Immunity
Meeting Report

Killer Cell Immunoglobulin-like Receptor Workshop: Insights into Evolution, Genetics, Function, and Translation

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The seventh killer cell immunoglobulin-like receptor (KIR) workshop was held at Tammsvik, Stockholm, Sweden, in the summer of 2011. This intimate and isolated setting brought together approximately 100 investigators, from a range of scientific disciplines, who are all actively working on KIRs in humans or closely related primate species.

Introduction

After the discovery of natural killer (NK) cells in the early 1970s, tumor immunologists turned to the mechanisms by which NK cells distinguish healthy cells from diseased cells. Persistent exploration led to appreciation that NK cells preferred targets cells that are lacking, or "missing," self-MHC class I (Kärre et al., 1986). Subsequent discovery of inhibitory MHC class I receptors on mouse and human NK cells (Moretta et al., 2006) gave an elegantly satisfying solution to the puzzle of the "missing-self" phenomenon. To everyone's surprise, the mouse Ly49 and human killer cell immunoglobulin-like receptors (KIRs) that recognize MHC class I were seen to be products of convergent evolution, forewarning the extraordinary diversity and species specificity of these primate-specific NK cell receptors. Recognizing the evolutionary chasm that separates mouse Ly49 from human KIR served to stimulate intensive, cooperative study of the human system for exploration of the biology and clinical relevance of the KIRs and their interactions with HLA class I.

In 2001, John Trowsdale held an informal gathering at Emmanuel College Cambridge, UK, for the small but growing number of workers in the field. Thus began the "KIR workshops" where, within a framework of short talks embracing all participants, the latest and mostly unpublished data are vigorously discussed. In the open atmosphere of these intimate meetings, many key contributions to KIR biology have first been reported, taking the research in diverse and complementary directions. These include evolution, population genetics, reproduction, infection, and, of course, cancer. This summer, the seventh KIR workshop was held at an isolated conference center outside of Stockholm, Sweden (Figure 1). In reporting here on the achievements of the workshop, we cover the latest advances and review the state of the art in this rapid evolving field of immunological research.

Evolution of KIRs

Comparison of KIR and MHC gene families in mammalian species demonstrates their rapid evolution under forces of natural selection and genetic drift (Parham, 2008). That structurally unrelated KIR and Ly49 receptors with analogous MHC class I binding function evolved independently highlights the importance and plasticity of this function. Whereas mice, rats, and horses express variable Ly49, cattle, monkeys, apes, and humans express variable KIRs. Strong species specificity to the evolution of KIR and Ly49 receptors probably reflects distinctive and competing selections imposed on NK cells by their contributions to immune defense and reproduction, functions necessary for individuals, populations, and species to survive.

From comparison of representative primate species, Laurent Abi-Rached (Stanford, USA) proposed a model for the emergence and diversification of KIR genes in primates. Whereas simian primates (monkeys, apes, and humans) have diverse KIRs, prosimian primates have a single nonfunctional KIR gene. Thus, expansion of one ancestral *KIR3DL* to become a diverse and rapidly evolving gene family occurred only in simian primates.

The ligand-binding specificities of human (and ape) KIRs are restricted to the A3/11, Bw4, C1, and C2 epitopes carried by subsets of HLA-A, -B, and -C allotypes. Old World monkeys, including rhesus macaques, have counterparts to HLA-A and HLA-B but not to HLA-C, as described by Peter Parham (Stanford, USA). Unexpectedly, nine rhesus macaque KIR3DL bound strongly to human HLA-C, but only weakly to HLA-A, and with intermediate strength to HLA-B. Emerging in hominoids from an HLA-B like ancestor, HLA-C has evolved under natural selection to become an inherently better KIR ligand than HLA-A or -B. Whereas every HLA-C allotype is a KIR ligand, balancing selection maintains only one-third of HLA-A and -B allotypes as KIR ligands, whereas two-thirds are dedicated T cell receptor ligands.

Continuing her analysis of KIR haplotype structure, Lisbeth Guethlein (Stanford, USA) showed that structural variation in orangutan and chimpanzee KIR haplotypes is restricted to the centromeric region of the KIR locus. In contrast, human KIR haplotypes uniquely display structural variation in both the centromeric and telomeric regions. Different gene-content motifs in the centromeric (Cen-A and Cen-B) and telomeric (Tel-A and Tel-B) regions combine to form the group A and B KIR haplotypes, which also are unique to humans.

Figure 1. The Tammsvik Conference Site Just outside Stockholm where the Seventh KIR Workshop Was Held

Population Genetics of KIRs

That all human populations have both group A and B KIR haplotypes points to them having important and complementary functions. Although their relative frequencies vary, balancing selection maintains the A and B KIR haplotypes even in small geographically isolated populations such as the Yucpa Amerindians (Parham, 2008).

HLA and KIR are functionally interacting gene families situated on chromosomes 6 and 19, respectively. Because both are exceptionally polymorphic, the combinatorial diversification of their functional interaction is enormous. Paul Norman (Stanford, USA) compared this potential in two distinctive African populations: the older, smaller Khoisan from southern Africa and the younger, larger Ghanaian from West Africa. Despite different demographic histories, they displayed similar functional potential. Individuals have at least 1, a mean of 8, and a maximum of 15 different HLA-KIR interactions. Although overall functional diversity was similar, a striking difference was a unique and dominant form of KIR2DL1 in the Khoisan that has C1 specificity instead of the C2 specificity generally associated with this KIR.

The close head-to-tail organization of the KIR genes facilitates nonhomologous recombination with evolution of new KIR haplotypes through gain and loss of genes. Developing new technology that types for both presence and copy number of KIR genes, James Traherne (Cambridge, UK) uncovered KIR haplotypes displaying unique examples of gene expansion or contraction, representing up to 4% of Caucasian KIR haplotypes.

By sequencing coding regions for the 14 functional KIR genes and determining their phase, Carolyn Hurley (Washington D.C., USA) defined allele-level KIR haplotypes in 76 European Americans, with gene content frequencies similar to those reported for other Caucasian panels. Three factors contribute to haplotype diversity: random association of centromeric and telomeric gene-content motifs, formation of new gene-content motifs, and mutation for generating new alleles of the individual KIR genes. Cynthia Vierra-Green (Minneapolis, USA) similarly described 1012 KIR haplotypes in a panel of 506 Caucasians. These haplotypes involved association of nine centromeric and eight telomeric motifs, with three centromeric (Cen-A1, -B1, and -B2) and two telomeric (Tel-A1 and -B1) motifs accounting for 972 (96%) of them. Such high-resolution KIR analyses are necessary for achieving precise definition of KIR variation in human populations and accurate assessment of disease and other clinical associations.

Regulation of KIR Repertoires

Transcription of the KIR locus occurs late in NK cell development. *KIR2DL4*, the central framework gene, is transcribed first, and the other KIR genes subsequently transcribed. As the result of a complex and only partially understood process, each KIR is expressed by a subset of NK cells and individual NK cells express different combinations of KIR. The variegated patterns of KIR expression are stabilized by methylation of the nonexpressed genes and produce a diverse NK cell repertoire. KIR genes can have two or more promoters, which produce competing sense and antisense transcripts that determine the frequency with which each KIR is expressed in the NK cell population.

In progenitor but not mature NK cells, Frank Cichocki (Minneapolis, USA) identified antisense transcripts originating from the second intron of the *KIR2DL1*, *KIR2DS1*, and *KIR3DL1/S1* genes. Induction of this antisense from the *KIR2DL1* gene shuts of production of the KIR2DL1 protein. Such results suggest intron 2 antisense transcripts serve to silence KIR genes in immature cells. Furthermore, Lutz Walter (Göttingen, Germany) and collaborators identified a potential binding site for micro-RNA miR-24 33–55 base pairs upstream of the canonical AUG start codon of KIR genes. Knockdown of endogenous miR-24 by an anti-miR-24 construct induced transcription of KIR genes in HeLa and 293 cell lines, pointing to a role for this miR in regulation of KIR expression.

Martin Ivarsson (Stockholm, Sweden), showed that KIR expression was detectable on fetal NK cells by the second trimester, particularly in the lung. The unique exposure of fetal lung NK cells to amniotic fluid raises the intriguing possibility that KIR-expressing NK cells in the fetal lung provide defense against infection arriving in the amniotic fluid.

Multi-parameter flow cytometry analyses of human NK cell receptor repertoires suggests all receptor combinations are allowed, implying an absence of negative selection that deletes NK cells with receptor combinations that are harmful or useless (Andersson et al., 2010). Accumulation of NK cells expressing self-specific KIRs might arise from viral imprinting during childhood. Indeed, CMV seropositive individuals exposed to hantavirus infection have expanded populations of highly differentiated $NKG2C^{+}$ NK cells that preferentially express self-reactive KIRs (Björkström et al., 2011). Similarly, Vivien Beziat (Paris, France) discussed how CMV drives expansion of similar NK cell populations in patients chronically infected with HCV or HBV.

After reviewing NK cell repertoire development in humans and mice, Petter Höglund (Stockholm, Sweden) concluded that the mouse Ly49 and human KIR repertoires are remarkably similar in their basic compositions. However, interactions with self-MHC class I caused greater repertoire skewing in the mouse.

$CD8^{+}$ T cells acquire KIRs during maturation from naive to fully differentiated effector cells. Their KIR repertoires are

dominated by a single KIR with no bias for or against KIRs reacting with self-MHC class I (Niklas Björkström: Stockholm, Sweden). Although NK cells are commonly assumed to cause the clinical effects correlating with epistatic interactions between KIR and HLA, this hypothesis remains largely untested. Consequently, contribution from KIR-expressing CD8 T cells cannot be dismissed.

KIR and Reproduction

Maternal uterine NK (uNK) cells function in reproduction by interacting with fetal trophoblasts that invade the uterus and convert small spiral arteries into voluminous channels that will nourish the growing fetus. Inadequate trophoblast invasion causes several common disorders of pregnancy: recurrent miscarriage, preeclampsia, and fetal growth restriction (Moffett and Loke, 2006). Emerging evidence points to a balanced trophoblast-NK cell interaction being crucial for achieving optimal depth of invasion. Modulating these cellular interactions are interactions uNK cell KIRs and HLA class I ligands of the trophoblast.

Ashley Moffett (Cambridge, UK) emphasized how trophoblasts express maternal and parental HLA-C, but no HLA-A or HLA-B. Pregnancies during which the fetus expresses the C2 epitope, particularly paternal C2, and the mother is homozygous for A KIR haplotypes are at risk for pregnancy disorder. Such observations implicate overly inhibitory interactions between maternal KIR2DL1 (an inhibitory receptor) and fetal C2 in disease. Consistent with this mechanism, the combination of fetal C2 and a maternal KIR B haplotype, which has *KIR2DS1* encoding the activating C2 receptor, is protective (Hiby et al., 2010). Extending the resolution of this analysis to the allele level, Olympe Chazara (Cambridge, UK) found that homozygosity for *KIR2DL1*003*, the commonest *KIR2DL1* allele, appears to be most strongly associated with pregnancy disorder.

Abnormally high or low birth weight associates with perinatal mortality, as well as maternal morbidity and mortality. Whereas implantation disorders correlate with fetal C2 and maternal KIR A haplotypes, Susan Hiby (Cambridge UK) found that high birth weight associates with the combination of fetal C2 and the telomeric region (Tel-B) of maternal KIR B haplotypes. Hence, interaction of fetal C2 with KIR2DS1, encoded in Tel-B, could cause overactivation of uNK leading to excessive invasion of spiral arteries and dangerously high birth weights. Overall, the spectrum of disease associations in pregnancy emphasizes the importance of a balanced uNK cell response during trophoblast invasion.

Pippa Kennedy (Cambridge, UK) compared telomeric KIR gene expression in blood and uterine NK cells from the same donor. Whereas KIR2DL1, KIR2DS1, and KIR2DS4 were expressed at high frequencies by uNK, KIR2DL5 was undetectable on uNK, although clearly present on blood NK cells. Inverse correlation of nonfunctional *KIR2DS4 del* with successful pregnancy suggests that functional *KIR2DS4*, a Tel-A gene encoding an activating receptor that recognizes some HLA-C, along with *KIR2DS1* in the Tel-B region, contribute to reproductive success. Supporting this theory is the fact that a high frequency of uNK cells expresses activating KIRs that recognize HLA-C.

Francesco Colucci (Cambridge, UK) described how mice, like humans, exhibit invasive placentation (Colucci et al., 2011). Furthermore, mouse trophoblast expresses paternal MHC class I that influences the uterine NK cell Ly49 receptor repertoire and decidual vascularization during implantation.

KIR and Infection

Defense against viruses is a major function of NK cells. Homozygosity for *KIR2DL3* and *HLA-C1* correlates with resolution of acute hepatitis C virus (HCV) infection (Khakoo et al., 2004). Building upon this foundation, Clair Gardiner (Dublin, Ireland) examined a unique cohort of >500 women infected with the same strain of HCV, a contaminant in the anti-D immunoglobulin they received as prophylactic treatment for preventing hemolytic disease of the newborn. Besides confirming the protective role of the *KIR2DL3* and *C1* combination, *KIR2DS3* and *C2* had the detrimental effect of favoring chronic infection. Becca Asquith (London, UK) reported that KIR2DL2 increased the protective role of HLA-B57 and HLA-C8 in HCV and HLTV-1 and the detrimental role of HLA-B54 in HTLV-1, suggesting that KIR2DL2 expression enhances virus-specific $CD8^+$ T cells.

Study of KIRs in human immunodeficiency virus (HIV) infection has mainly focused on viral control and progression to AIDS (Bashirova et al., 2011). Now investigating HIV transmission, Elizabeth Trachtenberg (Oakland, USA) reported that activating KIR genes, including *KIR2DS1*, *KIR2DS4*, and *KIR2DS5*, correlated with protection against HIV transmission. In contrast, *KIR3DL1* and *KIR3DS1*, which associate with slower progression to AIDS, were not protective. Thus, different KIRs may contribute to establishment and progress of infection.

Although it is well established that macaques have diverse KIRs, knowledge of their interactions with MHC class I is only now emerging. Using pig-tailed macaques, Bernard Lafont (Bethesda, USA) demonstrated functional interactions between KIR and MHC class I allotypes that inhibit NK cells. Meike Hermes (Göttingen, Germany) studied KIR expression in rhesus macaque NK cells and T cells by using a new panel of anti-macaque KIR antibodies. Investigation of SIV-infected rhesus macaques by Christina Albrecht (Göttingen, Germany) showed that rhesus macaque KIR3DL02 is associated with low viral load, whereas KIR3DL10*002, KIR3DS05, and KIR3DSw08 are associated with high viral load. Together, these findings should facilitate more incisive dissection of the role of KIRs and NK cells in SIV infection: the major experimental model for human HIV infection.

KIR and Therapeutic Interventions

When applying allogeneic hematopoetic stem cell transplantation (HSCT) as therapy for acute myeloid leukemia (AML), the gold standard is for the donor and recipient to be HLA identical. When this cannot be achieved, transplantation is performed across a range of HLA-A, -B, and -C mismatches. With certain types of mismatch, NK cells of donor origin respond to "missing-self" in the recipient. By attacking residual leukemia cells, these alloraective NK cells can reduce the incidence of relapse and improve patient survival. These effects, however, vary with transplant center and transplant protocol, as well as the HLA mismatch. Regardless of HLA type, transplants from donors with B KIR haplotypes reduce relapse and improve survival after HSCT, with the major effect coming from Cen-B haplotype (Cooley et al., 2010). This retrospective analysis is

stimulating prospective studies in which both HLA and KIR type will be considered when selecting transplant donors.

The variability in clinical outcomes presents a major challenge, one that demands knowledge and understanding of the variables contributing to beneficial NK cell alloreactivity. In particular, the processes involved in the reconstitution, maturation, and education of NK cell populations after HSCT remain largely unknown. During NK cell education, interactions between KIR and self-MHC class I determines how mature NK cells can respond to ''missing-self''-MHC class I (Anfossi et al., 2006; Höglund and Brodin, 2010). Whereas some studies, including results presented by Paul Fisch (Freiburg, Germany), report breaking of NK cell tolerance during the early posttransplantation period (Hsu et al., 2005), others find that functional NK cells depend upon continuing education by donor HLA class I, after transplant (Haas et al., 2011). In this context, Sarah Cooley (Minneapolis, USA) examined the dynamics of NK cell responses after adoptive transfer of haploidentical NK and described how uneducated, functionally immature donor NK cells gained function after interactions with recipient HLA class I. By contrast, when educated NK cells were transferred into a patient with no HLA class I ligands, they retained function, a possible consequence of the patients having received subcutaneous IL-2.

Clinical trials performed by Jeff Miller, Sarah Cooley, and collaborators (Minneapolis, USA) showed how adoptive transfer of haploidentical NK cells induced remission for 31% of patients with refractory AML, an effect correlating with expansion of donor NK cells. They also reported exciting, but preliminary, results from a trial combining adoptive NK cell therapy with a recombinant DNA-derived cytotoxic protein composed of diphtheria toxin and human interleukin-2a (Ontak, denileukin diftitox). With this regimen, in which regulatory T cells were depleted, a large majority of patients, with previously refractory AML, cleared disease and proceeded to allogeneic HSCT.

Last, but not least, Ariane Thielens (Marseille, France) described the development of a potentially therapeutic monoclonal human IgG4 (IPH2101) with broad specificity for KIR2DL1, KIR2DL2, and KIR2DL3, the inhibitory HLA-C-specific KIRs. Future strategies may combine such anti-KIR antibodies with adoptive transfer of highly activated NK cells to further improve the efficacy of treatment.

Concluding Remarks

Inescapable from this KIR workshop is the fact that variable KIR-HLA interactions are crucial modulators of lymphocyte function in human immunity, reproduction, and resistance to cancer. In the decade since the first KIR workshop, much knowledge of KIRs' place in NK cell biology has been attained. Less developed is understanding of the cellular and molecular mechanisms that underlie the immunogenetic correlations of KIR-HLA with disease and its treatment. An obvious limiting factor is that epidemiological and population studies are usually performed at the low KIR gene-content resolution achieved by commercial KIR typing kits. High-resolution allele-level analysis is essential at this stage in the study of KIRs, and several examples of it were presented at the workshop. By defining precisely KIR variation in human populations, high-resolution analysis will inform the simplifications necessary to translate KIR typing into clinical analysis and application. Without high-resolution data, any such simplifications will only be guesswork. Furthermore, because both KIR and HLA class I are highly variable, new tools are needed to study the range of their molecular interaction and the effects it has in diversifying human NK cell receptor repertoires and their responses to disease and pregnancy. Adding to the challenge is the underinvestigated contribution of the variable peptides that are bound to HLA class I and that are known to contact KIRs.

In his masterly ''wrapping up'' of the 7th KIR workshop, Klas Kärre (Stockholm, Sweden), alluded to the fact that KIR studies have provided new insights into the history of modern humans, including their origin and adaptation to environmental stress, such as epidemic infections. From a medical perspective, KIR studies provide a foundation for constructing new therapeutic interventions for alleviating a variety of diseases, including chronic viral infections and cancer. Although coming far since the first descriptions of KIRs in the 1990s, many questions remain unresolved and, undoubtedly, new questions will emerge. Exciting years and the eighth KIR workshop are still to come. The eighth workshop will be organized by Martin Maiers, Sarah Cooley and Jeffrey Miller in Minneapolis, USA.

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REFERENCES

Andersson, S., Malmberg, J.A., and Malmberg, K.J. (2010). Tolerant and diverse natural killer cell repertoires in the absence of selection. Exp. Cell Res. *316*, 1309–1315.

Anfossi, N., André, P., Guia, S., Falk, C.S., Roetynck, S., Stewart, C.A., Breso, V., Frassati, C., Reviron, D., Middleton, D., et al. (2006). Human NK cell education by inhibitory receptors for MHC class I. Immunity *25*, 331–342.

Bashirova, A.A., Thomas, R., and Carrington, M. (2011). HLA/KIR restraint of HIV: surviving the fittest. Annu. Rev. Immunol. *29*, 295–317.

Björkström, N.K., Lindgren, T., Stoltz, M., Fauriat, C., Braun, M., Evander, M., Michaëlsson, J., Malmberg, K.J., Klingström, J., Ahlm, C., and Ljunggren, H.G. (2011). Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J. Exp. Med. *208*, 13–21.

Colucci, F., Boulenouar, S., Kieckbusch, J., and Moffett, A. (2011). How does variability of immune system genes affect placentation? Placenta *32*, 539–545.

Cooley, S., Weisdorf, D.J., Guethlein, L.A., Klein, J.P., Wang, T., Le, C.T., Marsh, S.G., Geraghty, D., Spellman, S., Haagenson, M.D., et al. (2010). Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood *116*, 2411–2419.

Haas, P., Loiseau, P., Tamouza, R., Cayuela, J.M., Moins-Teisserenc, H., Busson, M., Henry, G., Falk, C.S., Charron, D., Socié, G., et al. (2011). NK-cell education is shaped by donor HLA genotype after unrelated allogeneic hematopoietic stem cell transplantation. Blood *117*, 1021–1029.

Hiby, S.E., Apps, R., Sharkey, A.M., Farrell, L.E., Gardner, L., Mulder, A., Claas, F.H., Walker, J.J., Redman, C.W., Morgan, L., et al. (2010). Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J. Clin. Invest. *120*, 4102–4110.

Höglund, P., and Brodin, P. (2010). Current perspectives of natural killer cell education by MHC class I molecules. Nat. Rev. Immunol. *10*, 724–734.

Hsu, K.C., Keever-Taylor, C.A., Wilton, A., Pinto, C., Heller, G., Arkun, K., O'Reilly, R.J., Horowitz, M.M., and Dupont, B. (2005). Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood *105*, 4878–4884.

Kärre, K., Ljunggren, H.G., Piontek, G., and Kiessling, R. (1986). Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature *319*, 675–678.

Khakoo, S.I., Thio, C.L., Martin, M.P., Brooks, C.R., Gao, X., Astemborski, J., Cheng, J., Goedert, J.J., Vlahov, D., Hilgartner, M., et al. (2004). HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science *305*, 872–874.

Moffett, A., and Loke, C. (2006). Immunology of placentation in eutherian mammals. Nat. Rev. Immunol. *6*, 584–594.

Moretta, L., Bottino, C., Pende, D., Castriconi, R., Mingari, M.C., and Moretta, A. (2006). Surface NK receptors and their ligands on tumor cells. Semin. Immunol. *18*, 151–158.

Parham, P. (2008). The genetic and evolutionary balances in human NK cell receptor diversity. Semin. Immunol. *20*, 311–316.