Cutting Edge: Heterozygote Advantage in Autoimmune Disease: Hierarchy of Protection/Susceptibility Conferred by HLA and Killer Ig-Like Receptor Combinations in Psoriatic Arthritis[1]

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Functionally relevant combinations of HLA and killer Ig-like receptor (KIR) genotypes influence resistance to several diseases in humans. Analysis of genetic data from such studies is challenging because it involves multiple linked and unlinked loci that exert their influence in an epistatic manner. We previously reported that subjects with certain activating receptors were susceptible to developing psoriatic arthritis (PsA), an effect that was strongest when HLA ligands for corresponding homologous inhibitory receptors were missing. In this study, we present a novel model in which susceptibility to PsA is determined by the overall balance of activating and inhibitory composite KIR-HLA genotypes. This model fits our knowledge of clonal NK cell expression of KIR and regulation of NK cell activity better than does the previous model, as reflected in a robust trend for increasing susceptibility to PsA with more activating genotypes. These data emphasize the remarkable influence of KIR/HLA combinations on this disease. The Journal of Immunology, 2004, 173: 4273–4276.

Several human diseases have been shown to associate with variability at the killer Ig-like receptor (KIR)[4] gene cluster, supporting a model whereby disease morbidity/mortality generates and maintains diversity at this locus (1–3). Although haplotypes containing multiple activating KIR may mediate protective NK cell responses against infectious disease (2) and enhance beneficial maternal-fetal interactions,[5] these same haplotypes may predispose to autoimmune pathogenesis. Indeed, activating KIR, in particular KIR2DS1 and KIR2DS2, have been associated with autoimmune disorders (2, 3).

KIR2DS1 and KIR2DS2 show 98% sequence similarity to KIR2DL1 and KIR2DL2/3 (KIR2DL2 and KIR2DL3 are alleles of the same gene and their products recognize the same HLA ligands), respectively, in their extracellular domains, but the inhibitory KIR2DL bind HLA-Cw ligands (HLA-Cw group 2 for KIR2DL1 and HLA-Cw group 1 for KIR2DL2/3) with significantly greater affinity than do the activating KIR2DS (4). Despite its low affinity, KIR2DS binding to HLA class I is likely to be functionally relevant under various circumstances. Genes encoding KIR2DL1 and KIR2DL2/3 are present in virtually all individuals, unlike their activating counterparts, KIR2DS1 and KIR2DS2, which are present in ~35% and 56% of European Americans, respectively.

We recently reported a strong association of KIR2DS1 and/or KIR2DS2 with development of psoriatic arthritis (PsA) (2). The effect was exacerbated in the absence of ligands for the corresponding inhibitory NK cell receptors, KIR2DL1 and KIR2DL2/3, respectively (odds ratio = 3.2, 95% confidence interval = 1.8–5.5, $p = 3 \times 10^{-5}$), compared with subjects lacking both KIR2DS1 and KIR2DS2). For example, individuals with KIR2DS1 were most strongly associated with PsA when the HLA-Cw group 2 ligands for KIR2DL1 were absent; the same was true of KIR2DS2 when HLA-Cw group 1 ligands for KIR2DL2/3 were absent. We concluded that the presence of an activating KIR was particularly detrimental when ligand for the corresponding inhibitory KIR was missing, in which case an inhibitory signal would not quench a potentially harmful activating signal.

We reconsidered the model tested in this study because theoretically, any inhibitory KIR-HLA ligand combination should be able to provide counteracting inhibition. The function of NK cells is regulated by positive and negative signals transmitted through paired activating and inhibitory receptors (5, 6). In vivo, NK cells are under the constitutive dominance of inhibitory receptors for self MHC class I ligands (7, 8). Thus, effector

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[4] Abbreviations used in this paper: KIR, killer Ig-like receptor; PsA, psoriatic arthritis.
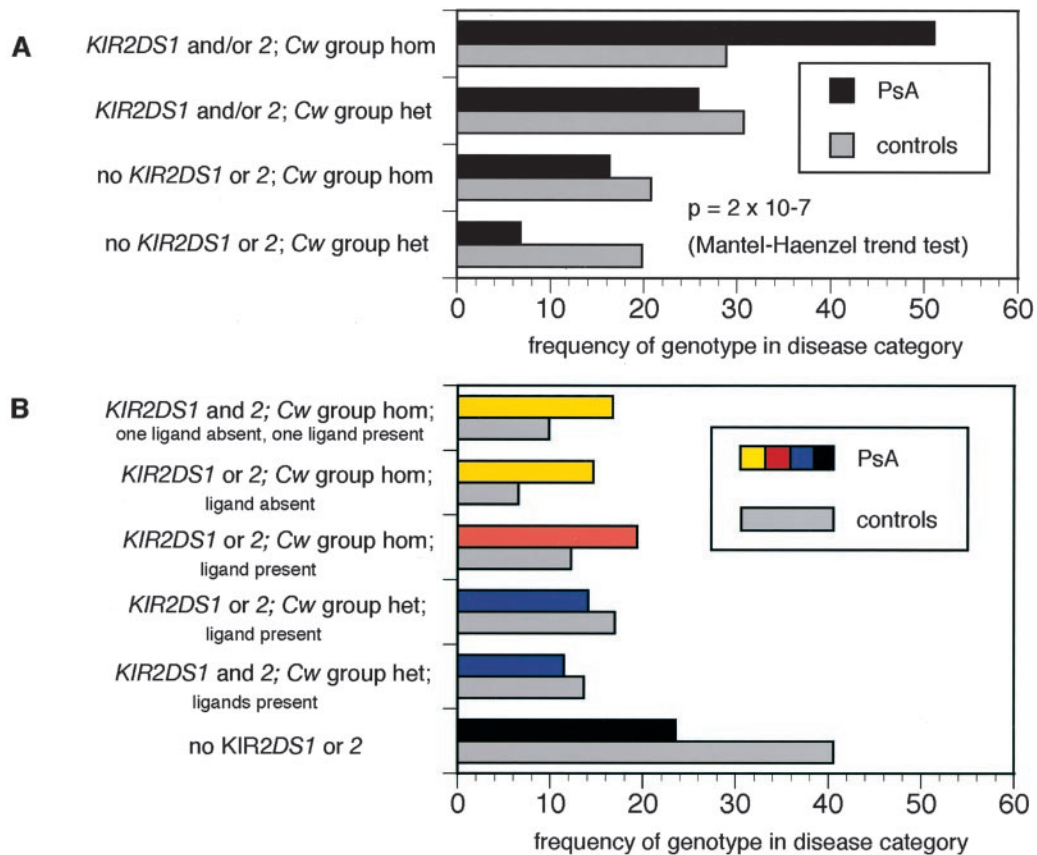
FIGURE 1. *A*, Trend toward increasing susceptibility to PsA with genotypes conferring increasing KIR-mediated NK cell activation. Subjects are divided into four groups according to the presence or absence of the activating *KIR2DS1* and/or *KIR2DS2*, and zygosity (hom, homozygosity; het, heterozygosity) for the *HLA-Cw* groups that serve as ligands for the inhibitory KIR2DL1 or 2DL2/3. The frequencies of each genotype among PsA patients (■) and controls (▨) are shown for each comparison group. Significance was determined from the Mantel-Haenzel trend test for a progressive difference in frequencies between groups, ranging from most susceptible (*top*) to most protected (*bottom*). The two *middle* groups were combined in the test for trend, as both represent intermediate susceptibility. *B*, Test of a genotypic group with different predicted PsA susceptibility under the two alternate models. Subjects predicted to be relatively protected under the old model but susceptible under the new model (red) are compared with subjects predicted to be susceptible under both models (yellow) and subjects predicted to be intermediate in terms of risk under both models (blue).

functions occur only when activating signals are able to overcome inhibitory signaling. This is achieved either by a predominance of activating receptor:ligand interactions or a lack of inhibitory receptor:ligand interactions (9). KIR are clonally expressed on NK cells in a stochastic manner such that each NK cell clone expresses only a portion of the genes within the genetic profile (8, 10). This predicts that, depending on the genotype, a given individual could have a relatively high frequency of NK cells that are: 1) primarily under the control of inhibitory receptors (most inhibition); 2) controlled by inhibitory and activating receptors fairly equally (relatively neutral); or 3) primarily under the control of activating receptors (most activation). In a similar vein, individuals who are missing ligands for some inhibitory receptors (as is the case among *HLA-Cw* group 1 or 2 homozygotes) will have fewer NK cells under inhibitory control than individuals who have all ligands present. Thus, an activating *KIR*, such as *KIR2DS1*, might be detrimental in terms of developing PsA if the ligand for either *KIR2DL1* or *KIR2DL2/3* is missing (i.e., homozygotes for either group of *HLA-Cw* ligands). Based on this reasoning, we propose a novel model that appropriately fits our understanding of KIR expression and function, and that shows more robust statistical support for the role of *KIR* in susceptibility to PsA than does our previous model.

Materials and Methods

Results and Discussion

We recently reported a strong association between *KIR* genotypes conferring NK cell activation and the development of PsA (2). Based on the model used in the analyses, we proposed that *KIR2DS1* and *KIR2DS2* (both activating receptors with unknown high affinity ligands) increase the risk of developing PsA, particularly when *HLA* class I ligands for the corresponding inhibitory *KIR* (*KIR2DL1* and *KIR2DL2/3*, respectively) were missing. However, based on further considerations (see *Introduction*), we now hypothesize that susceptibility to PsA increases progressively with increasing levels of KIR-mediated activation of NK cells, a phenomenon that increases both with the presence of certain activating KIR and with the absence of ligand for inhibitory KIR. The absence of ligand for inhibitory KIR occurs when individuals are homozygous for either *HLA-Cw* group 1 (ligands for KIR2DL2/3) or group 2 (ligands for KIR2DL1). Therefore, we tested for effects of *HLA-Cw*
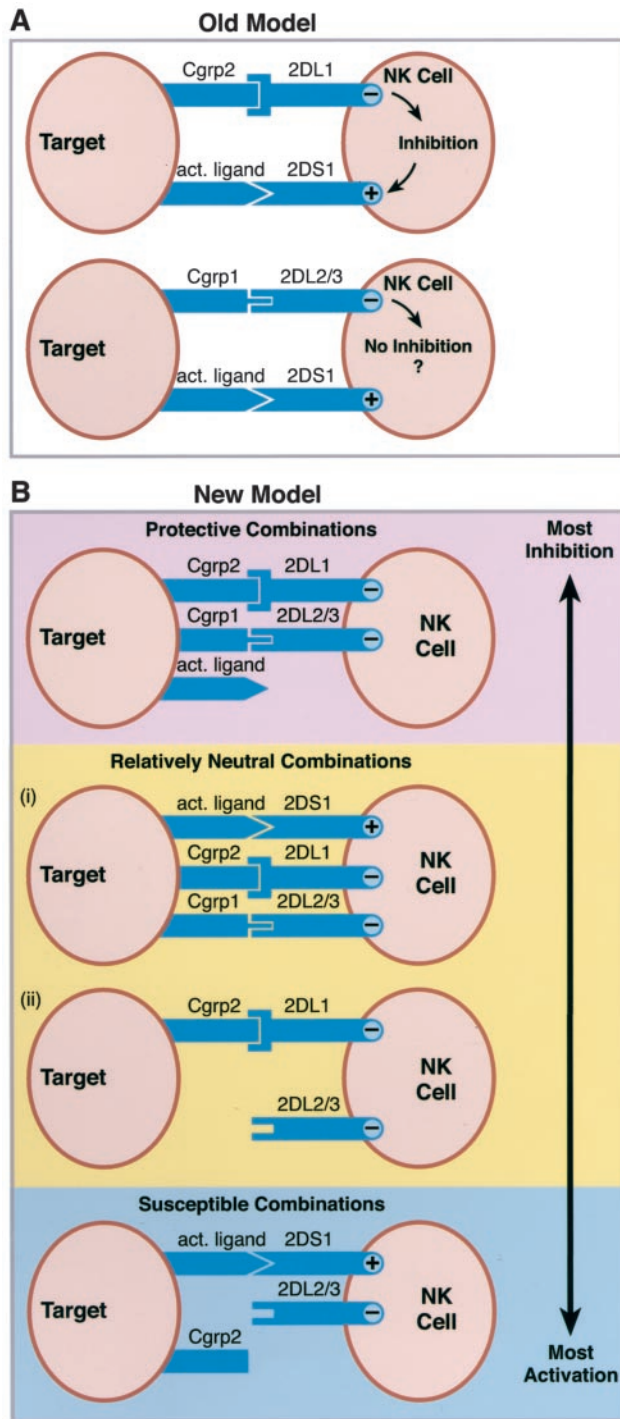
FIGURE 2. Susceptibility to PsA: old vs new model. *A*, Old model. The activity of an activating receptor (in this case, KIR2DS1) is quenched in the presence of ligand for the corresponding inhibitory receptor (KIR2DL1 + Cw group 2), but not in the presence of another inhibitory receptor-ligand combination (in this case, KIR2DL2/3 + Cw group 1). *B*, New model. The trend for susceptibility to develop PsA increases when genotypes are ordered by their ability to confer the most inhibition (protection) to the most activation (susceptibility). A putative protective genotype, *2DL1-2DL2/3; Cw* group 2-*Cw* group 1, characterized by the absence of activating *KIR2DS* in combination with heterozygosity for *HLA-Cw* group is shown in the *top panel* (pink). The *middle panel* illustrates two intermediate genotypes: *i*, *2DS1-2DL1-2DL2/3, Cw* group 2-*Cw* group 1, in which one or both activating *KIR2DS* are present in combination with *HLA-Cw* group heterozygosity; and *ii*, *2DL1-2DL2/3, Cw* group 2 in which no activating *KIR2DS* are present in combination with *HLA-Cw* group homozygosity (yellow). The *bottom panel* shows a putative susceptible genotype, *2DS1-2DL2/3, Cw* group 2, in which one or both activating *KIR2DS* are present, in combination with *HLA-Cw* group homozygosity (blue).

Table I. *Influence of KIR and HLA on psoriatic arthritis*

Allele/Gene	p
*HLA-B*27* (dominant)	0.0001
*HLA-Cw*0602* (dominant)	0.00003
KIR2DS1 and/or *KIR2DS2*	0.0001
HLA-Cw group homozygosity	0.005
KIR2DS1 and/or *KIR2DS2,* without ligand	0.80

group zygosity in combination with the presence/absence of *KIR2DS* genes based on genotypes expected to result in a range of NK cell activity from the most activating to the most inhibitory as follows: 1) *KIR2DS1* and/or *KIR2DS2*, *HLA-Cw* group 1 or 2 homozygous; 2) *KIR2DS1* and/or *KIR2DS2*, *HLA-Cw* group heterozygous; 3) no *KIR2DS1* or *KIR2DS2*, *HLA-Cw* group homozygous (in the test for trend, we combined groups 2 and 3, as there is no a priori reason why one should be more susceptible than the other); and 4) no *KIR2DS1* or *KIR2DS2*, *HLA-Cw* group heterozygous (Fig. 1*A*). A χ^2 test of 26.8 for trend ($p = 2 \times 10^{-7}$) was determined using this new model compared with $\chi^2 = 7.8$ ($p = 2 \times 10^{-5}$) in the old model (i.e., an activating *KIR* in the absence of ligand for corresponding inhibitory *KIR*).

Statistical support for the old model, although weaker than that for the new model, was still quite strong, probably because the levels of susceptibility hypothesized by the two models overlap somewhat. In fact, all individuals in the most susceptible group under the old model are also in the most susceptible group under the new model because an individual who lacks a ligand for either KIR2DL1 or KIR2DL2/3 (one of the defining characteristics of the most susceptible group in the old model) are necessarily homozygous for *HLA-Cw* group, because *Cw* group heterozygotes necessarily carry the ligand for both inhibitory receptors. In contrast, some individuals in the most susceptible group under the new model were not included in the most susceptible group under the old model. Specifically, under the old model, some individuals who were homozygous for *HLA-Cw* group were classified in an intermediate group. These included: 1) those who had *KIR2DS1* in the absence of *KIR2DS2* and were homozygous for *Cw* group 2 (i.e., the ligand for the corresponding KIR2DL1); and 2) those who had *KIR2DS2* in the absence of *KIR2DS1* and were homozygous for *Cw* group 1 (i.e., the ligand for the corresponding KIR2DL2/3). These individuals are classified under the most susceptible group in the new model, because they are homozygous for a *Cw* group and positive for *KIR2DS1* and/or *KIR2DS2*. Thus, the level of susceptibility of these particular genotypes allows direct determination of the model that best fits the genotypic data. As shown in Fig. 1*B*, individuals who are homozygous for *Cw* group are at increased risk of developing PsA regardless of whether ligand for corresponding inhibitory KIR is present or absent (compare the top three sets of bars, where yellow represents individuals susceptible in the old model, and where yellow and red combined represent individuals susceptible in the new model). All three sets of homozygous individuals show very similar levels of susceptibility to one another, but they are distinct from groups who are heterozygous for *Cw* group (blue bars). These data fit the prediction of the new but not the old model.

The primary difference between the old and new models is the hypothesis regarding the interaction between activating and

inhibitory receptors. In the old model (Fig. 2*A*), the assumption was made that only a corresponding inhibitory receptor could quench the activity of an activating receptor. Therefore, activation mediated by KIR2DS1, for example, could be quenched by KIR2DL1, but not by KIR2DL2 (Fig. 2*A*). The new model, in contrast, does not make that assumption; rather, it proposes that the trend for susceptibility to develop PsA increases when genotypes are ordered by their ability to confer the most inhibition (protection) to the most activation (susceptibility) (Fig. 2*B*).

We further tested five explanatory factors (*HLA-B*27*, *HLA-Cw*0602*, presence of *KIR2DS1* and/or *KIR2DS2*, presence of *KIR2DS1* and/or *KIR2DS2* in the absence of *HLA-Cw* group ligand for the corresponding inhibitory *KIR*, and *HLA-Cw* group homozygosity) in a multiple logistic regression (Table I). The presence of *KIR2DS1* and/or *KIR2DS2* is highly significantly associated with susceptibility to PsA, as are the established factors *HLA-B*27* and *HLA-Cw*0602* ($p \leq 0.0001$). *HLA-Cw* group homozygosity is also significantly associated with increased risk of developing PsA ($p = 0.005$), but the presence of *KIR2DS1* or *KIR2DS2* in the absence of *HLA-Cw* group ligand for corresponding inhibitory KIR showed no association ($p = 0.80$) in the multiple regression analysis. We also considered the possibility that, like heterozygosity for *HLA-Cw* group 1 and 2, *HLA-B Bw4*, the ligand for KIR3DL1, may provide additional protection among individuals with *KIR3DL1*. However, multiple logistic regression analysis indicated no protective effect of *HLA-B Bw4* among homozygotes for *Cw* group, heterozygotes for *Cw* group, or all subjects combined. Thus, protective inhibitory effects against PsA appear to be restricted to KIR2DL-mediated inhibition. In light of our current understanding of KIR expression and function, a model proposing that a combination of *KIR2DS1* and/or *KIR2DS2* plus *HLA-Cw* ligand group homozygosity (a situation where the inhibitory signal is diminished) confers susceptibility to PsA is clearly favored.

It is essential to continually pursue plausible models for data pertaining to complex genetic loci (such as *HLA* and *KIR*) in human diseases. If logical intelligent models are used in data analysis, the genetic studies will be an invaluable tool for directing future biological studies. One general model is unlikely to fit data derived from different types of diseases, and as our understanding of KIR biology advances, we must reconsider and possibly modify our genetic models. This approach will serve to strengthen and clarify the genetic effects of these polymorphic loci on human disease.

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