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The frequencies of Killer immunoglobulin-like receptors and their HLA ligands in chronic inflammatory demyelinating polyradiculoneuropathy are similar to those in Guillian Barre syndrome but differ from those of controls, suggesting a role for NK cells in pathogenesis

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ABSTRACT

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired inflammatory neuropathy, which has similar clinical and pathological features to Guillain–Barré Syndrome (GBS), but differs in time course. We investigated the frequency of genes encoding Killer immunoglobulin-like receptors and their HLA ligands in subjects with CIDP, in subjects with GBS and in healthy controls. There were no differences in KIR gene frequency among the 3 groups. The gene frequencies for HLA-B Bw4-I were significantly greater in CIDP than HC, but did not differ from GBS. The frequency of the combination of 3DL1/HLA-B Bw4I was greater in CIDP than HC, but did not differ from that of GBS. These data raise the possibility of NK cell function being an important factor in the pathogenesis of CIDP.

1. Introduction

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired disorder of peripheral nerves leading to progressive or relapsing weakness and sensory loss. Guillain–Barré Syndrome (GBS) is an acute post-infectious neuropathy, which has various subtypes. CIDP shares some pathological and electrophysiological features with demyelinating forms of GBS, but differs from GBS in its time course and response to steroid therapy (Dyck et al., 1982; McCombe et al., 1987; Hughes et al., 2006, 2007). Both diseases are thought to be autoimmune in origin, although differ from other autoimmune diseases by their male predominance and the lack of confirmed HLA association (Adams et al., 1979; Feeney et al., 1990; van Doorn et al., 1991; Blum and McCombe, 2014).

In CIDP there is segmental demyelination and remyelination of peripheral nerves and nerve roots, with onion bulb formation, combined with lymphocytic and macrophage infiltration (Bouchard et al., 1999). Analysis of T cells from nerve biopsies shows clonal expansions of T cells (Schneider-Hohendorf et al., 2012). There is also differential expansion of NK T cell receptors, suggesting a role for these CD1 restricted T cells that recognize lipid antigens (Illes et al., 2000). There are reports of T cells and antibody directed against peripheral nerve antigens in CIDP (Yan et al., 2001; Csurhes et al., 2005a,b; Inglis et al., 2007; Lim et al., 2014) but no single target antigen has been found. Abnormalities of immunoregulation have been described, namely reduced numbers and function of CD4 + CD25 + regulatory T cells in CIDP (Chi et al., 2008). These findings suggest a role for the adaptive immune system in CIDP.

In CIDP, the role of the innate immune system, which responds to infection and tissue damage (Clark and Kupper, 2005), is less well documented. NK cells are part of the innate immune system (Vivier et al., 2008). Studies of NK cells in CIDP are limited to a single study that found reduced numbers of circulating NK (Sanvito et al., 2009). Killer immunoglobulin-like receptors (KIR) are cell surface receptors on NK cells, interact with MHC class I molecules on T cells and are critical for NK cell signalling (Guinan et al., 2010). We recently reported that frequencies of KIR2DL2/HLA-C2 and KIR-3DL1/HLA-B Bw4-T frequencies in GBS are different from those of healthy controls (Blum et al., 2013a). Using similar methods, we have now investigated the frequency of carriage of KIR genes and their HLA ligands in subjects with CIDP, and compared these with the gene frequencies of healthy controls (HC) and patients with GBS, to look for abnormalities that could implicate NK cells in CIDP.

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Table 1
KIR genes in subjects with CIDP, compared with GBS subjects and healthy controls. Fisher test; Bonferroni correction for multiple tests (ND = not done).

	CIDP (n = 78) N (%)	Controls (n = 225) N (%)	p value (Fisher)	GBS (n = 195) N (%)	p value (Fisher)
KIR gene					
2DL1	74 (94.8)	214 (95.1)	0.95	182 (93.3)	0.78
2DL2	48 (61.5)	110 (48.9)	0.06	114 (58.5)	0.68
2DL3	70 (89.7)	200 (88.9)	1	160 (82.1)	0.14
2DL4	78 (100)	224 (99.6)	ND	192 (98.5)	ND
2DL5A	30 (38.5)	84 (37.3)	0.89	67 (34.4)	0.57
2DL5B	20 (25.6)	47 (20.9)	0.43	47 (24.1)	0.88
2DS1	35 (44.8)	92 (40.9)	0.59	83 (42.6)	0.78
2DS2	45 (57.7)	111 (49.3)	0.24	106 (54.4)	0.69
2DS3	23 (29.4)	56 (24.9)	0.45	59 (30.3)	1
2DS4	33 (42.3)	95 (42.2)	1	74 (37.9)	0.58
2DS5	26 (33.3)	80 (34.7)	0.78	63 (32.0)	0.49
3DL1	74 (94.8)	212 (94.2)	1	174 (89.2)	0.17
3DL2	78 (100)	223 (99.1)	ND	195 (100)	ND
3DL3	78 (100)	224 (99.5)	ND	194 (99.5)	ND
3DS1	31 (39.7)	88 (39.1)	1	75 (38.5)	0.89
Haplotype					
AA	20 (25.6)	73 (32.4)	0.31	53 (27.1)	0.88
BX	58 (74.4)	152 (67.6)		142 (72.9)	

2. Methods

2.1. Subjects

A cohort of patients (n = 78) with a confirmed diagnosis of CIDP was recruited from several hospitals in Queensland and from private neurologists. All patients fulfilled standard diagnostic criteria for CIDP (Koski et al., 2009). The CIDP cohort was compared with a previously reported cohort of patients (n = 195) with a confirmed diagnosis of GBS from several hospitals on the East Coast of Australia (Blum et al., 2013a,b). All subjects fulfilled standard diagnostic criteria for GBS (Asbury and Cornblath, 1990). As a control group healthy volunteers were recruited (n = 225), and matched for ethnicity. Ethical approval was obtained from the Human Research Ethics Committee; all subjects gave informed consent.

2.2. Laboratory methods

Highly pure genomic DNA was extracted either from the peripheral blood (Nucleospin, Macherey Nagel, Duren, Germany) or saliva (Oragene, DNAGenothek, Kanata, Canada). For KIR, typing the KIR Genotyping SSP Kit (Invitrogen, Carlsbad, USA) was used. For typing specific KIR associated HLA ligands the KIR-HLA Ligand kit (Olerup, Vienna, Austria) was used. This kit distinguishes HLA-CwAsparagin80 (HLA-C1) from HLA-CwLysine80 (HLA-C2) and HLA-BBw4-Threonine80 (HLA-BBw4-T) from HLA-BBw4+ Isoleucine80 (HLA-BBw4-I), and tests for the presence of HLA-ABw4. These kits do not provide complete HLA typing. The kits were used according to the manufacturer's instructions. PCR was performed followed by analysis with gel electrophoresis using a 2% agarose gel at 100 V and photographed using an ultraviolet analysing system (Biorad).

2.3. Statistical analysis

Data analysis was performed using Excel (Microsoft) and GraphPad Prism. Fisher test was used, with $p < 0.05$ regarded as statistically significant. Bonferroni correction was used for multiple tests, with adjustment of p divided by the number of tests regarded as significant (for KIR genes $p < 0.003$; for HLA ligands $p < 0.01$; for KIR/HLA–ligand interactions $p < 0.005$). The association of each polymorphism with the disease was measured by odds ratio (OR) and its 95% confidence interval (95% CI).

3. Results

The frequency of KIR genes and HLA genes in the different groups is shown in Table 1. There was no difference in KIR gene frequency among the groups. The frequency of HLA-B Bw4T and Bw4I genes differed significantly between CIDP and HC, but not between CIDP and GBS. Whilst the gene frequency of C1 and C2 was similar in all 3 groups, the heterozygous situation of C1/C2 was significantly more frequently found in CIDP than HC; no differences in the frequency of C1/C2 were detected between CIDP and GBS (Table 2). The gene frequency of the functional KIR/HLA pairs is shown in Tables 3 and 4. Whilst there were no statistically significant differences between subjects with CIDP and GBS, 3DL1/HLA Bw4I was more frequent in CIDP than HC.

4. Discussion

In this study the carriage of KIR and their HLA ligands was assessed in subjects with CIDP and compared to that in HC and subjects with GBS. The KIR/HLA system is critical for the function of NK cells (Guinan et al., 2010). No differences were found among the frequency of KIR genes in the 3 groups. However, the function of KIR depends on the presence of the appropriate HLA ligand, and in the absence of the ligand, no signalling occurs (Carrington and Martin, 2006). HLA gene frequencies differed between CIDP subjects and HC. Whilst in GBS HLA-C2 and HLA-B BwT4 were different (Blum et al., 2013a), HLA-B Bw4I was more frequent in CIDP than HC, with a trend to significance for HLA-B Bw4T. The heterozygous situation HLA-C1/C2 was more frequently seen in CIDP than HC.

Since the KIR/HLA interaction is required for NK cell signalling, these findings suggest a role for NK cells in the pathogenesis of both CIDP and GBS. NK cells have not been studied in depth in CIDP. One study showed decreased numbers of circulating NK cells in CIDP (Sanvito et al., 2009). Intravenous immunoglobulin administration, which is a standard therapy for CIDP and GBS (Eftimov et al., 2009), is associated with a decreased number of circulating NK cells and reduced NK-cell-mediated cytotoxicity (Bohn et al., 2011). NK cells are part of the innate immune response and we speculate that these could be activated by the infections or other illness that frequently trigger exacerbations of CIDP. There is an interaction between the innate immune response that could then trigger an adaptive immune response (Clark and Kupper, 2005).

Table 2
HLA genes in subjects with CIDP, compared with GBS subjects and healthy controls. Fisher test; Bonferroni correction for multiple tests with $p < 0.01$ regarded as significant.

HLA Gene	CIDP (n = 78) N (%)	Controls (n = 225) N (%)	p value (Fisher)	OR (95% CI)	GBS (n = 195) N (%)	p value (Fisher)	OR (95% CI)
HLA-C1	67 (85.9)	201 (89.3)	0.41	0.72 (0.33–1.56)	156 (80)	0.30	1.52 (0.73–3.15)
HLA-C2	50 (64.1)	113 (50.2)	0.03	1.77 (1.04–3.01)	130 (66.6)	0.78	0.89 (0.52–1.55)
HLA-B Bw4-T	36 (46.1)	67 (29.8)	0.01*	2.02 (1.19–3.43)	88 (45.1)	0.89	1.04 (0.61–1.766)
HLA-B Bw4-I	35 (44.8)	61 (27.1)	0.0047*	2.18 (1.28–3.74)	70 (35.9)	0.17	1.45 (0.85–2.48)
HLA-A Bw4+	30 (38.4)	63 (28)	0.09	1.61 (0.93–2.76)	69 (35.4)	0.67	1.14 (0.66–1.96)
C1/C1	27 (34.6)	110 (48.9)	0.03	0.55 (0.32–0.94)	65 (33.3)	0.88	1.06 (0.61–1.84)
C1/C2	42 (53.8)	93 (41.3)	0.01*	1.99 (1.16–3.41)	91 (46.7)	0.1	1.6 (0.93–2.76)
C2/C2	9 (12.5)	22 (9.8)	0.67	1.2 (0.53–2.74)	39 (20)	0.11	0.52 (0.24–1.12)

* $p < 0.01$.