

Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease

R. J. Boyton*[†] and D. M. Altmann[‡]

Lung Immunology Group, National Heart & Lung Institute, Sir Alexander Fleming Building, South Kensington Campus, Faculty of Medicine, Imperial College, London, UK,^{*} and Department of Respiratory Medicine, Royal Brompton and Harefield NHS Trust, London, UK,[†] and Department of Infectious Diseases and Immunity, Imperial College, Hammersmith Hospital, Du Cane Road, London, UK[‡]

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Correspondence: Dr Rosemary Boyton, MRC Clinician Scientist, Lung Immunology Group, National Heart and Lung Institute, Sir Alexander Fleming Building, South Kensington Campus, Faculty of Medicine, Imperial College, London SW7 2AZ, UK.

E-mail: r.boyton@imperial.ac.uk

Introduction

Between 1974 and 1977 a series of papers from Cantor, Wigzell, Playfair and others described a novel lymphocyte effector function mediated by cells that were granulocytic lymphocytes, yet were neither T cells nor B cells and did not use related families of antigen receptors [1–3]. Initially described in the mouse as a population capable of killing murine leukaemia virus tumours, the key features here were that targets could be either syngeneic or allogeneic, and killing was detected in the absence of prior immunization. Termed ‘natural killer’ (NK) cells, the criteria for inducing cytotoxicity by this population appeared rather blunt-edged and simple compared with the exquisite rules governing cytotoxic T cell (CTL) killing. Indeed, NK cell killing, often indicated by lysis of the myelogenous leukaemia cell line, K562, was relegated initially to the role of a control for ‘non-specific killing’ on the CTL assay 96-well plate.

The programme for control of NK cell activation and killing is now understood to be a highly complex system of diverse, inhibitory and activatory receptor-ligand interac-

Summary

Natural killer cells constitute a potent, rapid part of the innate immune response to infection or transformation, and also generate a link to priming of adaptive immunity. Their function can encompass direct cytotoxicity as well as the release of cytokines and chemokines. In humans, a major component of natural killer (NK) cell target recognition depends mainly on the surveillance of human leucocyte antigen (HLA) class I molecules by killer immunoglobulin-like receptors (KIR). Different KIR can transmit inhibitory or activatory signals to the cell, and effector function is considered to result from the balance of these contributing signals. The regulation of NK cell responses depends on a number of variables: KIR genotype, HLA genotype, heterozygosity *versus* homozygosity for these, whether there is cognate recognition between the HLA and KIR products carried by an individual, clonal variation between individual NK cells in KIR expression, and the specific modulation of HLA expression by infection, transformation or peptide binding. Different HLA/KIR genotypes can impart different thresholds of activation to the NK cell repertoire and such genotypic variation has been found to confer altered risk in a number of diseases including human immunodeficiency virus (HIV) susceptibility and progression, hepatitis C virus clearance, idiopathic bronchiectasis, autoimmunity and cancer.

Keywords: HLA, human, innate immunity, KIR, NK cell

tions, sensing changes in major histocompatibility complex (MHC) expression. A key step in the elevation of the NK cell from unsophisticated ‘null cell’ was the work of Karre and others, identifying ‘missing-self’ as the central concept in NK cell recognition [4,5]. In the ‘missing self’ hypothesis, engagement of inhibitory receptors by MHC class I or related molecules is necessary to inhibit NK cell activation. The contemporary update of this hypothesis incorporates lack of class I, with expression and binding of activation ligands [6]. In addition to the observations of NK cell killing of transformed haematopoietic cells, there are also many examples of recognition of viral infection [7,8]. This tied in with the observation from herpesviruses and a number of other families that some viral gene products have evolved mechanisms to subvert various aspects of MHC class I biosynthesis [9]. Today NK cell effector function is perceived to be an important first line of innate immunity across viral, bacterial and parasitic infections, as well as forming an important bridge for activation of the adaptive immune response [10]. Because NK cells share with CD8 $\alpha\beta$ T cells and $\gamma\delta$ T cells a *modus operandum* depending on MHC class

I recognition, Parham has postulated the existence of a common, ancestral, NK-like lymphocyte effector cell that must have appeared in vertebrates at the 'big bang' of adaptive immunity [11]. NK cell function can act either through cytotoxicity following release of perforin or granzymes, or by cytokine release, acting to prime dendritic cells.

NK cell receptors

The key families of receptors used by NK cells are the killer immunoglobulin-like receptors (KIRs), the Ly49 receptors and the CD94/NKG2 receptors. NKG2D receptors are expressed by all human NK cells and bind diverse ligands [12]. The Ly49 genes are C-type lectin-like and are crucial to NK cell recognition in the mouse, but appear to be of less importance to NK cell recognition in primates [13,14]. Humans have one Ly49 gene that is likely to be a pseudogene, although it is expressed in baboons [14]. The major NK recognition function in primates results from expression of KIR genes. The KIR genes are immunoglobulin superfamily members located in the leucocyte receptor complex on chromosome 19 [15–19]. The CD94/NKG2A lectin-like receptors are used by NK cells in both species. Ligands for KIR include human leucocyte antigen (HLA)-C allotypes, some HLA-A and B allotypes and, in the case of 2DL4, HLA-G [20]. The inhibitory CD94/NKG2A heterodimer recognizes HLA-E [21]. NKG2D is expressed as an activating receptor by all NK cells and binds several different ligands, including major histocompatibility complex class I chain-related gene A (MICA) and UL16 binding proteins (ULBPs) [22,23].

The KIR gene cluster on chromosome 19 contains up to 17 KIR genes or pseudogenes. These 17 genes show varying degrees of polymorphism. KIRs can possess either two or three extracellular Ig domains, as reflected in the 2-dimensional (2D) or 3D nomenclature. At the time of writing, each KIR gene has between 4 and 19 alleles. However, with the exception of limited examples, the impact of specific polymorphisms on function has not been investigated widely. In terms of the cytoplasmic tails of KIRs, they can be either long (designated 'L'), and containing immunoreceptor tyrosine-based inhibition motifs (ITIMs), or short ('S'), lacking ITIMs and mainly activating. Short-tailed receptors have a transmembrane lysine residue necessary for pairing with the immunoreceptor-based activation motif (ITAM)-containing adaptor, DAP12. KIR 2DL4 is an exception in that signalling is dependent upon association with an accessory protein, Fc ϵ RI- γ , which confers an activating signal via its ITAM [24]. The corresponding HLA class I ligands for the individual KIRs are known in many cases, although not for 2DL5 among the inhibitory receptors or 2DS1, 2, 3, 4, 5 and 3DS1 among the activating receptors.

KIRs are generally considered to function as membrane protein, cellular receptors. However, some sequenced cDNAs carry early stop codons, suggesting the release of KIRs as secreted proteins. Examples of this have been described for

2DS4 and 2DL4 [25–27]. The functional impact of such proteins, if they are indeed secreted, has not yet been characterized.

Combinations of KIRs together can be regarded as forming inherited haplotypes with different inherent balances between inhibition and activation [28]. KIR haplotypes have been categorized into two basic groups on the basis of gene content. Group A contains two activating KIR genes, KIR2DL4 and KIR 2DS4, and five inhibitory KIR genes, KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL2 and KIR3DL3. Group B haplotypes, on the other hand, have a variable number of KIR genes, many with activating function. More than 20 different B haplotypes have been characterized so far. Over and above the basic KIR gene make-up of A and B haplotypes, there is also the functional contribution of allelic polymorphism at each locus to consider [29].

A number of approaches including X-ray crystallography and tetramer binding studies have been used to characterize KIR/HLA interactions [30–32]. The contribution of HLA-bound peptide to this interaction has been difficult to define. Tananchai and colleagues investigated the interaction between HLA-Bw4 allotypes in the form of peptide-loaded tetramers and different alleles of the inhibitory receptor, KIR3DL1, expressed as a transfected receptor into Jurkat or Baf3 cell lines [30]. The data showed that KIR3DL1 allotypes can have different specificities of HLA class I binding. Furthermore, binding was sensitive both to HLA class I polymorphisms, as expected, but also to the identity of the bound peptide. Some KIR3DL1 allotypes could bind a range of Bw4⁺ alleles associated with a range of human immunodeficiency virus (HIV)- or cytomegalovirus (CMV)-derived peptides, while others were more constrained in their recognition pattern.

Evidence both from crystallography of HLA-C/KIR complexes and from alignment of KIR ligand sequences shows the importance in binding of the amino acid dimorphism at residue 80 of the α 1 helix in HLA-C alleles and of the 44 position in the D1 domain of KIRs [31,33]. On this basis, HLA-C alleles can be defined as either 'group 1' or 'group 2'. In group 1 are the 2DL2, 2DL3, and 2DS2 binders, C*01, C*03, C*07 and C*08. In group 2 are the 2DL1 and 2DS1 binders, C*02, C*04, C*05 and C*06. It is a basic tenet that the tuning of NK cell recognition must depend on inheritance of both the HLA and KIR genotype and, overall, on the outcome of counter-balancing inhibitory and activating signals [34–36]. However, because the HLA ligands and their KIR receptors are encoded on different chromosomes, it is possible to express a KIR with no corresponding expression of a relevant HLA class I ligand. Shilling and colleagues analysed the relationship between HLA genotype, KIR genotype and KIR expression, finding that KIR genotype was of greater importance than HLA for determining the expressed NK repertoire [37]. Individual NK cell clones from any given individual can show different patterns of receptor expression. An exhaustive analysis in 104 Japanese donors of

the relationship between HLA and KIR polymorphisms and homozygosity in relation to frequency and level of expression on NK cells was undertaken by Yawata and colleagues [29]. They found that different KIR3DL1 allotypes had different levels of expression and were expressed by different proportions of NK cells. There was a phenotypic effect of gene dose, as individuals with two low or high expressing 3DL1 allotypes had higher frequencies of positive NK cells than individuals with one. Interestingly, the presence of the cognate HLA ligand for a given KIR also increased the frequency of NK cells expressing that KIR, and decreased the frequency of NK cells expressing other inhibitory KIRs. Overall, NK cell ligand/receptor recognition can thus vary at multiple levels: KIR haplotype, KIR allelic polymorphisms, HLA-B and C polymorphisms and differences in clonal expression patterns.

KIRs and disease

A fascinating aspect of HLA/KIR evolutionary biology is the very extreme divergence in haplotype frequencies between human populations, presumed to indicate regional population differences in pathogen driven selection [11]. The AA genotype is found in around 56% of Japanese individuals and around 15% of Australian Aboriginal individuals. While these frequencies must also be considered in the context of the corresponding HLA class I allelic frequencies, they suggest that different populations may possess NK cell systems of inherently different functional programming. Carrington and colleagues proposed a model for ranking KIR/HLA combinations in terms of predicted, inhibitory or activating tendency of the NK cell programme [34,38]. At one extreme of this spectrum are AA haplotypes, with an inherent tendency to inhibition, at the other end, the BB haplotypes with an inherent tendency to activation. Genotypes carrying inhibitory receptors but lacking the HLA ligands would be predicted to show heightened activation, with fewer NK cell clones under inhibitory control. While this model appears to have validity with respect to analysis of disease risk, there is as yet little functional data validating the existence of such a spectrum. Thus, while the genotypic data argue a compelling case, for many of the diseases under discussion as yet we lack functional evidence as to the specific involvement of NK cells in pathogenic events. It is clear from the above discussion of individual and clonal variation in expression and distribution of KIRs on NK cells depending on genotype, KIR/HLA combinations, zygosity and peptide binding that filling in these gaps is going to prove challenging and the model will need to become more complex to take account of the functional data. Furthermore, any attempt to model the functional impact of KIR/HLA genotypes must take into account the fact that those individuals carrying truncated variants of 2DS4 and 2DL4 on A haplotypes will express no activating KIRs on the surface of their NK cells, yet appear to have normal innate immunity.

NK cells are known to play an important role in a wide range of disease settings and there have been many studies relating KIR genotypes to disease susceptibility [34–36,39]. The disease studies encompass several conditions in which NK cell function might be expected to play a role. This includes viral infection, autoimmune and inflammatory conditions, tumour immunity, pre-eclampsia and recurrent spontaneous abortion [Table 1].

HIV: susceptibility to infection, disease progression and respiratory pathogens

The interplay between innate and adaptive immunity impacts on several stages of HIV infection and disease progression. NK cells are implicated in many aspects of HIV immunity [40–42]. The first issue is defence against acute infection, then priming of the adaptive response through dendritic cells and the events associated with limiting disease progression [43,44]. Lastly, there is the question of mechanisms controlling AIDS-associated infections, particularly respiratory infections [45,46].

It has long been clear from *in vitro* studies that NK cells can lyse HIV infected targets and that defects in NK cell killing are associated with disease progression [40,47,48]. NK cells can impact on HIV infection through a number of different effector mechanisms: by lysis of infected cells following class I down-regulation or altered expression due to peptide loading, by antibody-dependent cell-mediated cytotoxicity, through effects of cytokine release on DC programming and the initiation of adaptive immunity through the release of chemokines [40,41]. Secretion of the chemokines, CCL3, CCL4 and CCL5 allows for competitive inhibition of CCR5 binding, so blocking entry of R5 HIV viruses to the cell [49].

HIV is known to down-regulate HLA class I expression and thus would indeed be expected to mark infected cells for NK cell lysis. However, it became clear that the virus could down-regulate HLA-A and B while sparing HLA-C, thus helping to evade NK cell recognition [47,48]. Immunogenetic studies of progression to AIDS have, for some years, pointed to an influence of HLA-B polymorphisms, particularly those encompassed by the Bw4 serotype [50]. Carrington and colleagues then characterized 1039 patients, stratified for disease by relating time from seroconversion to several indicators of progression, looking at HLA-B and KIR genotypes [43]. They found that the known, protective effect of HLA-Bw4 alleles was limited to those carrying the amino acid isoleucine at residue 80. The significance of this is that the HLA-B alleles carrying isoleucine at this position are more effective ligands for KIR3DL1 and possible KIR3DS1. When they then examined the KIR/HLA-B compound haplotypes, the individuals with KIR3DS1 in concert with HLA-Bw4 alleles carrying isoleucine at residue 80 had significantly reduced progression. This argued that carrying an 'activatory' NK cell programme was beneficial in limiting disease.

Table 1. Human leucocyte antigen (HLA) and killer immunoglobulin-like receptor (KIR) disease associations.

Disease	KIR / HLA association	Observation	References
Infection			
CMV	> 1 activating KIR in donor	Reduced risk of CMV reactivation in recipient following bone marrow transplantation	[63]
HCV	2DL3/2DL3 - HLA-C1/C1	Resolution of infection	[51]
	3DS1 - HLA-Bw4	Resolution of infection	
	3DS1 - HLA-B-Bw4 80I	Protection against the development of hepatocellular carcinoma	[64]
HIV-1	3DS1 - HLA-Bw4 80I	Delays progression to AIDS	[43]
	3DL1 - HLA-B*57alleles that contain Bw4 80I	Delays progression to AIDS	[65]
<i>Plasmodium falciparum</i>	3DL2*002	High NK cell response to <i>P. falciparum</i> -infected RBC	[66]
Idiopathic bronchiectasis	HLA-Cw*03	Susceptibility	[53]
	HLA-Cw*06	Protection	
	2DS1 and/or 2DS2 - HLA-C1/C1	Susceptibility	
Autoimmunity			
Behçet's disease	Altered 3DL1 expression	Associated with severe eye disease	[67]
IDDM	2DS2 - HLA-C1	Susceptibility	[61]
	Decrease in inhibitory KIR-HLA genotype combinations	Susceptibility	[60]
Psoriatic arthritis	2DS1/2DS2; HLA-Cw group homozygosity	Susceptibility	[68,38]
Rheumatoid vasculitis	2DS2; HLA-Cw*03	Susceptibility	[59]
Scleroderma	2DS2+ /2DL2-	Susceptibility	[58]
Spondylarthritides	3DL2 expression increased	May contribute to disease pathology	[62]
Acute coronary syndromes	<i>De novo</i> expression of 2DS2/DAP12 in CD4+ T cells	T cells acquire cytolytic capability that can bypass TCR triggering	[69]
Cancer			
Malignant melanoma	2DL2/2DL3; HLA-C1	Susceptibility	[70]
Cervical cancer	3DS1/absence of HLA-C2 and/or HLA-Bw4	Susceptibility	[71]
	Genotype 10 (2DL1/2/3/4, 3DL1/2/3, 2DS4 2DL5*002	Susceptibility	[72]
		Protection	
Nasopharyngeal carcinoma	EBV seropositive individuals with ≥ 5 activating KIR	Susceptibility	[73]
Leukaemia	2DL2	Susceptibility	[74]
	AB1 (AML) and AB9 (CML) KIR		
	3DL1/3DL1 +Bw4 (CLL)	Susceptibility	[75]
	3DL1/3DL1 - Bw4 (CLL)	Protection	[75]
	2DL2/2DL3 +Cw1 (myeloid leukaemia)	Susceptibility	
	2DL3/2DL3 +Cw1 (myeloid leukaemia)	Protection	[76]
Cutaneous T cell lymphoma	Expression of 3DL2 on malignant cells	May contribute to disease pathology	[77]
Reproduction			
Pre-eclampsia	Maternal AA KIR genotype; fetus HLA-C2	Susceptibility	[78]
Recurrent spontaneous abortion	Mothers lacking inhibitory KIRs with specificity for fetal HLA-Cw alleles	Susceptibility	[79]

AML: acute myeloid leukaemia; CML: chronic myeloid leukemia; CLL: chronic lymphoid leukaemia; CMV: cytomegalovirus; IDDM: insulin-dependent diabetes mellitus; NK: natural killer; RBC: red blood cells.

A number of immune correlates have been examined with a view to illuminating why some HIV-exposed female sex workers remain seronegative. A small cohort of this type was compared with seropositive sex workers in Côte d'Ivoire [44]. One might assume that the immunogenetics governing the ability to block HIV entry and resulting seroconversion

may not be the same as control of disease progression, as different NK cell effector mechanisms may operate. The study showed that the exposed but seronegative sex workers more commonly carried inhibitory KIR genes in the absence of their cognate HLA genes. This was proposed to have the effect of lowering the overall threshold for NK cell activation.

Hepatitis C

As in HIV infection, it would be expected that improved outcome in hepatitis C virus (HCV) infection might be associated with a reduced tuning of NK cells for inhibition. This was investigated in a sample of several hundred patients by Khakoo and colleagues [35,51]. Exposure to HCV can lead either to complete clearance of the virus or to chronic infection, with associated liver cirrhosis and carcinoma. The first point that was noted was that the group of patients who resolved infection had a higher frequency of HLA-C group 1 homozygosity. Furthermore, protection among HLA-C group 1 homozygotes also required that they be homozygous for KIR2DL3. The binding affinity of KIR2DL3 for HLA-C is lower than that of 2DL2 or 2DL1 for their ligands. Thus the finding implicates a beneficial effect of having NK cell inhibition which is at the lower end of the spectrum, presumably allowing more possibilities for stimulation through activating receptors. As with several other observations described here, the data fit a quantitative model for NK cell programming, such that there is probably a functional difference between heterozygosity and homozygosity both for KIRs and their ligands. It should also be noted that a study of chronic HCV in Swiss intravenous drug users did not detect any significant association between inhibitory KIR genotypes, their HLA-C ligands and resolution of infection. One potentially important difference from the earlier US study was that the Swiss patients were HIV co-infected [52].

Bronchiectasis

Relatively little is known about NK cells and KIRs in chronic bacterial infection. We recently analysed HLA-C and KIR genotypes in patients with the chronic, inflammatory, lung disease, idiopathic bronchiectasis [53]. This involves a dysregulated inflammatory response and recurrent bacterial infection resulting in progressive lung damage [54]. Thus it encompasses facets both of host responsiveness to respiratory bacteria and of inflammatory immunopathogenesis. The disease is considered to include a possible autoimmune component, as it can be seen in the context of systemic lupus erythematosus, rheumatoid arthritis and ulcerative colitis. Bacterial pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Pseudomonas* are often found in the lung, with persistent neutrophil trafficking. Colonization of the lower respiratory tract by pathogens is believed to cause a chronic inflammatory response characterized by neutrophil migration into the airways and secretion of oxidants and enzymes such as neutrophil elastase and myeloperoxidase.

Bronchiectasis is one of the clinical features of transporter associated with antigen processing (TAP) deficiency syndrome, which includes a wide range of pathology including recurrent bacterial pneumonia. Mutations in either or both TAP1 and TAP2 cause reduced cell surface expression HLA

class I expression, with resulting expansions of $\gamma\delta$ and NK cells [55]. While this constitutes a rare subset of bronchiectasis patients, it demonstrates the principle that bronchiectasis can occur in the context of dysregulated NK cell function. In UK patients with idiopathic bronchiectasis, HLA-Cw*03 is associated with increased susceptibility while HLA-Cw*06 is protective. The Cw*03 allele carries a 2.27-fold increased risk and the Cw*06 allele a 0.26-fold reduced risk. In general, HLA-C group 1 motifs are associated with increased susceptibility while group 2 motifs are protective. Importantly, HLA-C group 1 homozygosity confers markedly increased susceptibility. Individuals expressing only HLA-C group 1 with 2DS1 and/or 2DS2 stimulatory KIR are more susceptible to idiopathic bronchiectasis, while individuals with 'balanced' HLA-C/KIR genotype, where both HLA-C groups 1 and 2 are expressed with 2DS1 and SDS2 stimulatory KIRs, are protected. As discussed above, HLA class I bound peptides can be either permissive or prohibitive to KIR recognition. The peptides presented by healthy cells may be set just above the threshold for inhibition of NK cells, such that minor changes in the peptide pool, for example during bacterial infection, triggering NK activation. The effect of HLA-C homozygosity, which has been noted also in other disease associations, is not reconciled easily with a classical immune response gene effect of epitope presentation to the T cell receptor (TCR). It is more compatible with the notion that homozygous individuals who completely lack ligands for inhibitory receptors will have fewer NK cells under inhibitory control. Bronchiectasis patients include increased numbers of individuals carrying only HLA-C group 1 with 2DS1 and/or 2DS2 stimulatory KIR. Thus, in common with some autoimmune or inflammatory diseases, this places bronchiectasis among those associated with a highly activatory NK cell programme. As with the other diseases discussed here, however, the hypothesis currently rests on a genotypic association and now awaits functional studies. Interestingly, NK cell cytotoxicity is implicated in pathogenesis in a mouse model of *Pseudomonas* exotoxin A-induced disease [56].

Autoimmunity

While NK cells were initially described primarily in the context of tumour surveillance and viral immunity, there have been a number of important observations made about possible contributions to autoimmunity [34,39]. Here, a number of possible mechanisms have been proposed. It might be expected that if the existence of KIR/HLA genotypes that tune NK cells in favour of activatory interactions are advantageous in some infectious disease settings, these might in some cases tend to predispose to autoimmunity. Another possible axis for modulation of NK cells in autoimmunity comes from the fact that the activating receptor NKG2D can recognize endogenous, stress induced ligands [57]. Roles attributed to NK cells in various clinical and

experimental autoimmune disease include a pathogenic function through inappropriate activation, on one hand, and suppressive functions through lysis of dendritic cells or activated T cells on the other hand.

A number of associations have been identified between risk of autoimmune disease and proposed activating KIR or KIR/HLA genotypes accompanied by a lack of inhibition. Scleroderma, a disease of tissue fibrosis, inflammation and vascular injury was, in a small sample, associated with the presence of the activating KIR2DS2, in the absence of the corresponding inhibitory KIR2DL2 [58]. A striking example of such an effect is seen in the analysis of KIRs in patients with psoriatic arthritis. There is a strong effect on risk of carrying KIR2DS1 and/or KIR2DS2, this effect being enhanced in the absence of ligands for the inhibitory receptors, KIR2DL1 and KIR2DL2/3, respectively. In this context Nelson and colleagues suggested a model of susceptibility to PsA conferred by HLA-Cw ligand homozygosity such as to minimize inhibitory signals counterbalancing *KIR2DS1* and/or *KIR2DS2* [38]. A related scenario is proposed for susceptibility to rheumatoid vasculitis, where enhanced risk is associated with presence of the KIR2DS2 gene [59]. Similarly, in type I diabetes, disease is associated with an increase in KIR2DS2/HLA ligand pairs in the presence of diminished inhibitory interactions. The authors proposed a model whereby the level of KIR function is on the autoimmune T cell through augmentation of low affinity activation signals, rather than an effect on NK cell activation *per se* [60,61]. The spondyloarthritis show a strong association with HLA-B27, making it of interest to determine whether this may involve the interaction with KIR3DL2 [62]. Both NK cells and CD4 T cells from these patients show increased KIR3DL2 expression. Furthermore, the NK cells show an activated phenotype as measured by CD38 expression.

In summary, the NK cell has emerged from its past as a simple 'null' cell to be considered a key effector of innate immunity, bridging also with activation of the adaptive immune response. The rules governing the interplay between inhibitory and activatory ligand-receptor pairs in establishing the set-point for triggering NK cell function are being elucidated rapidly. Analysis of patient genotypes in a wide range of diseases has led to a model whereby genetic susceptibility to specific infectious diseases may be associated with genotypes thought to favour a tendency to NK cell inhibition. The flipside of this is that genotypes believed to favour a tipping of the balance to NK cell activation may predispose to some autoimmune diseases.

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