* and *Toxocara spp,* and the presence of T1DM in genetically susceptible individuals.

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