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Role of Natural Killer Cells in Antitumor Resistance

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Abstract

Kaneno R. Role of natural killer cells in antitumor resistance. ARBS Ann Rev Biomed Sci 2005;7:127-48. Natural killer cells constitute a population of lymphocytes able to non-specifically destroy virus-infected and some kinds of tumor cells. Since this lytic activity was shown by non-immunized animals the phenomenon is denominated natural killer (NK) activity and contrasts with specific cytotoxicity performed by cytolytic T lymphocytes (CTLs) because it does not depends on MHC-restricted peptides recognition. In fact, the main feature of most functional receptors of NK cells (NKRs) is their ability to be inhibited by different kinds of class I MHC antigens. In the middle of the 1950's, Burnet & Thomas forged the concept of tumor immunosurveillance and NK cells can be considered one of the main figures in this phenomenon both for effector and regulatory functions. In the present review the early studies on the biology of NK cells were revisited and both their antitumor activity and dependence on the activation by cytokines are discussed.

KEYWORDS: cell activation, cytotoxicity, natural killer, tumor immunology

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Table of Contents

1. Introduction

In the middle of the 1970´s Herberman *et al.,* (1975) and Kiessling *et al.*, (1975), showed that peripheral leukocytes of normal mice were able to lyse some lineages of tumor cells without a previous sensitization of the host. Since this lytic activity was shown by non-immunized animals the phenomenon was denominated natural killer (NK) activity as well as the effector cells (NK cells). It contrasts with specific cytotoxicity performed by cytolytic T lymphocytes (CTLs) because they are able to interact with target cells independently of peptides linked to major histocompatibility complex (MHC) antigens performing an MHC-unrestricted cytotoxicity.

Most natural killer activity is attributed to a population of cells morphologically defined as large granular lymphocytes (LGL) found in peripheral blood and lymphoid organs (Timonen *et al.*, 1979a, 1979b; Lotzova & Ades, 1988; Trinchieri, 1989). These cells are larger than typical small lymphocytes, showing a higher cytoplasma:nucleous ratio and large azurophilic cytoplasm granules (Timonen & Saksela, 1980). LGLs are non-T, non-B lymphocytes, since they do not express CD3 or TCR or any of the TCR chains (α, β, γ or δ) (Saksela *et al.,* 1979; Timonen *et al.,* 1979a, 1979b; Lotzova & Ades, 1988). B lymphocyte markers CD19 and surface Ig (Nishikawa *et al.*, 1990; Natajarian *et al.*, 2002) are also absent and these cells are non-adherent leukocytes and do not show phagocytic activity although they share the expression of CD16 with macrophages and neutrophils (Trinchieri, 1989; Natajarian *et al.*, 2002).

NK cells show a heterogeneous tissue distribution, and it was observed that cells from mesenteric lymph nodes of BALB/c mice show the most prolific NK activity (53.9% of lytic activity against Yac-1 target cells), followed by inguinal lymph nodes (30.4%), spleen and blood (26.4 and 25.3%, respectively) peritoneum (7%) and bone marrow (3.8%) (Herberman *et al.*, 1975).

Reynolds *et al.,* (1981) observed that in rats the main NK activity is presented by blood and spleen (22.1 and 20.5%), whereas peritoneum and bone marrow cells show low lytic activity (6.4 and 1.1%). These authors also observed that NK activity decreases with age, although the number of peripheral LGL had not been altered. NK activity is also under the influence of the host's genetic background; and different inbred strains of

mice present different level of lytic activity. It was demonstrated by Herberman, *et al.,* (1975) that some inbred strains of mice like B6C3F1 present high NK activity (39.0% of specific lysis of RBL-5 cells), whereas others like C3H have shown very low activity (2.6%). They also observed that *nude* mice show higher NK activity than their heterozygous counterparts both against RBL-5 and Yac-1 target cells.

Besides the high correlation between LGL and MHC-unrestricted lytic activity, it must be noted that not all LGLs show cytotoxic activity and not all MHC-independent lytic cells are morphologically identified as LGL (Trinchieri, 1989). Reynolds *et al.*, (1981) observed earlier that there is no correlation between the number of LGL and NK activity of lung infiltrating cells, indicating that the cells from this organ do not present an effective lytic activity as happens with thymus and bone marrow cells, suggesting the existence of cells in distinct stages of activation.

Isolation of LGL enriched cell suspension by Percoll density gradients (Reynolds *et al.*, 1981; Trinchieri, 1989) helped to achieve much knowledge of NK biology but the lack of a specific antibody made difficult the obtainment of highly purified suspension of these cells. At that time the isolation of tumoral clones of NK cells was fundamental to amplifying the knowledge on functional and phenotypic features of these cells.

2. Phenotypic Markers of NK Cells

NK cells constitute a heterogeneous population of lymphocytes with a non-specific cytotoxicity presenting a variety of surface markers, which are shared with other cell populations. This heterogeneity reflects on the phenotypic expression of several cell markers, which are evolved in antigen recognition, triggering lytic activity and cell regulation (Trinchieri, 1989; Cooper *et al.*, 2001a; Natarajan *et al.,* 2002) and most NK cells can be quantified or isolated through the recognition of some of these markers (West *et al.*, 1977; Trinchieri, 1989; Cooper *et al.*, 2001b).

Among these surface markers, CD16 is expressed by virtually all of the NK cells (West *et al.*, 1977), however it is not exclusive to these cells, since it is found in neutrophils, mature eosinophils and tissue macrophages, but is scarce on monocytes (Trinchieri 1989). CD16 is a low affinity receptor for the Fc portion of IgG (FcγRIII) but it cannot link to monomeric IgG. FcγRIII has molecular weight of 50 – 70 kDa, is highly glycosilated and is structurally different from the high-affinity receptor (FcγRI) expressed by monocytes and macrophages or FcγRII (low affinity) expressed by B lymphocytes and PMN (Trinchieri 1989). In this way, whereas the receptors on macrophages and neutrophils enhance the phagocytosis of antibody-linked structures, the Fc-receptors on NK cells promote the antibody-dependent cell cytotoxicity (ADCC) (Kay *et al.*, 1977), interfacing with the adaptive and natural defense mechanisms. These receptors are able to signal transduction since NK can lyse the anti-CD16 producing hybridomas (Lanier *et al.*, 1988). Although almost all NK cells express CD16, it had been observed that there is a $CD16^-$ NK cell subset possibly able to destroy some target cells distinct from those sensitive to

$CD16^+$ ones (Masucci *et al.*, 1980).

The ganglioside asialo GM-1 is another classical marker of NK cells and the polyclonal antibody against it frequently was used for phenotypic characterization of both human (Herberman *et al.*, 1977) and murine (Santoni *et al.*, 1979) NK cells. This marker is also not exclusive to NK cells and can be expressed by CTLs and activated macrophages (Kasai *et al.*, 1980; Young *et al.*, 1980; Suttles *et al.*, 1987).

The development of a variety of monoclonal antibodies allows the identification of many other antigens, especially in human and murine cells. The main surface marker for human NK cells that is largely used in clinical studies is the CD 56, an isoform of neuronal adhesion molecule (Lanier *et al.*, 1989), the function of which in NK cells appears to be associated with adhesion to target cells (Nitta *et al.*, 1989; Suzuki *et al.*, 1991). CD56 has molecular weight of 200-220 kDa and is recognized in 90% of human NK cells and a small percentage of T lymphocytes (Griffin *et al.*, 1983). According to Robertson & Ritz (1990), $CD56^+/CD3^-$ cells correspond to 15% of human lymphocytes and depending on the density of expression of this marker, they can be divided into 2 subsets identified as $CD56^{dim}/CD16^{bright}$ or $CD56^{bright}/CD16^{dim}$ (or $CD16^-$). Lanier *et al.* (1986a) showed that resting lymphocytes are featured as $CD56^{dim}$ and show high cytotoxic activity. Other authors suggest that these two populations perform distinct regulatory roles through the production of different cytokines (Cooper *et al.*, 2001a, 2001b). In fact, Cooper *et al.,* (2001b) observed that fresh $CD56^{bright}$ cells produce higher levels of IFN-γ, TNF-β and GM-CSF than $CD56^{dim}$, when stimulated with PMA or co-cultured with LPS –activated macrophages, suggesting that $CD56^{bright}$ NK cells are the main source of early IFN-γ required for rapid macrophage activation in the initial phase of the immune response.

Although NK cells are $CD3^-$ they share some markers with T lymphocytes, so that 30% of human and 86% of rat NK cells express CD8 antigens (Reynolds *et al.*, 1981), whereas murine cells are $CD8^-$ (Young *et al.*, 1986; Trinchieri, 1989). These cells also express CD2 antigens, probably using it for cell adhesion and for activation of the effector function after interaction with LFA-3 (Fletcher *et al.*, 1998). Although g and d chains of TCR (Tγδ lymphocytes) are also associated with the ability of MHC-unrestricted cytotoxicity, rearrangement of TCR genes is not observed in NK cells (Ritz *et al.*, 1985; Lanier *et al.*, 1986b), thus distinguishing them from Tγδ lymphocytes.

An important feature of NK cells is the expression of intermediary affinity IL-2 receptor (IL-2R). This IL-2R is a gb heterodimer and is distinct from the low-affinity a chain receptor and the high-affinity trimer receptor (αβγ) expressed by activated T lymphocytes (Nishikawa *et al.*, 1990). This feature allowed the exploitation of therapeutic potential of *in vitro* or *in vivo* stimulation of these cells by IL-2, as will be further discussed.

3. Activation-inhibition Balance of NK Effector Function

The main feature of most functional receptors of NK cells (NKRs) is their ability to react with different kinds of class I MHC antigens, which results in inhibitory effect on the cytotoxic activity. This observation was the base of the missing-self hypothesis

(Ljunggren & Kärre, 1990). According to this, whereas CTLs are only activated by contact with MHC-associated antigens, NK cells are naturally activated and are potentially able to destroy any self cell. However, this destructive activity of NK cells is suppressed by interaction of their receptors with any class I histocompatibility antigen. So, only the cells that had lost the expression of class I MHC antigens are still susceptible to NK mediated lysis, as observed in some tumor cells and virus infected cells (Zeidler *et al.*, 1997; Orange *et al.*, 2002). In fact, many studies have demonstrated that that human class I-deficient cells are susceptible to NK lytic activity, but became resistant after their transfection with HLA A, B or C alleles, whereas a similar phenomenon was observed with murine cells (Shimizu & DeMars, 1989; Storkus *et al.*, 1989; Orange *et al.*, 2002).

Human NK cells interact with histocompatibility molecules through a variety of membrane receptors and, according to their structure, they can be classified as Ig-like receptors (KIR and LIR) or lectin-like receptors (CD94/NKG2 and NKG2D) (Cooper *et al.*, 2001b; Natarajan, 2002), whereas mice express the lectin-like Ly-49 family as their main receptors for class I MHC molecules (Lowin-Kropf & Held, 2000; Makrigiannis *et al.*, 2001; Natarajan, 2002). Each type of receptor shows a different level of affinity for molecules codified by the several loci of class I MHC genes and any NK cell expresses at least one ligand for these MHC molecules, a fact that constitutes a mechanism to avoid autoreactivity.

The killer cell Ig-like receptor (KIR), also known as CD 158 is a family of molecules that react with different class I MHC structure (Natarajan *et al.*, 2002). In general, KIR3D receptors recognize HLA-A and B, whereas those of KIR 2D recognize HLA-C alleles (Middleton *et al.*, 2002). Minimal variations in each receptor allow them to recognize allotypic differences in MHC molecules. Although it is believed that the main function of KIRs is to scan the normal expression of class I MHC, the cognitive process is not totally independent from the peptide linked to the histocompatibility molecule. In fact it has been demonstrated that the substitution of amino acids in positions 7 and 8 of a peptide nonamer promotes the recognition of MHC-peptide by other receptors (Malnati *et al.*, 1995 Lowin-Kropf *et al.*, 2000).

The mechanism of KIR-induced signal transduction is not completely known but it has been shown that these receptors frustrate cytotoxicity through the recruitment of tyrosine-phosphatase SHP-1 (Lowin-Kropf *et al.*, 2000) for the immunore4ceptor tyrosine-based inhibition motifs (ITIM), inhibiting signal transcription (Burshtyn *et al.*, 1996; Campbell *et al.*, 1996).

Another family of NKRs is the leukocyte immunoglobulin-like receptor-1 (LIR-1), also known as ILT-3 or CD58h (Cella *et al.*, 1997; Fanger *et al.*, 1999), which recognizes most class I MHC structures without discriminating among allotypic variations. Hence, any NK cell expressing LIR-1 is inhibited by any target cell expressing any class I molecule and this effector cell only will be able to destroy target cells totally depleted of HLA antigens (Cella *et al.*, 1997; Fanger *et al.*, 1999).

Receptors CD94/NKG2 are lectin-like receptors, in which subunits CD94

and NKG2 are linked by disulphide bonds. These receptors are specific to non-classic class I MHC antigens that are codified by HLA-E locus (Soderstrom *et al.*, 1997). The subunit CD 94 is an invariant chain (Chang *et al.*, 1995) whereas the NKG2 subunit constitutes a multigenic family with at least 5 proteins named NKG2A (with a B variant chain), NKG2C and NKG2E, whose intracellular structure share a high degree of homology (Chang *et al.*, 1995; Soderstrom *et al.*, 1997) and are responsible for inhibitory signaling after interaction with MHC molecules.

Mice NK cells express Ly 49 (C-type lectin-like receptors) as the main ligand for class I MHC molecules, which perform the same role as human KIR molecules (Lowin-Kropf & Held, 2000; Makrigiannis *et al.*, 2001; Natarajan, 2002) when reacting with H-2K and H-2D structures (mouse class I antigens). Some KIR and Ly 49 receptors are linked to adaptor molecules with immunoreceptor tyrosine-based activation motifs (ITAM) for signal transduction. These receptors show short cytoplasmic chains that are associated with homodimeric adaptor molecules DAP 12 or KARAP, that contain cytoplasmic ITAM sequences, which promote cell activation via Syk and ZAP-70 (Tomasello *et al.*, 1998; Wu *et al.*, 2000). Therefore, some KIR and Ly 49 receptors can play an activating role after interaction with some MHC molecules.

NKG2D is the main activation C-type lectin-like receptors, showing just 20-34% homology with other NKG2 (A/B, C, E) chains (Diefenback *et al.*, 2000). NKG2D is a homodimeric structure expressed by NK, $CD8^+$ T cell, and $T\gamma\delta$ cells (Bauer *et al.*, 1999) that is bound to DAP-10 adaptor molecule that triggers tumor cell lysis (Wu *et al.*, 1999). In contrast with CD94/NKG2 that recognizes HLA-E antigens, NKG2D interacts with MIC A and MIC B that are class I-homologous structures (Menier *et al.*, 2002). MIC A and MIC B show the domains $\alpha1$, $\alpha2$ and $\alpha3$ of class I MHC molecules but fail to express both $\beta2$-microglobulin and peptides bound to the a chain (Wu *et al.*, 1999). MIC A and MIC B are scarce on normal cells but are presented in high density by epithelial tumor cells and, therefore, are important targets for NK cytotoxic function (Groh *et al.*, 1999; Natarajan *et al.*, 2002).

Since class I MHC antigens are able to be bound by both inhibitory and activator ligands of NK cells, the lytic activity of these cells appears to be the result of a fine balance of positive and negative signals triggered by such receptors. After the interaction between effector and target cells and if activator signals are more prevalent than the inhibitory ones, immunological synapses are formed between the cell surfaces and NK cell release the contents of cytoplasmatic granules. These granules are specialized lysossomes found only in NK and CTLs (Griffiths & Isaaz, 1993) and are full of enzymes and other compounds that have roles in the killing of target cells (Lieberman, 2003; Trambas & Griffiths, 2003).

Similarly to CTLs, in the early phase of cell:cell interaction, perforin monomers are released by the granules, which polymerize on target cell membrane forming transmembrane pores in the cell surface. Perforin shows strong homology with complement C 9 molecule (Shinkai *et al.* 1988) as well as the resulting pores (Sauer *et al.*, 1991), allowing

the loss of cell membrane integrity, influx of water, and cell death by osmotic lysis. During the degranulation of NK cells, they also release a series of many other compounds such as granzymes, calreticulin, catepsins and granulisins in the immunological synapses. These products penetrate the target cell through the perforin pores triggering its apoptosis by several mechanisms, according to the compound involved.

Granzymes (a name derived from granule enzymes) are the second most important of granular contents and they constitute a family of highly specific serine-proteases that still inactive in acidic medium of the granules (Lieberman, 2003) being activated after release in the target cell cytoplasm. Granzyme A and B are the more abundant compounds of granules and appear to be the main elements involved in the target destruction. Granzyme B has a large spectrum of substrate and can induce apoptosis of target cells by activation of the caspases cascade (Wolf *et al.*, 1999; Sharif-Askari *et al.*, 2001), lesion of mitochondria and cleavage of BID to cytochrome C (Heibein *et al.*, 2000; Alimonti *et al.*, 2001), DNA fragmentation due to derepression of CAD (Thomas *et al.*, 2000) or even by cleavage of nuclear membrane (Browne *et al.*, 2000; Zhang *et al.*, 2001).

Granzyme A induces cell death through a caspase-independent mechanism, promoting lesions on single-strand DNA and rapid loss of integrity of the plasmatic membrane (Irmler *et al.*, 1995; Suidan *et al.*, 1996). This enzyme can also destroy the nuclear membrane by acting on laminin and can destroy DNA by acting on histone (Lieberman & Fany, 2003). Other members of the granzyme family include granzymes C, D, E, F, G, H, K, and M; all of them are involved in cell destruction (Lieberman, 2003; Trambas & Griffiths, 2003).

Calreticulin and catepsins protect the effector cells from self-destruction. Calreticulin inhibits perforin and therefore is able to inactivate perforin polymerization on its own membrane, whereas the catepsins family has both granzyme–activating and -inactivating molecules, preventing self-destruction (Lieberman, 2003; Trambas & Griffiths, 2003). Granulisins released by NK cells appear to function especially against microorganisms whose cell walls are susceptible to this molecule (Lieberman , 2003); but this activity against cancer target cells remains unclear. The close interaction of target and effector cell membranes can vary from a few minutes up to hours and is called lethal hit, since even after the cell detachment the signals for programmed cell death are still in action (Lieberman, 2003; Trambas & Griffiths, 2003).

Other NK-induced cell-destruction mechanisms include the expression of FasL (Berke, 1997; Screpanti *et al.*, 2001), since many kinds of tumor cells frequently express higher density of Fas than normal cells. Binding of FasL to Fas molecules on tumor cells induces apoptosis signaling in these target cells by DNAse activation. TNF-related apoptosis-inducing ligand (TRAIL) is also able to induce cell death in a similar way (Kayagaki *et al.*, 1999). Although apoptosis-induction is accepted as the main mechanism for destruction of target cells, perforin appears to be essential to the effector function of any cytolytic cell, since animals knocked out for perforin are not able to destroy target cells even if the other granule compounds are normal (van den Broek *et al.*,

1995; Bossi & Griffiths, 1999; Hayakawa, *et al.*, 2002).

4. Role of NK Cells in Antitumor Resistance

In the middle of the 1950's, Burnet & Thomas forged the concept of immunosurveillance, establishing that any normal host is able to recognize and eliminate rising tumor cells even if it had not been immunologically manipulated (Burnet, 1970). Since then many immunologists have asked if the immune system can, in fact, recognize naturally rising tumor cells to prevent their development. Although some authors disagree on this concept, recent advances have added fuel to this discussion, giving new evidence of an effective antitumor surveillance. In this way, studies with experimental model of cancer have shown that animals depleted of some specific populations of cells (T lymphocytes, NK or NKT, for instance) as well as specific molecules such as INF-Tγ, TNF-Tα, IL-12, perforin and others, unequivocally show higher incidence of a variety of tumors. Conversely, the administration of some cytokines or induction of their *in vivo* production reduces the incidence of primary malignancies.

The antitumor role of NK cells was originally described due to their ability to lyse cells of some tumor lineages maintained in culture such as Yac-1 (Moloney virus-induced murine lymphoma) and K-562 (human chronic myeloid lymphoma) (Ortaldo *et al.*, 1977). The more relevant question was whether these cells were able to effectively play an *in vivo* role in antitumor activity, which had been partially answered through experimental models showing that naturally NK-lacking or artificially NK-depleted mice are less efficient than normal animals in eliminating transplantable tumor cells and show difficulty in preventing metastasis (Harning *et al.*, 1989; Arisawa *et al.*, 1990; Shibuya *et al.*, 1990; Chiodoni *et al.*, 2001).

In this aspect, Arizawa *et al.,* (1990) observed that nude BALB/c mice show higher resistance to metastasis of colon carcinoma cells (C26) than heterozygous counterparts. In their experiment, the authors observed that 2 days after the inoculation of tumor cells all the animals of heterozygous control group (BALB/c *nu*/+) had developed hepatic metastasis whereas any BALB/c *nu*/*nu* presented metastasis. Moreover, they observed that 66% of nude animals treated with anti-asialo GM-1 became susceptible to hepatic development of tumor cells. These data were corroborated by Chiodoni *et al.* (2001) who worked with the same experimental model and supported *in vivo* the early suggestion of Herberman *et al.,* (1975) that the higher NK activity of nude mice is associated with antitumor resistance.

Harning *et al.,* (1989) have demonstrated that i.v. inoculation of melanoma B16F10 in C57BL/6J beige mice that show low NK activity results in the appearance of a great number of pulmonary metastases, whereas normal mice are resistant to the phenomenon. However, this resistance is broken by treatment of normal animals with anti-NK monoclonal antibody (PK 136). Using the same model, Shibuia *et al.,* (1990) observed that administration of batroxobin, an analogue of trombin that induces metastasis of these cells, was able to significantly inhibit the occurrence of pulmonary metastasis.

They also observed that stimulation of NK activity with poly I:C resulted in a more intense level of inhibition of metastasis, whereas depletion of NK cells with anti-asialo GM-1 blocked the antimetastatic treatment.

Although experimental data have shown that NK activity can be important to inhibiting the occurrence of colon cancer metastasis, their efficiency in the immunosurveillance of this kind of cancer in humans cannot be very easily demonstrable. Aparicio-Pages *et al.*, (1989) observed that NK cells from patients with colorectal carcinoma show the same level of lytic activity as normal donors. However, cells obtained from the tumor mass show reduced lytic activity when compared with NK cells from peripheral blood or from intestinal mucous lymphoid tissue of the same patient. These observations suggest a local suppressive state, due to factors produced or induced by the tumor cells themselves.

Analyzing the melanoma infiltrating cell subsets, Simony *et al.,* (1991) observed numerous $CD8^+$ and $CD4^+$ T lymphocytes as well as NK cells in all evaluated samples (Golub *et al.*, 1982a, 1982b; Hersey *et al.*, 1982) suggesting the role of these cells in the antitumor responsiveness. Recent clinical studies by Ruggeri *et al.,* (1999, 2002) and Giebel *et al.,* (2003), have demonstrated that NK cells play an important antileukemia role in patients with acute mielogenous leukemia submitted to bone marrow transplantation from haploidentical donor. They observed that in some cases there is incompatibility between donor and recipient KIRs, that is, when recipient does not express inhibitory ligands for KIR molecules of donor NK cells, these effector cells show *in vivo* expansion in the host. Since they are not inhibited by leukemia cells they are able to substantially reduce the risks of tumor recidivism.

Although colorectal (Horny & Horst 1987; Adachi *et al.*, 1990), breast (An *et al.*, 1987; Balch *et al.*, 1990) and lung cancer tissues (Ishigami *et al.*, 2000; Villegas *et al.*, 2002) show a low number of infiltrating NK cells, their presence appears to have a prognostic value for patients with squamous cell lung carcinoma (Villegas *et al.*, 2002), gastric carcinoma (Ishigami *et al.*, 2000) and colorectal carcinoma (Coca *et al.*, 1997). Tissues obtained from metastasis also show reduced frequency or even absence of NK cells, whereas the patients submitted to treatment with cytokines show a marked increase in these effector cells ($CD56^+/CD3^-$), in the adjacent or infiltrating tumor. (Vujanovic *et al.*, 1996).

Considering that the action of NK cells results from the balance between the stimulatory and inhibitory signals triggered by their interaction with class I MHC molecules, it should be remembered that, similarly to other regulatory systems, inhibitory signals are more potent than the stimulatory ones. Because of this phenomenon, in some situations the NK cells need to receive additional stimulation by cytokines, whose *in vivo* production can determine quantitative increase in these cells and/or enhanced lytic activity, which can be fundamental to their antitumoral role. On the other hand, NK cells by themselves constitute an important source of a variety of cytokines, whose production can influence the systemic behavior of the immune system.

5. Action of Cytokines on the Antitumor Activity of NK Cells

Many studies have been developed to evaluate the interaction between cytokines and NK cells both through *in vitro* and *in vivo* models. One of the main early findings in this area was that IL-2 stimulates the MHC-independent cytotoxicity. Henney *et al.* (1981) have observed that NK cells show variable toxicity against several target cells and that previous incubation with IL-2 or type I interferon resulted in potent stimulation of lytic activity. These cells were called lymphokine-activated killer cells (LAK) by Grimm *et al.* (1982) and it was further observed that this function is fulfilled essentially by NK cells (Phillips & Lanier, 1986). It was also largely demonstrated that IL-2 triggers antitumor activity in both experimental and clinical studies (Sacchi *et al.*, 1991; Hayakawa *et al.*, 1994; Ueda *et al.*, 1999).

From the 80s through the middle 90s several clinical trials were conducted to evaluate the efficiency of antitumor immunotherapy with LAK cells. Arienti *et al.,* (1993) have observed that passive transference of IL-2-activated tumor infiltrating lymphocytes (TIL) to melanoma patients resulted in complete or partial cure of 33% of them. Continuous i.v. or intrasplenic infusion of high doses of IL-2 and further transference of LAK in 9 patients with hepatic metastasis also resulted in complete or partial response in 1/3 of them, who showed longer survival time (26-36 months) (Keilholz *et al.,* 1992). This group also observed that the treatment of patients with hepatic metastasis of melanoma by intraportal injection of IL-2 and LAK cells showed better results than systemic treatment, indicating that the cells found *in situ* present a more differentiated behavior than do systemic cells. Patients with cerebral tumors receiving IL-2/LAK therapy also have shown total or partial regression of tumor in 30% of cases, as demonstrated by tomography and clinical signals (Ibayashi *et al.*, 1993).

Although IL-2/LAK therapy has shown good results in some patients with a variety of tumors, this proportion was considered low and many of the patients showed several important adverse effects such as pulmonary dysfunction (Villani *et al.*, 1993), thrombocytopenia by destruction of progenitor megacariocytes (Guarini *et al.*, 1991), alterations of cloatting processes (Richard *et al.*, 1991), ischemia of the colon and severe diarrhea (Sparano *et al.*, 1991), besides minor symptoms such as cutaneous alterations (Gaspari, 1991). Considering these findings, IL-2/LAK-based immunotherapy proposed by Rosenberg's group (Grimm *et al.*, 1982; Barba *et al.*, 1989; Rosenberg *et al.*, 1989) was not considered a secure therapeutic method, due to the difficulty in achieving an efficient dose of IL-2/LAK with minimum adverse effects. Unfortunately, clinical trials for new therapeutic methods are usually tested in patients in an advanced stage of the disease who in general are resistant to conventional therapy. This fact makes it difficult to obtain conclusive data on the efficiency of experimental methods to establish secure and efficient doses for therapy.

Interleukin 15 (IL-15) is a cytokine whose structure shows some homology with IL-2 and has also been studied for its antitumor effect. This cytokine is a crucial

factor for the development of NK cells in the bone marrow (Mrozek *et al.*, 1996; Fehniger & Caligiuri, 2001) both in mice and humans, being produced by bone marrow stroma (Grabstein *et al.*, 1994; Mrozek *et al.*, 1996). IL-15 binds to a trimer receptor (IL-15R) expressed by 99% of NK cells, whose specific a chain is associated with the same b and g chains shared by IL-2R (Dunne *et al.*, 2001). *In vitro*, IL-15 drives the differentiation of progenitor cells towards NK (Mrozek *et al.*, 1996) and *in vivo* it is required for development and homing of NK, NKT, $CD8^+$ T, Tγδ and intestinal intraepithelial lymphocytes (IELs), as demonstrated through experimental studies with knockout mice (Willerford et al., 1995; Ohteki *et al.*, 1997; Suzuki *et al.*, 1997). In addition, IL-15 induces proliferation and supports the *in vitro* survival and effector functions of NK cells (Carson *et al.*, 1994, 1995) more intensively than IL-2 (Dunne *et al.*, 2001). Similarly to IL-2, IL-15 is able to induce IFN-γ production, enabling the possibility of its use in antitumor immunotherapy.

Besides IL-2, IL-12 is the main subject of study on antitumor effect, due to its potent NK stimulatory property (Kobayashi *et al.*, 1989; Ramani & Balkwill, 1989; D'Andrea *et al.*, 1992; Brunda *et al.*, 1993). Lieberman *et al.,* (1991) observed that IL-12 increases the NK and ADCC activity of human peripheral blood leukocytes against cells of human colon carcinoma. This cytokine acts on T lymphocytes and NK cells, enhances their generation and cytotoxicity and, in addition, induces the production of IFN-γ, for activation of TH1 branch and consequently of $CD8^+$ cytolytic T cells. Using the murine melanoma B16 model, Kodama *et al.*, (1999) observed that IL-12 depends essentially on the participation of NK cells that kill the tumor cells in a perforin-dependent way. Martinotti *et al.,* (1995) have observed that tumor mass of IL-12 treated C26-bearing mice are infiltrated by NK and $CD8^+$ cells. Curiously the authors noted that depletion of $CD4^+$ cells with monoclonal antibody increases the NK and CTL infiltration, rather than depressing the antitumor response. Although they could not explain it at that time (1995), the current concepts on T reg cells suggest that this effect was due to elimination of these $CD4^+$/CD25+ regulatory cells.

Studies with experimental Burkitt´s lymphoma show NK cells in the vicinity of infiltrating fine vessels (Yao *et al.*, 1999). Under treatment with IL-12 these cells became cytolytic for endothelial cells, probably due to release of IFN-γ and TNF-α, indicating that NK cells and IL-12 can prevent neovascularization. The antiangiogenic action of IL-12 however, is not dependent on NK cells, since this cytokine inhibits angiogenesis of both murine and human tumors in NK-depleted SCID mice (Duda *et al.*, 2000).

Several clinical studies have also investigated the importance of IL-12 in the antitumor response: For example Robertson *et al.,* (1999) observed that i.v. administration of this cytokine in cancer patients induces increased NK activity and lymphoproliferative responsiveness of T cells. However, it had not been observed in many kinds of clinical cancer and the employment of this cytokine as antitumor therapy did not achieve the expectation suggested by experimental studies.

TNF-α and type I interferons (IFN α/β) inhibit the hepatic metastasis of colon carcinoma C 26 in mice and this effect is attributed to the capacity of these cytokines

to enhance NK activity, both in isolated form and synergistically (Sanada *et al.*, 1990). Some authors observed that human rINF-a inhibit lung metastasis from original carcinoma and also attributed the effect to NK activation (Ramani *et al.*, 1989).

Interleukin 23 (IL-23) is another cytokine that shows a significant effect on NK cells, discovered by Oppmann *et al.,* in 2000. This cytokine is a hetorodimer formed by the subunits IL-12 p40 and a specific p19, the structure of which presents some similarity with IL-6, G-CSF and less homology with IL-12 p35 (Parham *et al.*, 2002). According to Parham *et al.*, (2002), IL-23 acts preferentially on memory T cells inducing their proliferation and production of IFN-γ and in this aspect is different from IL-12 that acts mainly on naïve T cells. Lo *et al.,* (2003) evaluated the antitumor effect of IL-23 by using C26 carcinoma cells transfected with IL-23 genes and observed that the *in situ* production of this cytokine reduces the tumorigenesis and metastasis. However, antimetastatic effect of IL-23 was observed to be less efficient than that of IL-12 perhaps because IL-12 is more efficient in triggering specific cellular response to tumor antigens.

IL-21 produced by CD4 T cells also show homology with IL-2, IL-4 and IL-15 (Parrish-Novak *et al.*, 2000) and promote *in vitro* NK expansion and differentiation from bone marrow progenitor cells (Parrish-Novak *et al.*, 2000, 2002). Inoculation of plasmidial DNA with IL-21 genes induces constant production of this cytokine and supports its high serum levels that are associated with inhibition of growth of B16 melanoma and MCA-205 fibrosarcoma. *In vivo* depletion of $CD4^+$ or $CD8^+$ cells do not affect the antitumor effect of IL-21 but depletion of NK completely abolishes the resistance against tumor growth, indicating the importance of these cells in the fight against melanoma.

Although NK cells can be insufficient to avoid tumor growth by themselves, even under cytokine activation, it must be considered that after destruction of some target cells, apoptotic bodies are generated, which can be endocytized by dendritic cells of macrophages with subsequent presentation of tumor antigens for T lymphocytes. In addition, early production of IFN-γ by NK cells could activate macrophages and dendritic cells, which in turn, can stimulate NK to play both the effector and regulatory roles in the antitumor response.

References

Adachi W, Usuda N, Sugenoya A, Iida F. Immune-competent cells of regional lymph nodes in colorectal cancer patients: II. Immunohistochemical analysis of Leu-7+ cells. J Surg Oncol 1990;45:234-41.

Alimonti JB, Shi L, Baijal PK, Greenberg AH. Granzyme B induces BID-mediated cytochrome c release and mitochondrial permeability transition. J Biol Chem 2001;276:6974-82.

An T, Sood U, Pietruk T, Cummings G, Hashimoto K, Crissman JD. *In situ* quantitation of inflammatory mononuclear cells in ductal infiltrating breast carcinoma. Relation to prognostic parameters. Am J Pathol 1987;128:52-60.

Aparicio-Pages NM, Verspaget HW, Pena SA, Lamers CB. Impaired local natural killer

cell activity in human colorectal carcinomas. Cancer Immunol Immunother 1989;28:301-4.

Arienti F, Belli F, Rivoltini L, Gambacorti-Passerini C, Furlan L, Mascheroni L, Prada A, Rizzi M, Marchesi E, Vaglini M. Adoptive immunotherapy of advanced melanoma patients with interleukin-2 (IL-2) and tumor-infiltrating lymphocytes selected in vitro with low doses of IL-2. Cancer Immunol Immunother 1993;36:315-22.

Arisawa Y, Kubota T, Suto A, Kodaira S, Ishibiki K, Abe O. Nude mouse resists hepatic metastasis of the allogeneic tumor, colon-26. Jpn J Surg 1990; 20: 487-90.

Balch CM, Riley LB, Bae YJ, Salmeron MA, Platsoucas CD, von Eschenbach A, Itoh K. Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. Arch Surg 1990;125:200-5.

Barba D, Saris SC, Holder C, Rosenberg SA, Oldfield EH. Intratumoral LAK cell and interleukin-2 therapy of human gliomas. J Neurosurg 1989;70:175-82.

Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999; 285:727–9.

Berke G. The Fas-based mechanism of lymphocytotoxicity. Hum Immunol 1997;54:1-7.

Bossi G, Griffiths GM. Degranulation plays an essential part in regulating cell surface expression of Fas ligand in T cells and natural killer cells. Nature Med 1999;5:90-6.

Browne KA, Johnstone RW, Jans DA, Trapani JA. Filamin (280-kDa actin-binding protein) is a caspase substrate and is also cleaved directly by the cytotoxic T lymphocyte protease granzyme B during apoptosis. J Biol Chem 2000;275:39262-6.

Brunda MJ, Luistro L, Warrier RR, Wright RB, Hubbard BR, Murphy M, Wolf SF, Gately MK. Antitumor and antimetastatic activity of interleukin 12 against murine tumors. J Exp Med 1993;178:1223-30.

Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res 1970;13:1-7.

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 inhibitor receptor. Immunity 1996;4:77.

Campbell KS, Dessing M, Lopez-Botet M, Cella M, Colonna M. Tyrosine phosphorylation of a human killer inhibitory receptor recruits protein tyrosine phosphatase 1C. J Exp Med 1996;184:93.

Carson WE, Giri JG, Lindemann MJ, Linett ML, Ahdieh M, Paxton R, Anderson D, Eisenmann J, Grabstein K, Caligiuri MA. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med 1994;180:1395-403.

Carson WE, Ross ME, Baiocchi RA, Marien MJ, Boiani N, Grabstein K, Kaligiuri MA. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells *in vitro*. J Clin Invest 1995;96:2578-82.

Cella M, Dohring C, Samaridis J, Dessing M, Brockhaus M, Lanzavecchia A, Collona M.

A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. J Exp Med 1997;185:1743–51.

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;25:2433–37.

Chiodoni C, Stoppacciaro A, Sangaletti S, Gri G, Cappetti B, Koezuka Y, Colombo MP. Different requirements for alpha-galactosylceramide and recombinant IL-12 antitumor activity in the treatment of C-26 colon carcinoma hepatic metastases. Eur J Immunol 2001;31:3101-10.

Coca S, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, Martos JA, Moreno M. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer 1997;79:2320-8.

Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001a;22:633-40.

Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56 (bright) subset. Blood 2001b;97:3146-51.

D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste M, Chan SH, Kobayashi M, Young D, Nickbarg E. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J Exp Med 1992;176:1387-98.

Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol 2000;1:119–26.

Duda DG, Sunamura M, Lozonschi L, Kodama T, Egawa S, Matsumoto G, Shimamura H, Shibuya K, Takeda K, Matsuno S. Direct in vitro evidence and in vivo analysis of the antiangiogenesis effects of interleukin 12. Cancer Res 2000;60:1111-6.

Dunne J, Lynch S, O'Farrelly C, Todryk S, Hegarty JE, Feighery C, Doherty DG. Selective expansion and partial activation of human NK cells and NK receptor-positive T cells by IL-2 and IL-15. J Immunol 2001;167:3129-38.

Fanger NA, Borges L, Cosman D. The leukocyte immunoglobulin-like receptors (LIRs): a new family of immune regulators. J Leukoc Biol 1999;66:231–6.

Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. Blood 2001;97:14-32.

Fletcher JM, Prentice HG, Grundy JE. Natural killer cell lysis of cytomegalovirus (CMV)-infected cells correlates with virally induced changes in cell surface lymphocyte function-associated antigen-3 (LFA-3) expression and not with the CMV-induced down-regulation of cell surface class I HLA. J Immunol 1998;161:2365-74.

Gaspari AA. Dermatologic changes associated with adoptive immunotherapy of cancer. Semin Dermatol 1991;10:178-87.

Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G, Maccario R, Bonetti

F, Wojnar J, Martinetti M, Frassoni F, Giorgiani G, Bacigalupo A, Holowiecki J. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood 2003;102:814-9.

Golub SH, D'Amore P, Rainey M Systemic administration of human leukocyte interferon to melanoma patients. II. Cellular events associated with changes in natural killer cytotoxicity. J Natl Cancer Inst 1982a;68:711-7.

Golub SH, Dorey F, Hara D, Morton DL, Burk MW. Systemic administration of human leukocyte interferon to melanoma patients. I. Effects on natural killer function and cell population. J Natl Cancer Inst 1982b;68:703-10.

Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, Beers C, Richardson J, Schoenborn MA, Ahdieh M. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science 1994;264:965-8.

Griffin JD, Hercend T, Beveridge R, Schlossman SF. Characterization of an antigen expressed by human natural killer cells. J Immunol 1983;130:2947-51.

Griffiths GM, Isaaz S. Granzymes A and B are targeted to the lytic granules of lymphocytes by the mannose-6-phosphate receptor. J Cell Biol 1993;120:885-96.

Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med 1982;155:1823-41.

Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc Natl Acad Sci USA 1999;96:6879–84.

Guarini A, Sanavio F, Novarino A, Gillio Tos A, Aglietta M, Foa R. Thrombocytopenia in acute leukaemia patients treated with IL2: cytolytic effect of LAK cells on megakaryocytic progenitors. Br J Haematol 1991;79:451-6.

Harning R, Koo GC, Szalay J. Regulation of the metastasis of murine ocular melanoma by natural killer cells. Invest Ophthalmol Vis Sci 1989;30:1909-15.

Hayakawa M, Hatano T, Ogawa Y, Gakiya M, Ogura H, Osawa A. Treatment of advanced renal cell carcinoma using regional arterial administration of lymphokine-activated killer cells in combination with low doses of rIL-2. Urol Int 1994;53:117-24.

Hayakawa Y, Kelly JM, Westwood JA, Darcy PK, Diefenbach A, Raulet D, Smyth MJ. Cutting edge: tumor rejection mediated by NKG2D receptor-ligand interaction is dependent upon perforin. J Immunol 2002;169:5377-81.

Heibein JA, Goping IS, Barry M, Pinkoski MJ, Shore GC, Green DR, Bleackley RC. Granzyme B-mediated cytochrome c release is regulated by the Bcl-2 family members bid and Bax. J Exp Med 2000;192:1391-402.

Henney CS, Kuribayashi K,. Kern DE, Gillis S. Interleukin-2 augments natural killer cell activity. Nature 1981;291:335-8.

Herberman RB, Bartram S, Haskill JS, Nunn M, Holden HT, West WH. Fc receptors on mouse effector cells mediating natural cytotoxicity against tumor cells. J Immunol

1977;119:322-6.

Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer 1975;16:216-29.

Hersey P, Hobbs A, Edwards A, McCarthy WH, McGovern VJ. Relationship between natural killer cell activity and histological features of lymphocyte infiltration and partial regression of the primary tumor in melanoma patients. Cancer Res 1982;42:363-8.

Horny HP, Horst HA. Lymphoreticular infiltrates in adenocarcinoma of the large intestine. Pathol Res Pract 1987;182:222-7.

Ibayashi Y, Yamaki T, Kawahara T, Daibo M, Kubota T, Uede T, Tanabe S, Hashi K. Effect of local administration of lymphokine-activated killer cells and interleukin-2 on malignant brain tumor patients. Neurol Med Chir 1993;33:448-57.

Irmler M, Hertig S, MacDonald HR, Sadoul R, Becherer JD, Proudfoot A, Solari R, Tschopp J. Granzyme A is an interleukin 1 beta-converting enzyme. J Exp Med 1995;181:1917-22.

Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, Aridome K, Hokita S, Aikou T. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer 2000;88:577-83.

Kasai M, Iwamori M, Nagai Y, Okumura K, Tada T. A glycolipid on the surface of mouse natural killer cells. Eur J Immunol 1980;10:175-80.

Kay HD, Bonnard GD, West WH, Herberman RB. A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. J Immunol 1977;118:2058-66.

Kayagaki, N., N. Yamaguchi, M. Nakayama, K. Takeda, H. Akiba, H. Tsutsui, Okamura H, Nakanishi K, Okumura K, Yagita H. Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells. J Immunol 1999;163:1906-13.

Keilholz U, Schlag P, Tilgen W, Brado B, Galm F, Gorich J, Kauffmann GW, Moller P, Schneider S, Hunstein W. Regional administration of lymphokine-activated killer cells can be superior to intravenous application. Cancer 1992;69:2172-5.

Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 1975;5:117-21.

Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. Exp Med 1989;170:827-45.

Kodama T, Takeda K, Shimozato O, Hayakawa Y, Atsuta M, Kobayashi K, Ito M, Yagita H, Okumura K. Perforin-dependent NK cell cytotoxicity is sufficient for anti-metastatic effect of IL-12. Eur J Immunol 1999;29:1390-6.

Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and

cytotoxic T lymphocytes. J Immunol 1986a;136:4480-6.

Lanier LL, Cwirla S, Federspiel N, Phillips JH. Human natural killer cells isolated from peripheral blood do not rearrange T cell antigen receptor beta chain genes. J Exp Med 1986b;163:209-14.

Lanier LL, Ruitenberg JJ, Phillips JH. Functional and biochemical analysis of CD16 antigen on natural killer cells and granulocytes. J Immunol 1988;141:3478-85.

Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. J Exp Med 1989;169:2233-8.

Lieberman J, Fany Z. Nuclear war: the granzyme A-bomb. Curr Op Immunol 2003;15:553–9.

Lieberman J. Cell death and immunity: The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal Nature Rev Immunol 2003;3:361-70.

Lieberman MD, Sigal RK, Williams NN 2nd, Daly JM. Natural killer cell stimulatory factor (NKSF) augments natural killer cell and antibody-dependent tumoricidal response against colon carcinoma cell lines. J Surg Res 1991;50:410-5.

Ljunggren HG, Kärre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 1990;11:237.

Lo CH, Lee SC, Wu PY, Pan WY, Su J, Cheng CW, Roffler SR, Chiang BL, Lee CN, Wu CW, Tao MH. Antitumor and antimetastatic activity of IL-23. J Immunol 2003;171:600-7.

Lotzova E, Ades EW. Natural killer cells: definition, heterogeneity, lytic mechanism, functions and clinical application. Highlights of the Fifth International Workshop on natural killer cells, Hilton Head Island, N.C., March 1988.

Lowin-Kropf B, Held W. Positive impact of inhibitory Ly49 receptor-MHC class I interaction on NK cell development. J Immunol 2000;165:91-5.

Lowin-Kropf B, Kunz B, Beermann F, Held W. Impaired natural killing of MHC class I-deficient targets by NK cells expressing a catalytically inactive form of SHP-1. J Immunol 2000;165:1314-21.

Makrigiannis AP, Pau AT, Saleh A, Winkler-Pickett R, Ortaldo JR, Anderson SK. Class I MHC-binding characteristics of the 129/j ly49 repertoire. J Immunol 2001;166:5034–43.

Malnati MS, Peruzzi M, Parker KC, Biddison WE, Ciccone E, Moretta A, Long EO. Peptide specificity in the recognition of MHC class I by natural killer cell clones. Science 1995;267:1016–8.

Martinotti A, Stoppacciaro A, Vagliani M, Melani C, Spreafico F, Wysocka M, Parmiani G, Trinchieri G, Colombo MP. CD4 T cells inhibit in vivo the CD8-mediated immune response against murine colon carcinoma cells transduced with interleukin-12 genes. Eur J Immunol 1995;25:137-46.

Masucci MG, Masucci G, Klein E, Berthold W. Target selectivity of interferon-induced human killer lymphocytes related to their Fc receptor expression. Proc Natl Acad Sci USA 1980;77:3620-4.

Menier C, Riteau B, Carosella ED, Rouas-Freiss N. MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. Int J Cancer

2002;100:63-70.

Middleton D, Curran M, Maxwell L. Natural killer cells and their receptors Transplant Immunol 2002;10:147–64.

Mrozek E, Anderson P, Caligiuri MA. Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. Blood 1996;87:2632-40.

Natarajan K, Dimasi N, Wang J, Mariuzza RA, Margulies DH. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. Annu Rev Immunol 2002;20:853-85.

Nishikawa K, Saito S, Morii T, Kato Y, Narita N, Ichijo M, Ohashi Y, Takeshita T, Sugamura K. Differential expression of the interleukin 2 receptor beta (p75) chain on human peripheral blood natural killer subsets. Int Immunol 1990;2:481-6.

Nitta T, Yagita H, Sato K, Okumura K. Involvement of CD56 (NKH-1/Leu-19 antigen) as an adhesion molecule in natural killer-target cell interaction. J Exp Med 1989;170:1757-61.

Ohteki T, Ho S, Suzuki H, Mak TW, Ohashi PS. Role for IL-15/IL-15 receptor beta-chain in natural killer 1.1+ T cell receptor-alpha beta+ cell development. J Immunol 1997;159:5931-5.

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715-25.

Orange JS, Fassett MS, Koopman LA, Boyson JE, Strominger JL. Viral evasion of natural killer cells. Nat Immunol 2002;3:1006-12.

Ortaldo JR, Oldham RK, Cannon GC, Herberman RB. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. J Natl Cancer Inst 1977;59:77-82.

Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W, Wagner J, O'Farrell AM, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick DM, Kastelein RA, de Waal Malefyt R, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002;168:5699-708.

Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, Johnston J, Madden K, Xu W, West J, Schrader S, Burkhead S, Heipel M, Brandt C, Kuijper JL, Kramer J, Conklin D, Presnell SR, Berry J, Shiota F, Bort S, Hambly K, Mudri S, Clegg C, Moore M, Grant FJ, Lofton-Day C, Gilbert T, Rayond F, Ching A, Yao L, Smith D, Webster P, Whitmore T, Maurer M, Kaushansky K, Holly RD, Foster D. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature 2000;408:57-63.

Parrish-Novak J, Foster DC, Holly RD, Clegg CH. Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses. J Leukoc Biol 2002; 72: 856-63.

Phillips JH, Lanier LL. Dissection of the lymphokine-activated killer phenomenon. Relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. J Exp Med 1986;164:814-25.

Ramani P, Balkwill FR. Action of recombinant alpha interferon against experimental and spontaneous metastases in a murine model. Int J Cancer 1989;43:140-6.

Reynolds CW, Timonen T, Herberman RB. Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. J Immunol 1981; 127: 282-7.

Richard V, Bernier M, Themelin L, Bron D, Sculier JP. Blood coagulation abnormalities during adoptive immunotherapy with interleukin-2 (r-Met Hu IL-2 [ala 125]). Ann Oncol 1991;2:67-8.

Ritz J, Campen TJ, Schmidt RE, Royer HD, Hercend T, Hussey RE, Reinherz EL. Analysis of T-cell receptor gene rearrangement and expression in human natural killer clones. Science 1985;228:1540-3.

Robertson MJ, Cameron C, Atkins MB, Gordon MS, Lotze MT, Sherman ML, Ritz J. Immunological effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. Clin Cancer Res 1999;5:9-16.

Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. Blood 1990;76:2421-38.

Rosenberg SA, Lotze MT, Yang JC, Aebersold PM, Linehan WM, Seipp CA, White DE. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann Surg 1989;210:474-85.

Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, Urbani E, Negrin RS, Martelli MF, Velardi A. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 1999;94:333-9.

Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097-100.

Sacchi M, Vitolo D, Sedlmayr P, Rabinowich H, Johnson JT, Herberman RB, Whiteside TL. Induction of tumor regression in experimental model of human head and neck cancer by human A-LAK cells and IL-2. Int J Cancer 1991;47:784-91.

Saksela E, Timonen T, Ranki A, Hayry P. Morphological and functional characterization of isolated effector cells responsible for human natural killer activity to fetal fibroblasts and to cultured cell line targets. Immunol Rev 1979;44:71-123.

Sanada E, Fuchimoto S, Orita K. Synergistic antiproliferative effect of the combination of natural human tumor necrosis factor-alpha and natural murine interferon-alpha/beta against colon-26 adenocarcinoma hepatic metastases in a murine model. Acta Med Okayama 1990;44:217-22.

Santoni A, Herberman RB, Holden HT. Correlation between natural and antibody-

dependent cell-mediated cytotoxicity against tumor targets in the mouse. I. Distribution of the reactivity. J Natl Cancer Inst 1979;62:109-16.

Sauer H, Pratsch L, Tschopp J, Bhakdi S, Peters R. Functional size of complement and perforin pores compared by confocal laser scanning microscopy and fluorescence microphotolysis. Biochim Biophys Acta 1991;1063:137-46.

Screpanti V, Wallin RPA, Ljunggren H-G, Grandien A.A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. J Immunol 2001;167:2068–73.

Sharif-Askari E, Alam A, Rheaume E, Beresford PJ, Scotto C, Sharma K, Lee D, DeWolf WE, Nuttall ME, Lieberman J, Sekaly RP. Direct cleavage of the human DNA fragmentation factor-45 by granzyme B induces caspase-activated DNase release and DNA fragmentation. EMBO J 2001;20:3101-13.

Shibuya M, Niitani H, Aoyama A, Kawachi S, Nukariya N, Baba M, Iizuka K, Sakai S, Ohtsuka M. Antimetastatic effect of defibrinogenation with batroxobin depends on the natural killer activity of host in mice. J Cancer Res Clin Oncol 1990;116:168-72.

Shimizu Y, DeMars R. Demonstration by class I gene transfer that reduced susceptibility of human cells to natural killer cell-mediated lysis is inversely correlated with HLA class I antigen expression. Eur J Immunol 1989;19:447-51.

Shinkai Y, Takio K, Okumura K. Homology of perforin to the ninth component of complement (C9). Nature 1988;334: 25-7.

Simony J, Rossi JF, Shishehian B, Ursule E, Radal M, Pujol H. Characterization of proliferative cells in malignant melanomas and their inflammatory infiltrates. Cancer Detect Prev 1991;15:183-7.

Soderstrom K, Corliss B, Lanier LL Phillips JH. CD94/NKG2 is the predominant inhibitory receptor involved in recognition of HLA-G by decidual and peripheral blood NK cells. J Immunol 1997;159:1072–5.

Sparano JA, Dutcher JP, Kaleya R, Caliendo G, Fiorito J, Mitsudo S, Shechner R, Boley SJ, Gucalp R, Ciobanu N. Colonic ischemia complicating immunotherapy with interleukin-2 and interferon-alpha. Cancer 1991;68:1538-44.

Storkus WJ, Alexander J, Payne JA, Dawson JR, Cresswell P. Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes. Proc Natl Acad Sci USA 1989;86:2361-4.

Suidan HS, Clemetson KJ, Brown-Luedi M, Niclou SP, Clemetson JM, Tschopp J, Monard D. The serine protease granzyme A does not induce platelet aggregation but inhibits responses triggered by thrombin. Biochem J 1996;315:939-45.

Suttles J, Schwarting GA, Hougland MW, Stout RD. Expression of asialo GM1 on a subset of adult murine thymocytes: histological localization and demonstration that the asialo GM1-positive subset contains both the functionally mature and the proliferating thymocyte subpopulations. J Immunol 1987;138:364-72.

Suzuki H, Duncan GS, Takimoto H, Mak TW. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2

receptor beta chain. J Exp Med 1997;185:499-505.

Suzuki N, Suzuki T, Engleman EG. Evidence for the involvement of CD56 molecules in alloantigen-specific recognition by human natural killer cells. J Exp Med 1991;173:1451-61.

Thomas DA, Du C, Xu M, Wang X, Ley TJ. DFF45/ICAD can be directly processed by granzyme B during the induction of apoptosis. Immunity 2000;12:621-32.

Timonen T, Saksela E, Ranki A, Hayry P. Fractionation, morphological and functional characterization of effector cells responsible for human natural killer activity against cell-line targets. Cell Immunol 1979a;48:133-48.

Timonen T, Ranki A, Saksela E, Hayry P. Human natural cell-mediated cytotoxicity against fetal fibroblasts. III. Morphological and functional characterization of the effector cells. Cell Immunol 1979b;48:121-32.

Timonen T, Saksela E. Isolation of human NK cells by density gradient centrifugation. J Immunol Methods 1980;36:285-91.

Tomasello E, Olcese L, Vely F, Geourgeon C, Blery M, Moqrich A, Gautheret D, Djabali M, Mattei MG, Vivier E. Gene structure, expression pattern, and biological activity of mouse killer cell activating receptor-associated protein (KARAP)/DAP-12. J Biol Chem 1998;273:34,115–9.

Trambas CM, Griffiths GM. Delivering the kiss of death. Nat Immunol 2003; 4: 399-403.

Trinchieri G. Biology of natural killer cells. Adv Immunol 1989;47:187-376.

Ueda Y, Yamagishi H, Tanioka Y, Fujiwara H, Fuji N, Itoh T, Fujiki H, Yoshimura T, Oka T. Clinical application of adoptive immunotherapy and IL-2 for the treatment of advanced digestive tract cancer. Hepatogastroenterology 1999;46:1274-9.

van den Broek MF, Kagi D, Zinkernagel RM, Hengartner H. Perforin dependence of natural killer cell-mediated tumor control in vivo. Eur J Immunol 1995;25:3514-6.

Villani F, Galimberti M, Rizzi M, Manzi R. Pulmonary toxicity of recombinant interleukin-2 plus lymphokine-activated killer cell therapy. Eur Respir J 1993;6:828-33.

Villegas FR, Coca S, Villarrubia VG, Jimenez R, Chillon MJ, Jareno J, Zuil M, Callol L. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. Lung Cancer 2002;35:23-8.

Vujanovic NL, Basse P, Herberman RB, Whiteside TL. Antitumor functions of natural killer cells and control of metastases. Methods 1996; 9: 394-408.

West WH, Cannon GB, Kay HD, Bonnard GD, Herberman RB. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J Immunol 1977;118:355-61.

Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity 1995;3:521-30.

Wolf BB, Schuler M, Echeverri F, Green DR Caspase-3 is the primary activator of apoptotic DNA fragmentation via DNA fragmentation factor-45/inhibitor of caspase-activated DNase inactivation. J Biol Chem 1999;274:30651-6.

Wu J, Cherwinski H, Spies T, Phillips JH, Lanier LL. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. J Exp Med 2000;192:1059–68.

Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, Phillips JH.. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 1999;285:730–32

Yao L, Sgadari C, Furuke K, Bloom ET, Teruya-Feldstein J, Tosato G. Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. Blood 1999;93:1612-21.

Young HA, Ortaldo JR, Herberman RB, Reynolds CW. Analysis of T cell receptors in highly purified rat and human large granular lymphocytes (LGL): lack of functional 1.3 kD beta-chain mRNA. J Immunol 1986;136:2701-4.

Young WW Jr, Hakomori SI, Durdik JM, Henney CS. Identification of ganglio-N-tetraosylceramide as a new cell surface marker for murine natural killer (NK) cells. J Immunol 1980;124:199-201.

Zeidler R, Eissner G, Meissner P, Uebel S, Tampe R, Lazis S, Hammerschmidt W. Downregulation of TAP1 in B lymphocytes by cellular and Epstein-Barr virus-encoded interleukin-10. Blood 1997;90:2390-7.

Zhang D, Beresford PJ, Greenberg AH, Lieberman J Granzymes A and B directly cleave laminins and disrupt the nuclear lamina during granule-mediated cytolysis. Proc Natl Acad Sci USA 2001;98:5746-51.