Human Immunology 77 (2016) 110–114

Contents lists available at ScienceDirect

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

Rapid Communication

Investigation of activating and inhibitory killer cell immunoglobulin-like receptors and their putative ligands in type 1 diabetes (T1D)

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ARTICLE INFO

Article history:
Received 31 December 2014
Revised 24 May 2015
Accepted 31 October 2015
Available online 2 November 2015

Keywords:
Killer immunoglobulin-like receptor
HLA class I ligands
Saudi population
Type 1 diabetes

ABSTRACT

Genetic and environmental factors play important roles in predisposing an individual to the development of type 1 diabetes (T1D). Several studies have investigated the role of killer cell immunoglobulin-like receptors (KIRs) and their HLA-class I ligands in susceptibility to T1D development, but only some of these studies have demonstrated an association. KIRs and their corresponding HLA class I ligands were investigated in Saudi patients with T1D compared with healthy controls. No significant differences in KIR gene distribution were observed between T1D patients and healthy controls. However, the homozygous C1/C1 ligand was considered a risk factor in predisposing individuals to T1D, whereas C2/C2 and HLA-Bw4 were considered protective factors against T1D. KIR2DL2/2DS2-C1C1 and KIR2DL3-C1C1 were significantly associated with T1D, and KIR2DS1-C2C2 and KIR2DL1-C2C2 were significantly less frequent in T1D patients. Stratification of KIR-HLA class I ligands in terms of the absence/presence of specific genotypes has different indications for susceptibility to T1D.

1. Introduction

Type 1 diabetes (T1D) is a multifactorial and polygenetic T cell-mediated autoimmune disease that leads to pancreatic B-cell destruction [1]. Natural killer (NK) cells are implicated in the development of T1D and might be important regulators and inducers of autoimmune diseases. NK cells, which are considered part of the innate immune system and can act against target cells after direct contact, can also kill islet cells [2,3]. NK cell activity is governed by a delicate balance between activating and inhibitory signals that arise from corresponding killer cell immunoglobulin-like receptors (KIRs), which are expressed on the cell surface and modulate NK and T cell functions by interacting with HLA class I-specific ligands on target cells [4]. Healthy human cells express HLA class I ligands, which interact with inhibitory KIRs to avoid spontaneous cell destruction, and failure of NK cells to recognize a suitable HLA ligand will activate these cells to kill their target [5,6]. KIR HLA class I-specific ligands include HLA-C group 1 (C1), HLA-C group 2 (C2) and HLA-Bw4 (Bw4) motifs that span residues 77–83 of the α1 domain; HLA-C is the most important ligand for NK cell regulation [7]. The different chromosomal locations of the KIR (residing on chromosome 19q13.4) and HLA (residing on chromosome 6p21) genes indicate the independent segregation of their existence within an individual, which is important for maintaining functional interactions and the immune response [2,8].

The presence of HLA molecules/genes is an important risk factor for susceptibility to T1D development [4], and certain combinations of HLA-KIR genotypes have been linked to susceptibility to autoimmune diseases. Further, several population studies have found associations between KIR/HLA class I ligands and T1D [1,3,4,9,10]. To our knowledge, no previous study on the role of KIRs and HLA class I ligands in T1D in the Saudi population has been published. Thus, this study aimed to investigate the distribution of certain KIRs and their corresponding HLA class I ligands in T1D patients compared with healthy controls in a Saudi population.

2. Materials and methods

KIRs and HLA class 1 alleles were defined in 106 unrelated Saudi individuals with T1D (39 individuals diagnosed prior to 5 years of age and 67 individuals diagnosed between 5 and 14 years of age); T1D diagnosis was based on WHO criteria (technical report series

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http://dx.doi.org/10.1016/j.humimm.2015.10.019
0198-8859/

727). In addition, 148 individuals who met the inclusion criteria of being healthy and unrelated [11] were randomly drawn from a pool of healthy donors at the Stem Cell Transplant Program of King Fahad Medical City (KFMC), Saudi Arabia, for inclusion as controls. The study was approved by the Hospital Institutional Review Board, and informed consent was obtained from the participants or their guardians prior to the time of blood collection. Blood samples were collected in containers containing EDTA, and genomic DNA was extracted using a MagNA pure compact instrument (Roche Diagnostics Ltd. Rotkreuz). DNA was measured using a NANODROP® 2000c spectrophotometer. KIR genes (KIR2DL1, KIR3DL2, KIR2DL2, KIR2DL3, KIR3DL1, KIR2DS1, KIR2DS2, KIR3DS1, KIR2DL5, KIR2DP1, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DL4, KIR3DL3 and KIR3DP1) were identified using a sequence-specific oligonucleotide probe (SSOP) method that utilized Luminex® technology, and HLA-A, HLA-B and HLA-C were genotyped using the Luminex method (One Lambda, Canoga Park, CA, USA). The details of the two procedures can be obtained from https://www.onelambda.com. The frequencies of individuals positive for each KIR gene were counted based on http://www.allelefrequencies.net. Individuals carrying one or more of the KIR2DL2, KIR2DL5, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3 and KIR2DS5 genes were grouped as B*x* haplotypes (*x* can be either an A or B haplotype), and individuals who did not carry any of these genes were grouped as A haplotypes. In addition, haplotype group ID profiles were obtained [12]. B*x* genotypes were classified into four subsets based on the presence or absence of centromeric (C) and telomeric (T) clusters: C4T4 (presence of both C and T), C4T*x* (presence of C and absence of T), C*x*T4 (absence of C and presence of T) and C*x*T*x* (absence of both) [13].

The frequency distribution of the C1, C2, A*03/11 and Bw4 ligands was also assessed. According to Mehers et al., C1 was assigned if an individual had a C*01, *03, *07, *08, *12 and *14 allele lineage, whereas C2 was assigned if an individual had a C*02, *04, *05, *06, *15, *17 or *18 allele lineage [14]. HLA-C alleles not possible to group into C1 or C2 (subtyped classifications were needed for HLA-C) were removed. Additionally, Bw4 was defined based on the HLA-allele database (http://hla.alleles.org), and only the Bw4 motif that presents within HLA-B was considered.

Using the SAS program [15], the frequency distributions for the KIR genes and their ligands were derived using a direct counting method, and Fisher's exact test and logistic regression methodology were applied. To determine significant differences between patients and healthy controls, the log of the odds (logit) for the presence of each KIR gene and its ligand was modeled as a function of each group, and the overall significance level was set at 0.05. Bonferroni corrections were applied whenever necessary to address multiple comparisons, and significant results are presented as corrected *p* values.

3. Results

The KIR gene frequency distribution results among Saudi patients with T1D and healthy controls are shown in Table 1. All tested KIR genes were represented in the patient and control samples, and no significant differences were observed in KIR gene frequencies between T1D patients compared with healthy controls. KIR haplotype variations, which depend on the presence of KIR genes within an individual, were calculated, and no differences were observed for the A and B*x* haplotype groups between the patients (20.8% and 79.2%, respectively) and controls (18.1% and 81.9%, respectively). Forty-six different KIR genotype groups were observed in the patients and controls, as shown in Fig. 1. For the HLA class I ligands for the KIR genes, a significantly higher frequency of homozygous C1C1 ligands was observed in patients with T1D (45% vs 15.9%, $p = 0.0002$, corrected $p = 0.0008$), whereas the frequency of homozygous C2C2 ligands was higher in healthy controls (36.4% vs 14%, $p = 0.0002$, corrected $p = 0.0008$).

Table 1
Frequency of KIR genes in children with type T1D compared with healthy controls.

Gene	All type 1 diabetes *n* = 106 (%)	Control *n* = 148 (%)
KIR2DL1	103 (97.2)	145 (98)
KIR2DL2	74 (69.8)	101 (68.2)
KIR2DL3	92 (86.8)	123 (83.1)
KIR3DL1	104 (98.1)	140 (94.6)
KIR3DL2	106 (100)	148 (100)
KIR2DS1	37 (34.9)	65 (43.9)
KIR2DS2	76 (71.7)	108 (73)
KIR3DS1	34 (32.1)	53 (35.8)
KIR2DL5	61 (57.5)	96 (64.9)
KIR2DP1	103 (97.2)	145 (98)
KIR2DS3	44 (41.5)	64 (43.2)
KIR2DS4	96 (90.5)	137 (92.6)
KIR2DS5	45 (42.5)	65 (43.9)
KIR2DL4	106 (100)	148 (100)
KIR3DL3	106 (100)	148 (100)
KIR3DP1	106 (100)	148 (100)

The Bw4 motif, which is the specific ligand for KIR3DS1 and KIR3DL1, was also found at a higher frequency in healthy controls (63.5% vs 41.5%, $p = 0.001$, corrected $p = 0.008$), and no difference was observed in the frequency of A*03/11 (ligand for KIR3DL2) between the patients and controls (18.9% vs 17.9%, $p = 0.84$).

An assessment the effect of KIR-HLA ligand genetic variation on susceptibility for T1D development is presented in Table 2. KIR2DL2-C1C1 and KIR2DS2-C1C1 combinations were present more frequently in T1D patients than controls (43.7% vs 19.4%, $p = 0.0008$, corrected $p = 0.0032$), and KIR2DL3-C1C1 was also present at a higher frequency in T1D patients than controls (46% vs 14.1%, $p = 0.0001$, corrected $p = 0.0004$). However, KIR2DS1-C1C1 had a higher frequency in controls (41.3%), and only one individual in each group of T1D patients was positive for KIR2DS1-C1C1.

Further, investigations were conducted on KIR-HLA ligands that exhibited significant differences between T1D patients and healthy controls. The presence of certain combinations, including KIR2DL2/3-C1C1, KIR3DL1-Bw4, KIR2DL1-C2C2, KIR2DL1 C2C2 + KIR3DL1-Bw4 or KIR2DL2/3-C1C1 + KIR3DL1-Bw4, was evaluated in each individual. As displayed in Table 3, higher frequencies with significant values were observed in T1D patients who exhibited KIR2DL2/3-C1C1 in the absence of KIR3DL1-Bw4 and KIR2DL1-C2C2 (p value = 0.0015, corrected p value = 0.0075) and KIR2DL2/3-C1C1 + 3DL1-Bw4 in the absence of 2DL1-C2C2 (p value = 0.001, corrected p value = 0.005), whereas 3DL1-Bw4 in the absence of 2DL2/3-C1C1 and 2DL1-C2C2 was significantly increased in healthy controls.

4. Discussion

Our results comparing T1D patients and controls in terms of KIRs genes frequencies are in agreement with Chinese and Basque populations [4,16]; however, Mehers et al. reported only a significant difference in the KIR2DL3 genes in a British population [14]. Significant differences were detected in HLA class 1 ligands. Accordingly, the homozygous C1C1 was considered a predisposing risk factor for T1D development, whereas the significantly increased frequencies of C2C2 and HLA-Bw4 in controls indicated these ligands might play a protective role against T1D in the Saudi population. Notably, the HLA-Bw4 motif in the HLA-B locus had a better effect on NK cell inhibition than the Bw4 motif in HLA-A [2]. No significant differences were observed in the heterozygous