Killer cell immunoglobulin-like receptors and exercise

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ABSTRACT

Exercise can alter human health in both beneficial (e. g. reduced risk of infection and of atherosclerosis) and adverse (e. g. anaphylaxis, exercise-induced asthma, and exacerbation of chronic illness) ways. Hitherto, the mechanisms linking exercise and health are not fully understood, but may rest on the capability of exercise to both increase circulating immune cells and modulate their activity. Natural killer (NK) cells, a major component of innate immunity, are one of the most sensitive populations of immune cells to exercise stress. NK cells play an important role in the detection and elimination of tumours and virus-infected cells. To mediate NK cell functions, there is an array of activating and inhibitory receptors with distinct specificities on their surface. Killer-cell immunoglobulin-like receptors (KIRs) which bind to MHC class I are a key example of receptors expressed by NK cells. The combination of MHC class I and KIR variants influences resistance to infections, susceptibility to autoimmune diseases, as well as complications of pregnancy. It is suggested that KIRs may also determine a considerable part of the effects of physical activity on human health. In this review we discuss KIRs in more detail, their role in the onset of human diseases, and the influence of acute exercise on KIR gene expression.

Key words: Killer cell immunoglobulin-like receptors (KIRs), NK cells, exercise, stress response

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INTRODUCTION

It is known that exercise as brief in duration as 6 min can mobilize leukocytes (44). Thus, such physical activity-related increase in circulating innate immune cells can happen many times in the daily lives of humans (10). The striking sensitivity of natural killer (NK) cells to exercise stress provides strong support that these cells may be implicated as a potential link between regular physical activity and overall health status (55). NK cells use many types of cell-surface receptors to recognize and to destroy virally-infected or malignantly transformed cells without prior sensitization (9, 37, 58). Inhibitory receptors of NK cells bind major histocompatibility complex (MHC) class I and thus protect healthy, class I-expressing cells from inappropriate NK cell aggression. Activating receptors specifically recognize various molecules that are upregulated on cells stressed by infection or malignant transformation, many of which are MHC class I related. In man, the largest family of receptors for MHC class I ligands expressed by NK cells (and small subsets of T cells) are the killer-cell immunoglobulin-like receptors (KIRs). The KIR family contains multiple inhibitory and activating members (3, 4). The combination of MHC class I and KIR variants influences resistance to viral infections, nonviral pathogens, susceptibility to autoimmune diseases, complications of pregnancy, as well as outcome of haematopoietic stem-cell transplantation (4, 19, 30, 37).

KIRs are categorized on the basis of structural features of the extracellular domain (2D or 3D reflecting the number of immunoglobulin-like domains) and the length of the cytoplasmic tail (L or S for long and short, respectively) (4, 25). KIR function can be predicted from the length of the cytoplasmic domain: long-tailed KIRs are generally inhibitory, whereas all short-tailed KIRs are activating. The only exception to this rule is KIR2DL4, which is a unique activating receptor with a long cytoplasmic domain.

Variability in organization of the *KIR* gene complex

Gene families that encode immunoglobulin-like receptors are located within the leukocyte-receptor complex. The boundaries of the *KIR* locus on chromosome region 19q13.4 are the *KIR3DL3* and *KIR3DL2* genes (17, 61, 67). Between these conserved genes lies a variable set of *KIRs*, commonly containing 7–12 genes. Numerous haplotypes with different content of *KIRs* are present in the human population (62, 67). Haplotypes with identical gene content are further differentiated by polymorphisms of the component genes (37). For some genes over 50 different alleles have been described (56). The consequences of variable gene content and allelic polymorphism are that unrelated individuals rarely have identical *KIR* genotypes and that ethnic populations differ markedly in their distribution of *KIR* genotype frequencies (37, 46).

Despite the extreme variability, some systematic features in the organization of the *KIR* gene complex can be defined. All haplotypes contain at least one *KIR* gene encoding an activating receptor (61). Among the stimulatory *KIR* genes, *KIR2DS4* is much more frequently found in the Caucasian population than any other stimulatory *KIR*. It is suggested that *KIR2DS4* carries out a specific function, which cannot be fully compensated by replacement with one of the other

stimulatory *KIRs*. Four *KIR* genes are held in common by virtually all haplotypes: *KIR3DL3, KIR2DL4, KIR3DL2*, and the pseudogene *KIR3DP1* (17, 61, 67). According to their gene content all haplotypes can be divided in two groups, A and B (Fig. 1) (61, 62). The simpler group A haplotypes have a common organization of seven genes and two pseudogenes but are distinguished by allele combination (62). In contrast to the A haplotypes, the B haplotypes have a more variable gene content. More than 20 different B haplotypes have been described, which in addition to genes that are present in group A haplotypes, include *KIR* genes that are unique to group B haplotypes: *KIR2DL5A (KIR2DL5B), KIR2DS1, KIR2DS2, KIR2DS3* and *KIR2DS5* (4, 61, 62). Genes *KIR2DL2* (an allele of *KIR3DL3*) and KIR3DS1 (an allele of *KIR3DL1*) are also specific to group B haplotypes. Most group-B-specific *KIR* genes encode activating receptors. In general, group B haplotypes contain more genes that encode activating *KIRs* than do group A haplotypes. All human populations have both group A and group B haplotypes, although their frequencies vary (37). In Caucasians group A and group B haplotypes are present at an approximately equal frequency (58% group A haplotypes, and 42% group B haplotypes). It is worth noting that the diversity of group A haplotypes is mainly due to allelic polymorphisms, including copy number variation, whereas group B haplotypes are both polymorphic and polygenic (56, 61). The variety of *KIRs* in copy number variation can lead to changes of transcripts levels through gene dosage (23, 56, 69). Such a high level of diversity probably reflects strong pressure from pathogens on the human NK/T cell immune response (19, 56).

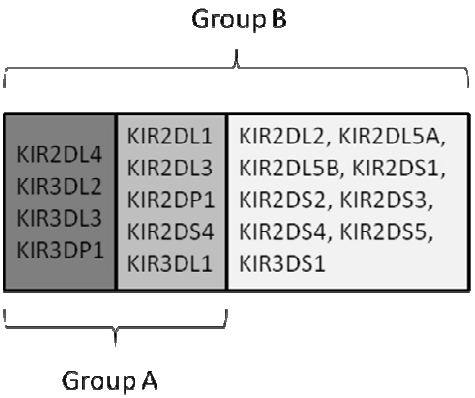

Figure 1. Group organisation of human *KIR* haplotypes. Activating *KIR* genes containing the gene name "S", inhibitory – "L", pseudogenes – "P". *KIR* genes, which are conservative for virtually all haplotypes, are in grey. Genes that can be present in both group A and group B *KIR* haplotypes are in light grey. Genes (and/or alleles) that are specific to group B *KIR* haplotypes are in white.

The *KIR* gene sequences, including intergenic regions, are highly conserved with exception of *KIR2DL4* (67). The high level of homology could facilitate non-reciprocal recombination, an evolutionary mechanism that can delete, duplicate or recombine genes (37). Such mechanisms may be behind a variation in number of immunoglobulin exons in some members of the *KIR* family, a generation of novel hybrid genes, as well as gain and loss of genes (4, 56, 67). Based on the genomic sequences, three hybrid genes exist: *KIR2DL5A/3DP1* (termed *KIR2DL5B*), *KIR2DL1/2DS1,* and *KIR2DL3/2DP1* (26, 56). Recombination processes may be facilitated by repeated elements, which exhibit dense clustering within *KIR* gene introns (56). It was suggested that such plasticity of the *KIR* complex allows a relatively rapid form of natural selection (4).

Regulation of *KIR* gene expression

KIR expression is restricted to NK cells and small subsets of T cells (2, 32, 63). The pattern of *KIR* gene expression is quite complex. The expression of a particular *KIR* (or its alleles) is largely independent of the expression of any other *KIRs* (7, 64). Moreover, each NK cell clone expresses only a portion of the set of KIRs encoded in a given individual's genome. The *KIR* genes are seemingly expressed stochastically, with the exceptions of *KIR2DL4*, which is expressed by all NK cells (37, 64). However, NK cell clones maintain a once established *KIR* expression pattern through multiple cell divisions (64). Thus, every NK cell stably expresses an apparently random combination of the available *KIR* genes. This combinatorial expression of genes is unique in human biology, and is essential to create a diverse and sensitive repertoire of NK cell specificities.

To comprehend how the *KIR* repertoire is generated and maintained, it is crucial to understand the regulation of *KIR* gene expression. Some explorations of *KIR* promoter regions were accomplished (52, 57, 68). Initially, close examination of the *KIR* region showed that the sequences upstream of the transcribed region are highly homologous (>91%), with the exception of the *KIR2DL4* gene, suggesting similar transcription regulation among the genes (59, 67). However, lately, *KIR* promoters were divided into four differently regulated groups, two of which control clonally expressed *KIR* genes, while one is unique for *KIR2DL4* (the only *KIR* gene transcribed in all NK cells), and one for the weakly expressed *KIR3DL3* (5, 52, 57). The differences in these promoters, including variations of transcription-factor binding sites, could explain altered patterns of expression. More recently it was established that *KIR* genes have two promoters: a distal promoter with weaker activity and proximal promoter (13, 23, 53). The latter one is bidirectional which leads to competing forward and reverse promoter activities resulting in a synthesis of sense and antisense transcripts, respectively. The *KIR2DL4* is unique because it is the only *KIR* gene lacking the repeat region and containing an activating element in the first intron (57, 67). It has been established that transcription starts with *KIR2DL4* which opens up the *KIR* locus, ensuring access of the transcription machinery to other *KIR* genes (37, 57). Then, using an unknown mechanism, NK cells express different combinations of *KIR* genes (64). It appears that transcription of *KIRs* occurs in a stochastic manner. However, the subset of *KIR* genes that are expressed by a particular NK cell becomes fixed through methylation in the 5' area of unexpressed *KIR* genes, and the pattern of expression is passed on to daughter cells during cell division (7, 43).

Cytotoxic T cells express *KIRs* in a similar manner to NK cells (49, 63, 65), but the transcriptional control of *KIR* expression differs between NK and T cells (68). This fact emphasizes the biological significance of *KIR* expression in T cells. Although *KIR* expression correlates with T cell differentiation – even naïve T lymphocytes have the transcriptional machinery to support the activation of the minimal *KIR* promoter – it was established that epigenetic mechanisms such as DNA methylation also play an important role in determining *KIR* expression in T cell subsets (24). It is noteworthy that signalling through KIRs expressed by T cells differs from KIR signalling in NK cells (50).

Human diseases and combination of MHC class I and KIR variants

NK cells play a role in the innate immune response that occurs in the early phase of infection. In particular, they are important for helping to clear viral infection (35). They kill infected cells, secrete inflammatory cytokines and interact with dendritic cells to determine a moment when an adaptive immune response should start (31). In functioning as NK cell receptors for MHC class I molecules, KIRs work together with the conserved lectin-like receptors CD94-NKG2A (NK group 2, member A; an inhibitory receptor) and CD94-NKG2C (an activating receptor) (64). All NK cells are non-responsive towards healthy autologous cells, a tolerance that involves the interaction of at least one autologous human leukocyte antigen (HLA) class I isoform with an inhibitory KIR or CD94-NKG2A. It is interesting to note that inhibitory signalling can not only prevent NK cell-mediated cytotoxicity, but also interfere with adhesion of NK cells to target cells (38). The balance of signals from activating and inhibitory receptors can be influenced by changes in surface expression levels of ligands on the target cells, which can alter the overall activation threshold of NK cells. Therefore, despite the supposed stochastic nature of KIR expression and the independent inheritance of *KIR* genes and genes encoding HLA, some regulatory link between the HLA repertoire and KIR expression evidently exists (4, 41, 69).

The lectin-like receptors have a broader view and recognize complexes of HLA-E and peptides cleaved from the leader sequences of HLA-A, HLA-B, HLA-C and HLA-G (30). Receptors of the KIR family are expressed on later stages of NK cell development than CD94-NKG2 (37). In contrast to CD94-NKG2, individual KIRs recognize distinct subsets of the classical human MHC-I molecules (30, 41). Together, the different inhibitory KIRs possess the capability to recognize 100% of the known HLA-C allotypes and subsets of HLA-A and HLA-B allotypes (41). The inhibitory KIR2DL2/2DL3 and the KIR2DL1 molecules are receptors for two mutually exclusive groups of HLA-C allotypes, HLA-C1 and HLA-C2, respectively (4, 32). HLA-C2 with KIR2DL1 is the combination expected to provide the strongest inhibition, and is apparently associated with lung cancer (1, 37). KIR3DL1 binds with HLA-B Bw4 allotypes (5, 34, 41). An increased frequency of KIR3DL1 and its ligand has been observed in kidney cancer patients compared with normal controls (1). Different alleles of *KIR3DL1* vary in terms of cell surface expression and strength of inhibitory signalling (4). KIR2DL4 interacts with HLA-G, which is upregulated in some tumour cells and under conditions of inflammation. KIR3DL2 is only known to recognize HLA-A3 and HLA-A11 allotypes (41). The ligands for KIR2DL5 and KIR3DL3 remain to be determined.

Based upon the high homology between the extracellular domains of activating and inhibitory KIR receptors (~99%), it was reported that activating KIRs recognize the same HLA molecules as their inhibitory counterparts, but with significantly weaker affinities (41, 51). However, the activating KIR-HLA affinities may be enhanced by specific peptides presented on the HLA molecules (41). Such enhancement has been observed for KIR2DS1 under its interaction with Epstein-Barr virus-infected cells, KIR3DL1 binding with HLA-B, and KIR3DL2 recognizing HLA-A3/-A11 (15, 51, 54). Interestingly, an activating signal generated by

a weaker interaction of KIR2DS1 with HLA-C2 can mute a stronger inhibitory signal from KIR2DL1 (37, 41). It appears that this effect may be explained by the same mechanism. Alternatively, activating KIRs may bind entirely distinct ligands and may be involved in the recognition of pathogen structures (41, 61). Thus, KIR2DS4 has been shown to recognize a non-MHC-I polypeptide on the surface of melanoma cells. Along this line, it was suggested that activating KIR receptors are involved in MHC-independent recognition of herpes simplex virus-infected cells (39).

A number of studies have reported associations between distinct *KIR*/*HLA* compound genotypes with susceptibility or resistance to viral infections. It was ascertained that homozygosity for both *KIR2DL3* and group HLA-C1 allotypes, providing lower inhibitory signals, is associated with increased resistance to hepatitis C virus infection (18). A lower frequency of *KIR2DL2* and/or *KIR2DL3* in combination with HLA-C1 ligands was found in patients with chronic hepatitis B compared with healthy controls (12). For infection with HIV, the progress to AIDS is slower in patients who have activating *KIR3DS1* in combination with HLA-B *Bw4-801* and an inhibitory *KIR3DL1*004* allele in combination with HLA-B *Bw4* (27, 29).

While haplotypes containing multiple activating *KIRs* may mediate a protective NK cell response against infectious disease, these same haplotypes may also predispose for autoimmune disease (28, 37). It has been found that activating *KIR2DS1* and/or *KIR2DS2* genes and group B *KIR* haplotypes are present in higher frequency in patients with certain autoimmune diseases than in healthy individuals (37). In the case of psoriatic arthritis, individuals carrying (activating) *KIR2DS1* and/or K*IR2DS2* genes show increased susceptibility to the onset of the disease, but only when one or both ligands of their homologous inhibitory receptors *KIR2DL1* and *KIR2DL2* (or *KIR2DL3*) are missing (28, 34). Absence of ligands for inhibitory KIRs could potentially lower the threshold for NK and/or T cell activation mediated through activating receptors, thereby contributing to pathogenesis. It was inferred that the trend for susceptibility to develop psoriatic arthritis increases when genotypes are ordered by their ability to confer the most inhibition (protection) to the most activation (34). Further, an influence of *KIR/HLA-C* gene combinations on type I diabetes and scleroderma was shown (37). Interestingly, acute coronary syndrome and rheumatoid vasculitis were associated with expression of *KIR2DS2* by clonally expanded populations of $CD4^{+}CD28^{null}$ T cells (70). In these diseases T cells expressing *KIR* genes are directly implicated in the disease mechanism. This fact brings up the question about a role of NK-cell responses for KIR-associated autoimmunity (37). It is noteworthy that an array of studies have described *KIR*/*HLA* compound genotypes that are associated with susceptibility to certain cancers (37, 41).

Since the interactions of KIRs with cognate HLA ligands can dramatically influence overall responsiveness of NK and T cells expressing these receptors, they have the potential to influence both the innate and adaptive immune response. Since we know that exercise has effects on both parts of the immune response, the question arises, what roles KIRs may play in the effects of physical activity on human health.

KIR gene expression and exercise

A rapid increase in circulating numbers of lymphocytes, in particular NK cells, with the onset of exercise is a well-documented phenomenon (10, 21, 44, 50) as is a change in the gene expression profile following exercise (6, 8, 11, 42, 48). However, there are only a handful of studies providing information about the effect of exercise on *KIR* genes.

In a recent study, Radom-Aizik et al. tested the alteration of gene expression in peripheral blood mononuclear cells of early- and late-pubertal girls using Affymetrix GeneChip technology (42). These authors found that four *KIR* genes, encoding three inhibitory receptors, KIR2DL3, KIR3DL1, and KIR3DL2, and one activating receptor KIR2DL4, had higher expression after exercise (2.3-3.0 fold). Blood samples were drawn before and after exercise consisting of ten 2-min bouts of constant-workrate cycle ergometry (the workrate was roughly halfway between the anaerobic threshold and peak oxygen uptake). Only insignificant differences in fold changes of *KIR* gene expression between the two groups of girls was observed. Earlier, Büttner et al. (6) had found that the *KIR2DS4* (activating) gene was upregulated more than 1.3 fold in their microarray analysis of exercise-induced changes of gene expression profiles of blood leukocytes. Only young men participating in leisure time sports were recruited for this study. The participants performed a strenuous treadmill exercise test at ~80% of maximal oxygen uptake (VO_{2max}) until exhaustion. In contrast to this work, Connolly et al. (8) reported down regulation of the *KIR2DS4* gene after 30 min exercise at ~80% of VO_{2max} in blood samples of untrained men. More recently, our laboratory has investigated the impact of high intensity exercise on gene expression by blood leukocytes. We examined the transcription response of male athletes after a ramp type treadmill test with an incremental step protocol, where the workrate is progressively increased until exhaustion, using GeneChip Human Gene 1.0 ST Arrays (unpublished data). In this kind of test, athletes perform at an exercise intensity above the anaerobic threshold for a rather long time (4-6 min). The results of our microarray analysis indicate that some genes of the *KIR* locus are upregulated more than 1.8 fold. Unfortunately, we cannot extract isolated data for individual *KIR* genes from our results due to the fact that *KIR*-specific probes on these arrays are common for several *KIR* genes.

Thus, so far, existing data are quite restricted and not entirely consistent. One can suggest that the dissimilarity of results may be caused by differences in gender and exercise intensity or duration. Thus, it was reported that the expression level of the *KIR3DL3* which is present in all haplotypes, was higher in females than in males (60).Thereto, the *KIR3DL3* transcript was detected in the $CD56^{bright}$ subset of NK cells as opposed to $CD56^{dim}$ NK cells. Consequently, a different mobilization of these two NK cell subsets during exercise (50) might result in various effects on *KIR3DL3* gene expression. Our preliminary exploration allows us to suggest that training levels of participants may also bias changes of *KIR* expression after exercise (47). It is important to note that global changes in NK cell numbers among total lymphocytes after exercise were not taken into account in all above mentioned studies. The number of NK cells may significantly vary in blood samples of different individuals both before and after exercise (14). In addi-

tion, the dissimilarity of results may be related to the high degree of polymorphism in the *KIR* gene family. As mentioned above, allelic variation was observed for most *KIR* genes (62) and some of the promoter polymorphisms lead to loss of transcription factor binding sites and affect the frequency of gene expression (23).

At this point we like to stress that exercise can obviously induce transcription of both, genes encoding activating and genes encoding inhibitory KIRs. Since KIRs are the major set of receptors determining the functional activity of NK cells, it is justifiable to infer that modulation of *KIR* expression by exercise may have the potential to influence the functional state of NK cells in both directions: activation or inhibition. This may seem to be a paradox or it could be arbitrary, merely reflecting the proposed stochastic nature of *KIR* expression. We would, however, argue that it also looks suspiciously like a mirror of the known dichotomous overall effects of exercise on the immune system. These are namely immune enhancement expressed as increased resistance to infection and certain cancers and immunosuppression expressed as increased susceptibility to infection following exhaustive exercise and as reduced chronic low grade inflammation with regular exercise. As we know, the effects of regular moderate exercise are highly beneficial to health, and there is solid evidence to suggest that NK cells may play an important role in this. After all, it is their task to kill virus infected cells and cancer cells, and improvement of NK activity through exercise has been documented *in vitro* (9, 16, 20, 33, 45). NK cells are also important producers of interferon (IFN)-γ, a cytokine which has the potential to amplify inflammatory cytokines (9). Suppression of IFN-γ release through exercise has also been shown (66). In addition, recently, persuasive evidence was provided that the *KIR* genotype predicts the capacity of NK cells to provide IFN-γ in response to various stimuli (19). Thus, in spite of the proposed stochastic nature of *KIR* expression it is tempting to speculate that the proven modulation of the functional state of NK cells through exercise may somehow be related to the observed modulation of *KIR* expression through exercise.

Thus, we like to hypothesize that, in the end, modulation of *KIR* gene expression by exercise may be involved in mediating the beneficial effects of chronic moderate exercise on our health. To test this hypothesis, the possible existence of discriminating regulatory mechanisms for different *KIRs* would be a key point to explore. When looking for possible triggers of *KIR* expression, different candidate molecules may be considered: (i) cytokines, which may be locally effective, although *KIR* gene expression seems to be largely independent of systemic cytokine levels (47); (ii) low molecular weight compounds/metabolites released into plasma upon exercise (22); (iii) proteins released from defective or stressed muscle, or, (iv) transcription factors activated through heat or hypoxia. In context of the latter it is noteworthy that a recognition site for heat shock transcription factor 1, which is involved in induction of heat shock proteins, was found in the *KIR2DL1/S1* promoter (56).

Knowledge of such mechanisms could greatly increase our understanding of the effects of physical exercise on chronic inflammatory diseases and might help to optimize exercise prescriptions to confer health benefits or even open up new opportunities to use exercise as adjunct to therapy in fighting infection, cancer or autoimmune disease.

Based on the structural complexity and high diversity of the *KIR* region, it seems that an individual's *KIR* repertoire may be very relevant for his or her gene expression response to exercise. A recent study revealed an influence of the *KIR* genotype on the ability of NK cells to respond to nonviral infections (19). Other studies emphasize the importance of distinguishing between alleles of *KIRs,* as well as between alleles of genes encoding their specific HLA ligands in disease studies (23, 29, 71). Therefore, the *KIR-HLA* compound genotype deserves consideration in *KIR*-exercise-disease associated research.

CONCLUDING REMARKS

Undoubtedly, much more work is needed to clarify the exact change of *KIR* gene expression patterns in response to physical activity and to determine what kind of conditions can influence this change (e.g. exercise intensity and duration, sex, puberty, training status, ageing). Two of the most intriguing questions also remain open: (i) what is the trigger of *KIR* expression changes in response to exercise and (ii) does the *KIR* genotype exhibit a great influence on the extent of *KIR* gene transcription activation? In the light of huge interest in the clinical role of NK cells and mounting evidence of the broad medical relevance of *KIRs*, to gain an insight into these questions is a fruitful task for the future studies. Changes in *KIR* gene expression caused by exercise may turn out to be a relevant immunotherapeutic marker reflecting peculiarities of the organism, which may be exploited for individual optimization of a programme of regular training or an adjunct exercise therapy.

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