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DO NATURAL KILLER CELLS PLAY A ROLE IN VIRUS INFECTIONS?

RAYMOND M. WELSH

Department of Pathology, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

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INTRODUCTION

Natural killer (NK) cells are functionally defined as lymphocytes which have the ability to lyse on contact certain types of tumor and normal cells with little evidence of specificity or antigenic memory (see reviews in refs. 14, 22 and 62). They have been found in virtually every examined vertebrate and even in some invertebrates. Thus, they are phylogenetically primitive, and for one reason or another have been preserved throughout evolution. Naturally occurring 'endogenous' NK cells lyse tumor cells, particularly of lymphoma origin, and extensive work has led to speculations that they may be mediators of 'immune' surveillance of tumors [14, 22, 62]. In the experimental mouse model the evidence is very good that NK cells function in some manner to reject tumor implants and curtail tumor metastases [10, 18, 19, 55], though it remains to be shown whether they inhibit spontaneous tumor formation.

THE EFFECTOR CELLS

Three features make NK cells of particular interest in virus infections: 1) they become cytolytically more active during virus infections; 2) they may be major producers of interferon; and 3) they possess receptors for the Fc portion of immunoglobulin G (IgG), granting them the ability to specifically lyse antibody-coated virus-infected cells. Each point will now be discussed in more detail.

Activation of NK cells during virus infections

NK cells become activated upon exposure to any of the three major types of interferon, i.e. type I fibroblast [8, 63], type I leukocyte [60], and type II immune [24, 59]. The activated cells display augmented lysis against normally sensitive cells and lyse most other types of primary normal and cultured cells which resist lysis by NK cells. Production of interferon has been shown to occur in various experimental virus infections in mice. In some systems, including infection with lymphocytic choriomeningitis

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(LCM) virus, activation of NK cells was also demonstrated [15, 34, 63, 68]. This activation appears to be predominantly due to type I interferon [8, 63], but T cells responding locally to viral antigens may also activate NK cells by secreting type II interferon [64].

Likewise, cultures of human or mouse leukocytes exposed to virus-infected cells or to virus alone synthesize interferon (predominantly type I leukocyte), and the NK cells in these preparations correspondingly become activated [36, 51, 65]. Hence, once might mistakenly infer the existence of a virus-specific cytotoxicity when in reality virus-induced interferon was causing the activation of a relatively non-specific cell.

Recently, it has become apparent that the interferon-mediated activation of NK cells may be bypassed by treating effector cells with lectins [4], alloantibody [4], and purified viral glycoproteins, such as those derived from mumps [13], measles, or LCM viruses (Casali, Sissons and Oldstone, personal communication). Treating effector cells with these reagents activates NK cells within 4 h without any apparent interferon synthesis. Although low level synthesis of interferon, escaping bioassay, cannot be excluded to occur in these systems, it is also possible that the agents act as analogs of interferon, binding to similar structures on the cell membrane and, thus, causing activation of the NK cell's lytic machinery.

It therefore appears that NK cells may be activated by three general mechanisms during a virus infection: by virus-induced interferon, by immune interferon associated with a T-cell immune response against the infection, or by viral glycoproteins directly.

Evidence that NK cells synthesize interferon

Since virtually all cells have the capacity to produce interferon under the proper conditions of stimulation, it is unclear which cells are the major interferon producers during a viral infection. Trinchieri et al. [61] reported that human peripheral blood lymphocyte (PBL) preparations enriched by fractionation for NK cells produced high levels of interferon when exposed to viruses or tumor cells. Saksela et al. [50] showed that highly purified human NK cell (large granular lymphocytes) preparations could be stained in immunofluorescence with an antibody to leukocyte interferon, after the lymphocytes had been exposed to K562 cells, which induce interferon production by the lymphocytes. Very little staining was found in the NK cell-depleted fractions. Treatment of mouse spleen cells with antibody to Ly5 and complement, a procedure known to selectively lyse mouse NK cells, prevented virus-induced interferon production in vitro [49]. The data collectively suggest that NK cells, in addition to being activated by interferon, may be major interferon-producing leukocytes during the course of a viral infection. The importance of this cannot be understated, because interferon can directly inhibit virus synthesis as well as modulate numerous functions of the immune system. Of interest is that human suppressor T cells and NK cells bear marked similarities and copurify [50]. It has been suggested that the inhibitory effect of T-suppressor (NK?) cells on cell proliferation is mediated by interferon [17].

Correlation of non-specific NK cell activity with virus-specific K cell-mediated lysis

Cells infected with virus express viral antigens on their membranes and hence can bind virus-specific antibodies. As a result of this, the cells become targets for antibody-dependent killer cells, henceforth called K cells. By virtue of the viral antigens that they carry on their membranes, virus-infected cells are also targets for specifically sensitized cytotoxic T-killer cells. Most laboratories now agree that NK and K cells, if not identical, belong to the same family of lymphocytes [14, 41, 42, 58]. K-lymphocyte killing of virus-infected cells has been examined most extensively in the human system and has been documented with a number of human viruses, including herpes simplex [53], mumps [12], measles [45], influenza [9], Epstein–Barr [44], respiratory syncytial [35], and vaccinia [46] viruses. K-cell activity in human PBL was found to parallel the rise in antiviral antibody titer following vaccination or acute infection [9, 35, 46]. Interestingly, antiviral K-cell activity has in general been easy to document in humans but not in mice, while virus-specific cytotoxic T-cell activity is easily demonstrable in mice but not in man. Perhaps, in humans, a potent system of cells with NK, K and/or T suppressor activity inhibits the cytotoxic T-cell response. In one model of herpes simplex virus-infected mice, cytotoxic T cells could not be demonstrated, unless the mice were first treated with cyclophosphamide, which blocks T-suppressor cell activity [47] but also reduces mouse NK-cell activity [7].

Most [16, 23, 37, 66] but not all [60] laboratories agree that K-cell activity is augmented by interferon treatment. The combination of increased antibody production, virus-induced and immune interferon production, and resulting K-cell activation could lead to an effective mechanism for curtailing a virus infection. The relative roles of complement-mediated lysis versus K cell-mediated lysis are not known.

THE TARGET CELLS

NK cells activated during virus infections are capable of lysing cells in uninfected syngeneic primary cultures [21, 39, 69], and while a high degree of non-specific cell damage may well occur during a virus infection, it would be advantageous for the host to preferentially eliminate virus-infected cells. The interactions of NK cells with virus-infected targets are actually quite complicated, as they depend on the virus, the target cell, the time of infection, etc. Analysis of these factors individually should highlight the complexity of the situation.

Interferon-mediated protection

Trinchieri and Santoli [60] made the interesting observation that interferon treatment of normal human fibroblasts renders them resistant to NK cell-mediated lysis. This was confirmed with tumor cells as targets [38] and has been extended to the mouse system [11, 66]. In fact, normal thymocytes as well as ascites tumor cells harvested from LCM

virus-infected or interferon-treated mice became resistant to NK cells [11, 66]. The mechanism of interferon-induced resistance is unclear. NK cells were found to bind to these target cells but failed to lyse them. Yet the interferon-treated targets competed poorly in cold target inhibition experiments [11, 60]. Our studies with interferon-treated L-929 cells indicated an increase in lipid-associated cell surface sialic acid as well as an increase in overall glycolipid synthesis. Whether these alterations relate to the resistance to lysis remains to be determined (Yogeeswaran, Gronberg, Fujinami, Kiessling and Welsh, submitted for publication).

Trinchieri and Santoli [11, 51, 59] proposed that the interferon protection mechanism could provide a means for selective lysis of virus-infected cells, because they found that uninfected, but not virus-infected cells were protected by the interferon. Presumably the virus-induced inhibition of host RNA and protein synthesis, required for interferon-mediated effects, precluded the protective state from developing. This is a plausable hypothesis in many circumstances, providing that the virus infection is sufficiently cytotoxic to keep interferon from performing its function.

Synergistic lysis of cells by virus and NK cells

Many virus infections are capable of inhibiting cellular macromolecular synthesis and eventually causing cell lysis. We have found that target cells treated with inhibitors of RNA (actinomycin D) or protein (cycloheximide, puromycin) synthesis become markedly more sensitive to NK cell-mediated lysis [28]. In addition to other effects, these drugs inhibit membrane repair processes [52]. It seems likely, then, that a virally 'poisoned' cell may also become more susceptible to NK cell-mediated lysis.

Lysis associated with enhanced NK cell binding to targets via viral glycoproteins

A number of virus infections alter the membranes of infected cells in a manner which results in enhanced binding of NK cells as well as of lymphocytes in general. This enhanced binding is presumably due to the insertion into the membrane of viral glycoproteins such as hemagglutinins, which avidly bind to cell surfaces. Hence, an NK cell may preferentially bind to and lyse a virus-infected cell. There are numerous reports concluding that NK cells preferentially lyse virus-infected cells, but many of these do not take into consideration the non-specific interferon activation mechanisms, particularly those occurring in long (16 h) cytotoxicity assays. Even in short-term assays, it is difficult to discriminate between enhanced lysis due to enhanced binding and enhanced lysis due to, for instance, a glycoprotein-mediated NK cell activation (see previous section on NK cell activation during virus infections). Enhanced binding of endogenous NK cells concomitent with enhanced lysis of targets in short-term assays has been reported for measles [1], Epstein–Barr [3] and Sendai [65, 70] viruses. In studies using previously activated NK cells and short-term assay we paradoxically have observed virus-infected cells to be commonly more resistant to lysis, but we have also observed enhanced sensitivity to lysis

under other circumstances [65]. For instance, both Sendai virus-infected L-929 and Vero cells avidly bound to NK cells, but they differed in regard to sensitivity to lysis by activated NK cells. Sendai virus-infected Vero cells were more sensitive to lysis, while Sendai virus infected L-929 cells were actually very resistant [65]. Obviously, the issue of selective NK cell-mediated lysis of virus-infected cells is complex and varies from system to system.

Virus-induced membrane alterations

Theoretically, a virus infection could affect the expression of the putative NK target antigen. Herpes simplex virus-infected Vero cells resist NK cell-mediated lysis and do not bind to NK cells, indicating a lack of expression of the target binding site [65]. Cells persistently infected with measles virus bind NK cells avidly, but this could not be inhibited by antibody to measles virus, suggesting an increase in the expression of the cellular target binding site [1].

EVIDENCE THAT NK CELLS PLAY A ROLE IN VIRUS INFECTIONS IN VIVO

Little evidence has been presented showing a role for NK cells in virus infections, and this is a field requiring a great deal of further research. After intravenous injection in mice, LCM virus-infected L-929 cells, labeled with ^{125}I-labeled iododeoxyuridine, were rejected more rapidly than uninfected cells (Biron and Welsh, unpublished). Also, LCM virus-infected mice rejected similarly labeled uninfected tumor cell implants more efficiently than uninfected mice [56]. NK cell-deficient beige mice were reported to have reduced efficiently in rejecting such implants, suggesting that NK cell-mediated lysis may be involved [18, 56] (Biron and Welsh, unpublished). These results are consistent with studies showing that athymic nude mice reject virus-infected tumor implants [29, 48]. However, bg/bg mice and their normal bg/+ littermates synthesize similar levels of LCM virus after intraperitoneal infection [67]. However, beige mice do have some degree of NK cell activity, and differences may be difficult to be shown in these types of experiments due to the ability of virus-induced interferon to activate bg/bg NK cells.

Some promising but indirect data come from genetic studies in mice suggesting that the susceptibility to virus infection is linked with the hemopoietic histocompatibility (Hh) system [6]. The effector cell in this system, which is based on the ability of mice to reject low levels of bone marrow allografts, is similar to the NK cell regarding dependence on the bone marrow, independence of the thymus, age of maturation, resistance to X-irradiation, and other properties [20]. Requirements for such a bone marrow effector cell have been linked to resistance of mice to Friend virus [25, 40], herpes simplex virus [31, 33], and mouse hepatitis virus [30, 57].

Evidence of a role for NK cells in human infections is lacking, though two tested humans suffering from severe disseminated herpes simplex virus (type I) infections had low NK activity [5], and patients suffering from an X-linked lymphoproliferative syn-

drome had very low levels of NK cell activity in conjunction with severe EB virus infection [54]. The ease with which antiviral K-cell activity is detected in humans, in conjunction with difficulties to demonstrate virus-specific cytotoxic T-cell activity, would suggest that the antibody-dependent K-cell function of NK cells may be of vital importance, at least in human virus infections. Yet, the clinical evidence suggests that humans with agammaglobulinemia are not particularly susceptible to virus infections while patients with T-cell deficiencies are highly susceptible [43]. This seems to contradict the K-cell hypothesis.

Obviously, much more work needs to be done before conclusions can be made regarding the role of NK cells in virus infections. However, their potentials for synthesizing interferon and for mediating both non-specific and virus-specific cytotoxicity in vitro renders them prime candidates as effectors in antiviral immunity.

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