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KIRs and their HLA ligands in Remitting–Relapsing Multiple Sclerosis

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ABSTRACT

Killer Immunoglobulin-like Receptor (KIR) genes may affect both resistance and susceptibility to autoimmune disorders, but their role in the pathogenesis of Multiple Sclerosis (MS) is still unclear. To evaluate the involvement of KIRs and their HLA ligands in the development of MS we performed genotyping of HLA -A, -B, -Cw, -DRB1 and KIRs loci in 121 RRMS patients and 103 healthy controls (HC). Results evidenced a possible protective role of the activating KIR2DS1 gene ($p_y = 0.001$; OR:0.38), enhanced in the presence of its ligand group HLA-C2 ($p_y = 0.0001$; OR:0.23). Our data suggest that the presence of functional compounds of activating KIR receptors together with their HLA ligands, allowing the immunomodulatory function of NK cells, may have a protective role against the disease.

1. Introduction

Multiple Sclerosis (MS) is an autoimmune disease of the CNS with a multi-factorial aetiology involving both environmental (Dean et al., 1976) and genetics factors (Sadovnick et al., 1996). Many genes have been screened with respect to mutations and polymorphisms associated with the disease itself (Weinshenker and Kantarci, 2000) or with clinical characteristics such as disease course (Barcellos et al., 2003) and response to therapy (Fusco et al., 2001). To date, the HLA class II haplotype DRB1*1501-DQA1*0102-DQB1*0602 is the only well-established factor associated with MS susceptibility (Gödde et al., 2005), though recent studies reported a protective effect of some HLA class I alleles, such as the HLA-A*02 and Cw*05, independently of HLADRB1*1501 (Bergamaschi et al., 2010; Brynedal et al., 2007; Yeo and The International Multiple Sclerosis Genetics Consortium, 2007).

Since the 1980s a great deal of evidence has been accumulated, suggesting that NK cells might play a role in the regulation of EAE and MS (Matsumoto et al., 1998; Takahashi et al., 2001; Takahashi et al., 2004; Xu et al., 2005; Zhang et al., 1997), though the mechanism by which NK cells could mediate immune regulation remains unclear (Shi and Van, 2006). NK cells activity reflects a delicate balance between activating and inhibitory signals, delivered by cell surface receptors belonging to many families. In humans, one of the key receptor families are the Killer Immunoglobulin-like Receptors (KIRs), that act by detecting shared allelic determinants of HLA class I molecules on cell surface of target cells (Khakoo and Carrington, 2006). To date, 17 distinct KIR genes or pseudogenes loci have been identified on chromosome 19q13.4, which encode both inhibitory and activating receptors expressed by NK. Notably, KIR receptors are also expressed by some T lymphocyte subpopulations, mainly TcR $\alpha\beta^+$ $CD8^+$ and TcR $\gamma\delta^+$, modulating TcR-dependent activation (Martinez-Rodriguez et al., 2010). The KIR genes cluster is highly polymorphic, with individual genes exhibiting allelic variability; combinations of KIR genes can be regarded as forming inherited haplotypes with widely differing balances between activating and inhibitory types. The polymorphism of the KIR loci parallels that of the MHC, facilitating the adaptation of the immune system to a dynamic, challenging environment (Bashirova et al., 2006). HLA class I genes map to chromosome 6, unlinked to the KIR genes on chromosome 19. Thus, the inheritance and expression of the genes encoding the receptors and their ligands are physically independent of one another. It is therefore possible that a certain KIR, its ligand, or both might be absent in a given individual, resulting in a functionally null situation (Kulkarni et al., 2008). KIR haplotypic diversity and interactions between KIR receptors and their appropriate ligands on target cells

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(Bw4 and C1/C2 motifs on HLA class I molecules in some instances) result in the production of positive/negative signals regulating NK and $CD8^+$ T cell subsets function (Middleton et al., 2005), with the functional consequence of differential susceptibility to diseases. This background suggests that diseases can be modified by specific KIR–ligand interactions and a variety of HLA-KIR compounds have been implicated in disease pathogenesis as: resistance to viral infections (Martin et al., 2002), inflammatory disorders (Yen et al., 2001), cancers (Arnheim et al., 2005; Butsch Kovacic et al., 2005) and several autoimmune diseases (Boyton and Altmann, 2007). Concerning KIR and susceptibility to MS, a recent study showed a possibly protective role of the KIR ligand HLA-Bw4 against MS in Norwegians (Lorentzen et al., 2009).

To investigate the influence of KIR genes on MS susceptibility in Italian population and to assess whether genetic interactions between specific KIR genes and their HLA class I ligands may contribute to the pathogenesis of MS, we examined the presence/absence of KIR receptors and their putative HLA ligands in a homogeneous group of Remitting–Relapsing Multiple Sclerosis (RRMS) patients.

2. Materials and methods

2.1. Patients and controls

A homogeneous group of 121 patients (76 female, 45 male), with a definite diagnosis of RRMS according to Poser's criteria (Poser et al., 1983) was recruited at the Multiple Sclerosis Unit of IRCCS S Maria Nascente Don C. Gnocchi, Milan and at the Department of Neurological Science, Federico II University, Naples, Italy. As control group, 103 healthy subjects (HC) (56 female, 47 male), matched for age and regional origin with the MS patients were enrolled for this study. All the subjects were of Italian origin, included in the study upon signing an informed consent. The study was approved by the Institutional Review Board of Fondazione Don Gnocchi, Milan.

Clinical characteristics of patients are shown in Table 1. Onset was defined as the first episode of focal neurological dysfunction indicative of MS, and disease duration was estimated as the number of years from onset of the disease to the last clinical assessment (median 12 years). Disability was measured by the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983), whereas the Multiple Sclerosis Severity Score (MSSS), obtained correlating EDSS scores with disease duration, was used to evaluate disease progression (Roxburgh et al., 2005). Median value of therapeutic treatment duration was reported as well, 58 patients underwent Interferon therapy, 47 were treated with Copaxone, whereas 16 subjects received other or no treatments.

2.2. HLA and KIR genotyping

Genomic DNA was isolated from peripheral blood using standard procedures. Both serological and molecular low resolution typing (two digits), of HLA A, B, Cw, was performed, while DRB1 locus was investigated by high resolution typing (four digits). Polymerase chain reaction, using sequence specific primers and/or sequence specific oligonucleotides (PCR-SSP/SSO) methods, was performed using commercial kits according to the manufacturer's instructions with reagents that defined all of the most common HLA alleles: HLA-A(14 alleles), HLA-B (27 alleles), HLA-Cw (14 alleles) and HLA-DRB1 (13 alleles) (BAG, Lich, Germany; Biotest, Dreieich, Germany; Innogenetics, Gent, Belgium; One Lambda, Los Angeles CA, USA). KIR genotyping was done by PCR-SSP method using commercial kit (Invitrogen, Carlsbad CA, USA), able to detect the presence or absence of the 17 KIR genes or pseudogenes reported in Table 2. Detection of the HLA and KIRs alleles recognized by the specific primers was done after amplification in GeneAmp PCR 9700 thermocycler (Applied Biosystem, Foster City CA, USA) and gel electrophoresis on 2% agarose gel. In order to avoid possible discrepancies in HLA and KIR genotyping owed to two different performing typing laboratories, inter-group analysis was done with 100% of concordance.

2.3. KIR haplotypes and ligands

A and B KIR haplotypes were reported in this paper as described (New Allele Frequency Database: http://www.allelefrequencies.net) (Middleton and Gonzelez, 2010). Briefly, when KIR-2DL2, -2DL5, -3DS1, -2DS1, -2DS2, -2DS3, -2DS5 are present, genotype is taken as having B. If none of these genes is present, genotype is considered as AA, thus the genotypes have been labeled as AA or BX, where X can be either an A or a B haplotype. This is because of the difficulty, without family studies, of distinguishing in the presence of a B haplotype whether the other haplotype is A or B (Middleton and Gonzelez, 2010).

Ligand groups were defined as follows: i) KIRs 2DL1 and 2DS1 have as their ligand the C2 epitope (Asparagine at pos 77, Lysine at pos 80) present in HLA-Cw*02, -Cw*04, -Cw*05, -Cw*06, -Cw*17, -Cw*18; ii) KIRs 2DL2, 2DL3 and 2DS2 have as their ligand the C1 epitope (Serine at pos 77, Asparagine at pos 80) present in HLA-Cw*01, -Cw*03, -Cw*07, -Cw*08, -Cw*13, -Cw*14, -Cw*16. In addition, it was reported ligand for 2DL4 as HLA-G, for 3DL1 (and 3DS1) as HLA-Bw4 motif, for

Table 1
Clinical characteristics of MS patients.

Clinical variables	Values
Number	121
Sex (M/F)	45/76
Median age at onset	26 (12–50)
Median disease duration	12 (2–39)
Median EDSS	3.0 (0–9.5)
Median MSSS	3.4 (0.1–9.9)
Interferon therapy: years duration median value (range)/N	5 (1–12)/58
Copaxone therapy: years duration median value (range)/N	5 (0.1–19)/47
Other treatments	16

Table 2
Distribution of KIR genotype and KIR haplotype in 121 RRMS patients and 103 Controls. N: absolute number; (%): percentage in brackets; p_y: p value with Yates correction; p_c: p value corrected for degree of freedom; nd: not determined; df: degree of freeedom; OR: Odds ratio, CI: confidence interval.

KIR gene	RRMS(121) N(%)	HC(103) N(%)	p value	OR (95%CI)
2DL1	119 (98.3)	101 (98.1)	nd	
2DL2	66 (54.5)	60 (58.3)	$p_y = 0.67$	0.86 (0.49-1.51)
2DL3	108 (89.3)	91 (88.3)	$p_y = 0.99$	1.10 (0.44-2.71)
2DL4	120 (99.2)	101 (98.1)	nd	
2DL5	46 (38.0)	57 (55.3)	$p_y = 0.013$ $p_c = 0.21$	0.49 (0.28–0.87)
2DS1	30 (24.8)	48 (46.6)	$p_y = 0.001$ $p_c = 0.016$	0.38 (0.21–0.69)
2DS2	64 (52.9)	59 (57.3)	$p_y = 0.60$	0.84 (0.48–1.47)
2DS3	33(27.3)	42 (40.8)	$p_y = 0.056$ $p_c = 0.80$	0.54 (0.30–0.99)
2DS4*001/2	45 (37.2)	16 (15.5)	$p_y = 0.0005$ $p_c = 0.008$	3.22 (4.61–6.49)
2DS4*003/7	90 (74.4)	86 (83.5)	$p_y = 0.14$	0.57 (0.28–1.17)
2DS5	26 (21.5)	32 (31.1)	$p_y = 0.13$	0.61 (0.32–1.16)
3DL1	116 (95.9)	97 (94.2)	$p_y = 0.78$	1.44 (0.37–5.62)
3DL2	121 (100)	103 (100)	nd	
3DL3	121 (100)	102 (99.0)	nd	
3DP1*001/2/4	12 (9.9)	12 (11.7)	$p_y = 0.84$	0.83 (0.33–2.10)
3DP1*003	117 (96.7)	100 (97.1)	$p_y = 0.99$	1.10 (0.44–2.71)
3DS1	38 (31.4)	43 (41.7)	$p_y = 0.14$ $p_c = 0.05$ 16df	0.64 (0.36–1.15)
KIR haplotype				
AA	35 (28.9)	25 (24.3)	$p_y = 0.52$	1.27 (0.67–2.41)
BX	86 (71.1)	78 (75.7)		