Immunobiology 219 (2014) 859–865

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

DC-SIGN polymorphisms are associated to type 1 diabetes mellitus

Ronaldo Celerino da Silva[a,b], Nathália de Alencar Cunha Tavares[b], Ronald Moura[a,b], Antônio Coelho[a,b], Rafael Lima Guimarães[a,b], Jacqueline Araújo[c], Sergio Crovella[a,b], Lucas André Cavalcanti Brandão[b,d], Jaqueline de Azevêdo Silva[b,*]

[a] Department of Genetics, Federal University of Pernambuco (UFPE), Recife, Pernambuco, Brazil
[b] Laboratory of Immunopathology Keizo Asami (LIKA), Federal University of Pernambuco (UFPE), Recife, Pernambuco, Brazil
[c] Pediatric Endocrinology Unit of Clinical Hospital, Federal University of Pernambuco, Recife, Pernambuco, Brazil
[d] Pathology Department, Federal University of Pernambuco (UFPE), Recife, Pernambuco, Brazil

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 10 July 2014
Accepted 15 July 2014
Available online 22 July 2014

Keywords:
T1DM
SNPs
DC-SIGN

ABSTRACT

Type I diabetes mellitus (T1DM) is an autoimmune disorder featured by raised glucoses levels. It has been hypothesised that raised glucose levels in T1DM might be recognised as PAMPs, leading to immune response by overloading the cell receptors for pathogens recognition. DC-SIGN is a transmembrane protein, present in dendritic cells (DC) and macrophages: it has an important role in inflammatory response and T cells activation. Notably, DC-SIGN activation and triggering of the immune response depend on the type of ligand, which may lead to a pro or anti-inflammatory pathway. In our association study, we analysed the SNPs rs4804803 (−336 A>G) and rs735239 (−871 A>G), both at *DC-SIGN* promoter region, in 210 T1DM patients and 157 healthy controls, also looking for a correlation with the age of onset of the disease. We found that the allele G and genotypes G/G and A/G of SNP-871 (rs735239), as well as the alleles G-G (rs735239-rs4804803) and genotypes combined AA-GG (rs735239-rs4804803) were associated with protection of T1DM development. We did not find association between these variations with the age of onset of the disease and the presence of other autoimmune disorders. Our results suggest that SNPs in DC-SIGN promoter region can be associated to protection for T1DM in the Northeast Brazilian population.

Introduction

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease and its hallmark is the infiltration of auto reactive T cells into the pancreas leading to impaired production of insulin by the beta-cells (Gillespie, 2006). T1DM can be diagnosed at any age, however it is the most common chronic disease of childhood with higher incidence between 5 and 7 years of age and near puberty (Atkinson et al., 2013).

T1DM is considered a multifactorial disease with genetics and environment playing crucial roles in its development and yet both factors are still not completely elucidated (Pugliese, 2010; Stankov et al., 2013; Todd, 2010). Even though studies revealed that human histocompatibility (HLA) complex gene contributes 40–50% of the overall susceptibility (Nejentsev et al., 2007; Noble et al., 1996; Steck et al., 2005), several non-HLA loci are associated with type 1 diabetes susceptibility, such as CTLA-4, PTPN22 and IL2RA (Pugliese, 2010; Steck et al., 2005). It is known that T1DM acts through immune system, altering the homeostasis and leading to self-tolerance breakdown (Pugliese, 2010). When considering environmental influences on T1DM development it has been proposed the role of some pathogens in disease triggering, specially Enteroviruses such as the Coxsackievirus B (CVB) (Atkinson et al., 2013; van Belle, 2011).

Innate immune system acts as the first line of host defence, including dendritic cells (DCs) and macrophages, which recognise a wide range of pathogen-associated molecular patterns (PAMPs) and trigger the immune response (Lloyd et al., 2007; Mahla et al., 2013). DCs, in the pancreas, absorb released β-cell-derived antigens, migrate to lymph nodes and activate naïve islet-specific CD4 and CD8T cells that, upon DCs signal, differentiates in inflammatory (Th1), anti-inflammatory (Th2) or regulatory cells (Tregs). The activated T cells migrate to the pancreas, infiltrating around

* Corresponding author at: Laboratory of Immunopathology Keizo Asami (LIKA), Federal University of Pernambuco (UFPE), Av. Moraes Rego, 1235, Recife CEP 50760-901, Brazil. Tel.: +55 81 21268484; fax: +55 81 21268484.
E-mail addresses: j.azvedo@gmail.com, j.azvedo@icloud.com (J. de Azevêdo Silva).

http://dx.doi.org/10.1016/j.imbio.2014.07.011
0171-2985/

the islets. Initially, the infiltrating are mostly Th2 and Tregs cells that subsequently become more abundant leading to β cells destruction (Morel, 2013).

Sugars, i.e. monosaccharides, oligosaccharides, polysaccharides, glycoproteins and glycolipids, are involved in cell signalling, recognition and communication. The surface glycoproteins are key components in modulating the immune response through cell recognition and interaction (Lloyd et al., 2007; Mahla et al., 2013). Furthermore, many key components of the innate immune system have evolved to recognise sugars such as microbial polysaccharides and oligosaccharides and interestingly, the most important characteristic in T1DM is the elevated serum glucose levels. These facts led to the hypothesis that the raised glucose levels in T1DM might be recognised as PAMPs, leading to immune response and overloading the cell receptors for pathogens recognition (Ilyas et al., 2011).

Dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN) is a type II transmembrane protein, present mainly in dendritic cells (DC), but also in macrophages, and encoded by *DC-SIGN* (*CD209*) gene, mapping on chromosome 19p13.3 (Khoo et al., 2008). This receptor has an important role in inflammatory response and T cells activation (Zhou et al., 2006). DC-SIGN is responsible for cell adhesion and pathogens arrest, which recognises self-glycoproteins intercellular adhesion molecule 2 and 3 (ICAM2 and ICAM3). The DC-SIGN interaction with ICAM3 allows the interaction from peptide–MHC complexes by T cells and steadies the connexion DC–T-cell membrane, supporting the engagement of the T-cell receptor (Van Kooyk and Geijtenbeek, 2003).

Expression of DC-SIGN is known to be affected by polymorphisms in the DC-SIGN gene. Some in silico and *in vitro* studies have associated SNPs in promoter region of DC-SIGN with genic expression modulation (Alagarasu et al., 2013; Sakuntabhai et al., 2005; Wang et al., 2011). The rs735239 with the A allele impairs the binding site for the Oct-1 transcription factor (Alagarasu et al., 2013), while rs4804803 affects the binding site for the Sp1 transcription factor, with the A allele related with increased expression *in vivo* (Sakuntabhai et al., 2005; Wang et al., 2011).

In this study we assessed two functional polymorphisms in the promoter region of DC-SIGN gene, namely rs735239 (position −871) and rs4804803 (position −336), looking for their possible association to T1DM in a Brazilian population.

Subjects and methods

Patients and controls

In this study we enrolled 210 T1DM children (111, 53% females and 99, 47% males), from Northeast Brazil, aged 0–18 years at diagnosis with mean age at onset 7.3 years ±4.06 SD. All the patients in this study attended to three paediatric endocrinology services of public healthcare system in Recife, Brazil ("Instituto Medicina Integral de Pernambuco Professor Fernando Figueira", "Hospital da Restauração" and "Hospital das Clínicas"). The T1DM patients were diagnosed according to American Diabetes Association criteria (1997) and classified as T1DM clinical presentation (Gabir et al., 2000).

For the control group, were enrolled 153 healthy unrelated volunteers, with no history of autoimmune or chronic diseases, with 102 females (66.4%) and 53 males (33.6%), from 16 to 72 years old and mean age 38.8 years ±14.7 SD, from the same geographical region of the patients. The free consent term from patients and controls, or their legal responsible, was obtained. This study was approved by the local ethics Committee (IMIP Number: 762/2006 and 1717/2010).

Autoimmune thyroid and celiac disease diagnosis

From the 210 T1DM children, 17.1% (36/210) were AITD positive (T1DM AITD+CD−), 4.5% (9/210) CD positive (T1DM AITD−CD+) and about 1% positive for both AITD and CD (T1DM AITD+CD+). Autoimmune thyroid disease was diagnosed using anti-thyroperoxidase (Anti-TPO) antibodies with chemio-luminescence (Immulite anti-TPO Ab, Diagnostic products Co, Los Angeles, USA). Patients with positive for anti-TPO (titre exceeding 35 IU/ml, accordingly to manufacturer's suggestion) were considered as AITD (Sinclair, 2006). Celiac disease was diagnosed using the anti-transglutaminase antibodies (anti-tTg), using the ELISA Eu-tTG kit (Eurospital, Trieste, Italy) following manufacturer's instructions. Patients presenting 10 AU (absorbance units) for anti-tTg antibodies were considered positive for CD (Husby et al., 2012).

SNP selection and DC-SIGN genotyping

Genomic DNA from all subjects was isolated from whole blood using the Wizard genomic DNA purification kit (Promega, Madison, MA, USA) following manufacturer's instructions. The evaluated SNPs in this study were the rs4804803 (−336 A>G) and rs735239 (−871 A>G), both in the promoter region. Genotyping was performed using commercially available fluorogenic allele specific probes (C_1999340-10 and C_989421_10 Taqman Probes, Applied Biosystems, Foster City, CA, USA) using the ABI7500 Real Time PCR platform (Applied Biosystems, Foster City, CA, USA). Allelic discrimination followed as recommended by the manufacturer and analysed using the SDS software 2.3 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Genotype, allele frequencies and Hardy–Weinberg equilibrium were calculated using the SNPStats tool (http://bioinfo.iconcologia.net/SNPstats). The Fisher Exact Test was applied to evaluate the statistical significance for all comparisons. The Haploview Software, version 4.2, was used for haplotypes associations. "SNPassoc" R package (R software, version 2.12.2), developed for genetic studies, was used for evaluating the association between SNPs and age of onset (González et al., 2007). Bonferroni's correction test was applied and the p-value <0.025 were considered as statistically significant. The post hoc statistical power analysis was performed with the "G*power" software (version 3.1), with an alpha-error probability of 0.05.

Results

The allele and genotype frequencies of the *DC-SIGN* promoter SNPs in T1DM patients and controls are shown in Table 1. All polymorphisms were in Hardy–Weinberg equilibrium, except for the SNP-336 (rs4804803) in T1DM and T1DM AITD−CD subjects. The *DC-SIGN* genotype and allele frequencies were differently distributed within patients and controls healthy (Table 1), being considered statistically significant p-value <0.025 after Bonferroni's correction.

The SNP 871 A>G (rs735239), the G allele was more frequent in healthy controls (42%) than in T1DM patients in general (27%, OR = 0.52, CI95% = 0.37–0.73 and p-value = $8.35e^{-05}$) and T1DM AITD−CD− (26%, OR = 0.50, CI95% = 0.35–0.96, p-value = 0.0001), being associated with protection to T1DM. The 871A/G genotype was also significantly more frequent in healthy controls (50%) than in patients T1DM in general (36%, OR = 0.43, CI95% = 0.26–0.70, p-value = 0.0004) and T1DM AITD−CD− (34%, OR = 0.40, CI95% = 0.23–0.68, p-value = 0.0004) by codominant model. Similarly, the G/G genotype (rs735239) was significantly