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Natural Killer cell education in human health and disease

Jeanette E. Boudreau[1,2] and **Katharine C. Hsu**[3,4,*]

[1]Department of Microbiology and Immunology, Dalhousie University, Halifax, Canada

[2]Department of Pathology, Dalhousie University, Halifax, Canada

[3]Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]Weill Cornell Medical College, New York, NY, USA

Abstract

Natural killer (NK) cells maintain immune homeostasis by detecting and eliminating damaged cells. Simultaneous activating and inhibitory input are integrated by NK cells, with the net signal prompting cytotoxicity and cytokine production, or inhibition. Chief among the inhibitory signals for NK cells are “self” human leukocyte antigen (HLA) molecules, which are sensed by killer immunoglobulin-like receptors (KIR). Through a process called “education”, the functional capabilities of each NK cell are counterbalanced by their sensitivity for inhibition by co-inherited “self” HLA. Since genes for HLA and the killer immunoglobulin-like receptors (KIR) that bind them are polymorphic, polygenic and independently segregate, NK education and function differ even between related individuals. In this review, we describe how variances in NK education, reactivity and sensitivity for inhibition impact reproductive success, infection, cancer, inflammatory and autoimmune diseases.

Education for NK potential – activation, inhibition, and regulation

Natural killer (NK) cells were initially characterized for their ability to lyse target cells lacking “self” major histocompatibility class I expression (MHC) [1]. Recognition of “missing self” in humans enables distinction of self from non-self tissues, and detection of diseased cells with downregulated human leukocyte antigen (HLA) expression, which occurs in some viral infections and cancers [2–4]. This “missing self” reactivity of NK cells is not, however, absolute. Rather, the avidity of interactions between NK receptors and HLA creates a spectrum of functionality among the NK cells both within and between individuals, where the reactive potential of each NK cell, endowed by a process of “NK education,” is counterbalanced by its dominant sensitivity for inhibition by co-inherited “self” HLA [5–7]. This process is called NK “education” (Figure 1).

*Correspondence: hsuk@mskcc.org (K.C. Hsu).

In humans, NK education occurs most through interactions between HLA class I molecules and inhibitory receptors, such as the CD94/NKG2A heterodimer and, more prominently, the killer immunoglobulin-like receptors (KIR), as extensively reviewed (Table 1) [3,8–10]. While several models have been advanced to describe how NK education occurs, they generally agree that cumulative interaction between inhibitory receptors and HLA ligands dictates the level of response of an NK cell to activating signals, such as stress ligands, inflammatory cytokines, and Fc receptor engagement [6,11,12]. The HLA and KIR gene families represent the most polymorphic and polygenic receptor-ligand pair in the human genome, having co-evolved to enable diverse NK cell responsiveness [13,14]. Genes for *KIR* and *HLA* segregate independently, yielding diverse compound genotypes [15]. Functional interactions between co-inherited KIR and HLA drive NK education, which enables a unique form of immunologic diversity with a minimum number of germline-encoded genes [15]. Finally, immunologic experiences can further modulate NK cell functions, through establishment of memory-like or adaptive NK cell populations via epigenetic remodelling [16,17].

Allelic variation influences the overall avidity of receptor-ligand interactions by controlling surface expression densities and binding affinities [18–20]. We and others have investigated the impact of allelic variation on human health for KIR3DL1 and its ligand HLA-Bw4, the most diverse and evolutionarily most ancient KIR-HLA partnership [21–23]. HLA-B alleles can be divided based on the Bw6 or Bw4 epitope encoded at positions 77–83, with further division of Bw4 into Bw4-80I or Bw4-80T subtypes based on a dimorphism (isoleucine vs threonine) at position 80. KIR3DL1 alleles encompass the activating KIR3DS1 alleles, and inhibitory alleles expressed at high, low and null cell surface densities [24,25]. Interestingly, the null KIR3DL1 allele is expressed exclusively intracellularly, which prevents inhibition by Bw4-expressing target cells, but permits education [26,27]. The remaining subtypes of Bw4 and KIR3DL1 mediate education and inhibition, with the strongest outcomes occurring between the densely expressed and strong binding KIR3DL1-high and Bw4-80I allotypes [18].

Binding to “self” HLA is not required for survival and maintenance of NK cells, which exhibit a spectrum of HLA binding from none to weak to strong, as a result of cumulative contribution from multiple inhibitory receptor types, their expression levels, and affinities to HLA [18–20]. “Uneducated” NK cells, non-binding and weakly-binding NK cells are beneficial in disease states where HLA expression persists, but set with a higher threshold for reactivity to avoid auto-aggression [10,26]. Nevertheless, in the presence of activating stimuli, such as antibodies bound to target cells for antibody-dependent cellular cytotoxicity (ADCC), or a cytokine-conditioned pro-inflammatory microenvironment, uneducated NK cells can readily be recruited for target cell killing [28,29].

Though considerations of NK education most often concern inhibitory KIR, it is now understood that other receptor-ligand interactions titrate the NK reactivity/inhibition balance. The inhibitory CD94/NKG2A and LIR-1 both bind conserved regions of HLA and increase NK response potential by providing additional binding to “self” HLA [30,31]. Conversely, NK education can also be reduced by activating receptors in the context of high ligand exposure: KIR2DS1+ NK cells, normally responsive to HLA-C2+ targets, are

refractory to stimulation when they are collected from HLA-C2 homozygous individuals, having been rendered tolerant to self [32]. KIR2DS2 and KIR3DS1 have likewise been reported to bind HLA-C1 and HLA-F, respectively [33–35]; whether these interactions contribute to education and tolerance has not been described.

Altogether, educated and uneducated NK cells establish a complementary system of responsiveness to enable reactivity against HLA-sufficient and HLA-deficient target cells, with important implications in reproduction, autoimmune, infectious and malignant disease (Table 2). In this review, we focus on interactions between KIR and HLA focusing on their combined influence on human health.

NK education in pregnancy

Successful pregnancy requires invasion of the fetal trophoblast and formation of spiral arteries, facilitated by NK cells. These processes must be carefully balanced: extensive invasion is associated with high birth weight and high-risk delivery; poor invasion is associated with preeclampsia, low birth weight and recurrent miscarriage [36].

Uterine NK cells (uNK) are the most abundant lymphocytes in the decidua, and are key players in successful pregnancy [37–39]. Through interactions with HLA expressed on extravillous trophoblast cells (EVT), uNK cells facilitate trophoblast invasion and placentation [37]. EVT lack expression of HLA-B and HLA-A; but exhibit HLA-E and HLA-C [40,41], whose cognate NKG2A and KIR2D receptors are overexpressed on uNK cells [38,40,42,43]. Moreover, uNK cells are enriched for educated populations: HLA-C1+ and HLA-C2+ mothers demonstrate increased frequencies of uNK cells expressing cognate KIR2DL3 and KIR2DL1, respectively [43,44]. In this way, maternal NK cells are equipped to enable tissue remodeling while remaining sensitive to inhibition, creating an optimal molecular environment for trophoblast invasion.

Fetal HLA-C2 of paternal origin in KIR2DS1+, HLA-C2-negative mothers exhibit high birth weight and successful placentation [36,38,45]. In contrast, KIR2DS1 is underrepresented and HLA-C2 is enriched among women experiencing recurrent miscarriages [46]. Similarly, the more inhibitory KIR-A haplotype, which lacks KIR2DS1, and HLA-C2 are enriched in women experiencing recurrent miscarriage and in their partners [47–49]. KIR-A haplotypes and fetal HLA-C2 of paternal origin are similarly enriched among patients with pre-eclampsia, where placental development and fetal growth are compromised due to poor invasion of the trophoblast [36,50,51]. Together, these studies suggest that uNK education that favors maternal NK responsiveness over inhibition may enable successful placentation and spiral artery formation.

NK education in autoimmune and inflammatory disease

The etiopathology of autoimmune and inflammatory diseases, including the role(s) of NK cells, remains largely to be determined, but is associated with activation of T and B lymphocytes against self proteins and persistent inflammation mediated by innate and adaptive cells. The available evidence suggests that NK cells fulfill both beneficial and

detrimental roles, implying that inter-patient variability, possibly in NK education, could underlie the pathology of inflammatory and autoimmune disease.

Across a spectrum of inflammatory disorders, including Kawasaki disease, rheumatoid arthritis, multiple sclerosis (MS), systemic lupus erythematosus and type I diabetes, decreased numbers and function of circulating NK cells precede disease flares [52–58], suggesting that NK cells are important for controlling inflammation. In apparent contrast, NK cells are indispensable in the priming phase for subsequent development of experimental myasthenia gravis, and IFN-γ produced by NK cells exacerbates experimental autoimmune encephalopathy and diabetes in mouse models [59], suggesting that the role for NK cells could differ between diseases and/or patients.

In patients responding to modern treatments for MS including mitoxantrone, daclizumab or natalizumab – therapies that control lymphocyte expansion – selective enrichment and maturation of the NK population and its cytotoxic capacities are observed in responding patients [58,60–62]. In patients treated for MS and systemic lupus erythematosus, antibodies aimed at depleting auto-aggressive B cells, including alemtuzumab and rituximab, function by recruiting NK cells for antibody-dependent cellular cytotoxicity (ADCC) [63,64]. Their success is associated with enrichment of NK cells, and depletion of the NK population eliminates the efficacy of treatment in experimental models [63,64]. Several studies have now reported a protective role of KIR2DS1 against MS, enhanced by the presence ofo its HLA-C2 ligand [65–67], a configuration that supports tolerance of NK cells bearing this activating receptor[32]. Altogether, these observations support a regulatory role for NK cells in preventing autoimmune and inflammatory disease, but the mechanism(s) through which this occurs and the contribution of NK education remains largely to be investigated.

NK education in infectious disease

Given the prominent role for T cells in detecting viral antigens loaded onto HLA molecules, it is not surprising that many viruses have evolved to reduce surface expression of HLA. Although this creates a target for educated NK cells, viral adaptations, including encoding HLA mimics or selective HLA downregulation facilitate virus spread throughout populations.

In HIV infection, downregulation of HLA is limited to the HLA-A and HLA-B loci, with HLA-C expression persisting or even upregulated [68–70]. To NK cells educated by KIR3DL1 and HLA-B, HIV infected cells therefore present themselves as missing-self targets, and killing proceeds in a manner reflective of education strength [18,68]. As a result, HIV-infected individuals harbouring compound genotypes of KIR3DL1 and HLA-Bw4, particularly combinations of high density and high-affinity receptor-ligand, proceed more slowly to AIDS than those lacking the HLA-Bw4 epitope [71].

Graded control of HIV infection occurs, correlated to the extent of NK education and the sensitivity of KIR3DL1^{+} NK cells to missing self targets [71]. We recently demonstrated that the high-avidity combination of HLA-Bw4 molecules exhibiting isoleucine at position 80 (Bw4-80I) and KIR3DL1 receptors expressed at high surface densities show greater

degranulation and IFN-γ production in response to HLA-negative K562 target cells compared with all other combinations of KIR3DL1 and HLA-Bw4 [18]. The latter still exhibited greater reactivity that that of KIR3DL1$^+$ NK cells collected from individuals lacking HLA-Bw4 epitopes, however. Importantly, this hierarchy predicted direct cytotoxicity of NK cells against HIV-infected autologous CD4$^+$ T cells, a finding consistent with hierarchical control of HIV infection based on a patient's compound KIR3DL1 and HLA-B genotypes.

An alternate viral strategy to evade detection by education is to trigger inhibition of NK cells by maintaining normal HLA expression. Both Zika virus and HIV induce upregulation of HLA-C expression on infected cells [70,72]. Since HLA-C interacts primarily with NK cells and not with T cells, and the vast majority of HLA-C molecules exhibit at least one HLA-C1 or HLA-C2 epitope [73], this strategy enables escape from immune surveillance through HLA-C-mediated inhibition of KIR2DL-expressing NK cells. Finally, adaptive NKG2C+ NK cells expanding in response to human cytomegalovirus (HCMV) infection exhibit self-specific KIR[74]. Whether education of this population is beneficial to controlling the virus is not known, but the expression of an activating receptor on an educated NK cell population may help to overcome HLA-driven inhibition.

Viruses may also employ molecular mimicry to avoid detection, whereby expression of virally-encoded HLA-like proteins by infected cells trigger inhibitory signaling in NK cells. For example, HCMV encodes an HLA-like protein, UL-18, that triggers inhibition of NK cells through the conserved leukocyte inhibitory receptor-1, but does not present antigen to T cells [75,76]. In this way, the HCMV has evolved to evade recognition by both major cytolytic lymphocyte subsets.

NK education in cancer

Although HLA expression is often assumed to be low on tumour cells, recent findings reveal that HLA expression persists at some detectable level and/or can easily be induced on the majority of tumours, a conclusion empirically supported by success observed with immune checkpoint inhibitor therapy for multiple different cancers [77,78]. We and others have demonstrated that HLA expression is upregulated in the presence of inflammation, including that driven by cancer therapy itself [29,79]. It is the exceptional tumour that exhibits loss of HLA expression at the genetic level and usually under severe immunological pressure [80], and the majority of studies support a prominent role for uneducated NK cells in cancer control.

A potent role of NK cells in cancer control is evident in patients with acute myelogenous leukemia (AML) receiving hematopoietic cell transplantations (HCT). A significant "graft-versusleukemia" (GVL) effect occurs during HCT in some patients, where donor-derived NK cells insensitive to inhibition by the HLA expressed on a recipient's leukemia cells mediate potent cancer control [81–83]. Initially, this was thought to reflect killing of "missing self" targets by educated NK cells following HLA-mismatched HCT in which the recipient lacked HLA ligands present in the donor, but surprisingly, a GVL impact has also been observed in patients undergoing HLA-matched HCT. Mechanistically, in the pro-

inflammatory pro-stimulatory post-HCT environment, lack of HLA binding of inhibitory KIR expressed on uneducated cells permits NK reactivity against leukemia [28]. As a result, patients lacking one or more of the inhibitory KIR-binding HLA ligands, a so-called "missing ligand" benefit, exhibit lower relapse and improved overall survival compared with those expressing all three KIR ligands.

We recently demonstrated that subtype variability for KIR3DL1 and HLA-B can approximate a "missing ligand" benefit in AML recipients of HLA-matched HCT even if all KIR ligands are present in the patient. Specifically, donor KIR3DL1 and HLA-B subtype combinations predictive of weak or no inhibition are associated with improved AML control after HCT in comparison to combinations predictive of strong inhibition [26]. This may provide a novel opportunity for the transplant physician to select among HLA-matched donors for those carrying KIR3DL1 subtypes unlikely to be inhibited by the tumour to prevent AML relapse after transplant. It is noteworthy that the combinations most beneficial in AML control are the opposite to those beneficial in HIV infection, highlighting the fundamental importance of HLA expression on the diseased cell in determining which NK subpopulation and education level will have the most clinical impact.

The same allele subtype variability has now been demonstrated to impact the success of treatment for other cancers, including those treated with monoclonal antibodies, including rituximab and anti-GD2 [84,85]. In patients bearing all three KIR ligands, these antibody therapies are less effective, as educated NK cells are inhibited from performing cytotoxicity against antibody-tagged tumour cells. As in patients with AML, non-engaging KIR-HLA combinations enable cytotoxicity to proceed, leading to better tumour control and overall survival in these patients.

Manipulating NK education to restore health

Recognizing the power of NK education in determining the outcomes of disease and treatment, many laboratories are now focused on developing NK-based immunotherapies and immunodiagnostics, where they have several advantages. Unlike T cells, NK cells do not generate a graft-versus-host response; therefore, HLA matching is not required for adoptive transfer. Good manufacturing protocols exist for expansion of NK cells *ex vivo,* and NK education is maintained during both expansion and following adoptive transfer [86–89]. Because NK cell education is defined by a relatively restricted number of receptor and ligand subtypes, it may be possible to establish off-the-shelf approaches using NK-modifying agents and/or NK cell banks to precisely control and redirect NK cell function. These approaches, however successful they may be at *ex vivo* activation and expansion, cannot fully control for the dominant effects of *in vivo* inhibition of the transferred NK cells by HLA expression on the recipient's diseased cells.

Interrupting NK cell inhibition as a strategy to enhance NK cell function against HLA-expressing virus infected and malignant cells has therefore become a goal of many laboratories. To minimize KIR3DL1-mediated NK cell inhibition following HCT in patients with AML, we are undertaking a prospective trial to select among HLA-matched donors for those whose KIR3DL1 allele subtypes enable weak or no inhibition (clinicaltrials.gov

NCT02450708). This approach is highly feasible: For 93% of patients in a pilot test, we identified at least one donor whose KIR3DL1 allele subtypes predicted for weak or no inhibition by the donor and recipient HLA-B [26].

An alternative approach is to block inhibition with antibodies targeted against inhibitory receptors. The anti-KIR antibody, 1-7F9/IPH2101, is being tested for this purpose. Though initial studies in mice demonstrated a promising improvement in lymphoma control [90], clinical trials of this same antibody as a single agent in the treatment of multiple myeloma and AML have not demonstrated a substantial benefit of anti-KIR antibodies [91,92]. Approaches to combine this antibody with others to increase NK cell activation, alterations in antibody dosing or schedule, or modifications to prevent binding to activating receptors may be required to unleash the clinical potential of this and other antibodies targeting inhibitory receptors. Whether these antibodies can be employed to promote NK aggression against virally-infected cells or support successful pregnancy; or antibodies against activating receptors may be beneficial in autoimmune diseases, has not been explored.

Conclusions

Variable interactions between KIR and HLA, driven by gene and allele-level diversity, strongly influence NK cell education with impacts on human health. No one pattern of NK education (or co-inheritance of particular KIR and HLA genes) can be universally classified as beneficial or detrimental, as their benefits are disease-specific. Understanding how NK education contributes to successful pregnancy, disease development, resolution and successful treatment may identify *KIR* and *HLA* genes as biomarkers for prognosis and their proteins as targets for precision therapy.

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Highlights

a. NK cell education is endowed by independently-segregating polygenic *KIR* and *HLA* which, combined, enable diverse effector functions

b. Education counterbalances each NK cell's effector potential with sensitivity for inhibition

c. Uneducated and educated NK cells have roles in successful pregnancy, disease resistance and susceptibility

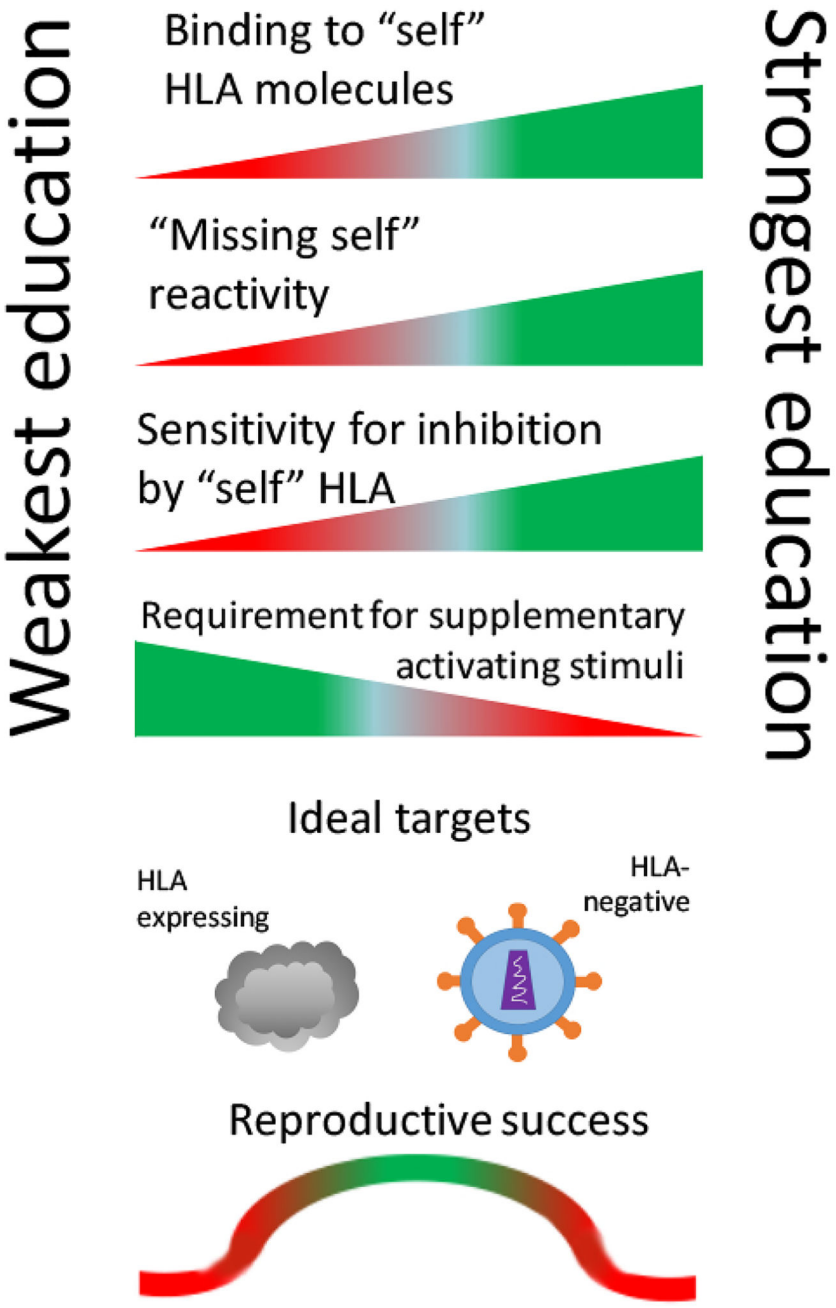

Figure 1. NK education and impacts on human health

NK cell education represents a continuum of reactivity and inhibition, determined by interactions with co-inherited HLA. Cells capable of high avidity binding exhibit the most potent "missing self" reactivity and sensitivity for inhibition by "self" HLA class I molecules. Uneducated NK cells do not bind "self" HLA molecules, leading to weak "missing self" capabilities, but also insensitivity for inhibition. NK education determines the threshold for NK reactivity, with the strength of NK education inversely correlated with the requirement for additional activating input (i.e. pro-inflammatory signals or bound antibodies). NK education equips populations to detect damaged cells exhibiting a variety of

HLA phenotypes, with uneducated NK cells as the best effectors against HLA-expressing targets (i.e. most cancers) and educated NK cells as the best effectors against HLA-negative targets (i.e. certain viral infections). Successful pregnancy is fostered by specialized uterine NK cells (uNK), educated to enable trophoblast invasion and placentation, while managing fetal growth.

Table 1

Receptor-ligand partnerships contributing to NK cell education

HLA epitope	**Cognate receptor**		
HLA-C1 (Asn77)	KIR2DL2 KIR2DS2	(major),	KIR2DL3,
HLA-C2 (Lys80)	KIR2DL1, KIR2DS1	KIR2DL2	(minor),
HLA-Bw4	KIR3DL1		
HLA-F (open conformation)	KIR3DS1		
HLA-A11	KIR3DL2		
HLA-E, presenting HLA leader sequences	NKG2A		
pan HLA	LIR-1		

Table 2

Associations of NK education in human health and disease

KIR-HLA combination	Education status	Health/Disease state	Impact
KIR2DL1 + HLA-C2	Educated	Pregnancy	Increased risk of pre-eclampsia, stillbirth and low birthweight (especially with fetal HLA-C2) [50,93,94]
		Infectious disease	Enriched among individuals acquiring Dengue virus infection [95]
		Infectious disease	Enriched among HCMV seropositive NKG2C+ cells in persons with HCMV [74]
KIR2DL1 without HLA-C2	Uneducated	Infectious disease	Lower reactivation of HCMV after kidney transplant [96]
		Cancer	Protective against AML relapse following allogeneic HCT in patients with AML (missing ligand) [83,97]
KIR2DL2 + HLA-C1	Strongly educated	Autoimmune disease	Protection against ulcerative colitis [98]
		Inflammatory disease	Enriched in patients with Kawasaki disease [99]
		Cancer/infectious disease	Associated with younger age of onset for hepatocellular carcinoma in persons infected with hepatitis B [100]
		Infectious disease/cancer	Enriched among persons infected with high-risk human papillomavirus and patients developing invasive cervical cancer [101]
		Cancer	Protection against relapse of hematological malignancy in patients receiving umbilical cord blood transplantation [102]
KIR2DL2 + HLA-C2	Uneducated – weakly educated	Infectious disease	Lower reactivation of HCMV after kidney transplant [96]
		Autoimmune disease	Increased susceptibility to type I diabetes [103]
KIR2DS1 + HLA-C2	Educated (tolerogenic)	Pregnancy	Enriched in patients with recurrent miscarriage [49]
		Autoimmune disease	Protective against multiple sclerosis [67]
KIR2DS1, without HLA-C2	Uneducated (reactive)	Pregnancy	Enriched in patients with failed pregnancy after *in vitro* fertilization [105]
		Cancer	Protection against AML relapse after hematopoietic cell transplantation [104]
		Cancer	Protection against relapse of hematological malignancy in patients receiving umbilical cord blood transplantation [102]
KIR2DL3 + HLA-C1	Educated	Infectious disease	increased resolution of HCV infection compared with 2DL2+C1 or 2DL1+C2; possibly due to weaker inhibitory signals [106]
		Infectious disease	Resistance to Ebola infection [107]

KIR-HLA combination	Education status	Health/Disease state	Impact
		Infectious disease	Higher incidence of cerebral malaria in infected persons [108]
		Infectious disease	Underrepresented among individuals acquiring Dengue virus infection [95]
		Infectious disease	Enriched among patients resolving hepatitis B infection; underrepresented in patients with chronic hepatitis B infection [109]
		Infectious disease	Enriched among HCMV seropositive NKG2C+ cells in persons with HCMV [74]
		Infectious disease/cancer	Enriched among persons infected with high-risk human papillomavirus and patients developing invasive cervical cancer [101]
		Cancer	Protection against relapse of hematological malignancy in patients receiving umbilical cord blood transplantation [102]
KIR2DS2 + HLA-C1		Infectious disease	Protective against HCV and Dengue virus [110]
KIR2DL3 without HLA-C1	Uneducated	Autoimmune disease	Protection against ulcerative colitis [98]
		Cancer	Protection against AML relapse in patients receiving hematopoietic cell transplantation [102,104,111]
KIR3DL1 + HLA-Bw4	Educated	Infectious disease	High affinity pairs (KIR3DL1-high + HLA-Bw4-80I) protect against HIV progression to AIDS [71]
		Infectious disease	Enriched among individuals acquiring Dengue virus infection [95]
		Cancer	low affinity pairs are associated with the protection against relapse patients with AML after hematopoietic cell transplantation [26]
		Cancer	Enriched in patients with kidney cancer and non small cell lung carcinoma compared with healthy controls [112]
KIR3DL1 without HLA-Bw4	Uneducated	Cancer	Protection against AML relapse after hematopoietic cell transplantation [26,83,97]
		Cancer	Improved overall survival and cancer control in patients treated with 3F8 antibody (Bw4-negative and weak binding pairs of KIR3DL1 + HLA-Bw4) [29,84,85]
		Infectious disease/cancer	Higher likelihood of hepatitis C-associated hepatocellular carcinoma [113]