PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 2000 February; 6(1):1-11
World Journal of Gastroenterology
 ISSN 1007-9327

Reviews

On the cell biology of pit cells, the liver-specific NK cells

Luo DZ, Vermijlen D, Ahishali B, Triantis V, Plakoutsi G, Braet F, Vanderkerken K and **Wisse E**

Subject headings pit cells; hepatic natural killer cells; large granular lymphocytes

INTRODUCTION

\Natural killer (NK) cells are functionally defined by their ability to kill certain tumor cells and virus-infected cells without prior sensitization[1]. NK cells comprise about 10% to 15% of lymphocytes in the peripheral blood and most of these cells in human and rat have the morphology of large granular lymphocytes (LGL)[2]. However, recent studies have demonstrated that small agranular lymphocytes, lacking CD3 expression, have cytolytic activity comparable to NK cells[3]. These variations may be related to the stage of NK cell differentiation or heterogeneity[4]. Moreover, some cytotoxic T lymphocytes (CTL) also display LGL characteristics[4]. Besides NK cells in peripheral blood, NK cells are also found in tissue compartments, such as the spleen, lung, intestine, lymph nodes, bone marrow and liver[4]. NK cells in the liver, also called pit cells[5], constitute a unique, resident NK population in the liver sinusoids. Their immunophenotypical, morphological and functional characteristics are different from blood NK cells[6]. Presently, several names, such as pit cells, hepatic NK cells and LGL are used to describe pit cells, referring to different aspects of their morphology or function[6]. We prefer to use the first-given name of pit cells for these cells in the liver, because it is not related to the ever varying levels of function and morphology or a person (like Kupffer cells)[7]. In addition, liver-associated lymphocytes (LAL), in some instances, are used to describe the total population of lymphoid cells in the liver[8,9]. However, LAL contain about 30% T lymphocytes, 3% B lymphocytes besides 43% pit cells in a human liver washout[9]. Rat liver washouts contain 43% T lymphocytes, 16% of B lymphocytes, 3.3% of monocytes and 26% pit cells[10].

The present review will discuss the biological relevance of pit cells with emphasis on rat liver.

IDENTIFICATION, STRUCTURE AND TISSUE DISTRIBUTION OF PIT CELLS

Pit cells were firstly described in 1976 by Wisse *et al*[5]. The name pit cell was introduced because of the characteristic cytoplasmic granules, which in Dutch language are called pit, resembling the pits in a grape[5]. The hypothesis that pit cells might possess NK activity was suggested by Kaneda *et al*[11], because of their morphologic resemblance to LGL. The deve lopment of a method for the isolation and purification of pit cells from rat liver and the evidence of pit cells possessing spontaneous cytotoxicity against NK sensitive YAC-1 lymphoma cells confirmed these cells to be hepatic NK cells[12,13].

Pit cells exist in the liver sinusoids and often adhere to endothelial cells (Ec), although they incidentally contact Kupffer cells (Kc) (Figure 1). They face the blood directly. Pseudopodia of pit cells can penetrate the fenestrae of the Ec, and enter the space of Disse and can directly contact the microvilli of hepatocytes[5,14]. Their appearance in the space of Disse is not a c ommon feature[15]. By morphological investigation, the frequency of pit cells in liver tissue is about an average of 1 pit cell per 10 Kupffer cells. The number of pit cells, in untreated rats, is therefore estimated to be (1.4-2) $\times 10^6$ cells per gram liver weight[12]. By immunohistoc hemistry, using mAb 3.2.3 against NKR-P1A (a specific marker of NK cells), the

Dian Zhong Luo[1,3], David Vermijlen[1], Bülent Ahishali[1], Vasilis Triantis[1], Georgia Plakoutsi[1], Filip Braet[1*], Karin Vanderkerken[2*] and Eddie Wisse[1]

[1]Laboratory for Cell Biology and Histology, [2]Department of Hematology and Immunology, Free University of Brussels (VUB), Brussels-Jette, Belgium

[3]Department of Pathology, Guangxi Medical University, Nanning, China

*Filip Braet and Karin Vanderkerken are postdoctoral research fellows of the Fund for Scientific Research Flanders.

Dian Zhong Luo, male, born on 1955-07-07 in Guilin, Guangxi, graduated from Guangxi Medical University in 1982, got Master degrees in 1987 in Guangxi Medical University and in 1994 in Free University of Brussels, Professor of Pathology of Guangxi Medical University, and now is following Ph.D. program in Medical Sciences in Free University of Brussels, Belgium, having more than 30 papers published.

Supported by grants 3.0053.92,3.0050.95, 9.0038.96,1.5.411.98 from the National Foundation for Scientific Research (FWO) and grants 194.322.1740,195.332.1310,196.322.0140 and OZR.230 from the Research Council of the Free Univ ersity of Brussels.

Correspondence to: Prof. Dr. Eddie Wisse, Laboratory for Cell Biology and Histology, Free University of Brussels (VUB), Laarbeeklaan 103, B-1090 Brussels, Belgium
Tel. (32)-2-477 4404, Fax. (32)-2-477 4405
Email. wisse@cyto.vub.ac.be

Received 1999-09-22 **Accepted** 1999-11-15

number of pit cells in frozen sections of rat liver is about 13.7 per mm^2[16]. After intravenous injection of biological response modifiers (BRM), the number of pit cells increases 4-fold to 6-fold in rat liver treated with zymosan[17] and 43-fold with interleukin-2 (IL-2)[18]. The surplus of pit cells is considered to originate from local proliferation and from the bone marrow[17,18]. Pit cells were found to be more numerous in the periportal than in the pericentral region of the liver lobule[11,16].

Pit cells have essentially the same morphology as NK cells from blood and other organs, *i.e.* LGL (Figure 1). LGL morphology is characterized by a relatively large size, the presence of granules in the cytoplasm, a pronounced asymmetry of the cell and an indented or kidney shaped nucleus of high density[10]. Pit cells in the rat are about 7μm in diameter and vary in shape, while possessing well developed pseudopodia. They show a pronounced polarity with an eccentric nucleus and most organelles lying at one side of the nucleus.

The most conspicuous organelles are the electron-dense granules. These granules have several characteristics. They are azurophilic, therefore, Giemsa staining of a cell smear or cytospin preparation reveals the presence of the granules in pit cells with light microscopic examination. As measured by electron microscopy, the granules differ in size between different pit cell subpopulations (LD an d HD pit cells, see next page), but within one cell type the granules are very homogeneous with respect to size, shape and electronic density[19]. The granules are membrane bound and range in size between 0.2μm in LD pit cells an d 0.5μm in LAK cells. These granules contain a number of lysosomal enzymes, such as acid phosphatase[12,20]. Although perforin and granzymes, which have been isolated from NK cell granules[21,22], are not yet been identified in pit cell granules, it is believed by analogy that these molecules are present in the granules of pit cells.

Rod-cored vesicles are small inclusions, ranging in diameter from 0.17 μm to 0.2 μm, and are exclusively found in LGL[11]. They contain a straight rod structure which is 30 nm-50 nm in length, that bridges the entire diameter of the vesicle[11,20]. Rod-cored vesicles derive from and distribute preferentially around the Golgi apparatus. Possibly rod-cored vesicles may also contain cytotoxic factors functioning in natural cytotoxicity[15].

Pit cells also exist in human and mouse liver, but the identification of pit cel ls in human and mouse liver is more difficult than in rat because they contain a lower number and smaller size of the typical dense granules and very few rod-cored vesicles[9,23,24]. On the other hand, 5% to 25% of human pit cells contain 'parallel tubular arrays' (PTA), that were also reported in human blood NK cells and considered as a characteristic of these cells[6,23].

SURFACE PHENOTYPE OF PIT CELLS

Extensive phenotypic analysis has shown that no unique NK cell marker has been identified yet, but expression of a set of differentiation antigens in the absence of antigen-specific receptors of T and B lymphocytes serves to identify NK cells. NKR-P1 was first identified in the rat[25] and has now been shown to be expressed also by mouse and human NK cells[26,27]. NKR-P1 is a type II membrane glycoprotein of the C-type lectin superfamily[28]. The N KR-P1 genes are located on mouse chromosome 6[29], human chromosome 12 p 12-p13[27], and rat chromosome 4 in a region designated as the 'NK gene complex' (NKC)[28]. NKR-P1 antigen is present on 94% of rat LGL and serves as a triggering structure on these cells[25]. NKR-P1 is consider ed to be a useful marker for NK cell identification[25]. However, a subset of T lymphocytes and polymorphonuclear leukocytes also express NKR-P1[25,27]. CD56 and CD16 are expressed, either alone or in combination, on the majority of human NK cells and are most extensively used as human NK cell 'markers' for clinical and basic research purposes[2]. Other surface antigens expressed on NK cells are: CD2, CD8, CD11a-c, CD18, CD45, CD54, CD56, CD58 and CD69[2,4,30,31].

Most surface antigens found on rat pit cells are similar to that found on spleen or peripheral blood NK cells (Table 1)[13,16,32]. All LGLs from a rat liver washout, in preparations for light and electron microscopy, were found to express NKR-P1[16] (Figure 2) as recognized by using the monoclonal ant ibody (mAb) 3.2.3[25]. CD11a is present on 90% of rat pit cells, which is different from rat peripheral blood NK cells (54%)[32]. Approximately 90% of rat pit cells express CD18, 35% express CD54 and 80% express CD2[32]. Asialo-GM1, which is expressed by all rat blood NK cells[19], is present on 36% of LD pit cells and 70% of HD pit cells[13]. CD8, a marker of NK cells and cytotoxic T lymphocytes[2], is present on all rat pit cells[13]. However, the composition of CD8 in NK cells and T lymphocy tes is different. Most CD8$^+$ NK cells express CD8á/CD8á homodimers rather than the CD8á/CD8â heterodimers prevalent on cytotoxic T cells[33]. In addition, rat pit cells do not express T cell receptor and CD5 antigen (a pan T cell marker)[13,15].

LAL from human liver contain about 35% CD56$^+$ cells, in which three subsets are found: ① CD3$^+$/ CD16$^-$, ② CD3$^-$/ CD16$^-$, ③ CD3$^-$/ CD16$^+$[8]. Moreover, all the CD56$^+$ LAL express CD11a and CD18, and partly express CD2, CD11b, CD11c, CD54 and CD58[8,34,35].

Table 1 Characteristics of LD, HD pit cells and peripheral blood NK cells

Item	LD pit cell	HD pit cell	Blood NK cell
Morphology*			
Size(μm^2)	27.7	24.5	24.8
Rod-cored vesicles per cell	1.0	0.8	0.5
Microvilli per cell	5.2	5.9	7.1
Size of granules (μm^2)	Smaller(0.09)	Intermediate(0.1)	Larger(0.14)
Number of granules per cell	Higher(50)	Intermediate(20)	Lower(1 0)
Surface antigens+			
CD2	80	80	80
CD8	100	100	40
CD11a	90	90	54
CD18	90	90	90
CD54	35	35	35
Asialo-GM1	36	70	100
NKR-P1	95	95	94
Functional features			
NK activity	High	Intermediate	Low
P815 cell killing	Yes	No	No

*Data from reference 19.
+Approximate % of cells that express antigen; data summarized from referen ces 2, 13, 16, 19, 25, and 32.

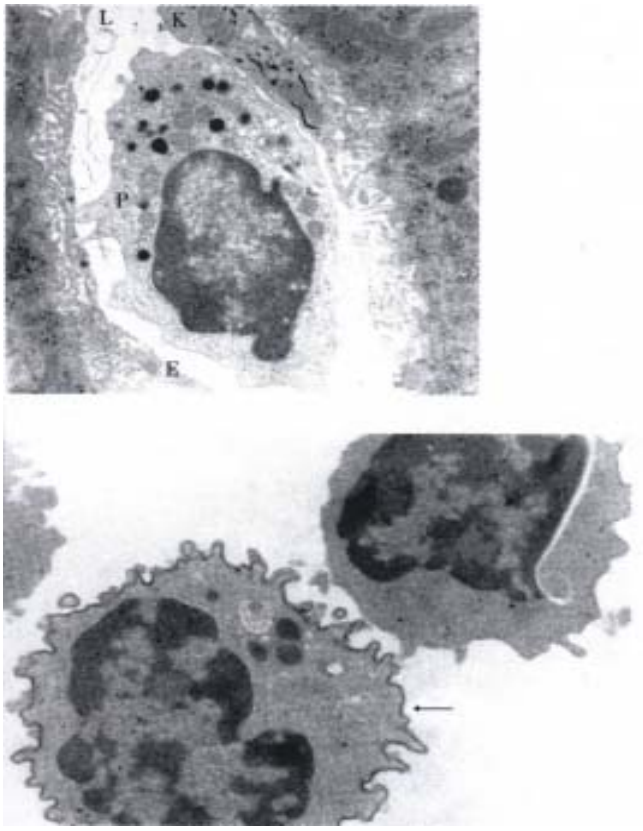

Figure 1 Transmission electron micrograph of a pit cell in a rat hepatic sinusoidal lumen (L). The pit cell shows polarity with an eccentric nucleus. The cytoplasm is abundant and contains characteristic electr on-dense granules and other organelles lying mainly on one side of the nucleus. The cell contacts an endothelial cell (E) and a portion of a Kupffer cell (K) with a positive peroxidase reaction product in the rough endoplasmic reticulum. Bar = 1 μm. (from Hepatology,1988;8: 46-52, with permission)

Figure 2 Immuno-transmission electron micrograph showing a 3.2.3 positive LGL (pit cell) (arrowhead) and a 3.2.3 negative agranular cell. The $3.2.3^+$ pit cell shows characteristic electron-dense granules in the cyt oplasm and immunoperoxidase reaction product on the surface. Bar = 1 μm.(from Hepatology, 1995;21: 1690-1694, with permission)

ISOLATION AND PURIFICATION OF PIT CELLS

Our isolation method for rat pit cells is based on a washout technique[12] followed by purification, based on the magnetic negative-selection of cells using mAbs against surface antigens and receptors found on T and B cells[6,36]. Since pit cells are apparently not heavily anchored in the liver sinusoids, the cells could be washed out by this non- enzymatic, high-pressure (50cm water) perfusion of the liver via the portal vein with phosphate-buffered saline supplemented with 0.1% EDTA[12]. The washout was collected from the vena cava. The erythrocytes, granulocytes and cell debris in the washout were removed by Ficoll-Paque gradient centri fugation. The mononuclear cells recovered from the interface of Ficoll-Paque gr adient contained T cells, pit cells, B cells, monocytes, and a few Ec and Kc. Ad herent monocytes and B cells in this population could be selectively removed in a nylon wool column[12]. Pit cells were further purified by magnetic cell sorting[36]. With this system, a highly purified population of pit cells was obtained by negative selection, *i.e.* by elimination of remaining monocytes, T and B cells using specific antibodies and immunomagnetic beads. By this method, we obtained pit cells with a purity of more than 90% and a viability of more than 95% (Figure 3). Moreover, this non-enzymatic method does not destroy cell surface molecules.

Alternatively, pit cells could be isolated by enzymatic methods[5]. Howe ver, this method is time consuming, labor-intensive and only provides pit cells with 30% purity and 90% viability[6,12].

HETEROGENEITY AND ORIGIN OF PIT CELLS

A considerable set of data indicates that rat pit cells constitute a heterogeneo us population. Based on the cell density, pit cells can be separated into a low density (LD) and high density (HD) fraction by 45% iso-osmotic Percoll gradient centrifugation[19]. These two cell populations have been shown to differ immunophenotypically, morphologically and functionally from each other and fro m blood LGL (Table 1)[19,37]. LD pit cells (Figure 4A) contain more rod-cored vesicles and more, but smaller granules than blood NK cells (Figure 4B)[19,37]. The number of rod-cored vesicles and granule composition (number and size) of HD pit cells are intermediate between LD pit cells and blood NK cells[19,37]. Immunophenotypically, almost all blood NK cells are asia lo-GM1 positive, and 70% of HD pit cells are strongly positive, whereas only 36 % of LD pit cells are weakly positive[19]. Furthermore, functional differences have been observed among these three populations. The LD pit cells are five to eight times more cytotoxic against YAC-1 cells and

colon carcinoma cells than blood NK cells[37]. The HD pit cells have intermediate cytotoxic activity between LD pit cells and blood NK cells[37]. In addition, LD pit cells are able to lyse LAK-sensitive P815 mastocytoma targets, which are resist ant to normal blood NK cells and hepatic HD pit cells[37].

Pit cells are considered to originate from blood NK cells[38,39]. Several evidences support the concept that blood NK cells immigrate into the hepatic sinusoids to become HD pit cells, which further differentiate into LD pit cells. Importantly, the characteristics and functions of HD pit cells are intermediate between blood NK cells and LD pit cells[19]. Kinetic experiments with sublethal total body irradiation (700cGy) showed that blood NK cells and HD pit cells were depleted in about one week after irradiation, whereas LD pit cells totally disappeared at two weeks after irradiation[39]. Shielding of the liver gave similar results and splenectomy did not affect pit cell number[39]. By using intravenous anti-asialo-GM1 antiserum injection, blood NK and HD pit cells totally disappear ed within one week of treatment, whereas LD pit cells disappeared from the liver one week later[39]. The direct evidence for LD pit cells originating from asialo-GM1 positive precursors (blood NK and HD pit cells) was given by the adoptive transfer of fluorescent-labeled HD pit cells into syngeneic rats[39]. After three days, 5% of labeled cells were recovered in the LD fraction and these cells displayed typical LD pit cell morphology[39]. These observations also indicate that the life span of pit cells in the liver is about two weeks[6,39].

The mechanism behind the migration of blood NK cells to the liver sinusoids is not fully understood. Several adhesion molecules were found to be involved in the process[32]. Rat blood NK and pit cells express LFA-1 (CD11a/CD18) and CD2 (LFA-2) adhesion molecules[32]. Their ligands, CD54(ICAM-1) and CD58 (LFA-3) were found to be present on liver Ec[40]. After intravenous injection of antibodies against CD2, CD11a and CD18 into rats, the number of pit cells in the liver decreased significantly, indicating that the interactions of LFA-1/CD54 and CD2/CD58 are involved in the recruitment of pit cells in the liver[32].

Once marginated in the liver sinusoids, blood NK precursors further differentiat e into HD pit cells, then into LD pit cells. The microenvironment of the liver sinusoid is believed to be responsible for this differentiation process[41]. Vanderkerken *et al* found that Kc were selectively eliminated 3 days after intravenous injection of liposomes containing the cytotoxic drug dichloromethy lene diphosphonate[41]. The number of HD pit cells declined 3 days after the injection. Conversely, the LD pit cell population showed no change in number after 3 days, but a decline of about 80% was seen 7 days after the injection[41]. These data indicate that pit cells constitute a Kupffer cell-dependent population and that Kc play an essential role in the differentiation of pit c ells in the liver. However, it remains unclear what factor(s) secreted by Kc is responsible for this differentiation. On the other hand, other conditions present in the liver microenvironment, *i.e.* Ec and their secreted factors, may work synergically with Kc to contribute to pit cell differentiation, since coincuba tion of HD pit cells with Kc failed to induce the full differentiation of HD into LD pit cells[41].

FUNCTIONS OF PIT CELLS

NK cells were initially defined as lymphoid cells capable of mediating spontaneo us killing of target cells, including tumor and virus- infected cells[1]. Such NK cytotoxic activity is mediated without prior sensitization and any obvious stimulation or activation[1]. In addition to this natural spontaneous pathway of tumor killing, NK cells can mediate antibody-dependent cellular cytotoxicity (ADCC) by a mechanism involving CD16, an IgG Fc receptor[42]. Most human and mouse NK cells express CD16[2]. Rat NK cells contai n genes with a high level of homology to human and murine Fc receptors[43] and are able to display ADCC[44]. Unfortunately, no antibodies against rat CD16 are available yet.

Although the cytotoxic function of NK cells is spontaneous, it can be significantly augmented by several cytokines[2]. One of these, IL-2, has been shown to play a central role in the regulation of NK cells, including augmenting NK cell cytotoxicity, expanding NK cell antitumor spectrum and inducing NK cell proliferation[4].

NK cytotoxic (i.e. cytolytic) activity is usually determined by measuring the release of radiolabeled chromium from target cells after been exposed to effector cells[2]. A new assay using flow cytometry to assess NK cell activity, in which various dyes are used to differentiate viable from dead target cells, has recently been described[45]. Initial studies have shown that this method is quick, reliable, and correlates well with the standard ^{51}Cr release assay[45].

Besides the cytotoxic function, NK cells can produce various cytokines[46], regulate the growth of hemopoietic tissues and bone marrow transplants[47], and participate in the resistance to microbial pathogens[2].

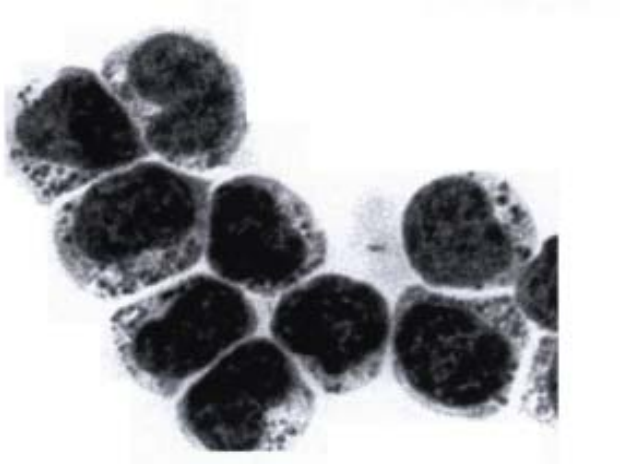

Figure 3 Light micrograph of an isolated and purified pit cell population in a May-Grünwald-Giemsa-stained cytospin. The cells contain cytoplasmic granules, which can be used to recognize and count the number of pit cells in freshly isolated liver-associated lymphocyte population. Bar = 5 μm.

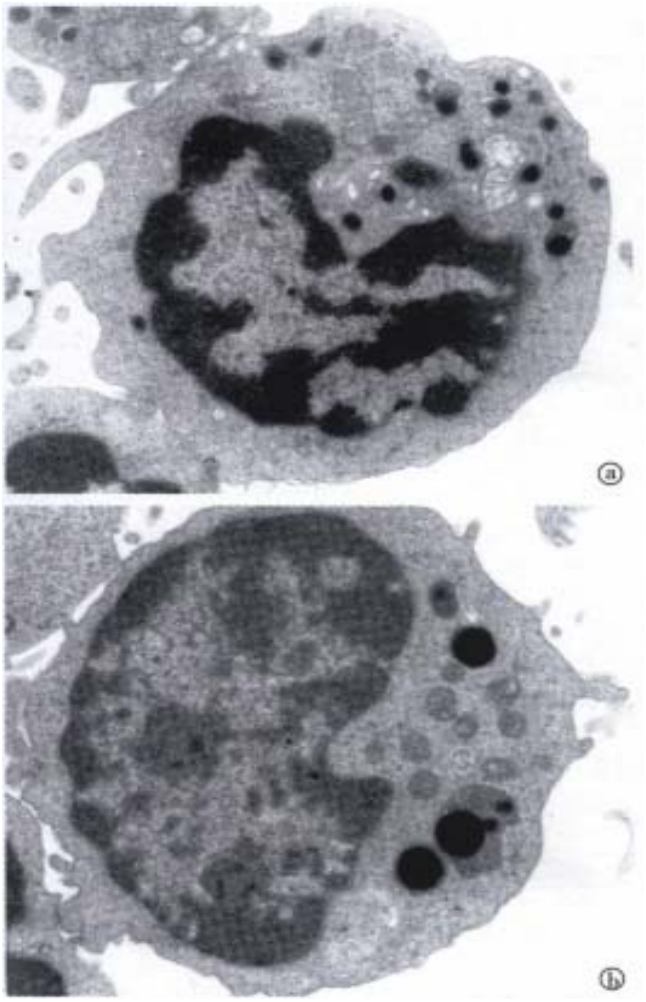

Figure 4 Transmission electron micrographs of a typical LD pit cell and a blood NK cell (B).
(A) The main morphological characteristic of LD pit cells, compared to HD cells and blood NK cells, is the presence of numerous small cytoplasmic granules.
(B) Note the few, but large granules in blood NK cell. Bar = 1 μm. (from Hepatology, 1990;12:70-75, with permission)

Most investigations on pit cell functions focus on cytotoxic activity. Rat pit cells have high spontaneous cytotoxic activity against various tumor cell lines, such as YAC-1, P815, CC531s, DHD-K12, L929, 3LL, and 3LL-R[10]. Comp ared with blood NK cells, pit cells are four to eight times more cytotoxic against YAC-1 and CC531s cells, and are able to kill the NK-resistant but lymphokine activated killer (LAK)-sensitive P815 cells (Figure 5)[19,48]. This evidence seems to support the idea that pit cells become activated once they become liver residents. However, it is not understood yet what kind(s) of factor(s) is (are) responsible for the activation of pit cells in the liver, although it has been shown that pit cells are dependent on the presence of a healthy Kc population[41]. Furthermore, NK activity in the liver could be augmented by BRM, like *Propionibacterium acnes* or maleic anhydride divinyl ether[24]. Interestingly, an increase in function seems to coincide with a large increase in the number of LGL[17,24]. IL-2 treatment results in a dramatic accumulation of pit cells in the hepatic sinusoids *in vivo*[18], induces HD cell proliferation and augments liver HD pit cell cytotoxic activity *in vitro*[49]. In contrast, IL-2 treatment does not induce liver LD pit cell proliferation[49].

MOLECULAR MECHANISMS IN NK CELL-MEDIATED CYTOTOXICITY

It is believed that the cytotoxic function of NK cells is mediated by multiple pathways, and each pathway, in principle, encompasses a cascade of events, including recognition of target cells, binding of effector cells to target cells (conjugation), activation of effector cells, delivery of the lethal signal to tar get cells, and effector cell detachment and recycling[2,4,50]. Although the precise mechanisms of individual steps in this process have not been fully elucidated, significant progress has been made recently in identifying a number of molecules participating in NK cell-mediated cytotoxicity.

CONJUGATION

The prerequisite of NK cell killing is the binding of one or more effector cells to a target cell, that is, conjugation[6]. Several adhesion molecules on the NK cell, such as CD2, CD28 and LFA-1, and on the target cell, such as CD58, B7 and CD54, participate in this process, and some of them may also possess co-stimulatory or even triggering capacity in the cytotoxic cascade[28,50-52].

CD2 is an adhesion molecule of the immunoglobulin (Ig) superfamily expressed on T cells and NK cells[53]. Approximately 80% of rat pit

cells express CD_2[32]. Although CD2 is a well-known activation structure on T cells[53], mAbs against CD2, depending on experimental conditions, either induce[54,55] or inhibit NK activity[31,56]. Anti-CD2 mAb had no effect on the binding of pit cells to rat colon carcinoma cells (CC531s), or on the cytotoxicity against CC531s[57]. However, the anti-CD2 mAb enhanced the cytolytic function in rat pit cells against $Fc\gamma R^+$ P815 target cells[57]. The ligand of CD2 is another adhesion molecule, CD58 (LFA-3) that is widely expressed on various cell types[53]. Transfection of CD58 into murine cell lines increased the lysis of these targets by some human $CD2^+$ NK cell clones[58]. However, expression of CD58 alone is insufficient to confer cells sensitive to NK cell-mediated lysis, indicating that CD2 may serve as a costimulatory receptor that augments, but not initiates, the primary activation of NK cells[58].

The interaction between β2 integrins (CD11a-c/CD18) and ICAMs (in tercellular adhesion molecules) has been found to be important in the binding of NK cells to their targets[31,51,56]. β2 integrins are heterodimers containing a common β-chain (CD18) and one of three different β-chains (CD11a,CD11b,CD11c). β2 Integrins are expressed only in leukocytes, including NK[56] and pit cells[32]. Besides the effect on the binding to target cells, LFA-1 (CD11a/CD18) also participates in signal transduction in NK cells required for NK cell activation[59]. Cross-linking of LFA-1 on NK cells with its antibody is known to induce a calcium influx, phosphoinositide turnover, tumor necrosis factor-α (TNF-α) production[59], and to inhibit the target cell killing by NK cells[60]. LFA-1 was also found to be involved in pit cell-mediated cytotoxicity. The antibody against LFA-1 inhibits not only the binding of pit cells to target cells, but also the killing of target cells by pit cells[57]. Taken together, this information suggests that LFA-1 on effector cells may have a dual function of binding to target cells and of triggering cytolysis.

Studies have shown that conjugation between NK cells and target cells is essential but not sufficient for NK activity[50]. After conjugation, further recognition events mediated by triggering and inhibitory receptors on NK cells are required to trigger NK cell cytotoxic activity[2,51].

NK CELL RECEPTORS INVOLVED IN MHC CLASS I RECOGNITION

NK cell-mediated cytotoxicity was originally thought to be spontaneous and major histocompatibility complex (MHC) class I-unrestricted. However, increasing evidence indicates that NK cells preferentially kill cells lacking MHC class I. Expression of MHC class I on a number of target cells is correlated with target cell resistance to natural killing[61-65]. Masking of MHC class I by a n mAb, enhances pit cell-mediated cytotoxicity against CC531s cells, indicating that MHC class I on CC531s cells protects these cells from being killed by rat pit cells[66].

An explanation for these observations is, that the cytotoxic activity of NK cells is regulated by positive and negative signals from triggering and inhibitory membrane receptors. The final outcome, *i.e.* triggering of cytotoxic activity or inhibition of cytotoxicity, appears to depend on the balance between the positive and negative signals[51,67]. An increasing number of triggering and inhibitory receptors have been described in recent years[28,68,69]. In hibitory receptors on NK cells recognize MHC class I, and these generally inhibit the lysis of MHC class I^+ cells[28,67-71]. Three receptor families , Ly49, CD94/NKG2 and Killer-cell inhibitory receptors (KIRs), are reported to be involved in the recognition of MHC class I molecules on target cells[28]. The Ly49 family is the product of at least nine highly related genes (Ly 49A-Ly49I) present on mouse chromosome 6 in the NKC[72]. Ly-49 homologies have been identified on rat chromosome 4 in the 'NKC'[73], but have not been found in human. The Ly-49 molecules are type II membrane glycoprotei ns and belong to the C-type lectin superfamily[72]. Ly-49 receptors recognize a trimeric MHC class I complex composed of the H-2D or H-2K heavy chain, β2-microglobulin, and a bound peptide. However, the composition of the bound peptide does not appear to influence the interaction to a large ex tent[68]. Most, but not all, Ly-49 receptors contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains[28,67]. Ly-49 receptors containing the ITIM sequence inhibit NK cell effector function[28,67], whereas Ly-49 lacking ITIM, such as Ly-49D and Ly-49H, can activate NK cell-mediated cytotoxicity when the receptor is ligated by ant i-Ly49D mAb[74].

KIRs, the inhibitory receptors recognizing MHC class I in human NK cells, are monomeric type I glycoproteins that contain Ig domains[67]. They are enc oded by genes located on human chromosome 19q13.4[28]. Two subfamilies of KIRs can be identified by the number of Ig-like domains in the extracellular regions of the molecules[28]. The KIR3D subfamily contains three Ig-like domains, whereas the KIR2D contains two Ig-like domains[28]. A remar kable feature of both KIR2D and KIR3D is the heterogeneity in the length of the cytoplasmic domains. KIRs with long cytoplasmic

domains, *i.e*. KIR2DL (p58) and KIR3DL (p70), contain two ITIM sequences that are responsible for the inhibitory function of these molecules[67,75]. KIRs containing short cytoplasmic domains, *i.e*. KIR2DS (p50) and KIR3DS, lack ITIM and potentially activate NK activity[76,77]. Both KIR2D and KIR3D molecules bind to HLA class I trimers, composed of a class I heavy chain, β^2 microglobulin, and a bound peptide[28].

In addition to KIRs, human NK cells also express another type of receptor capable of recognizing MHC class I, namely CD94/ NKG2[78-80]. This receptor is a heterodimer and is composed of CD94 glycoprotein that is disulfide-bonded to either a NKG2A or a NKG2C subunit[58]. CD94 and NKG2 genes are pre sent on human chromosome 12p12.3 - p13.1 in the 'NKC'[81]. Both CD94 and NKG2 molecules belong to the C-type lectin superfamily[81]. CD94 lacks a cytoplasmic domain, thus lacking intrinsic signal transduction capacity[81]. However, CD94 is required for the transport and membrane expression of the NKG2A or NKG2C glycoproteins[78,80]. Since NKG2A possesses an ITIM sequ ence in the cytoplasmic domain and NKG2C lacks an ITIM, the CD94/ NKG2A complex acts as inhibitory receptor, whereas CD/NKG2C complex acts as a noninhi bitory ceceptor for MHC class I on NK cells[28,68].

Triggering NK cell receptors

Several membrane molecules are described to serve as triggering receptors on NK cells, including CD16, NKR-P1, NK-TR1, 2B4, P38 and Lag3[28,51,69]. Only CD16 and NKR-P1 can be regarded as 'established' triggering receptors, while the role of the others is still undefined or controversial[51]. However, CD16 is responsible and necessary for ADCC and is not involved in natural killing activity[28].

NKR-P1, a marker of NK cells[25], is expressed by rat[25], mous e[82] and human NK cells[27], including pit cells[16]. There are three homologous NKR-P1 genes, NKR-P1A, NKR-P1B and NKR-P1C, in mice and rats[26,82,83], while only one human NKR-P1 gene has been found[27]. MAbs against mouse and rat NKR-P1 were found to trigger NK cell-mediated lysis of FcR^+ target cells, termed re-directed ADCC[25]. This action also involves a rise in intracellular Ca^{2+} levels[84] and cytokine production[85]. Furthermore, mAbs to NKR-P1 stimulate phosphoinositide turnover[84], arachidonic acid generation[86] and granule exocytosis[25]. NKR-P1 on pit cells is involved in pit cell -mediated cytotoxicity against FcR^+ P815 target, but not in FcR^- CC531s tar get killing[66]. However, the function of NKR-P1 on human NK cells appears more complex. Treatment of human NK cells with anti-NKR-P1 mAb gives cont roversial results, such as activation, inhibition or no effect, depending on the NK cell population studied[27,87]. The conditions determining the outco me of the engagement of NKR-P1 in human NK cells are not known. When human NKR-P is compared with the corresponding rat and mouse proteins, it was found that all rodent NKR P1 has the C×CP motif that interacts with phosphorylated P56[1ck][88], whereas human NKR-P1 lacks this motif[28].

THE TWO MAJOR PATHWAYS OF NK CELL-MEDIATED CYTOTOXICITY

CTL and NK cells, including rat pit cells, kill target cells by one of two disti nct mechanisms or both: necrosis and apoptosis[19,48,50,89]. Necrosis or cytolysis is characterized by swelling of the cell and organelles, and results in disruption and leakage of the cell membrane and in lysis[6]. Cell membrane damage is a key event in cytolysis and release of the cytoplasmic contents possibly leads to an inflammatory response *in vivo*[6]. The ^{51}Cr-release assay is thought to reflect this type of damage[6].

Apoptosis or programmed cell death is morphologically recognizable by membrane blebbing, chromatin condensation, nuclear fragmentation, shrinking, condensation of cells and their organelles, and fragmentation of the cells into apoptotic bodies (Figure 6). The cellular remains are phagocytosed by neighboring cells or macrophages. When phagocytosing cells are absent, apoptotic bodies progress to secondary necrosis[6,90].

Recent studies have demonstrated that NK cell-mediated apoptosis can mainly be implemented by two pathways, *i.e*. the perforin/granzyme (granule exocytosis) pathway and the Fas/FasL (Fas ligand) pathway[91,92]. NK cell-medi ated lysis is believed to be mainly based on granule exocytosis[91], whereas Fas-mediated necrosis has been recently reported when caspases are blocked[93].

The Fas pathway of apoptosis is mediated by the interaction of CD95 ligand (CD95 L, FasL) with the apoptosis-inducer CD95 (Fas/APO-1) molecule expressed on target cells[91,94,95]. CD95 is a member of the tumor necrosis factor (TN F) and nerve growth factor (NGF) receptor family[95,96]. CD95 is widely expressed on lymphoid and nonlymphoid tissues, and some tumor cells[89,95]. The expression of CD95 can be up-regulated by interferon γ (IFN-γ) in various cell lines[96,97]. The cytoplasmic tail of CD95 con tains a motif called 'death domain', that is essential for transmitting the apoptotic signal[98].

CD95L is a type II transmembrane protein of the TNF family[95]. CD95L is expressed by activated T cells, NK cells and pi t cells[89,95,99,100]. The binding of CD95L to its receptor CD95 induces apoptosis of CD95-bearing cells[94]. It is demonstrated that CD95/ CD 95L plays an important role in the killing of virus-infected cells and tumor ce lls by CTL and NK cells[98]. Although CD95 is expressed on CC531s cells and CD95L is expressed on rat pit cells, pit cell-mediated CC531s apoptosis was found to be exclusively implemented by the perforin/granzyme exocytosis pathway[89].

The perforin/granzyme pathway is a Ca^{2+} dependent pathway and is mediat ed by the pore-forming protein perforin and granzymes, especially granzyme B, both of which are stored in NK cell granules[92]. After the contact between effector and target cells, perforin and granzymes are released in a directed manner into the intercellular space between these cells. Perforin alone induces lysis without inducing apoptosis, *i.e.* fragmentation of target cell DNA. Gr anzymes play a critical role in the rapid induction of DNA fragmentation by CTL, NK cells and pit cells (Figure 7)[89,101]. Entrance of granzymes into target cells is postulated to occur through pores produced in the target cell me mbrane by perforin. Recent studies have shown that granzyme B is endocytosed by target cells independently of perforin, possibly through saturable high affinity cell surface binding sites. In the absence of perforin, granzyme B shows a cytoplasmic localization. When perforin is added, granzyme B relocalizes to a nuclea r position, rapidly inducing apoptosis[102,103]. These data indicate that the cooperation of the two molecules is necessary to induce apoptosis including DNA fragmentation.

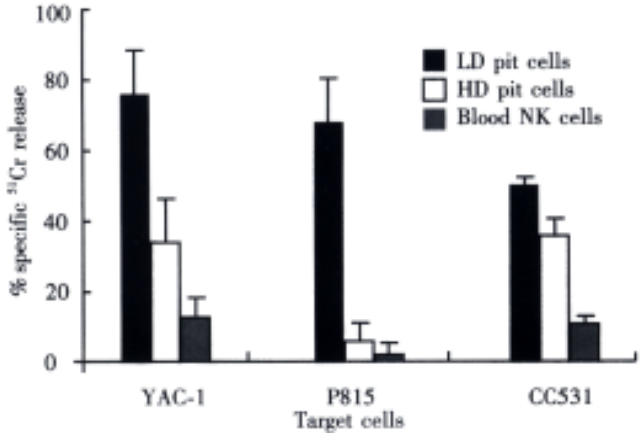

Figure 5 Comparison of cytolysis between rat blood N K, HD and LD pit cells. The ratio of freshly isolated effector cells to target cells was 20:1.The cytolysis was measured in a 4 hour ^{51}Cr-rele ase assay for YAC-1and P815 cells and a 16 hour ^{51}Cr-release assay for CC531s cells. The data show that LD pit cells are more cytotoxic against YA C-1, P815 and CC531s than HD cells and blood NK cells. Values were means±SD of three to five independent experiments. (Hepatology,1990;12:70-75, with permission)

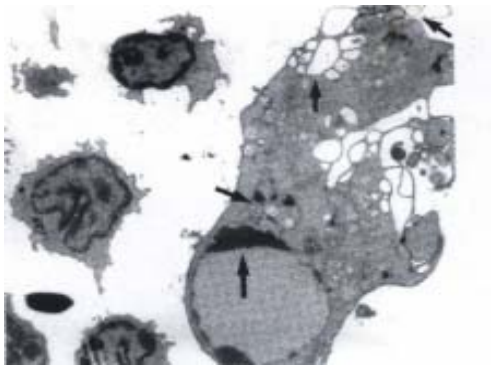

Figure 6 Transmission electron micrograph of an apoptotic CC531s cell (T) coincubated with pit cells (E) for 3 hours. The apoptotic CC531s cell (T) shows vacuolization (large arrowhead), blebbing of the cell surface (small arrowhead), chromatin condensation (thin arrow), and fragmentation of the nucleus (thick arrow). Bar:2μm. (Hepatology,1999;29:51-56, with permission)

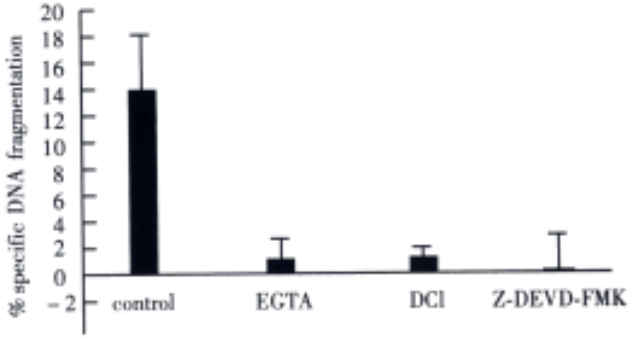

Figure 7 The involvement of the perforin / granzyme pathway in pit cell-induced CC531s apoptosis. The ratio of freshly isolated pit cells to CC531s cells was 10:1. Apoptosis was measured in a 3 hour DNA-fr agmentation assay. EGTA is a Ca^{2+} chelator that blocks granule exocytosis and the action of perforin. DCI is a granzyme inhibitor. Z-DEVD-FMK is a inhibitor of caspase 3. These treatments completely inhibit pit cell-induced CC531s apoptosis. Values were mean±SD of three independent experiments. (Hepatology, 1999;29:51-56, with permission)

SUMMARY

There is s growing evidence that pit cells are highly active, liver-specific NK cells. Pit cells are located in the liver sinusoids and can be easily isolated and purified by liver sinusoidal lavage and a magnetic separation method. Furthermore, pit cells can be separated into a LD and HD fraction by 45% iso-osmotic Per coll gradient centrifugation. These two populations are shown to differ morphol ogically, phenotypically and functionally from each other and from blood NK cells. LD pit cells contain more rod -cored vesicles and more, but smaller granules than blood NK cells although both of them share LGL morphology. Phenotypically, LD cells have a higher expression of LFA-1 and a lower expression of asialo-GM 1 molecules compared to blood or spleen NK cells. Functionally, pit cells are more cytotoxic against several tumor cell lines as

compared to blood NK cells, and are able to kill-NK- resistant but LAK-sensitive P815 cells. These data indicate that pit cells are a kind of naturally activated NK cells and their cytotoxic function is comparable to IL-2 *in vitro* activated NK cells. The characteristics of HD cells are intermediate between LD pit cells and blood NK cells. Pit cells most probably originate from blood NK cells, although they show mitosis in the liver after certain stimuli. The recruitment of pit cells in the liver is mediated by adhesion molecules. A major challenge is to achieve a better understanding of the mechanisms of pit cell cytotoxicity and the cooperation between pit cells and other cells in the liver, *i.e.* Kc, Ec and LAL. Moreover, since pit cells are located in a strategic position in the hepatic sinusoids, they represent a first line of cellular defense against metastasing colon cancer cells. The role of pit cells in a number of liver pathologies deserves more attention.

ACKNOWLEDGEMENTS We thank Carine Seynaeve and Marijke Baekeland for their excellent technical support and Chris Derom for her photographic support.

REFERENCES

1 Trinchieri G. Biology of natural killer cells. *Adv Immunol*, 1989; 47:187-376
2 Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. *Blood*, 1990;76:2421-2438
3 Inveraldi L, Witson JC, Fuad SA, Winkler Pickett RT, Ortaldo JR, Bach FH. CD3 negative “small agranular lymphocytes” are natural killer cells. *J Immunol*, 1991;146:4048-4052
4 Lotzova E. Definition and functions of natural killer cells.*Nat Immun*,1993;12:169-176
5 Wisse E, van’t Noordende JM, van der Meulen J, Daems WTh. The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. *Cell Tisses*,1976;173:423-435
6 Wisse E, Luo D, Vermijlen D, Kanellopoulou C, De Zanger R, Braet F. On the function of pit cells, the liver-specific natural killer cells. *Sem Liver Dis*,1997;17:265-286
7 Wisse E, Braet F, Luo D, Vermoesen A, Jans D, Crabbé E, De Zanger R. On the tumoricide function of pit cells, the NK cells of the liver. In: Vidal-Vanaclocha F ed. Functional hererogeneity of the liver tissue. New York: Springer Verlag, Medical Intelligence Unit, 1997:207-235
8 Winnock M, Lafon ME, Boulard A, Ferrer AM, Saric J, Dubuisson L, Bioulac-Sage P, Balabaud C. Characterization of liver-associated natural killer cells in patients with liver tumors.*Hepatology*, 1991;13:676-682
9 Hata K, Zhang XR, Iwatsuki S, Van Thiel DH, Herberman RB, Whiteside TL. Isolation, phenotyping, and functional analysis of lymphocytes from human liver.*Clin Immunol Immunopath*,1990; 56:401-419
10 Bouwens L. Isolation and characteristics of hepatic NK cells. In: Bouwns L ed. NK cells in the liver. New York, Astin: Springer Verlag, Medical Intelligence Unit, R.G. *Landes Company*, 1995: 1-19
11 Kaneda K, Wake K. Distribution and morphological characteristics of the pit cells in the liver of the rat. *Cell Tissue Res*,1983; 233:485-505
12 Bouwens L, Remels L, Baekeland M, Van Bossuyt H, Wisse E. Large granular lymphocytes or “pit cells” from rat liver: isolation, ultrastructural characterization and natural killer activity.*Eur J Immunol*,1987;17:37-42
13 Bouwens L, Wisse E. Immuno-electron microscopic characterization of large granular lymphocytes (natural killer cells) from rat liver.*Eur J Immunol*, 1987;17:1423-1428
14 Wisse E, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestratae, the sinusoidal wall and the space of Disse.*Hepatology*,1985;5:683-692
15 Bouwens L, Wisse E. Pit cells in the liver.*Liver*,1992;12:3-9
16 Luo D, Vanderkerken K, Bouwens L, Kuppen PJK, Crebbe E, Wisse E. The number and distribution of hepatic natral killer cells (pit cells) in normal rat liver: an immunohistochemical study. *Hepatology*,1995;21:1690-1694
17 Bouwens L, Wisse E. Tissue localization and kinetics of pit cells or large granular lymphocytes in the liver of rats treated with biological response modifiers. *Hepatology*,1988;8:46-52
18 Bouwens L, Marinelli A, Kuppen PJK, Eggermont AMM, Van De Velde CJH, Wisse E. Electron microscopic observations on the accumulation of large granular lymphocytes (pit cells) and Kupffer cells in the liver of rats treated with continuous infusion of interleukin-2. *Hepatology*,1990;12:1365-1370
19 Vanderkerken K, Bouwens L, Wisse E. Characterization of a phenotypically and functionally distinct subset of large granular lymphocytes (pit cells) in rat liver sinusoids.*Hepatology*,1990;12:70-75
20 Kaneda K. Liver-associated large granular lymphocytes: morphological and functional aspects. *Arch Histol Cytol*,1989;52:447-459
21 Liu CC, Perussia B, Cohn ZA, Young JDE. Identification and characterization of a pore-forming protein of human peripheral blood natural killer cells. *J Exp Med*,1986;164:2061-2076
22 Kamada MM, Michon J, Ritz J, Holldack J, Serafin WE, Austen KF, MacDermott RP, Stevens RL. Identification of carboxypeptidase and tryptic esterase activities that are complexed to proteoglycans in the secretory granules of human cloned natural killer cells.*J Immunol*,1989;142:609-615
23 Bouwens L, Brouwer A, Wisse E. Ultrastructure of human hepatic pit cells. In: Wisse E, Knook DL, Decker K, eds. Cells of the hepatic sinusoid. The Kupper Cell Foundation, PO Box 5815, 2280 HV Rijwijk, The Netherlands,1989;2:471-476
24 Wiltrout RH, Mathieson BJ, Talmadge JE, Reynolds CW, Zhang SR, Herberman RB, Ortaldo JR. Augmentation of organ-associated natural killer activity by biological response modifiers. Isolation and characterization of large granular lymphocytes from the liver.*J Exp Med*,1984;160:1431-1449
25 Chambers WH, Vujanovic NL, DeLeo AB, Olszowy MW, Herberman RB, Hiserodt JC. Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine activated killer cells.*J Exp Med*, 1989;169:1373-1389
26 Giorda R, Trucco M. Mouse NKR P1:a family of genes selectively coexp ressed in adherent lymphokine activated killer cells. *J Immunol*,1991;147:1701-1708
27 Lanier LL, Chang C, Philips JH. Human NKR-P1A: a disulfide linked homodimer of the C type lectin superfamily expressed by a subset of NK and T lymphocytes. *J Immunol*,1994;153:2417-2428
28 Lanier LL. NK cell receptors.*Annu Rev Immunol*,1998;16:359-393
29 Yokoyama WM, Ryan JC, Hunter JJ, Smith HRC, Stark M, Seaman WE. cDNA cloning of mouse NKR-P1 and genetic linkage with Ly 49: identification of a natural killer cell gene complex on mouse chromosome 6. *J Immunol*, 1991;147:3229-3236
30 Siliciano RF, Pratt JC, Schmidt RE, Ritz J, Reinherz EL. Activation of cytolytic T lymphocyte and natural killer cell function through the T11 sheep erythrocyte binding protein. *Nature*, 1985; 317:428-430
31 Timonen T, Gahmberg CG, Patarroyo M. Participation of CD11a-c/CD18,CD2 and RGD binding receptors in endogenous and interleukin-2-stimulated NK activity of CD3 negative large granular lymphocytes.*Int J Cancer*,1990;46:1035-1040
32 Luo D, Vanderkerken K, Bouwens L, Kuppen PJK, Baekeland M, Seynaeve C, Wisse E. The role of adhesion molecules in the recruitment of hepatic natural killer cells (pit cells) in rat liver. *Hepatology*,1996;4:1475-1480
33 Baume DM, Caligiuri MA, Manley TJ, Daley JF, Ritz J. Differential expression of CD8á and CD8â associated with MHC-restricted and non-MHC restricted cytolytic effector cells.*Cell Immunol*, 1990;131:352-365
34 Garcia-Barcina M, Winnock M, Huet S, Dubuisson L, Neaud V, Bidaurrazaga I, Bernard P, Bedin C, Saric J, Bioulac-Sage P, Balabaud C. Expression of cell-adhesion molecules on liver-associated lymphocytes and peripheral blood lymphocytes in patients with benign or malignant liver diseases. In: Knook DL, Wisse E, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation, Rijswijk,

The Netherlands, 1993;4:508-511
35 Garcia-Barcina M, Winnock M, Bidaurrazaga I, Huet S, Bioulac-Sage P,Balabaud C. Detection of cell-adhesion molecules on human liver-associated ymphocytes.*Immunology*,1994;82:95-98
36 Kanellopoulou C, Seynaeva C, Crabbé E, Baekeland M, Vermijlen D, Vermoesen A, Braet F, De Zanger R, Wisse E. Isolation of pure pit cells wit a magnetic cell sorter and effect of contaminating T cells on their cytolytic capability against CC531. In: Wisse E, Knook DL, Balabaud C, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation, PO Box 2215,2301 CE Leiden, The Netherlands, 1997;6:471-473
37 Vanderkerken K, Bouwens L, Wisse E. Heterogeneity and differentiation of pit cells or large granular lymphocytes of the rat. In: Wisse E, Knook DL, Decker K, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation, PO. Box 5815, 2280 HV Rijswijk, The Netherlands, 1989;2:456-461
38 Vanderkerken K, Bouwens L, Monden K, Van den Berg K, De Neve W, Wisse E. Kinetics of rat hepatic natural killer cells. In: Wisse E, Knook DL, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation, PO Box 430,2300 Leiden, The Netherlands, 1993;4:483-486
39 Vanderkerken K, Bouwens L, De Neve W, Van den Berg K, Baekeland M, Delens N, Wisse E. Origin and differentiation of hepatic natural killer cells (pit cells).*Hepatology*,1993;18:919-925
40 Lukomska B, Garcia-Barcina M, Gawron W, Winnock M, Bioulac-Sage P, Balabaud C, Olszewski WL. Adhesion molecules on liver associated lymphocytes and sinusoidal lining cells of human livers. In: Wisse E, Knook DL, Wake K, eds. Cells of the hepatic sinusoid. The Kupffer Cell Fundation, PO Box 430, 2300 Leiden, The Netherlands, 1995;5:99-102
41 Vanderkerken K, Bouwens L, Van Rooijen N, Van den Berg K, Baekeland M, Wisse E. The role of Kupffer cells in the differentiation process of hepatic natural killer cells.*Hepatology*,1995;22:283-290
42 Perussia B, Starr S, Abraham S, Fanning V, Trinchieri G. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J Immunol*,1983;130:2133-2141
43 Zeger DL, Hogarth PM, Sears DW. Characterization and expression of an Fc-gamma receptor cDNA cloned from rat natural killer cells.*Proc Natl Acad Sci USA*,1990;87:3425-3429
44 Song ES, Young K, Sears DW. Rat and human natural killers exhibit contrasting immunoglobulin G subclass specificities in antibody-dependent cellular cytotoxicity reflecting differences in their Fc receptors (Fc gammaR). *J Leukoc Biol*, 1990;48:524-530
45 Chang L, Gusewitch GA, Chritton DB, Folz JC, Lebeck LK, Nehlsen-Cannarella SL. Rapid flow cytometric assay for the assessment of natural killer cell activity. *J Immunol Methods*, 1993;166:45-54
46 Perussia B. Lymphokine-activated killer cells, natural killer cells and cytokines.*Curr Opin Immunol*,1991;3:49-55
47 Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, Rimm AA, Ringden O, Rozman C, Speck B, Truitt RL, Zwaan FE, Bortin MM. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*,1990;75:555-562
48 Bouwens L, Wisse E. Hepatic pit cells have natural cytotoxic (NC) activity against solid tumor-derived target cells. In: Wisse E, Knook DL, Decker K, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation, PO Box 5815, 2280 HV Rijwijk, The Netherlands, 1989;2:215-221
49 Vanderkerken K, Bouwens L, Baekeland M, Wisse E. Characterization of a liver specific population of large granular lymphocytes (LGL) or pit cells.In:Wisse E, Knook DL, McCuskey RS, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation. PO Box 430,2300 Leiden, The Netherlands, 1991;3:291-294
50 Berke G. The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects. *Ann Rev Immunol*, 1994;12:735-773
51 Timonen T, Helander TS. Natural killer cell-target cell interactions. *Curr Opin Cell Biol*,1997;9:667-673
52 Malorni W, Iosi F, Zarcone D, Grossi CE, Arancia G. Role of adhesion molecules in the mechanism of non-MHC (major histocompatibility complex) restricted cell-mediated cytotoxicity.*Scanning Microsc*,1993;7:323-332
53 Springer TA. Adhesion receptors of the immune system. *Nature*, 1990;346:425-434
54 Anasetti C, Martin PJ, June CH, Hellstrom KE, Ledbeter JA, Rabinovitch PS, Morishita Y, Hellstrom I, Hansen JA. Induction of calcium flux and enhancement of cytolytic activity in natural killer cells by cross-linking of the sheep erythrocyte binding protein (CD2) and the Fc-receptor (CD16).*J Immunol*,1987;139:1772-1779
55 Van De Griend RJ, Bolhuis RLH, Stoter G, Roozemond RC. Regulation of cytolytic activity in CD3 and CD3+ killer cell clones by monoclonal antibodies (anti-CD16, anti-CD2, anti CD3) depends on subclass specificity of target cell IgG FcR. *J Immunol*, 1987;138:3137-3144
56 Robertson MJ, Caligiuri MA, Manley TJ, Levine H, Ritz J. Human natural killer cell adhesion molecules: differential expression after activation and participation in cytolysis. *J Immunol*,1990;145:3194-3201
57 Luo D, Vermijlen D, Vanderkerken K, Kuppen PJK, Seynaeve C, Eddouks M, Baekeland M, Wisse E. Involvement of LFA-1 in hepatic NK cell (pit cell)mediated cytolysis and apoptosis of colon carcinoma cells.*J Hepatol*,1999;31:110-116
58 Lanier LL, Corliss B, Phillips JH. Arousal and inhibition of human NK cells.*Immunol Rev*,1997;155:145-154
59 Melero I, Balboa M, Alonso JL, Yagüe E, Pivel J, Sanchez-Madrid F, Lopez-Botet M. Signaling through the LFA-1 leucocyte integrin actively regulates intercellular adhesion and tumor necrosis factor-α production in natural killer cells. Eur J Immunol,1993;23:1859-1865
60 Smits KM, Kuppen PJK, Eggermont AMM, Tamatani T, Miyasaka M, Fleuren GJ. Rat interleukin-£²-activated natural killer (A-NK) cell-mediated lysis is determined by the presence of CD18 on A-NK cells and the absence of major histocompatibility complex class I on target cells. *Eur J Immunol*, 1994;24:171-175
61 Giezeman-Smits KM, Kuppen PJK, Ensink NG, Eggermont AMM, Stals F, Wonigeit K, Fleuren GJ. The role of MHC classI expression in rat NK cell-mediated lysis of syngeneic tumor cells and virus-infected cells. *Immunobiology*, 1996;195:286-299
62 Carlow DA, Payne U, Hozumi N, Roder JC, Czitrom AA. Class I (H-2K-b) gene transfection reduces susceptibility of YAC-1 lymphoma targets natural killer cells. *Eur J Immunol*,1990;20:841-846
63 Piontek GE, Taniguchi K, Ljunggren HG, Gr-nberg A, Kiessling R, Klein G, K-rre K. YAC-1 MHC class I variants reveal an association between decreased NK sensitivity and increased H-2 expression after interferon treatment or *in vivo* passage. *J Immunol*, 1985;135:4281-4288
64 Kraus E, Lambracht D, Wonigeit K, Hünig T. Negative regulation of rat natural killer cell activity by major histocompatibility complex class I recognition.*Eur J Immunol*,1996;26:2582-2586
65 Storkus WJ, Howell DN, Salter RD, Dawson JR, Cresswell P. NK susceptibility varies inversely with target cell class I HLA antigen expression. *J Immunol*,1987;138:1657-1659
66 Luo D, Vermijlen D, Vanderkerken K, Kuppen PJK, Seynaeve C, Eddouks M, Wisse E. Participation of CD45 on pit cells and MHC class I on target cells in rat hepatic NK cell (pit cell) mediated cytotoxicity against colon carcinoma cells. In: Wisse E, Knook DL, eds. Cells of the hepatic sinusoid. The Kupffer Cell Foundation. PO Box 430,2300 Leiden, The Netherlands, 1999;7:in press
67 Burshtyn DN, Long EO. Regulation through inhibitory receptors: lessions from natural killer cells. *Trends in Cell Biol*,1997;7:473-479
68 Lanier LL. Follow the leader: NK cell receptors for classical and nonclassical MHC class I.*Cell*,1998;92:705-707
69 Yokoyama WM. Natural killer cell receptors. *Curr Opin Immunol*, 1995;7:110-120
70 Yokoyama WM, Daniels BF, Seaman WE, Hunziker R, Margulies DH, Smith HRC. A family of murine NK cell receptors specific for target cell MHC class I molecules.*Sem Immunol*,1995;7:89-101
71 Trinchieri G. Recognition of major histocompatibility complex class I antigens by natural killer cells. *J Exp Med*,1994;180:417-421
72 Yokoyama WM. The Ly-49 and NKR-P1 gene families encoding lectin-like receptors on natural killer cells: the NK gene complex. *Annu Rev Immunol*,1993;11:613-635
73 Dissen E, Ryan JC, Seaman WE, Fossum S. An autosomal dominant locus, NKa, mapping to the Ly-49 region of a rat natural killer (NK) gene complex, controls NK cell lysis of allogeneic lymphocytes. *J Exp Med*, 1996;183:2197-2207
74 Mason LH, Anderson SK, Yokoyama WM, Smith HRC, Winkler-Pickett R, Ortaldo JR. The Ly-49D receptor activates murine natural killer cells. *J Exp Med*,1996;184:2119-2128
75 Burshtyn DN, Scharenberg AM, Wagtmann N, Rajagopalan S, Berrada K, Yi T, Kinet JP, Long EO. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitory receptor.*Immunity*, 1996;4:77-85
76 Biassoni R, Cantoni C, Falco M, Verdiani S, Bottino C, Vitale M, Conte R, Poggi A, Moretta A, Moretta L. The human leukocyte

antigen (HLA)-C-specific "activatory" or "inhibitory" natural killer cell receptors display highly homologous extracellular domains but differ in their transmembrane and intracytoplasmic portions.*J Exp Med*,1996;183:645-650
77 Moretta A, Sivori S, Vitale M, Pende D, Morelli L, Augugliaro R, Bottino C, Moretta L. Existence of both inhibitory (p58) and activatory (p50) receptor for HLA-C molecules in human natural killer cells.*J Exp Med*,1995;182:875-884
78 Lazetic S, Chang C, Houchins JP, Lanier LL, Phillips JH. Human NK cell receptors involved in MHC class I recognition are disulfide liked heterodimers of CD94 and NKG2 subunits.*J Immunol*, 1996;157:4741-4745
79 Carretero M, Cantoni C, Bellon T, Bottino C, Biassoni R, Rodriguez A, Perez-Villar JJ, Moretta L, Moretta A, Lopez-Botet M. The CD94 and NKG2A C-type lectins covalently assemble to form a natural killer cell inhibitory receptor for HLA class I molecules. *Eur J Immunol*,1997;27:563-575
80 Brooks AG, Posch PE, Scorzelli CJ, Borrego F, Coligan JE. NKG2A complexed with CD94 declines a novel inhibitory NK cell receptor. *J ExpMed*,1997;185:795-800
81 Chang C, Rodriguez A, Carretero M, Lopez-Botet M, Phillips JH, Lanier LL. Molecular characterization of human CD94: a type II membrane glycoprotein related to the C-type lectin superfamily. *Eur J Immunol*,1995;25:2433-2437
82 Ryan JC, Turck J, Niemi EC, Yokoyama WM, Seaman WE. Molecular cloning of the NK1.1 antigen, a member of the NKR-P1 family of natural killer cell activation molecules.*J Immunol*,1992; 149:1631-1635
83 Giorda R, Rudert WA, Vavassori C, Chambers WH, Hiserodt JC, Trucco M. NKR-P1, a signal transduction molecule on natural killer cells. *Science*, 1990;249:1298-1300
84 Ryan JC, Niemi EC, Goldfien RD, Hiserodt JC, Seaman WE. NKR-P1, an activating molecule on rat natural killer cells, stimulates phosphoinositide turnover and a rise in intracellular calcium.*J Immunol*,1991;147:3244-3250
85 Arase H, Arase N, Saito T. Interferon ã production by natural killer (NK) cells and NK1-1+T cells upon NKR-P1 cross-linking. *J Exp Med*,1996;183:2391-2396
86 Cifone MG, Roncaioli P, Cironi L, Festuccia C, Meccia A, D'Alo S, Botti D, Santoni A. NKR-P1A stimulation of arachidonate-generating enzymes in rat NK cells is associated with granule release and cytotoxic activity. *J Immunol*,1997;159:309-317
87 Poggi A, Costa P, Morelli L, Cantoni C, Pella N, Spada F, Biassoni R, Nanni L, Revello V, Tomasello E, Mingari MC, Moretta A, Moretta L. Expression of human NKR-P1A by CD34+ immature thymocytes: NKR-P1A-mediated regulation of proliferation and cytolytic activity.*Eur J Immunol*,1996;26:1266-1272
88 Turner JM, Brodsky MH, Irving BA, Levin SD, Perlmutter RM, Littman DR. Interaction of the unique N-terminal region of tyrosine kinase P56-1ck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. *Cell*, 1990;60:755-765
89 Vermijlen D, Luo D, Robaye B, Synaeve C, Baekeland M, Wisse E. Pit cells (hepatic natural killer cells) of the rat induce apoptosis in colon carcinoma cells by the perforin/granzyme pathway.*Hepatology*, 1999;29:51-56
90 Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. *Br J Cancer*, 1972;26:239-257
91 Moretta A. Molecular mechanisms in cell-mediated cytotoxicity. *Cell*, 1997;90:13-18
92 Kagi D, Ledermann B, Burki K, Zinkernagel RM, Hengartner H. Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis *in vivo*. *Annu Rev Immunol*,1996;14:207-232
93 Vercammen D, Brouckaert G, Denecker G, Van de Craen M, Declercq W, Fiers W. Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. *J Exp Med*, 1998;188:919-930
94 Berke G. The CTL's kiss of death. *Cell*, 1995;81:9-12
95 Nagata S, Golstein P. The Fas death factor. *Science*,1995;267: 1449-1456
96 Itoh N, Yonehara S, Ishii A, Yonehara M, Mizyshima SI, Sameshima M, Hase A, Seto Y, Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell*,1991;66:233-243
97 Watanabe-Fukunaga R, Brannan CI, Itoh N, Yonehara S, Copeland NG, Jen kins NA, Nagata S. The cDNA structure, expression and chromosomal assignment of the mouse Fas antigen.*J Immunol*, 1992;148:1274-1279
98 Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science*,1998;281:1305-1308
99 Oshimi Y, Oda S, Honda Y, Nagata S, Miyazaki S. Involvment of Fas ligand and Fas mediated pathway in the cytotoxicity of human natural killer cells. *J Immunol*,1996;157:2909-2915
100 Lee RK, Spielman J, Zhao DY, Olsen KJ, Podack ER. Perforin, Fas ligand, and tumor necrosis factor are the major cytotoxic molecules used by lymphokine activated killer cells.*J Immunol*, 1996;157:1919-1925
101 Shresta S, MacIvor DM, Heusel JW, Russell JH, Ley T. Natural killer and lymphokine-activated killer cells require granzyme B for the rapid induction of apoptosis in susceptible target cells. *Proc Natl Acad SciUSA*,1995;92:5679-5683
102 Jans DA, Jans P, Briggs LJ, Sutton V, Trapani JA. Nuclear transport of granzyme B (fragmentin 2). Dependence on perforin *in vivo* and cytosolic factors *in vitro*. *J Biol Chem*,1996;271:30781-30789
103 Shi L, Mai S, Israels S, Browne K, Trapani JA, Greenberg AH. Granzyme B (GraB) autonomously crosses the cell membrane and perforin initiates apoptosis and GraB nuclear localization.*J Exp Med*,1997;185:855-866

Edited by Wu XN
Proofread by Miao QH