Brief Genetics Report

KIR in Type 1 Diabetes

Disparate Distribution of Activating and Inhibitory Natural Killer Cell Receptors in Patients Versus HLA-Matched Control Subjects

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Killer cell immunoglobulin-like receptors (KIRs) modulate natural killer cell and T-cell function by interacting with HLA class 1 ligands on target cells. Both KIR and HLA are highly polymorphic. We studied the influence of KIR and HLA class 1 genes on the susceptibility to develop type 1 diabetes. The results showed increased numbers of activating KIR genes in patients compared with control subjects ($P = 0.049$). The combination of the activating KIR2DS2 gene, together with its putative HLA ligand, was present more frequently in patients than in diabetes high-risk HLA-matched control subjects ($P = 0.030$). Moreover, our results imply that an increase in activating KIR2DS2-HLA ligand pairs combined with a lack of inhibitory KIR-HLA ligand pairs is associated with an additional risk to develop type 1 diabetes in individuals with diabetes high-risk HLA alleles ($P = 0.035$). We propose that the genetic imbalance between KIR and their HLA class 1 ligands may enhance the activation of T-cells with a low affinity for pancreatic self-antigens, thereby contributing to the pathogenesis of type 1 diabetes. *Diabetes* 52: 2639–2642, 2003

Killer cell immunoglobulin-like receptors (KIRs) are membrane glycoproteins of the Ig superfamily expressed by natural killer cells and subsets of T-cells. KIR molecules modulate cell function upon recognition of HLA class 1 (1–3). KIR genes are organized in a highly polymorphic, multigene family (4) with considerable allelic polymorphism (5,6) and are divided into haplotype groups A and B on the basis of their gene content. Common to both groups of haplotypes are the framework genes KIR3DL3, KIR2DL4, and KIR3DL2 (7). The group A haplotypes are relatively homogeneous and may contain only one activating KIR gene. The A haplotype, which is found most frequently in Caucasians, lacks all functional 2DS-activating genes and is therefore referred to as an inhibitory haplotype. The B haplotypes are more diverse and may contain up to six activating KIR genes (8). The variation of KIR haplotypes largely depends on the number of activating KIR genes because inhibitory KIR genes display a more conserved distribution among both A and B haplotypes (9). As a consequence of this genetic variation, the repertoire of KIR expression by natural killer cells and T-cells varies between individuals.

The relation of KIR and type 1 diabetes has yet to be studied. Type 1 diabetes results from T-cell–mediated autoimmune destruction of the pancreatic β-cells in genetically predisposed individuals (10). It is conceivable that T-cell function can be disturbed by an imbalance in the regulatory capacities of KIR molecules, which may facilitate the development of autoimmune diseases. We studied the association between KIR and HLA class 1 genes with susceptibility to type 1 diabetes in a large cohort of patients with juvenile-onset diabetes and control subjects using a PCR-based approach. We hypothesized that autoimmunity in type 1 diabetes could result from increased expression of activating KIR-ligand pairs leading to increased activation, from expression of inhibitory KIR without their ligand present leading to a lack of inhibition and/or regulation, or from a combination of these.

RESEARCH DESIGN AND METHODS

Genomic DNA samples. The case group consisted of 149 unrelated individuals with juvenile-onset type 1 diabetes (<14 years of age) who were collected consecutively upon diagnosis by pediatricians in the southwest region of the Netherlands (11). The control group consisted of 207 unrelated, nondiabetic, randomly selected Dutch individuals. DNA was extracted from peripheral blood with a standard salting-out procedure. All subjects were HLA typed at class 1 using a PCR–sequence-specific oligonucleotide probe (Dynal Biotech) and typed at HLA class 2 using local standard PCR for sequence-specific polymorphisms.

KIR genotyping. We performed KIR genotyping on the DNA of all subjects. We typed for KIR genes responsible for inhibitory functions (2DL1, 2DL2,

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KIR, killer cell immunoglobulin-like receptor.

TABLE 1
KIR gene frequencies in patients and control subjects

KIR	Diabetes	Control subjects
n	149	207
Inhibitory		
2DL1	94.6	97.6
2DL2	55.7	48.4
2DL3	91.9	92.3
2DL5	50.3	46.9
3DL1	96.0	96.1
Activating		
2DS1	36.2	35.7
2DS2	55.7	47.8
2DS3	24.8	27.1
2DS4	40.9	42.0
2DS5	32.9	27.1
3DS1	38.9	33.3
KIR1D	81.9	81.6

Data are %.

2DL3, 2DL5, and 3DL1) and activating signals (2DS1, 2DS2, 2DS3, 2DS4, 1D, 2DS5, and 3DS1). The PCR–sequence-specific polymorphism primers used for the detection of KIR loci were based on primer sites that have been previously described (12,13). The conditions for PCR amplification and additional primer sets that are used in this study are available on the journal's website (online appendix available from http://diabetes.diabetesjournals.org). In cases of unique profiles or previously unreported profiles, typing was repeated at least once.

Statistical analysis. KIR frequency differences between disease and control groups were tested by a two-tailed Fisher's exact test. Activating KIR-ligand pair gene frequencies between case and control subjects were tested for a progressive difference using a 2×3 test for trend. Both case and control groups were also stratified for diabetes high-risk HLA (individuals positive for DQ2 and/or DQ8) to correct for the strong linkage between HLA class 1 and 2 loci. The analyses described above were also performed in the HLA high-risk group from both panels.

RESULTS AND DISCUSSION

To establish KIR gene frequencies, we performed KIR genotyping on patients and control subjects (Table 1). All of the KIR genes tested were represented in both patients and control subjects. The KIR gene frequencies in our control cohort confirmed those described for previous cohorts of healthy control subjects. The KIR gene frequencies in patients were not statistically different from those in control subjects. We hypothesized that type 1 diabetic patients would possess more activating KIR genes than healthy control subjects. The number of activating KIR genes per phenotype may influence disease susceptibility through a gene dosage effect. The distribution of activating KIR genes (the presence of at least one copy of any given activating locus per phenotype) was compared in patients and control subjects (Fig. 1). In general, individuals from either cohort possessed up to five activating KIR genes. Individuals who possessed one or no activating KIR gene were more frequently control subjects than patients. In contrast, individuals with two or more activating KIR genes were more frequently patients than control subjects (65.8 vs. 55.1%, respectively, $P = 0.049$).

Any effect of KIR on disease susceptibility might depend on the presence of putative HLA ligands within an individual. We established frequencies for both activating and inhibitory KIR genes in the presence of an individual's predicted HLA ligands (Table 2). Individuals positive for activating KIR2DS2 in the presence of group 1 (Asn^{80}) HLA-C alleles were more frequently patients than control subjects (49.0 vs. 41.1%, respectively), whereas individuals positive for inhibitory KIR2DL1 in the presence of group 2 (lys^{80}) HLA-C alleles were less frequently patients than control subjects (45.6 vs. 55.6%, respectively). Furthermore, individuals positive for inhibitory KIR3DL1 in the presence of HLA-Bw4 (Ile^{80}) alleles were less frequently patients than control subjects (17.4 vs. 26.6%, respectively). These differences did not reach statistical significance. No differences were detectable between patients and control subjects for activating KIR2DS1, KIR3DS1, and inhibitory KIR2DL2/KIR2DL3 in the presence of their HLA ligands.

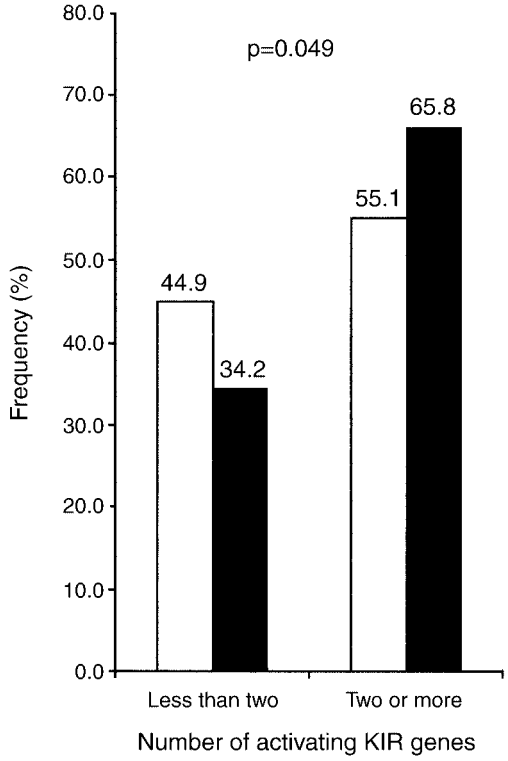

FIG. 1. Distribution of activating KIR loci among phenotypes in patients and control subjects. Phenotypic activating KIR combinations in patients ($n = 149$, ■) and in control subjects ($n = 207$, □) are shown. Percentages for the phenotypic distributions are shown above the bars. The *P* value was determined using two-tailed Fisher's exact test.

Both case and control groups were subsequently stratified for diabetes high-risk HLA (individuals positive for DQ2 and/or DQ8) to correct for the strong linkage between HLA class 1 and 2 loci (Table 2). The frequencies of individual KIR genes were comparable with those in subjects not stratified for disease-predisposing HLA. However, the frequent appearance of KIR B haplotypes was more pronounced in diabetes high-risk HLA-matched individuals (data not shown, $P = 0.022$). The results in the diabetes high-risk HLA group show that individuals positive for KIR2DS2 in the presence of group 1 HLA-C alleles are significantly more often patients than control subjects (48.9 vs. 33.7%, respectively, $P = 0.030$). The results that were obtained for KIR2DS1, KIR3DS1, KIR2DL1, KIR2DL2/KIR2DL3, and KIR3DL1, combined with their putative HLA ligands, were similar to those in subjects not stratified for disease-predisposing HLA.

It is conceivable that distinct combinations of KIR genes

TABLE 2
KIR gene frequencies in combination with their putative HLA ligands

KIR (HLA ligand)	Diabetes	Control subjects	Diabetes high-risk HLA*	
			Diabetes	Control subjects
n	149	207	135	95
Inhibitory				
2DL1 (HLA-C, Lys^{80})	45.6	55.6	45.9	55.8
2DL2/2DL3 (HLA-C, Asn^{80})	88.6	84.5	91.1	87.4
3DL1 (Bw4, Ile^{80})	17.4	26.6	17.8	27.4
Activating				
2DS1 (HLA-C, Lys^{80})	16.8	18.4	17.0	18.9
2DS2 (HLA-C, Asn^{80})	49.0	41.1	48.9	33.7†
3DS1 (Bw4, Ile^{80})	6.0	8.7	5.2	7.4

Data are %. Inhibitory KIR and activating KIR genes are shown top to bottom. The inhibitory KIR2DL2 and KIR2DL3 segregate as alleles of a single locus. The predicted HLA ligands are shown between parentheses. *Both disease and control groups were stratified for diabetes high-risk HLA (individuals positive for DQ2 and/or DQ8). †*P* was determined using two-tailed Fisher's exact test: $P = 0.030$.

may have a combined effect on type 1 diabetes susceptibility. We divided the activating KIR2DS2-ligand pairs of diabetes high-risk HLA-matched individuals according to their inhibitory KIR2DL1 and KIR3DL1-ligand pairs as described above (Table 3). Individuals who were positive for activating KIR2DS2 in the presence of its predicted ligand and lacking both inhibitory KIR2DL1 and KIR3DL1-ligand pairs were more frequently patients than HLA-matched control subjects (54.6 vs. 43.7%, respectively, $P = 0.035$). No differences were detected between those patients and control subjects who possessed activating KIR2DS2-ligand pairs and lacked either inhibitory KIR2DL1- or KIR3DL1-ligand pairs. None of the patients possessed all three of the investigated KIR-ligand pairs as compared with 9.4% of the control subjects.

The function of KIR on effector cells is highly dependent on the HLA molecules expressed on target cells. Given the high degree of polymorphism of both the KIR and major histocompatibility complex gene complexes, a given individual can express the receptor or the ligand only, or both receptor and ligand. Our results suggest that activating KIR2DS2 might contribute to the pathogenesis of type 1 diabetes by influencing the immune response when it is present with group 1 HLA-C ligands. A decrease in function of inhibitory KIR will abrogate the regulation of the activating KIR function and may predispose for additional risk of developing type 1 diabetes. Indeed, the inhibitory KIR2DL1 and KIR3DL1 genes were present less frequently with their putative HLA ligands in patients than in control subjects. These effects do not likely result from linkage between HLA class 1 and 2 because the association was obtained in patients and control subjects matched for diabetes high-risk HLA. It is conceivable that a genetic imbalance between activating and inhibitory KIR genes is fundamental for this effect.

TABLE 3
Influence of inhibitory KIR-ligand pairs on activating KIR2DS2-ligand pair frequencies in patients and control subjects

KIR	Diabetes	Control subjects
n	66	32
$2DS2^+$, $2DL1^-$, $3DL1^-$	54.6	43.7
$2DS2^+$, $2DL1^-$, $3DL1^+$	13.6	9.4
$2DS2^+$, $2DL1^+$, $3DL1^-$	31.8	37.5
$2DS2^+$, $2DL1^+$, $3DL1^+$	—	9.4

Data are %. Both patients and control subjects were matched for diabetes high-risk HLA. $^+$, present in combination with its putative HLA ligand; $^-$, present without its putative HLA ligand. A trend towards progressive differences in frequencies was found between three groups (no inhibitory KIR, one inhibitory KIR, and two inhibitory KIRs); $P = 0.035$.

The gene frequencies of KIR loci observed in our control group closely resembled those found in previous studies that investigated the KIR repertoire in Caucasian populations from Australia (14), Ireland (15), and the U.K. (16). Similar results for KIR gene frequencies were obtained in our patients with juvenile-onset type 1 diabetes. The phenotypic distribution of activating KIR genes in individuals between the two groups confirms the prevalence of the B haplotype over A haplotypes in the diabetic cohort. It is conceivable that KIR B haplotypes more effectively costimulate natural killer cell and/or T-cell responses against pathogens. However, a gene dosage effect of the number of activating KIR genes may also tend toward undesirable autoimmune pathogenesis.

How could KIR-expressing cells play a role in initiating autoimmunity? The KIR repertoire determines self-tolerance in natural killer cells, which allows these cells to detect the loss of cell-surface HLA class 1 expression. However, not all T-cells express inhibitory KIR for autologous HLA class 1. This lack of specific inhibitory KIRs seems especially apparent on αβ T-cells, which may exhibit a distinct pattern of KIR expression that is different from that of γσ T-cells and natural killer cells (17). Activating KIRs can enhance class 2 restricted T-cell responses, making such cells particularly responsive to small quantities of antigens or peptide epitopes with low binding affinity with their HLA restriction element. It has been proposed that this costimulation of T-cells sustains the immune response for an extended period (18). Furthermore, recognition of HLA class 1 by activating KIRs on both natural killer and T-cell subsets may affect the immune response through the secretion of interferon-γ and other possible T-helper 1 cytokines (19). KIRs may also be important in the regulation of peripheral tolerance of T-cell subsets. In the context of the pathogenesis of type 1 diabetes, costimulation by activating KIRs, combined with insufficient regulation of T-cell autoreactivity by inhibitory KIR, may facilitate the activation of autoreactive T-cells that are targeting β-cells by breaking tolerance for islet self-antigens.

A genetic imbalance between activating and inhibitory KIR genes may influence the pathogenesis of autoimmune diseases, either via upregulated activation or lack of inhibition or both. Recently, various studies related the influence of activating KIR with HLA, which may facilitate immune responses in the absence of or during downregulation of inhibitory KIR-ligand pairs. $CD4^{+}CD28^{null}$ T-cell clones isolated from rheumatoid arthritis patients with vasculitis were found to express the activating KIR2DS2 receptor often present in the absence of opposing inhibitory KIRs (20). The activity of KIR2DS2 in conjunction with the appropriate HLA-C ligand may favor the activation of autoreactive T-cells that target endothelial cells. Furthermore, both the activating KIR2DS1 and KIR2DS2 receptors have been associated with psoriatic arthritis, an inflammatory arthritis associated with psoriasis (21). This association was only found when the HLA ligands for their homologous inhibitory receptors were absent.

Although recognition of specific HLA molecules by inhibitory KIRs has been firmly established (22), activating KIRs bind their putative HLA ligands with very low affinity (23). Yet, peptides bound in the groove of HLA molecules may influence recognition by activating KIRs (24). Viral peptides bound to HLA-C molecules may abrogate recognition by inhibitory KIRs (25). Conversely, it is conceivable that specific HLA-peptide complexes may augment interaction with activating KIRs.

Our results suggest that autoreactivity in type 1 diabetes is associated with increased frequencies of activating KIRs in the absence of regulation by inhibitory KIRs. Activating KIR molecules may enhance rapid induction of T-cell–mediated immune responses in the presence of their specific HLA ligand. Costimulation by activating KIRs may facilitate activation of T-cell subsets in which the T-cell receptors interact weakly with low amounts of self-peptide, thereby contributing to the initiation of type 1 diabetes.

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