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Factors Influencing the Control of Virus Infections by Natural Killer Cells

RAYMOND M. WELSH, ROBERT J. NATUK, KIM W. MCINTYRE, HYEKYUNG YANG, CHRISTINE A. BIRON, and JACK F. BUKOWSKI

1. Introduction

It has long been speculated that natural killer (NK) cells play a role in natural resistance to and regulation of virus infections.[1] Reviewed elsewhere in this volume is evidence that suggests a role for NK cells in cytomegalovirus (CMV), herpes simplex virus, and influenza virus infections. Proving definitively that NK cells provide resistance to viruses has been virtually impossible in man and difficult in animal models, many of which are currently beset with contradictory studies. Perhaps the most accurate conclusion from the available evidence is that NK cells regulate some but not all virus infections and that virus dose and inoculation routes and host species, age, and target organ may all influence the relative importance of the NK cells.

2. NK-Resistant and NK-Sensitive Viruses

In our experience the lymphocytic choriomeningitis virus (LCMV) and murine CMV (MCMV) infections of mice represent extremes for NK-

RAYMOND M. WELSH, ROBERT J. NATUK, KIM W. MCINTYRE, HYEKYUNG YANG, CHRISTINE A. BIRON, and JACK F. BUKOWSKI • Department of Pathology, University of Massachusetts Medical School, Worcester, Massachusetts 01655.

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resistant and NK-sensitive viruses. LCMV is a relatively noncytopathic virus which establishes a lifelong persistent infection of mice infected *in utero* or an immunizing infection regulated by cytotoxic T cells in mice infected as adults.[2] Depletion of NK cell activity in adult mice by antiserum to asialo GM_1[3] or by cyclophosphamide[4] does not influence LCMV synthesis. Homozygous beige mice, which have an NK cell defect, synthesize amounts of LCMV comparable to their NK-sufficient heterozygous littermates early in infection.[5] Reduced clearance of the virus is seen later in infection, correlating with a defective CTL response.[5,6] Adoptive transfers of NK cell-containing adult leukocytes into 5-day-old suckling mice that have low NK cell activity do not protect the suckling mice from LCMV.[7] Furthermore, there are no age- or strain-dependent resistance factors to LCMV that correlate with NK cell activity.

The situation with MCMV is entirely different. This is a relatively cytopathic virus which can establish a persistent infection in the salivary gland and a latent infection in leukocytes. Antibody to asialo GM_1 enhances MCMV infection in both acutely and persistently infected adult mice.[3,8] The biological response modifier OK432 enhances NK cell activity and induces a resistance to MCMV which can be abrogated by antiserum to asialo GM_1.[9] Beige mice are very sensitive to MCMV, and in bone marrow chimeras, resistance to infection is correlated with donor bone marrow cells from heterozygous but not homozygous (NK cell-deficient) mice.[10] There is a general correlation between NK cell activity and resistance to MCMV in a variety of strains of mice,[11] and suckling mice are very sensitive to MCMV, with resistance developing with age in parallel to the maturation of the NK cell response.[12] Adoptive transfer of adult leukocytes into baby mice protects the recipients from MCMV. Depletion of NK cells but not other leukocyte populations eliminates the protective effect, and partial purification of the NK cells enriches for the protective effect.[7] Furthermore, a cloned large granular lymphocyte (LGL) cell line mediating NK cell activity protected both suckling and irradiated adult mice from MCMV but not from LCMV.[7] LCMV is therefore NK-resistant, and MCMV appears to be NK-sensitive, but the NK-sensitivity of MCMV may be unusually profound, compared to other viruses.[13–18]

3. Age-Dependent Resistance

Resistance to virus infections often increases with age and may sometimes depend on the maturation of the NK cell response. However, in our hands the adoptive transfers of adult leukocytes into suckling mice, which were used to demonstrate an antiviral role for NK cells in the MCMV infection, have not shown a profound role for NK cells in other infections. Adult leukocytes did not protect recipients at all from the NK-resistant LCMV infection (Table I) but did protect against several other vi-

TABLE I
Protection of Suckling Mice from Viruses by Adult Leukocytes[a]

Virus	Donor cell number	Recipient organ	PFU/organ: No leukocytes (control)	PFU/organ: Adult leukocytes	PFU/organ: Adult NK-depleted leukocytes
LCMV	5×10^7	Spleen	4.7 ± 0.1	4.4 ± 0.1	ND[b]
MCMV	5×10^7	Spleen	4.6 ± 0.1	3.2 ± 0.1	4.1 ± 0.1
HSV-1	1.7×10^7	Spleen	4.5 ± 0.2	3.0 ± 0.1	3.1 ± 0.2
	5.6×10^6		4.5 ± 0.2	3.2 ± 0.2	3.1 ± 0.3
	1.8×10^6		4.5 ± 0.2	4.6 ± 0.2	4.5 ± 0.3
VSV	5×10^7	Spleen	3.0 ± 0.4	<2	<2
	5×10^7	Liver	4.0 ± 0.9	<2	<2
	5×10^7	Brain	7.7 ± 0.5	<2	<2
PV	5×10^7	Spleen (Exp 1)	4.1 ± 0.1	3.3 ± 0.4	4.0 ± 0.1
		(Exp 2)	3.7 ± 0.1	3.5 ± 0.1	3.7 ± 0.1
		(Exp 3)	3.7 ± 0.1	3.2 ± 0.1	3.7 ± 0.1

[a]Spleen leukocytes from 4- to 10-week-old C57BL/6 mice were injected IP into 4- to 6-day-old mice 1 day prior to IP infection with virus. Organs were titrated by plaque assay for virus 2–3 days postinfection. Mice injected with antibody to asialo GM_1 served as donors for NK cell-depleted leukocytes. Results are expressed as $\log_{10}$ plaque-forming units ± SE. See references 3 and 19 for further details.
[b]ND = Not determined.

ruses, including MCMV, herpes simplex virus (HSV) type 1, vesicular stomatitis virus (VSV), and, to a lesser extent, Pichinde virus (PV). Depletion of NK cell activity in the donor leukocytes ablated the protective effect against the NK-sensitive MCMV and inhibited the very modest protective effect against PV. This suggests that PV may be sensitive to NK cells but much less so than MCMV. Consistent with this observation are data indicating that antibody to asialo GM_1 causes a modest but significant two- to fourfold enhancement of PV synthesis in adult mice, but a 10- to 1000-fold enhancement of MCMV synthesis and no enhancement of LCMV synthesis[3] (Table II). Work suggesting that NK cells play a role in regulating murine HSV[13,14] and VSV[16–18] infections was not supported by this adoptive transfer model (Table I).

Donor leukocytes protected mice against HSV-1, but depletion of NK cell activity in the donor cells did not inhibit the protective effect (Table I). In addition, whereas 5×10^7 adult leukocytes were required to protect suckling mice from the NK-sensitive MCMV, one-tenth that number protected against HSV-1. Further investigation of the HSV-1 system revealed that virtually any tested adult leukocyte or even cultured cell lines, such as L-929 cells, mediated protection.[19] Earlier reports had indicated that adult but not suckling mouse macrophages protected suckling mice from

TABLE II
Replication of Viruses in NK Cell-Depleted Mice[a]

Virus	Mouse	Anti-asialo GM_1	PFU/spleen	% Lysis vs. YAC-1 cells
PV (Exp 1)	Adult	−	4.7±0.1	24
		+	5.3±0.1	−1.3
(Exp 2)		−	4.0±0.2	20
		+	4.9±0.2	−1.0
LCMV	Adult	−	5.7±0.2	63
		+	5.6±0.2	0.5
MCMV	Adult	−	1.7±0.2	30
		+	4.6±0.1	0.2
HSV-1	Adult	−	5.2±0.3	38
		+	5.2±0.3	1.1
MHV	Adult	−	1.7±0.3	48
		+	3.4±0.1	16
MCMV	5-day-old beige	−	3.8±0.3	22
	5-day-old normal	−	3.6±0.3	19
	5-day-old normal	+	3.7±0.2	−1.5
MCMV	Adult beige	−	3.9±0.2	9.5
	Adult beige/+	−	1.4±0.1	51

[a]C57BL/6 normal (unless otherwise stated) or beige mice were injected IP with 20 μl anti-asialo GM_1 followed 4–6 h later by an IP challenge with virus. Spleens were titrated for virus 2–3 days later, and spleen cells were assayed for NK cell activity. Results are expressed as $\log_{10}$ pfu/spleen ± SE. Effector-to-target ratios of 100:1 or 50:1 were employed in cytotoxicity assays.

HSV-1,[20] but in our hands spleen cells protected even after depletion of macrophages. A series of investigations by Kirchner and co-workers has suggested that the IFN made during the first round of viral replication (early IFN) is crucial to the outcome of the infection.[21–23] Resistant strains of mice tended to make higher levels of early IFN than did susceptible strains.[21] The peritoneal fluid of suckling mice receiving adoptive transfers of leukocytes generated higher levels of early IFN after HSV-1 infection than did fluid from mice not receiving leukocytes.[19] Antibody to IFN completely abrogated the protective effect of the leukocytes. Furthermore, high doses of antibody to asialo GM_1 *in vivo* abrogated the early IFN response and prevented the protective effect of donor leukocytes. Lower levels of this antibody, which still depleted NK cell activity but not early IFN production, had no effect on virus titers[19] (Table II). Prophylactic IFN treatment was highly effective in preventing HSV-1 infection of suckling mice.[19] It therefore seems that the primary mechanism for adult leukocytes to provide resistance to HSV-1 in suckling mice is by

enhancing the early IFN response. It is possible that a similar mechanism could be occurring with VSV, a very IFN-sensitive virus.

Age-dependent resistance of mice to at least one strain of mouse hepatitis virus (MHV), MHV3, is consistent with an NK cell role, as it develops at 3 weeks of age.[15] With MHV3 several factors seem to be involved in resistance shown in this adoptive transfer system: a thy-1.2.-bearing (T) cell, a plastic adherent cell (macrophage), and a bone marrow cell from a mouse at least 3 weeks of age. This bone marrow cell shares similarity to NK cells in a variety of properties including culture lability, ^{89}Sr sensitivity, and stimulation by IFN inducers.[15] This strongly suggests a role for NK cells in age-dependent resistance to MHV3. Depletion of NK cell activity in adult mice with antibody to asialo GM_1 enhances the synthesis of MHV strains A-59[3] (Table II) and MHV-Y,[26] consistent with an NK cell role.

Thus, the mechanism of age-dependent resistance is complex, involving a variety of factors, of which NK cells may be only one. MCMV may be unusual in that NK cells are the only age-dependent factor with great significance. This may explain why the adoptive transfer results are so clear. Even in this model, however, there are perplexing inconsistencies. For instance, we have been unable to detect any differences in the synthesis of MCMV between suckling normal and homozygous C57BL/6 beige mice, which have an NK cell defect (Table II). In contrast, the growth of MCMV in adult beige mice is much higher than in normal adult mice[8,10] (Table II). Suckling mice have very low NK cell activity but develop significant levels of NK cell activity upon viral infection. A possible explanation for the lack of difference in MCMV synthesis in normal and beige suckling mice is that there is less of a differential between beige and normal suckling mouse NK cell activity after virus infection, whereas adult mice have a major difference[8] (Table II). However, to our great surprise, depletion of NK cell activity in suckling mice with antibody to asialo GM_1 did *not* influence MCMV titers, even though it ablated all detectable NK cell activity (Table II).

We are therefore left with the paradox that transfer of adult NK cells into baby mice renders protection against MCMV, but depletion of the suckling mouse's own NK cells has no effect on MCMV synthesis. It is difficult to find a suitable explanation for this. It seems that NK cells from the suckling mice are inadequate to control MCMV infection, even though they get activated. Perhaps their numbers are too few, their level of activation is not high enough, or there is an age-dependent defect in another function contributing to their antiviral effects.

4. Site of Virus Replication

The injection route and site of viral replication may play a role in the relative importance of NK cells in mediating antiviral effects. Although

NK cell activity is normally present predominantly in the spleen and peripheral blood, potent responses are seen in other organs that are sites of virus infection. For instance, NK cell activity is found in the cerebrospinal fluid after intracranial inoculation,[27] in the lung after intranasal[28] or intratracheal[29] inoculation, and in the peritoneal cavity after intraperitoneal inoculation.[30] Hepatotropic viruses stimulate high levels of NK cell activity in the liver,[31] and viruses that grow to high levels in the bone marrow stimulate NK cell activity therein.[32]

The increased levels of NK cell activity in organs are often associated with significant increases in NK cell number. The IFN induced during a virus infection *in vivo* stimulates the blastogenesis and proliferation of NK cells.[33–35] This is accompanied by significant increases in the number of LGLs[31,33,36] and the frequency of LGL-bearing blast morphology[37] in virus-infected organs. NK/LGLs respond chemotactically to extracts from virus-infected organs,[36] and the LGLs with blast morphology respond chemotactically much better than do resting LGLs.[38] It thus appears that virus-infected organs stimulate an accumulation of NK/LGLs by releasing factors chemotactic for blast LGLs and by synthesizing IFN, which induces NK cell blastogenesis.

This accumulation and enhanced activity of NK cells at virtually any examined site of virus infection should seemingly allow NK cells to mediate antiviral effects in most of the body's organs. Again, however, the situation may not be so simple. When the injection route is IV or IP, the titers of MCMV are greatly elevated in beige mice or in normal mice depleted of NK cell activity with antibody to asialo GM_1.[3,8,10] Dissemination of the virus into the lung is enhanced by NK cell depletion.[8] In contrast, upon instranasal inoculation with MCMV, there are no differences in virus titers between normal, beige, and NK cell-depleted mice.[8] The reason for this is not known. In this model there is substantial replication of MCMV within the lung before the virus disseminates elsewhere. It would seem, then, that whereas NK cells may inhibit the spread of MCMV *to* the lung, they do not inhibit MCMV replication *within* the lung. An obvious explanation would be that there is a defect in the activity or number of lung NK cells, but several reports indicate that they do get activated during virus infections.[28,29] This has been well documented with influenza virus and is reviewed elsewhere in this volume. Antibody to asialo GM_1 enhances the synthesis of influenza in the lung following intratracheal inoculation,[39] but other reports indicate that influenza virus pathogenesis is normal in lungs from beige mice[28] and from NK cell-depleted normal mice[40] after intranasal inoculation. Perhaps whether the inoculation route is intranasal versus intratracheal is significant.

In another study, ^{125}IUDR-labeled L-929 cells infected with LCMV were examined for clearance from the lung after intravenous injection.[41] Cells injected intranasally are initially trapped in the lung, and clearance of radiolabeled cells from the lung has been taken as an indication of *in vivo* cytotoxicity. Several studies have indicated that NK cells mediate *in*

vivo lysis of radiolabeled YAC-1 cells, the prototype mouse NK-sensitive target.[42–44] The virus-infected cells were lysed *in vivo* more rapidly than uninfected cells, and hydrocortisone and cyclophosphamide abrogated the lysis. However, this lysis was not blocked by antibody to asialo GM_1, which markedly inhibited the *in vivo* lysis of YAC-1 cells.[41] This suggests the existence of an antiviral cytotoxic cell within the lung that may not be an NK cell. Whether this represents an NC- (natural cytotoxic) type of killing mechanism[45] is unclear. However, one lot of antiserum to asialo GM_1 that was highly cytotoxic to macrophages did inhibit the virus-associated *in vivo* lysis, suggesting that alveolar macrophages may be involved (Biron and Welsh, unpublished). We are left to conclude that the issue of antiviral natural cytotoxicity associated with the lung may be very complex.

Another potential complication in organ-dependent NK cell accumulation is that there may be a finite number of NK cells for which different organs compete. For example, intraperitoneal injection of mice with MHV results in a high number of NK cells within the peritoneal cavity and an intermediate number in the liver (Fig. 1). Intravenous infection stimulates very high levels of NK cells within the liver but few in the peritoneal cavity. Inoculation by both routes also results in high levels of NK cells in the liver but not the peritoneal cavity. Thus the liver and

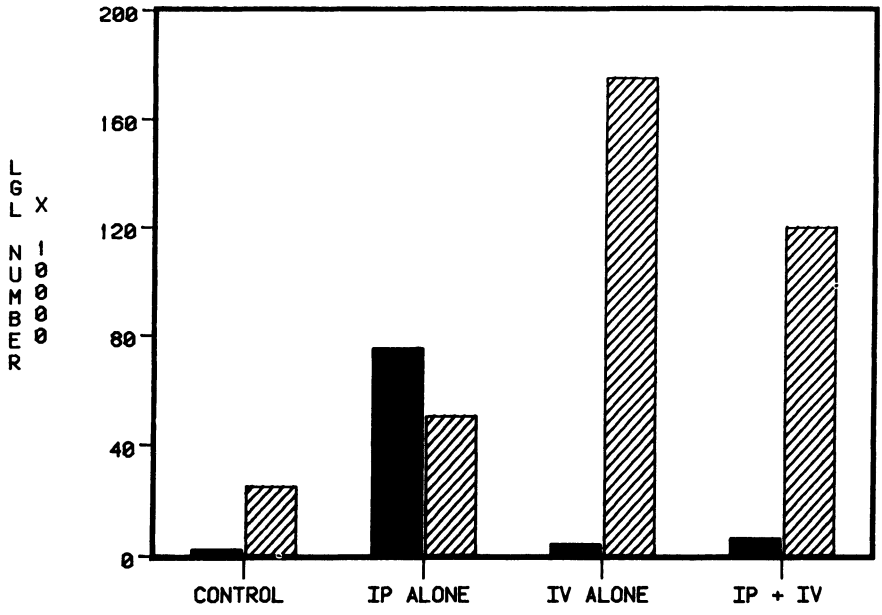

FIGURE 1. *In vivo* compartmentalization of NK/LGL responses. C57BL/6J mice were infected with 4×10^4 PFU of mouse hepatitis virus (MHV) either IP or IV or with 2×10^4 PFU MHV both IP and IV. At day 3 postinfection, peritoneal exudate cells (PEC) and liver leukocytes were prepared, and total LGL at each site (PEC, solid bars; liver, hatched bars) were enumerated as described.[31,36]

perhaps other visceral organs appear to compete with the peritoneal cavity for the attraction of NK cells. Similar results have been seen with virus-specific cytotoxic T cells in the LCMV infection.[31] The significance of this is that NK cells may not be able to mediate antiviral effects in certain organs if they cannot accumulate within them because of competition from other infected organs.

5. Interferon-Mediated Effects on Target Cells

Interferon protects target cells from NK cell-mediated lysis,[46] presumably by inhibiting the target cell from triggering the release of cytotoxic factors from bound NK cells.[47–49] As a virus infection progresses *in vivo*, cells in the body become progressively more resistant to NK cells[44,50,51] and, probably because of IFN-mediated up-regulation of class I major histocompatibility antigens, progressively more sensitive to cytotoxic T cells.[51] Infection of target cells by cytopathic viruses often renders these cells resistant to IFN-mediated effects, probably because of virus-induced inhibition of cellular RNA and protein synthesis, which IFN requires to stimulate most of its effects, including protection against NK cells.[44,46] Protection of target cells by IFN may be an important mechanism of homeostasis that prevents the IFN-mediated activation and proliferation of NK cells from causing a potent autodestructive effect.

It has been hypothesized that a selective IFN-mediated protective effect against uninfected but not virus-infected cells may provide a mechanism by which NK cells selectively lyse virus-infected cells *in vivo*.[46,47,52] *In vitro* studies with the NK-resistant LCMV and NK-sensitive MCMV are consistent with this hypothesis, as IFN protects LCMV-infected but not MCMV-infected cells from lysis by activated NK cells.[53] Within a given organ the degree of efficiency of this antiviral selectivity may depend on how sensitive the uninfected cells in the tissue are to IFN. NK cells could be less efficient in mediating an antiviral effect in tissue that responds poorly to the protective effect of IFN. This would cause NK cells to release cytotoxic factors after binding to uninfected targets. A further extension of this line of thinking deals with the demonstrated fact that some cells bind to NK cells much better than other cells.[54] In tissue in which high avidity binding occurs between NK cells and normal tissue, the antiviral effects of NK cells may be inhibited by their adsorption to those uninfected targets.

6. Mechanisms of Selective Lysis of Virus-Infected Target Cells by NK Cells

Numerous publications attest that cultures of virus-infected cells are lysed more readily than uninfected cells when exposed to NK cells *in vi-*

TABLE III
Binding of Purified NK Cells to Virus-Infected Targets[a]

Target	Infection	% Lysis	% Bound targets
Mouse embryo fibroblasts (MEF)	None	28	58
	VV-1d	32	54
	VV-2d	9.2	29
	MCMV-1d	15	36
	MCMV-2d	5.6	22
L-929	None	30	50
	VV-1d	26	42
	VV-2d	16	17
	MCMV-1d	6.5	32
	MCMV-2d	4.9	15

[a]Target cells were infected with MCMV or VV at an MOI of 3 or left untreated. These cells were then used in ^{51}Cr-release assays and target cell–binding assays[56] using purified poly I:C–activated spleen NK cells[79] as effectors at an effector-to-target ratio of 5:1. Binding assays were run at 4°C for 0.5–3 h; cytotoxicity assays were run for 4 h.

tro.[55] This has led many to speculate that NK cells selectively "recognize" virus-infected targets. Actually, the lysis of virus-infected cells is quite complex and can be the result of either of two equally complex phenomena—the activation of NK cells by virus-dependent mechanisms, and the innate sensitivity of virus-infected cells to lysis. This is an important distinction, as we have commonly found that virus-infected targets are often more resistant than uninfected targets to lysis when exposed to already activated NK cell populations. We have seen this effect with HSV-1, vaccinia virus (VV), Sendai virus, Sindbis virus, VSV, and MCMV,[53,56] and this phenomenon has also been seen by other laboratories.[11,57] Furthermore, adding to the complexity is a recent observation in our laboratory that the susceptibility of virus-infected cells to NK cell-mediated lysis changes with the duration of the infection, such that a virus such as VV may enhance sensitivity to lysis early after infection but may inhibit sensitivity to lysis late in infection, possibly because of the loss of NK cell receptors on the cell membrane (unpublished; Table III).

6.1. NK Cell Activation

Two mechanisms have been proposed for the activation of NK cells on incubation with virus-infected targets. The first involves a protein synthesis-independent triggering of NK or NK-like cells via viral glycoproteins. The second is associated with a protein synthesis-dependent step involving NK cell activation by virus-induced IFN. These will be discussed separately.

Purified glycoproteins from mumps,[58] Sendai,[59] measles,[60] LCMV,[60] and influenza[61] viruses have all been shown to activate human NK or NK-like cells. These proteins share a common property of binding to cell membranes, but they differ in their enzymatic activities. For example, both the hemagglutinin and the neuraminidase of influenza virus stimulate activity.[61] The glycoproteins act on the effector cell but are presumed to direct killing to virus-infected target cells that express them. There is controversy over the nature of the effector cell, with some evidence that a $CD3^+$ T cell mediates much of the killing, with some variation caused by the target cell type.[62,63] The mechanism behind this phenomenon has not been elucidated. Curiously, viral glycoprotein activation of mouse NK cells has not yet to our knowledge been shown.

The original observations on selective lysis of virus-infected cells correlated the degree of lysis with the amount of IFN secreted into the culture fluid.[64] The source of IFN was either the virus-infected target cell, leukocytes responding to virus infection, or leukocytes responding directly to virus-infected cell membranes. Recent evidence has suggested that a DR (IA)-antigen expressing cell may be an accessory cell for NK cell activation, perhaps by producing IFN in this system.[65] In some systems antibody to IFN blocks lysis. An interesting dichotomy in lysis was shown with measles virus-infected targets.[66] Virus-selective lysis seen in a 4 h assay occurred in the absence of detectable IFN and was not blocked by antibody to IFN. However, elevated lysis seen in an overnight assay did correlate with IFN production and was blocked by antibody to IFN. In several other systems, an antibody to IFN did not block lysis, even in an overnight assay.[63,67–70] This does not, however, rule out a possible role for IFN, particularly if there is close contact between an NK effector cell and an IFN-producing accessory cell.[65] A more convincing experiment to rule out a role for IFN is the addition of RNA or protein synthesis inhibitors to the assay. If these fail to block the elevated killing, then IFN mediation is unlikely. This is because RNA and protein synthesis are required both for IFN production and for IFN-induced effects. In some systems such inhibitors have been used without blocking the virus-selective lysis.[68,69]

6.2. Innate Sensitivity of Virus-Infected Cells to Lysis

Although we often find that virus-infected cells are frequently more resistant to lysis, it is clear that in several systems, virus-infected cells have enhanced sensitivity to lysis. This could be mediated at several levels: (1) recognition or binding, (2) triggering of or stimulating the release of cytolysins from bound NK cells, and (3) susceptibility to released cytolysins. These will be discussed separately.

6.2.1. Recognition or Binding

Much has been written about virus-infected cells preferentially being "recognized" by NK cells. This is an attractive hypothesis to explain virus-selective killing, because viral glycoproteins on the target cell surface usually bind well to cell membranes and can often mediate cell–cell adhesion or fusion. There is little evidence that "recognition" is a major mechanism for virus-selective lysis, however. NK cells bind well to most cell types, so, except for the rare target that normally does not bind to NK cells (such as P815 in the mouse system),[54,71] enhanced binding is unlikely to make the difference between sensitivity and resistance.

Enhanced binding in target binding cell assays has been seen in several viral systems,[56,71] often correlating with enhanced killing, but reduced killing has also been seen in the presence of enhanced binding.[56] For example, Sendai virus-infected L-929 cells bind well to NK cells and can be used to deplete NK cells from leukocyte populations, but these targets are quite resistant to lysis.[56] The best correlation that can be made is the invariably reduced killing one sees when virus infections inhibit the binding of NK cells. Reduction in binding may occur as a result of obscuring or reducing the concentration of the target-binding structure. We have observed such reduced binding late in infections with HSV-1,[56] MCMV, and VV (Table III). Alternatively, a virus infection may enhance the binding of non-NK cells to targets. An example of this is the MHV-A59 infection of 3T3 cells, which causes these cells to rosette B cells,[72] which in this system mediate lysis. Lysis of these MHV-infected cells by NK cells is low until B cells are removed from the leukocyte population.

The conclusion is often made on the basis of cold-target competition assays that NK cells bind preferentially to virus-infected cells. This is an inadequate technique to measure binding, as this assay is more a measure of the inactivation of NK cells resulting from the release of cytolysins stimulated by the signal transduction or "triggering" event.[47–49] IFN-treated target cells bind to NK cells[44,47,48] but fail to cold-target-inhibit,[44,46,47,56] presumably because their cytolysins are not released.[47,49] This is discussed in the next section.

6.2.2. Triggering and Release of Cytolysin

This may be an important mechanism for the selective lysis of virus-infected cells, and an obvious mechanism may involve a stimulation or signal transduction mediated by viral glycoproteins. Other virus-induced membrane changes could occur as well. For example, vesicular stomatitis virus (VSV)-infected cells, depending on the system, may be selectively lysed by NK cells. Antibody to VSV does not block this elevated lysis, and target cells transfected with and expressing the gene for the VSV G pro-

tein are *not* more sensitive to lysis.[71] Cells infected with mutants in VSV G, M, or L genes are also not sensitive to lysis. This suggests that part of the enhanced sensitivity of infected cells may involve cellular alterations resulting from a productive virus infection. In contrast, it is reported that the lysis of HSV-1-infected target cells can be blocked by an antibody to a surface glycoprotein,[73] so different results are found in different systems.

6.2.3. Susceptibility to Released Cytolysins

Virus infections themselves cause cytopathic effects, and it is likely that the cytotoxic effects of the virus may act synergistically with the cytotoxic effect of cytolysins released from NK cells. It is becoming apparent that NK cells may be involved in the regulation of picornavirus infections and that cells infected with Coxsackie B or encephalomyocarditis viruses can be quite sensitive to NK cells[63,74] (E. Godeny, C. Gauntt, L. White, and R. Smith, personal communications). Picornaviruses do not insert glycoproteins into the plasma membrane, and, although they may mediate some membrane alterations,[75] the increased sensitivity to NK cells could be happening at the intracellular level. These viruses are potent inhibitors of cellular protein synthesis, and metabolic inhibitors of RNA and protein synthesis have been shown to enhance the sensitivity of uninfected target cells to NK cell-mediated lysis.[76,77] Since these inhibitors can inhibit membrane repair, it has been suggested but not proved that membrane repair alterations by inhibitors of protein synthesis such as these drugs or virus infections could be the cause of increased sensitivity to NK cell-mediated lysis.[76]

7. Use of NK Cells to Treat Virus Infections

Recent results in our laboratory indicate that culture-derived NK cells may be useful in controlling virus infections. Lymphokine-activated killer (LAK) cells were generated by incubating mouse spleen leukocytes in culture for 5 days with 100–1000 units of human recombinant interleukin 2. This protocol has been used in murine antitumor studies.[79] Injection of as few as 5×10^5 LAK cells into suckling mice protected them against MCMV, the NK-sensitive virus, but not against LCMV, the NK-resistant virus (Table IV). This protective effect was dramatic and required far fewer LAK cells than that reported for antitumor effects.[78] Furthermore, in contrast to the tumor studies, no *in vivo* IL-2 injections were required to produce the antiviral effect. *In vitro* cytotoxicity against YAC-1 targets was mediated predominantly by NK-1.1$^+$ leukocytes, which represented 14% of the LAK cell preparation. Injection of NK-1.1$^+$ LAK cells into suckling mice protected them against MCMV. These experi-

TABLE IV
Prophylaxis against Virus Infections by LAK Cells[a]

Exp.	Inhibition of virus synthesis		
	Donor cells	Virus	Log_{10} reduction in spleen titers
1	5×10^7 Adult leukocytes	LCMV	0
	4×10^6 LAK cells	LCMV	0
2	5×10^7 Adult leukocytes	MCMV	1.8
	4×10^6 Adult leukocytes	MCMV	0.6
	4×10^6 LAK cells	MCMV	>3.8
	1×10^6 LAK cells	MCMV	>2.6
	5×10^5 LAK cells	MCMV	>1.9
	2.5×10^5 LAK cells	MCMV	0
3	2×10^5 NK-1.1$^+$ LAK cells	MCMV	2.5
	2×10^5 Unseparated LAK cells	MCMV	2.4

[a]LAK cells were generated by *in vitro* culture of 1.5×10^7 spleen cells in 5 ml RPMI-1640 for 5 days with 100 U/ml human recombinant IL-2. Adoptive transfers of LAK cells and adult leukocytes into suckling mice were performed as described in Table I.

ments suggest that it may be possible to culture autologous NK cells in IL-2 and use them to treat human virus infections.

That human virus infections are regulated by NK cells can be surmised from the murine studies, although definitive experiments to prove this hypothesis are lacking. The most convincing data available are with the herpes group of viruses, some of which are summarized elsewhere in this volume. Whether human CMV (HCMV) is as uniquely sensitive to NK cells as is its murine counterpart is uncertain. Several reports suggest that human NK cells may selectively lyse HCMV-infected target cells *in vitro*.[65,80,81] HCMV infection is generally most severe under conditions associated with NK cell deficiency, such as congenital or neonatal infections, or in adults immunosuppressed by other viral infections, cancer, or drugs.[82] In one study with bone marrow transplant recipients, the severity of HCMV infection inversely correlated with the levels of NK activity before onset of disease.[83]

HCMV pneumonitis is a frequent, if not the major cause of infectious disease death in transplant recipients and in patients with AIDS. If HCMV is as susceptible to NK cells as MCMV, one could predict a possible therapeutic effect of LAK (activated NK) cells on that infection. Intravenous injection would deliver these cells into the lung, where they might mediate an antiviral effect.

Understanding the complexities behind the mechanisms of the antiviral effects of NK cells should help in designing appropriate regimens for the ultimate treatment of virus-infected patients with NK cells.

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