Bull Yamaguchi Med Sch 58(3-4):37-43, 2011

Genotyping of Human Killer Cell Immunoglobulin-like Receptor Genes in Japanese Patients with Psoriatic Arthritis, Generalized Pustular Psoriasis or Psoriasis Vulgaris

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(Received January 12, 2012, accepted February 7, 2012)

Abstract The interaction of HLA-C with killer cell immunoglobulin-like receptors (KIRs) on natural killer cells and some natural killer-T cells together with a strong association between HLA (human leukocyte antigen), especially HLA-Cw*06, and psoriasis indicates that this is an immune-mediated disorder. The association between KIRs and psoriatic arthritis (PA) and generalized pustular psoriasis (GPP) was investigated by typing 14 KIR genes from Japanese patients with GPP, PA or psoriasis vulgaris (PV) using PCR. Frequencies of KIR2DS1 and KIR2DL5, which was in linkage disequilibrium with KIR2DS1, were significantly increased in PV cases when compared with controls. We report an increase in KIR2DS1 and KIR2DL5 in Japanese patients with GPP and PV. The KIR haplotype (KIR2DS1+2DS2+2DS3) in PA cases occurred more frequently than in GPP and PV cases. Thus, KIR2DS1 may be a common factor in susceptibility to psoriasis, although the variation in the frequencies of HLA-C and KIRs in cases of PV, GPP, and PA indicates that other genetic factors are also involved.

Key words: susceptibility to psoriasis, human leukocyte antigen, natural killer cells, killer cell immunoglobulin-like receptors

Introduction

Psoriasis is a chronic inflammatory skin disorder characterized by red, scaly plaques occurring in 1% to 2% of Caucasians and about 0.1% of Japanese. Psoriasis is classified by symptoms into three types: psoriasis vulgaris (PV), psoriatic arthritis (PA) and generalized pustular psoriasis (GPP). Cases can progress from one type to another and, although the cause of the disease is still unknown, several studies point to a combination of strong genetic predisposition and environmental factors.[1-3] Psoriasis is strongly associated with the presence of certain specific human leukocyte antigen (HLA) alleles, such as HLA-Cw*06 and Cw*07.[4] Although extensive studies have focused on T lymphocytes, natural killer (NK) cells and natural killer-T (NKT) cells have more recently been implicated in psoriasis.[5,6] An NK/NKT cell line induced psoriatic plaques in a severe combined immunodeficiency (SCID) mouse model.[7,8] IFN-γ was the trigger for the development of psoriasis.[9] Furthermore, NK and NKT cells were found in plaques from psoriatic skin specimens.[10] NK cell function is regulated by activating and inhibitory cell surface receptors. HLA-C ligand is recognized by NK and NKT cells through the killer cell immunoglobulin-like receptors (KIR)[11,12] and, therefore, KIR may also be related to the development of psoriasis. KIR genes are found on chromosome 19q13.4.[13] and are either activating or inhibitory depending on their structure. The activating receptors carry a short (S) cytoplasmic tail (KIR2DS and KIR3DS) and the inhibitory receptors carry a long (L) cyto-

plasmic tail (KIR2DL and KIR3DL). Group 1 HLA-C alleles (HLA-Cw*01, Cw*03, Cw*07, Cw*08, Cw*12, and Cw*14) carrying an asparagine (N80) are recognized by KIR2DL2 and KIR2DS2. Group 2 HLA-C alleles (HLA-Cw*02, Cw*04, Cw*05, Cw*06, Cw*15 and Cw*17) carrying a lysine (K80) are recognized by KIR2DL1 and KIR2DS1.[7,11,12] Because the HLA and KIR loci are located on different chromosomes, individuals can inherit both the ligand and the receptor. These HLA/KIR combinations could thereby lead to differences in NK/NKT cell activation thresholds.

We reported previously that KIR2DS1 and KIR2DL5 have been associated with susceptibility to PV.[14] In this study, we investigated the susceptibility of Japanese patients to psoriasis by typing 14 KIR genes using PCR with sequence-specific primers (PCR-SSP) in patients with PA, GPP or PV and compared them with healthy controls.

Subjects and Methods

Clinical samples

Samples of peripheral venous blood were collected from 28 unrelated Japanese patients (16 males and 12 females) with PA, 11 patients (9 males and 2 females) with GPP, 100 patients (74 males and 26 females) with PV and 60 healthy individuals (31 males and 29 females) after receiving their informed consent. Authorization was obtained from the Ethics Review Committee of Gene Analysis Research, Yamaguchi University School of Medicine and University Hospital. PA was diagnosed according to criteria proposed by Moll and Wright.[15] PA cases consisted of 20 patients with polyarticular asymmetrical arthritis, 5 patients with sero-negative arthritis indistinguishable from rheumatoid arthritis, 2 patients with arthritis mutilans, and 1 with ankylosing spondylitis. No cases of either spondylitis or classical arthritis involving predominantly distal interphalangeal joints were found. GPP is a rare and intractable disease characterized by the development of sterile pustules over the whole body. GPP was diagnosed using criteria proposed by the Japanese Association of Dermatology.[16] Clinical information for the variables among the patients with PV, PA, or GPP is presented in Table 1. Control samples were also obtained from 60 unrelated healthy controls.

PCR

DNA was extracted from 5 ml of whole blood samples using a QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, Germany) and stored at −20 ℃.

DNA samples were genotyped using PCR-SSP for the following KIR: 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1. PCR primers and conditions were as reported previously and was performed using a Gene

Table 1 Characteristics of the study participants with psoriasis

Variable	PA (N=28)	GPP (N=11)	PV (N=100)
Age (years)	47 ± 18	53 ± 15	49 ± 21
Sex (male: female)	16:12	9:2	74:26
Smoking status			
Never smoker	24/28 (86%)	9/11 (82%)	79/100 (79%)
Former or current smoker	4/28 (14%)	2/11 (18%)	21/100 (21%)
Type 2 diabetes mellitus	None	None	8/100 (8%)
Clinical severity measured by body surface area(%)	20 ± 16	61 ± 12[a,b]	16 ± 10

PA: psoriatic arthritis, GPP: generalized pustular psoriasis, PV: psoriasis vulgaris
a: GPP vs PA : p <0.001 (by t-test)
b: GPP vs PV : p <0.001 (by t-test)

Amp PCR system 9700 (Applied biosystems, Foster City, CA, USA).[17] Briefly, approximately 100 ng of genomic DNA was used per reaction in 20 μL TaKaRa EX Taq buffer with 2 mM $MgCl_2$, 200 mM dNTPs and 0.5 U TaKaRa EX Taq polymerase (Takara Bio Inc., Shiga, Japan). An initial denaturation step of 2 min at 95 ℃ was followed by 10 cycles of 20 s at 94 ℃, 10 s at 65 ℃ and 1 min 30 s at 72 ℃ and a further 20 cycles of 20 s at 94 ℃, 20 s at 61 ℃ and 1 min 30 s at 72 ℃. The human growth hormone (GH1) served as a positive control for the PCR. All primers were synthesized and validated by Hokkaido System Science Co., Ltd. (Hokkaido, Japan). PCR products were electrophoresed on 1.2% agarose gels using ethidium bromide and each positive sample was genotyped. The phenotype frequency of each KIR was calculated as the percentage of positive numbers among all specimens. Frequencies of combined activating KIRs were examined because it has been reported that activating KIRs might enhance the development of PA.[5]

DNA typing of HLA-C genes

HLA-C alleles were identified by PCR-SSP as described previously.[18] Briefly, 10 μg of genomic DNA was used to amplify each HLA-C gene by PCR with specifically designed primers. Alleles were assigned by the reaction patterns of the sequence-specific oligonucleotide probes (One Lambda, Inc., Canoga Park, CA, USA).

Statistical analysis

The differences in the phenotype frequencies between the case and control subjects were assessed using either the two-sided Fisher's exact test or χ^2 test. Student's *t*-test was used to examine the statistical significance of the mean values of affected body surface area.

Results

As shown in Table 1, there were more male patients with the three types of psoriasis (PA, PV, or GPP) than female patients. GPP patients exhibited the greatest affected body surface area with more cutaneous lesions compared with those of the patients with either PA or PV ($p<0.001$, respectively). There was no statistical significance associated with smoking status or development of type 2 diabetes mellitus among the PA, PV, or GPP cases.

A significant increase at the 5% level was observed for KIR2DS1 in 64% of the GPP samples and 46% of the PV samples compared to 30% for the controls ($p=0.043$, $p=0.048$, respectively). KIR2DL5 was also significantly increased at the 5% level in 64% of GPP cases and 47% of PV cases compared to 30% of the control group ($p=0.043$, $p=0.046$, respectively) (Table 2).

The three activating KIRs (KIR2DS1, KIR2DS2 and KIR2DS3) occurred more frequently in PA samples (11%) compared with those of GPP (0%), PV (4%) and controls (3%). However, the haplotype frequency was not significantly increased ($\chi^2=1.9$, $p=0.16$) (Table 3).

The frequency of Group 2 HLA-C genes in PV samples (16%) was significantly ($p=0.044$) increased compared with that of the controls (5%). HLA-C DNA typing in patients with PA was not done because of the lack of genomic DNA. Since HLA-C ligands (Group 2) for the corresponding inhibitory KIR (KIR2DL1) were missing in all GPP cases, it is possible that activating KIR will enhance the development of GPP (Table 4).

Discussion

This study showed that KIR2DS1 and KIR2DL5 were significantly increased in patients with GPP and PV. According to the KIR haplotype model based on family studies and genomic sequencing by Hsu *et al.*,[19] all haplotypes with KIR2DS1 also have KIR2DL5 upstream, but they do not always coexist. This haplotype structure indicates that an increase in KIR2DS1 leads to a secondary increase in KIR2DL5. The frequencies of KIR2DS1 and KIR2DL5 were nearly identical in the three groups in this study and all individuals with KIR2DS1 also possessed KIR2DL5. The slightly increased frequency of activating KIR haplotype (KIR2DS1+2DS2+2DS3) seen in PA cases may be correlated with an increased risk of developing PA, as suggested by Martin *et al.*[5]

Table 2 Summary of phenotype frequency of killer cell immunoglobulin-like receptors (KIR) in Japanese patients with psoriatic arthritis (PA), generalized pustular psoriasis (GPP) or psoriasis vulgaris (PV) compared to healthy controls

KIR		PA (N=28)			GPP (N=11)			PV (N=100)			Control (N=60)		
		+	−	%(+)	+	−	%(+)	+	−	%(+)	+	−	%(+)
2DL	1	26	2	93	11	0	100	93	7	93	59	1	98
	2	8	20	29	1	10	9	27	73	27	10	50	17
	3	28	0	100	11	0	100	99	1	99	59	1	98
	4	26	2	93	11	0	100	99	1	99	60	0	100
	5	11	17	39	7	4	64a	47	53	47c	18	42	30
3DL	1	27	1	96	11	0	100	87	13	87	58	2	97
	2	27	1	96	11	0	100	99	1	99	60	0	100
	3	28	0	100	11	0	100	100	0	100	60	0	100
2DS	1	11	17	39	7	4	64b	46	54	46d	18	42	30
	2	8	20	29	0	11	0	20	80	20	10	50	17
	3	5	23	18	2	9	18	15	85	15	4	56	7
	4	27	1	96	11	0	100	85	15	84	57	3	95
	5	7	21	25	6	5	55	34	66	34	14	46	23
3DS	1	9	17	32	6	5	55	38	62	38	15	45	25

Abbreviations:
[a]: p =0.043, GPP vs control.
[b]: p =0.043, GPP vs control.
[c]: p =0.046, PV vs control.
[d]: p =0.048, PV vs control.
p values are for the two-sided Fisher's exact test.

Table 3 Haplotype frequency of activating killer cell immunoglobulin-like receptors (KIR2DS1, 2DS2 and 2DS3) in Japanese patients with psoriatic arthritis (PA), generalized pustular psoriasis (GPP) or psoriasis vulgaris (PV) compared to healthy controls

KIR haplotype	PA	GPP	PV	Control
KIR2DS1+2DS2+2DS3	3/28 (11%)	0/11 (0%)	4/100 (4%)	2/60 (3%)

Table 4 Phenotype frequencies of HLA-C in generalized pustular psoriasis (GPP) or psoriasis vulgaris (PV) compared to healthy controls

HLA-C	GPP	PV	Control
Group 1 HLA-C (HLA-Cw*01,- Cw*03,- Cw*07,- Cw*08,- Cw*12,- Cw*14)	11/11 (100%)	84/100 (84%)	57/60 (95%)
Group 2 HLA-C (HLA-Cw*02,- Cw*04,- Cw*05,-Cw*06,-Cw*15,-Cw*17)	0/11 (0%)	16/100* (16%)	3/60 (5%)

Group 1 indicates HLA-C Ser77Asn80 and Group 2 indicates HLA-C Asn77Lys80.
* p <0.05 (two-sided Fisher's exact test) vs control.

More samples are required to elucidate the statistical significance of the increased risk of developing PA with this KIR haplotype.

The specificity of inhibitory KIR for HLA-C ligands is mainly dictated by the presence of asparagine or lysine at position 80 of the HLA-C molecule.[20] KIR2DS1 recognizes Group 2 HLA-C (Cw*02, Cw*04, Cw*05, Cw*06, Cw*15 and Cw*17), while KIR2DS2 interacts with Group 1 HLA-C (Cw*01, Cw*03, Cw*07, Cw*08, Cw*12 and Cw*14). Group 2 HLA-C (including HLA-Cw*06) was strongly associated with PV, whereas Group 1 HLA-C (including HLA-Cw*07) was associated with GPP. In this study, we show that KIR2DL5 was increased in patients with GPP. Although ligands for KIR2DL5 remain unclear, inhibitory KIR2DL1, observed in all of the 11 GPP cases, is missing the corresponding Group 2 HLA-C ligands. Thus, inhibitory KIR2DL1 cannot sufficiently suppress the function of immunocytes. This might contribute to the pathogenesis of GPP by influencing NK or NKT cell activity, and by increasing the severity of cutaneous lesions as measured by affected body surface area.

Several human diseases are associated with KIR gene variability. KIR2DS2 is significantly increased in rheumatoid arthritis patients with vasculitis compared to those without vasculitis or healthy controls.[21] Furthermore, KIR2DS2 was increased in patients with scleroderma but only in the absence of the inhibitory receptor KIR2DL2.[22]

Th17 lymphocytes have been shown to be important in the development of PV.[1,23] NK cells, macrophages and plasmacytoid dendritic cells produce key cytokines (TNF-α, IFN-α, IFN-β, IFN-γ, IL-1β and IL-6) that activate myeloid dendritic cells. These cells present antigens and secrete mediators such as IL-12 and IL-23, leading to the differentiation of Th17 and Th1 cells. T cells secrete mediators (IL-17 and IL-22) that activate keratinocytes and induce production of antimicrobial peptides, proinflammatory cytokines, chemokines and S100 proteins. These mediators feed back into the proinflammatory disease cycle shaping the inflammatory infiltrate.[1]

The striking clinical and histological differences in GPP compared to PA and PV indicate that it may be a disease of distinct etiology. It has been reported recently that GPP is caused by abnormal IL-36A signaling resulting from IL36RN (previously known as IL1F5) loss-of-function mutations, although no significant association was observed with a variant located in IL36RN in PV and PA.[24] In a preliminary experiment concerning IL36RN mutations, the variant of the corresponding single nucleotide polymorphism (338 C→T and 142 C→T) was not absolutely observed (data not shown). Further research is required to provide conclusive evidence of a genetic component to GPP.

In conclusion, KIR2DS1 may be a common denominator in the development of the different types of psoriasis, although the variation in the frequencies of HLA-C and KIRs in cases of PV, GPP and PA indicates that other genetic factors might also be involved.

Acknowledgments

This work was supported by a Grant from the Ministry of Health, Labour and Welfare (Research on Measures for Intractable Diseases) and by Grants-in-Aid for Scientific Research (22591223) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflict of Interest

The authors state no conflict of interest.

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