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Natural Killer Cell Receptors: Functional Roles

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RECEPTORES DE CÉLULAS ASESINAS NATURALES: RELEVANCIA FUNCIONAL

RESUMEN

El Sistema inmune adaptativo, con sus receptores inmunoglobulínicos y receptores de células T, ha cautivado el enfoque de la mayor parte de la investigación inmunológica y opacado la importancia de los receptores expresados por células del sistema inmune innato. Las células Asesinas Naturales (NK) han desarrollado dos sistemas de receptores principales para llevar a cabo su función, ambos sistemas emplean receptores tanto inhibitorios como activatorios, e incluyen a miembros de la Superfamilia Inmunoglobulina–similes al igual que Receptores Lectina tipo C-símiles. Los receptores Inmunoglobulina–símiles KIR son receptores polimórficos de superficie celular capaces de reconocer a moléculas clásicas de HLA de clase I y modular de manera alternativa la repsuesta inmune a células infectadas o tumorales. Los receptores Lectina-símiles recientemente han demostrado poseer la capacidad de reconocer a moléculas no-clásicas del Complejo Mayor de Histocompatibilidad (MHC) tales como HLA-E y la cadena relacionada a clase I de MHC (MICA). La extensión de la diversidad y de la complejidad organisacional que estos receptores geneticamente pre-establecidos y no-rearreglantes poseen, ha sido recientmente demostrada. Los receptores de células NK se han visto implicados en una gran variedad de escenarios clínicos incluyendo a la resistencia/susceptibilidad a infecciones por patógenos, el reconocimiento, eliminación y vigilancia antitumoral, al igual que como elementos importantes para el desenlace del transplante de organos sólidos y de células hematopoyéticas. Las relaciones funcionales que existen entre el MHC y los receptores de células NK nos brindan una mejor comprension de la respuesta inmune a incursiones patogénicas, neoplasias asi como en la transplantación clínica.

PALABRAS CLAVE: KIR / NK / NKG2.

ABSTRACT

Adaptive immunity, with its rearranging immunoglobulin and T-cell receptors, has caught most of the attention of current immunological research and overshadowed the importance of the receptors expressed by cells of the innate immune system. Natural Killer cells have evolved two main receptor systems to carry out their functions, both of them involving activating and inhibitory receptors, and include members from the Immunoglobulin-like superfamily as well as lectin-like receptors. Killer Immunoglobulin-like receptors (KIR) are polymorphic cell surface molecules present on Natural Killer (NK) cells which recognise classical HLA class I molecules and in doing so provide an alternative means of modulating the immune response to infected or tumoural cells. Lectin-like receptors have been shown to bind to non-classical MHC molecules such as HLA-E and the MHC class I-related chain (MICA). These genetically defined, non-rearranging receptors have recently begun to show the extent of the potential variability encoded within them as well as the complexity of their organisation. NK cell receptors are involved in a great variety of clinical scenarios ranging from resistance/susceptibility to pathogen infections, tumour surveillance, recognition and elimination, and as important elements in solid organ and haematopoietic cell transplant outcome. The functional relationships that exist between the MHC and NK receptors provide a better understanding of the immune responses related to pathogen incursions, malignancies and clinical transplantation.

KEY WORDS: *KIR / NK / NKG2.*

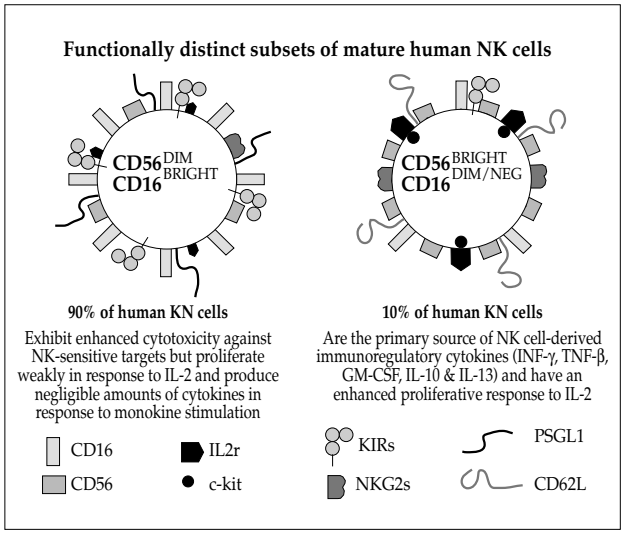

Figure 1. *Two functionally distinct subsets of human NK are recognised. These subsets have differences in the cell surface expression density of CD56 and CD16, adhesion molecule expression and MHC-receptor repertoire. These differences allow for differential trafficking, proliferative responses and cytotoxic activity. PSGL: (P-selectin glycoprotein ligand).*

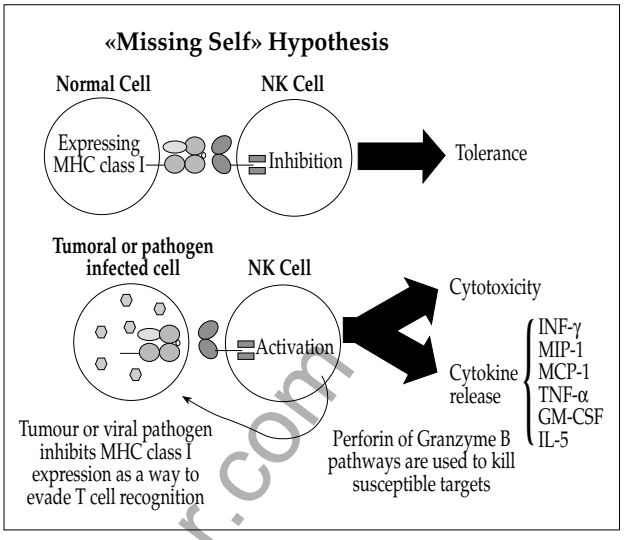

Figure 2. *According to the «Missing Self Hypothesis» NK cells fail to recognise an appropriate MHC-ligand to inhibit the otherwise activating stimuli presented by the recognition of other «unspecific» ligands on the surface of the target cell. This stimulates the NK cell to produce cytokines, chemokines and to release lytic granules.*

NATURAL KILLER CELLS

Natural Killer (NK) Cells are bone marrow derived peripherally circulating cytolitic lymphocytes slightly more voluminous than B or T cells, which comprise approximately 10% of all peripheral blood lymphocytes. Phenotypically NK cells express CD56 cell surface molecules and lack rearranging antigen receptors as well as CD3[1, 2]. Two distinct NK cell subsets defined by the cell surface expression density of CD56 have demonstrated distinctive functional roles (Fig. 1). NK cells are derived from $CD34^+$ hematopoietic pregenitor cells and require cytokines present in the bone marrow environment to mature. NK development requires NK cell progenitors to adopt a $CD34^+IL\text{-}2/IL\text{-}15R\beta^+CD56^-$ intermediate phenotype which then develops into a mature $CD56^+$ NK cell in response to IL-15. Whether this is also true for the $CD56^{dim}$ population of NK cells remains unknown[3]. These two subsets show differences in the expression of IL–2r, c-kit receptor tyrosine kinase expression, Major Histocompatibility Complex (MHC)-receptor repertoire and adhesion molecule expression. Such differences allow for differential proliferative responses, cytotoxic activities and trafficking profiles[4]. The majority of NK cells are $CD56^{dim}$ and $CD16^{bright}$ and represent the effector population responsible of natural cytotoxicity and Antibody-Dependent Cellular Cytotoxicity (ADCC)[5]. Unlike T cells, natural killing is not MHC restricted in the classical sense but influenced by MHC class I expression on target cell surface according to the «missing self hypothesis»[6] (Fig. 2), in which NK cells eliminate MHC class I-deficient target cells which have lost or downregulated the expression of the cognate MHC-receptor ligands due to oncogenic, viral pathogenic or other cellular incursions[7, 8]. Although such cytotoxicity is restricted to MHC class I-deficient hematopoietic tissues, NK cells readily kill virus-infected cells that have maintained their expression of MHC class I molecules, possibly by recognising pathogen specific epitopes on the cell surface[5].

NATURAL KILLER CELL RECEPTORS

NK cell cytotoxicity is regulated by at least two families of receptors that recognise classical MHC class I molecules on the surface of target cells and enable them to discriminate between healthy cells and pathogen infected or tumour cells by monitoring the expression levels of MHC molecules. These two NK receptors are structurally distinguished as belonging to the Immunoglobulin (Ig) superfamily, such as KIRs or as members of the C-type lectin-like domain (CTLD) superfamily, such as CD94/NKG2s. The extracellular part of lectin-like receptor resembles the carbohydrate recognition domain of a C-type lectin, whereas that of a KIR receptor is made up of immunoglobulin-like domains. Both superfamilies include both inhibitory and activating receptor variants, which have the capacity to inhibit or activate NK cell activity (cytotoxicity and/or cytokine

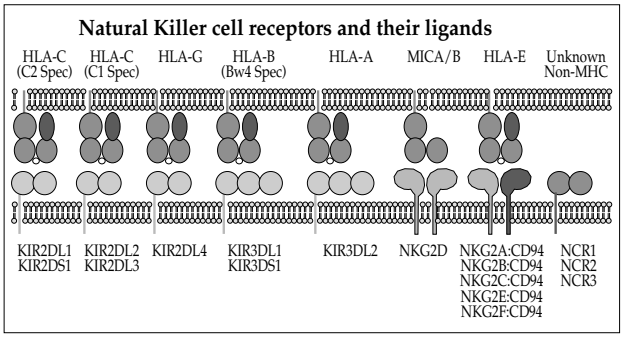

Figure 3. *The ligands for most KIRs and NKG2s have been described in detail and the crystalographic structures of some NK receptor-ligand interactions have been published*[21, 125-128]. *Although NCR1 has been shown to recognise pathogen derived proteins for Sendai Virus and Human Influenza Virus hemagglutinins, the specificity of NCR2 and NCR3 have not been described. Whether these receptors also have the potential to recognise normally expressed host ligands is also unknown.*

release) as a consequence of binding to their cognate MHC-ligands. In addition to their distinctive structures, these two families complement each other's MHC-specificities (Fig. 3). CD94/NKG2 lectin–like receptors recognise HLA-E and MICA, whereas KIR molecules recognise specific HLA-A, -B and -C allotype subsets as well as HLA-G ligands[8, 9]. Unlike the rearranging B and T cell receptors, NK cell receptors of the lectin and immunoglobulin families, are preformed and non-rearranging, their variability being a direct consequence of the genetically defined subset of genes present for each family and later modulated during NK cell development into complex combinatorial expression patterns[10, 11]. It is this preformed receptor repertoire which constitutes the hallmark of innate immunity and which allows NK cells to control pathogen incursions or cellular transformation early on during the prolonged period required for the clonal expansion of antigen-specific B and T cells[12]. NK cell MHC-receptors are encoded by two large and dense immune gene complexes located on different chromosomes. The natural killer complex (NKC) which contains the genes encoding the lectin-like family of receptors is located on mouse chromosome 6 and human chromosome 12. The leukocyte receptor complex (LRC), which contains the KIR encoding genes, is located on human chromosome 19. The importance of CTLDs in human innate immunity resulted from observations that NKG2D binds to the stress-induced MICA and MICB. The CTLD family of receptors include NKG2A, NKG2B, NKG2C, NKG2D, NKG2E and NKG2F. In humans, NKG2A, -B, -C, -E and -F form heterodimers in conjunction with CD94 and give rise to both activating and inhibitory proteins. NKG2A, -B and -C complexes with CD94 recognise HLA-E, an MHC molecule which presents nonameric peptides derived from leader sequences of other HLA class I molecules[5]. Such interaction confers CTLD receptors the ability to monitor the global MHC class I repertoire. Although mice have an apparently orthologous organisation of CD94 and NKG2D, KIR genes are exclusive to primates and no mouse homologue of a KIR gene has been reported, however mice have evolved a CTLD molecule to fulfil the function of KIR proteins, called Ly49[13]. Given the current knowledge regarding NK cell receptors, it seems very unlikely that a single NK receptor will be responsible for the diverse biological properties attributed to NK cells[14]. Recent findings, however, have described three non–MHC–class–I–specific activating receptors belonging to the Ig-superfamily but not related to KIRs, termed NKp46, NKp44 and NKp30 (Human Genome Organisation Gene Nomenclature Committee approved gene symbols: NCR1, NCR2, NCR3 respectively, for Natural Cytotoxicity-triggering Receptors)[15]. Unlike KIRs and CTLD receptors, NCRs are exclusively expressed on NK cells and seem to be the main receptors involved in NK cell-mediated tumour lysis[13]. KIRs are by far the most polymorphic receptors present on NK cells.

KILLER IMMUNOGLOBULIN-LIKE RECEPTORS (KIR)

KIRs are polymorphic cell surface molecules present on NK cells and a small (8%) population of T cells known as Natural Killer T cells (NKT). They recognise HLA class I molecules and in doing so provide an alternative means of modulating the immune response to damaged or foreign cells[16]. KIR proteins possess characteristic Ig-like domains on their extracellular regions which are involved in classical MHC class I ligand binding and transmembrane and cytoplasmic regions defining the type of signal which is transduced to the NK cell. KIR proteins can have two or three Ig-like domains. In the current nomenclature used to describe KIR genes, the number of Ig-like domains present are indicated by a 2D for two domain KIRs or 3D for three domain KIRs; the presence of a short or long cytoplasmic tail being indicated by an S or L, respectively, at the end of the name. Two domain KIR proteins are subdivided into two large groups depending on the origin of the membrane distal Ig-like domains present. Type I KIR2D proteins (KIR2DL1, -2DL2, -2DL3, -2DS1, -2DS2, -2DS3, -2DS4 and -2DS5) possess a membrane-distal Ig-like domain similar in origin to the KIR3D D1 Ig-like domain encoded mainly by the fourth exon of the corresponding KIR genes, and lack a D0 domain. Type II KIR2D proteins, KIR2DL4 and -2DL5, possess a membrane-distal Ig-like domain similar in origin and structure to the D0 domain present in KIR3D proteins encoded mainly by the third exon of the gene, however lack a D1 domain. KIRs control the response of

human NK cells by delivering inhibitory or activating signals upon recognition of MHC class I ligands on the surface of potential target cells[17]. KIR proteins can possess short or long cytoplasmic tails. Long cytoplasmic tails usually contain Immune Tyrosine-based Inhibitory Motifs (ITIMs) which transduce inhibitory signals to the NK cell. Short cytoplasmic tail KIRs possess a positively charged amino acid residue (lysine) in their transmembrane region, which allows them to associate with a DAP12 signalling molecule capable of generating an activation signal[11]. The existence of KIRs with MHC binding properties was suggested as a consequence of observations relating to NK cell killing of HLA class I–deficient B lymphoblastoid cell lines which could be reversed by transfecting these cell lines with certain HLA class I genes[18-20]. Two-domain KIRs recognise HLA-C allotypes while three domain KIRs recognise HLA-B allotypes. KIR2DL1 exhibits C2 specificity and recognises HLA-C allotypes with Asn^{77} and Lys^{80} (for example: HLA-Cw4, HLA-Cw2, HLA-Cw5 or HLA-Cw6). KIR2DL2 has a C1 specificity and recognises HLA-C allotypes with Ser^{77} and Asn^{80} (for example: HLA-Cw3, HLA-Cw1, HLA-Cw7 or HLA-Cw8)[21]. KIR3DL1 recognises HLA-B allotypes with a Bw4 motif on their a-helix (for example: HLA-B13, HLA-B38 and HLA–B51), and KIR3DL2, has been shown to recognise HLA-A molecules. Although KIRs with specificity for HLA–A[22], -B[23], -C[24] and –G[13] allotypes have been defined; for the other KIR, the specificity currently remains unknown. The genetic factors determining KIR repertoire diversity are related to the type of KIR genes present on any given individual. Recent studies, however, have demonstrated the role that other immune cell surface molecules, such as HLA molecules and NKG2 related receptors, have in determining the predominantly expressed KIR in any given cell.

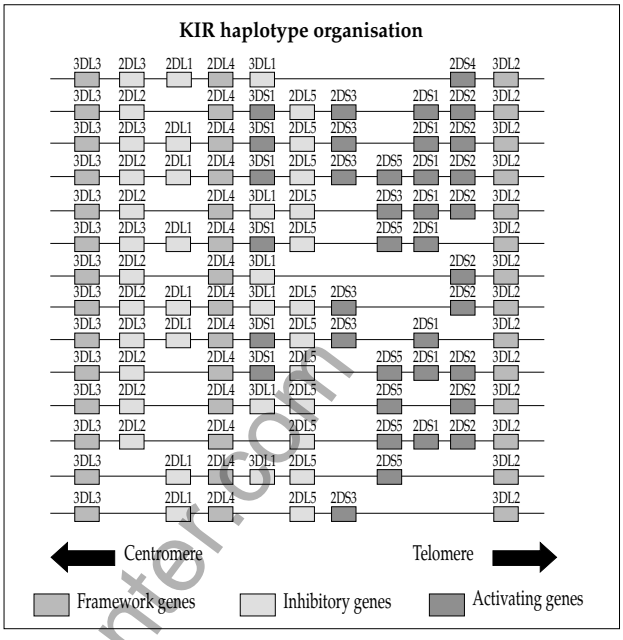

Figure 4. *KIR genes are encoded within a 1 MB stretch of DNA in chromosome 19 known as the LRC. All KIR haplotypes are flanked on their centromeric ends by KIR3DL3 gene and in their telomeric end by KIR3DL2. Together with KIR2DL4 located in the centre of this region, these three genes represent the framework genes so called because they are present in all human individuals. Each KIR gene spans approximately 14 Kb and is separated from neighbouring genes by 2 Kb intergenic sequences. NOTE: the order in which KIR genes are organised within a haplotype has not been entirely determined for some KIR genes. Figure adapted from Trowsdale[26].*

KIR GENES

The KIR gene family consists of 14 genes and 3 pseudogenes encoded within a 150 Kb region of the LRC on chromosome 19 (19q13.4). The LRC constitutes a large (1 Mb), dense cluster of rapidly evolving immune genes of relatively recent evolutionary origin[25]. The LRC and its centromeric prolongation, the extended-LRC[9], contain genes encoding cell surface molecules with distinctive immunoglobulin-like extra-cellular domains[26, 27]. KIR genes are organised into haplotypes, which have been defined in family segregation studies[16]. The number of KIR genes present in any given haplotype may vary[8, 16]. All known KIR haplotypes are flanked at their centromeric end by KIR3DL3 and at their telomeric end by KIR3DL2, together with the centric KIR2DL4, constitute the framework genes[9, 28]. The framework genes limit two regions of variable KIR gene content where the remaining KIR genes are arranged in a head to tail fashion approximately 2 Kb apart from each other. KIR haplotypes show extensive haplotypic diversity characterised by variability in the number of genes present (Fig. 4), which can range from 7 to 11 KIR genes[16, 29]. Most KIR genes are approximately 14 Kb long and divided into 9 exons. The KIR proteins are encoded as follows: exon 1 together with exon 2 encode for the leader sequence of KIR proteins, exon 3 encodes for the membrane distal (D0) Ig-like domain (in the case of KIR3Ds and type II KIR2Ds), exon 4 encodes the middle (D1) Ig-like domain (present in type I KIR2Ds but absent in type II KIR2Ds), exon 5 encodes the membrane-proximal (D2) domain of all known KIR proteins, exon 6 encodes the stem region (classically absent in KIR3DL3), exon 7 encodes the carboxyl end of the stem region, the entire transmembrane region and the amino end of the cytoplasmic region, exon 8 encodes the first 18 amino acids of the cytoplasmic region and, finally, exon 9 with its varying lengths, encodes the remaining portion of the cytoplasmic region of KIR proteins. KIR genes have

been shown to be polymorphic and more than 91 sequences representing alleles of the seventeen gene loci have been described. Unlike HLA class I and class II, in which most of the polymorphism of functional significance is restricted to one or two exons, KIR polymorphism is evenly distributed throughout the KIR gene. KIR nucleotide sequences are arranged into groups or KIR loci based on the number of extracellular Ig-like domains, length of the cytoplasmic tail and sequence similarity[11]. Recent segregation studies carried out in families have shown how KIR sequences previously thought to be different genes based on cytoplasmic tail length differences may represent alleles based on the inheritance behaviour observed[16]. This is the case of KIR3DS1 and KIR3DL1, which differ by only 6-12 amino acid residues. Interestingly, no interaction of KIR3DS1 with Bw4 motif bearing HLA-B alleles has been found to date[11]. The way in which allelic polymorphism further diversifies the haplotypic variations shown in figure 4 has recently been demonstrated in high-resolution studies[16]. The extent of such diversity makes the possibility of finding a KIR matched unrelated individual very low. Whether the polymorphism of KIR genes translates into functionally distinct proteins responsible for certain biological advantages remains unknown.

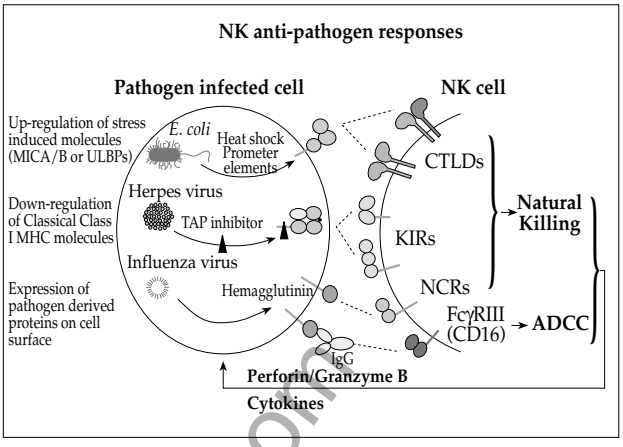

Figure 5. *NK cells can limit the magnitude of initial pathogen incursions in three ways. By recognising stress induced molecules expressed by infected cells typically by C-type lectin-Like Domain receptors, By failing to recognise «self» as a consequence of pathogen induced CTL evasion strategies, and by directly recognising pathogen derived proteins on the surface of infected cells.*

ROLE OF NK CELLS AND NK RECEPTORS IN PATHOGEN INCURSIONS

NK cells have been demonstrated to be critical elements in the early immune response to a large variety of intracellular pathogens. Of particular interest are the anti-viral responses, which have been extensively studied[30, 31]. Although limited studies have reported the importance of NK responses against cells infected with intracellular bacteria such as *Listeria, Salmonella* and *Legionella* in humans, recent developments have shown that NK cells play an important role in the innate immune response to microbial pathogens. The role of NK cell responses in such bacterial infections has been further supported by experimental NK dependent lysis of bacteria infected–cells and bacterial growth-inhibition[32, 33] as well as by the description of NK selective deficiencies associated with recurrent polymicrobial infections[12, 34, 35]. A role for NK cells in antibacterial responses has been demonstrated experimentally for *Toxoplasma gondii*, *Listeria monocytogenes* and *Leishmania major* in murine models[3, 36-38]. NK cells have been shown to participate in anti-pathogen responses in four ways (Fig. 5). The first of which is classically described as the result of the downregulation of MHC class I molecules which intracellular pathogen-infected cells undergo as a consequence of a direct cytopathic effect or the presence of pathogen «stealth» gene products which specifically interfere with the MHC class I processing[39, 40]. In vitro experiments have demonstrated the downregulation of HLA-C molecules (known to serve as ligands to the inhibitory receptors KIR2DL1 or KIR2DL2/KIR2DL3) in Herpes virus-infected cells and the subsequent triggering of isolated NK cell cytotoxic responses against them. The use of this pathway by NK cells has been further substantiated by the finding of viral-TAP inhibitor ICP47 of HSV (Herpes Simplex Virus) and the MHC class I-destroying US11 protein of HCMV (Human Cytomegalovirus). Bacterial downregulation of mononuclear phagocyte cell surface MHC expression has also been described in *Salmonella*, *Yersinia* and *Chlamydia pneumoniae infections*[41-43]. As expected for rapidly evolving viruses subjected to Cytotoxic T Lymphocyte (CTL) selective pressures, Human Immunodeficiency Virus (HIV-1) has also devised a way to elude CTL responses by downregulating host-cell's MHC expression[44, 45]. The *nef* gene product of HIV-1, is known to decrease HLA-A and –B expression levels but not those of HLA-C by accelerating the surface endocytosis rate in a highly selective manner which depends on the cytoplasmic tail region of the class I proteins involved[44]. NK receptors specific for HLA-A and HLA-B allotypes have been defined (KIR3DL2 and KIR3DL1, respectively) and as at least one of them (KIR3DL2) is known to be a structural gene which is present in all individuals[29, 46]. Based on this, one should expect to find NK cell clones capable of generating potent responses against these HLA-A and -B deficient HIV-infected cells. Other interesting aspects regarding HIV-1 infection

and KIRs are those related to the epistatic interactions that exist between KIR3DS1 and HLA–Bw4 allotypes bearing Isoleucine at position 80. Studies in HIV infected individuals have also demonstrated that the presence of the HLA-B specific KIR3DS1 activating receptor in combination with HLA-Bw4 alleles with an isoleucine at position 80 is associated to a delayed progression to AIDS presumably as a consequence of the activation of NK cells and subsequent elimination of HIV–infected cells. However, whether this represents a true functional association between these two loci which might be specific or not for HIV infections or the result of interactions between other genes in linkage disequilibrium with KIR3DS1 remains unclear[47]. These studies have shown that this activating KIR in combination with HLA-B molecules that express a Bw4 motif, are associated with a delayed progression to AIDS in HIV–1 infected individuals, however, KIR3DS1 seems to be linked to a faster progression to AIDS when present in the absence of alleles expressing a Bw4 motif. These results have conclusively demonstrated a role for KIR in the recognition of virally infected cells[48].

A second way in which NK cells can limit pathogen incursions is by secreting cytokines which modulate the subsequent adaptive immune response. IFN-γ production by NK cells plays a critical role at activating macrophages and at inducing resistance to intracellular pathogen infections in other cells[49-54]. NK cells have the potential to recognise unspecific «danger signals» expressed on the cell-surface of stressed cells. An example of this type of recognition involves NKG2D binding of the nonclassical MICA and MICB[55-57] and the GPI-linked UL16 Binding Proteins (ULBPs)[58] belonging to the extended MHC class I family. The expression of ULBPs or MICA/B molecules on the surface of NK resistant target cells confers susceptibility to NK dependent lysis. Such interactions result in the activation of NK cells and stimulate cytokines and chemokines production and release, proliferation, cytotoxic activity and upregulating the expression of other activating receptors on the NK cell surface[59-61]. Nevertheless, some pathogens and in particular HCMV, have evolved strategies to evade NK cell recognition and activation by producing ULBP and MICA/B mimicking proteins such as UL16, which blocks this interaction and enables the virus-infected cell to evade NK cell lysis. Although NK cells exhibit aggressive cytotoxic activity against susceptible targets without the need of costimulatory cytokines, their exposure to IFN-α, IFN-β or IL-12 has been shown to increase such cytotoxicity 20- to 100-fold[62-68]. IL-12 together with TNF-α can also stimulate NK cells to produce large amounts of IFN-γ, a cytokine known to play a crucial role at limiting some infections. The fact that NK cells constitute the main source of IFN-γ during the first days of infection and before an effective CTL response has been achieved has been demonstrated experimentaly in viral[64, 69, 70], bacterial[71, 72] and parasitic[73, 74] infections.

The third way by which NK cells contribute to anti-pathogen incursions is thought to be a consequence of the direct recognition of pathogen derived structures on the surface of the infected cell. The use of this «direct recognition» pathway by NK cells has been clearly supported by clinical and experimental findings. The recent discovery of NK receptors (NCR1) capable of recognizing pathogen-derived structures (Influenza Virus hemagglutinin and hemagglutinin–neuraminidase of the Sendai Virus) present on the cell–surface of infected cells has expanded the potential functional roles of NK cells and receptors[75]. A similar finding related to anti-bacterial responses evolved from observations of healthy individuals who had been in close contact with *Mycobacterium tuberculosis* infected patients and who had never developed a positive tuberculin skin test, suggesting a possible innate immune response prior to CTL recognition of the pathogen. Subsequent studies revealed the existence of NK cell-mediated lysis of *Mycobacterium tuberculosis* infected monocytes which had not downregulated their expression of MHC class I molecules, a response which did not seem to be a consequence of enhanced production of IL-18 or IFN-γ[38]. The description of these NK cell pathogen-specificities raises the question whether certain NK receptors are involved in the recognition of other pathogens of clinical relevance or whether the extensive polymorphism of the NK receptor families that have been described so far is the result of pathogen pressures and as such confer susceptibility or protection to them.

A fourth way in which NK cells have the ability to eliminate pathogen infected cells is through ADCC. NK cells recognise the Fc portion of IgG antibody molecules, present on the surface of infected cells, through FcγRIII (CD16)[5]. This receptor forms part of the Ig-superfamily and is also expressed on macrophages and mast cells and as such does not represent a cytotoxic pathway exclusive of NK cells.

ROLE OF NK CELLS AND NK RECEPTORS IN IMPLANTATION BIOLOGY

HLA-G and KIR2DL4 represent a unique NK receptor/ligand interaction which has shown to play an important role in embryonic implantation. HLA-G exhibits limited polymorphism[2] and its expression is restricted to fetal extravillous cytotorophoblasts (FECs). FECs also express HLA-E and HLA-C molecules but not HLA-A and HLA-B[76, 77]. HLA–G protects the semi-allogeneic embryonic tissue

against maternal NK cells present in the decidua. Although current knowledge has shown that the best candidate for HLA-G binding is KIR2DL4, HLA-G has also been shown to inhibit NK cells through Immunoglobulin-like transcripts (ILT-2)[78] and by CD94:NKG2A recognising HLA-E presenting the leader peptide of HLA-G[79]. The unique ability that KIR2DL4 has at recognising a nonclassical HLA class-I molecule is thought to be the result of this KIRs characteristic divergent structure[11]. This KIR is encoded by a framework gene and as such is thought to be expressed by all NK cells[80]. As NK cells are the predominant leukocytes present in implantation sites during the first trimester, a role for this particular interaction regarding maternal tolerance to the foetus has been proposed. Recent findings have suggested an activating role for this KIR by stimulating IFN-γ production[81]. Interestingly, IFN-γ has been shown to promote angiogenesis in maternal decidua, a requirement for implantation. NK cells have been shown to be less permisible to FEC invasion of maternal decidua in the presence of anembryonic pregnancies which result in first trimester fetal rejections. The mechanism by which NK cells recognise such anembryonic pregancies is thought to be the result of either the lack or downregulation of KIRs specific for FEC-expressed HLA-C allotypes or the upregulation of activating CTLDs[82]. Conversely, it is possible that certain NK repertoires may influence the susceptibility to other types of implantation disorders such as eclampsia. Eclampsia is the life threatening situation that evolves as a consequence of abnormal invasion of the maternal decidua by FECs. Although some studies relating to KIRs in this particular scenario have ruled out any association between gene content and clinical outcome[83], decidual NK cells have been shown to possess distinctive phenotypes and NK receptor profiles in comparison to those present in peripheral NK cells within the same individual[82].

ROLE OF NK CELLS AND NK RECEPTORS IN TUMOUR SURVEILLANCE

Natural killer cells were described two decades ago as functionally capable of lysing certain tumour cells[84-87]. Tumour immunity has shown to require the participation of potent lymphocyte effector responses. Both NK cells and CTLs, once activated, possess similar lytic pathways to carry out their functions, although triggered by different antigen receptors. NK cells use at least three structurally different receptors for this purpose: KIRs, NKG2s and NCRs[88-90] which mediate cytotoxicity via perforin and granzyme B[91-93]. A unified signal cascade triggered by susceptible target cell recognition has been postulated for a common signal pathway that leads to the mobilisation of lytic granules containing perforin and granzyme B towards the immune synapse[94]. NK cells exhibit spontaneous cytotoxic activity against tumour cell lines expressing unspecific inflammatory »stress-induced» ligands[93, 95] (which bind to activating NK cell receptors) as well as by recognising the absence of MHC class I molecules on the surface of the tumour cell[1, 93, 96]. These immunoevasive strategies constitute an attempt to escape immune detection by CTL and include the downregulation of MHC class I molecules on their cell surface, production of immunosupressive cytokines (such as TGF-β) and the increase of the levels of expression of Fas ligand[39]. Tumour cells that lack appropriate MHC class I molecule expression induce NK cell infiltration, cytotoxic activation, cytokine production and induction of transcription of IFN-γ in NK cells[39]. Of special oncological interest is the lectin-like NKG2D homodimer, which associates with the Phosphatidyl inositol 3 kinase activator DAP10. This NK receptor is broadly expressed on NK cells, γδT cells, macrophages and $CD8^+$ αβT cells[95]. This receptor has the ability to interact with a diverse family of MHC class I-related ligands not involved in peptide presentation, which are induced by cellular stress (such as MICA, MICB and ULBPs). Although the expression of these NKG2D ligands is low on the normal adult tissues, the increased expression of MIC has been widely documented in many epithelial carcinomas[58, 97, 98]. Ectopic expression of this ligand has demonstrated to elicit NK cell mediated cytotoxicity and cytokine production. IL-2 activated NK cells are of special interest in relation to tumour immunotherapy. These cells have been shown to infiltrate established lung and liver solid tumours and induce their regression. A further stimulation in such patients with an MHC class I expression inhibitory such as that based on TAP inhibition by infected cell protein (ICP)47 could possibly contribute to making this therapy more efficient[39]. Of similar immunotherapeutic potential is the concept of deleting inhibitory signals to optimise NK and NKT cell responses, even those activated with stimulatory cytokines, such as IL-2. This approach has demonstrated to be a powerful tool at eradicating tumours when the tumour burden is minimal as that occurs after cytoreductive therapy. This same approach could theoretically be used to elicit vigorous NK cell mediated antiviral responses[99]. New immunotherapeutic strategies should consider the way in which innate immunity might be able to control the development and nature of adaptive immunity by means of dendritic cell (DC)-NK cell interactions. The activation of pattern recognition receptors on DCs enables them to activate NK cells in the vicinity and consequently guide tumour recognition and lysis. The apoptotic cell bodies which result from such lysis are then taken up, transported

and processed by the DCs and presented to T cells, thus affecting the outcome of the subsequent adaptive immune responses[100]. Enhancement of the antineoplastic cytotoxicity of NK cells and infusion of selected NK cells as alternatives to CTL seem to be very promising in the treatment of haematological patients with low tumour burden (e.g., after stem cell transplantation or cytoreductive therapy)[26]. The recently described natural cytotoxicity triggering receptors (NCR1-3) have also been shown to play a crucial role in antitumoural responses[13].

ROLE OF NK CELLS AND NK RECEPTORS IN HAEMOPOIETIC TRANSPLANTATION

The relevance of NK cell function in the transplantation setting is fundamentally based on the fact that both KIR and NKG2 receptors have been shown to bind to specific MHC ligands, the role that MHC molecules play in this same setting having being described extensively during the last decades for heart[101, 102], kidney[103], cornea[104], lung[105], bone[106] and hematopoietic stem cell[107] transplants. The behaviour of NK cells in this setting is tightly regulated by a large number of structurally and functionally distinct receptors capable of generating activating or inhibitory signals. The existence of human NK cell receptors capable of mediating specific allorecognition was established in 1990[108]. Although a function for NK cells has been described in mediating graft-versus-host disease (GvHD) in bone–marrow transplantation, it remains unclear whether NK cells play a role in the rejection of solid organ transplants[40]. Other studies have shown that, at least under standard immunosuppressive therapy, alloreactive NK cells did not seem to play a major role in acute hepatic allograft rejection[109]. Studies regarding the behaviour of NK cells in an HLA haplotype-mismatched Haemopoietic Stem Cell Transplantation (HSCT) setting have produced controversial results regarding the level of KIR matching necessary to allow for optimal engraftment and potent Graft-versus-Leukaemia (GvL) responses while maintaining the level of GvH reactions to a minimum. Probably the most striking of which was the absence of GvHD despite donor–versus-recipient KIR alloreactivity. A finding that seems to suggest that alloreactive NK clones are either eliminated or survive in a state of anergy as has been shown to happen in MHC class I-deficient mice[110]. Another possible explanation is the opposite sense of the hybrid resistance murine model, in which F1 generation NK cells reject parental haematopoietic cells, but tolerate solid organ grafts of the same origin. These findings, coupled to the observed failure of the host MHC repertoire to educate or select a compatible KIR repertoire in this same HSCT setting, seem to indicate that NK cell sensitivity to HLA class I polymorphism might be restricted to haematopoietic cells. The rapid donor-derived NK cell reconstitution of the stem cell transplant recipient strongly suggests that large-scale maturation of the engrafted stem cells plays a much more important role than the expansion of mature NK cells present in the stem cell graft at replenishing the NK population[111]. This same study addressed the GvL potential of donor–versus–recipient alloreactive NK cells and demonstrated a significant GvL effect against all myeloid leukaemias but only against a minority of lymphoblastic leukaemias, possibly as a consequence of adhesion molecule expression differences amongst these tumours. As a consequence of this same donor NK alloreactivity, most of the host lymphocytes mediating rejection were killed, which led to a decrease in the number of rejection events observed in this cohort of patients, similarly, no myeloid relapses were observed whereas relapse did occur in the NK-resistant Acute Lymphoblastic Leukaemia (ALL) patients. This transplant setting provides an overall view of NK cell interactions of bulk populations, however provide very little information on the function of individual NK cells during target engagement[92]. Typing for the presence of particular KIR genes may be indicated for stem cell donor-recipient pairs for whom an HLA class I mismatch is unavoidable[112]. Umbilical cord blood transplants (UCBT) have now been widely accepted as an alternate source of stem cells for patients with malignant haematological and genetic disorders. The incidence of GvHD after UCBT has been noted to be lower than that resulting from other sources used. Recent findings seem to suggest that the low incidence of GvHD observed in UCBT recipients may be partially due to early NK cells suppressing the activity of effector cells known to cause GvHD or by regulating the activity of Antigen Presenting Cells (APCs)[113].

ROLE OF NK CELLS AND NK RECEPTORS IN IMMUNE TOLERANCE

Discrimination of self by the immune system's lymphocytes is just as essential to the preservation of our own tissues as recognising nonself material, especially important once an immune system develops aggressive strategies to destroy other cells, such as CTLs and NK cells, which are easily activated[110]. In the case of T cells, the presence of differentiation antigen specific clones which express inhibitory NK receptors in healthy individuals further supports the notion that inhibitory receptors control T cell tolerance to some peripheral antigens[114]. For the NK cells, signalling through MHC-specific inhibitory receptors might be a possible mechanism

by which they remain self-tolerant. Inhibitory receptors transduce their signals to the NK cell by means of SH2-containing protein tyrosine phosphatase (SHP-1). A reduction in SHP-1 activity has been associated to NK abnormalities, which result in defective natural killing. An important role for SHP-1 in self-tolerance induction[92, 115] has been suggested based on the possibility that both inhibitory and activating receptors might share a common SHP-1 pathway. Similarly, the blocking of the MHC-KIR interaction is sufficient to enable NK cells to kill normal cells, further supporting the importance of the inhibitory receptors at avoiding NK autoaggression. This hypothesis is also supported by the fact that every single human NK cell expresses at least one inhibitory receptor (which may be either KIR or NKG2) with specificity for a self–MHC molecule. Although MHC class I molecules do not seem to be required for the generation of a mature NK cell population tolerant to self, it has been shown to influence individual NK cell KIR repertoires[10]. Perhaps the best studied scenario in which NK cells have been linked to tolerance is that of implantation biology. During implantation, a fine balance has to be achieved in order to FEC invasion of the uterine decidua that will ultimately ensure an adequate blood perfusion for the developing embryo. The control of such invasion is thought to rely on a distinctive population of NK cells ($CD3^-$ $CD16^-$ $CD56^{bright}$), which accumulate in the decidua basalis at the implantation site and come into close contact with invading FECs. The result of these interactions which ultimately decides the fate of the developing embryo. Another NK receptor which might play an important role at inducing NK cell tolerance is the CTLD heterodimer CD94/NKG2 receptor, which has shown to bind specifically to HLA-E molecules. This is mainly based on the observation that the recruitment of HLA-E at the surface of a transfected mouse cell by the addition of synthetic peptide ligands provides protection from lysis by NK cells expressing this CTLD[110].

CONCLUSION

Although adaptive immunity with its rearranging receptors has been the focus of the great interest in immunological research, NK cell receptor biology and interactions have the potential to explain many events related to immune response to pathogens, antitumoural surveillance and transplantation issues that can not be addressed in the isolated context of MHC. As has been described in the previous sections NK cells have evolved highly specialised mechanisms to recognise normal healthy cells from those cells which have suffered malignant transformation or pathogen infection. Two extensively studied pathways for doing so have been described, those related to KIR and those related to CTLD receptors. These structures enable the NK cells to recognise a wide range of MHC determinants with locus and allele specificity, and in doing so, monitor the expression of most MHC molecules independently. Although initially thought to have redundant specificities for MHC molecules, recent studies[11] seem to indicate that KIRs and CTLDs have complementary specificities, where the first receptor system seems to monitor MHC diversity through the direct survey of polymorphic MHC motifs and the second receptor system is mainly involved in recognising conserved MHC peptides while ignoring the detailed MHC allele polymorphisms. The consequence of such approaches being the generation of a complex and polymorphic system for KIR genes and a relatively conserved system for the CTLDs. However, this oversimplistic view of the NK receptors has recently been challenged by the discovery of pathogen specific responses involving KIRs, which have been further supported by the description of increased susceptibility to specific bacterial and viral infections[34] in NK deficient patients. In a similar way, NK cell receptors have been linked to other non-malignant pathologies which seem to hint at their involvement in certain autoimmune[26] and inflammatory disorders which include rheumatoid arthritis[116], sarcoidosis[117], endometriosis[118], vascular leak syndrome[119], psoriasis[120] as well as possible roles in human diseases of the nervous system[121]. Similarly, the manipulation of KIR gene expression may allow new strategies to be developed in transplant settings to allow for adequate GvL effects or to modulate graft rejection[40]. KIR and NK cell receptor based strategies might also provide potentially useful approaches as antimicrobial agents in patients with a great variety of intracellular pathogens which include Herpes virus infections and in the near future may even provide an alternative therapeutical adjuvant in HIV infected patients[70, 122-124].

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