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The role of interferon in the NK cell killing of virus-infected target cells

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SUMMARY

Spleen cells from uninfected CBA mice are more cytotoxic for Sendai virus-infected L929 cells than for uninfected cells and the lymphocytes responsible have the properties of NK cells. Preincubation of spleen cells with culture supernatants from Sendai virus-infected L929 cells increases the cytotoxicity for uninfected target cells. This increase in cytotoxicity can also be produced by pretreatment with purified mouse interferon. The enhancing effect of both the infected culture supernatants and purified interferon can be neutralized with anti-interferon serum. It is concluded that the preferential killing of Sendai virus-infected L929 cells by NK cells is dependent on the induction of interferon and that interferon will increase NK cell cytotoxicity for uninfected target cells.

There has been widespread interest in the ability of certain lymphoid cells from unsensitized mice to kill lymphoid target cell lines (Beverley & Knight, 1979). The cells responsible for this innate cytotoxicity have been designated NK (natural killer) cells (Kiessling, Klein & Wigzell, 1975). NK cells differ from other lymphoid cell types in that they carry no θ antigen or surface immunoglobulin, have no adherent or phagocytic properties, and are resistant to X-irradiation (Welsh, 1978*a*). Their presence is not ubiquitous amongst mice; certain inbred strains exhibit high NK cell activity while in other strains NK cell cytotoxicity is undetectable (Petrànyi, Kiessling & Klein, 1975). During studies of the role of cell-mediated immunity in Sendai virus infection in mice a ^{51}Cr release assay was used to investigate the cytotoxicity of spleen cells for virus-infected and uninfected target cells. Spleen cells from CBA mice with no prior experience of Sendai virus were more cytotoxic for virus-infected L929 cells than uninfected cells (Anderson *et al.* 1977). Further investigation revealed that the cytotoxic lymphocytes were similar to the NK cells described by other workers as having activity against tumour cells (Anderson, 1978). There seemed to be two possible mechanisms by which preferential NK cell killing of virus-infected cells could occur. First, cells with new surface antigens, produced as a result of virus infection, may be more susceptible to NK cell cytotoxicity. Second, virus-infected cells may liberate some substance(s) which act on the NK cells and increase their cytotoxic capacity. L929 cells are capable of synthesizing large amounts of interferon on infection with

Newcastle disease virus (Lancz & Johnson, 1969) and Sendai virus is known to be a potent inducer of interferon (Lee & Ozere, 1965). Furthermore, other workers have demonstrated that interferon can induce NK cell activity expressed as cytotoxicity for tumour cell lines (Gidlund *et al.* 1978; Welsh, 1978*b*). Therefore, the possible role of interferon in NK cell cytotoxicity was investigated.

Cytotoxicity was measured using the principles of the ^{51}Cr release assay previously described (Anderson *et al.* 1977). Spleen cell suspensions from 8-week-old CBA mice with no prior experience of Sendai virus infection were prepared in medium 199 plus 5% fetal calf serum. The suspensions were reacted overnight at 37 °C with either uninfected or Sendai virus-infected monolayers of L929 cells labelled with ^{51}Cr. The spleen cell:target cell ratio used was 50:1. ^{51}Cr release was measured in samples from eight replicate wells and expressed as a percentage of the total ^{51}Cr released on incubation of the target cells with distilled water. The results quoted represent the mean of the replicates $\pm$ 95% confidence limits.

In order to test the effect of cell culture supernatants on NK cell cytotoxicity the spleen cells ($2{\cdot}5 \times 10^7$ cells/ml medium) were preincubated for 5–60 min at 33 °C in either medium from control, uninfected L929 cell cultures or medium from Sendai virus-infected cultures (SS). The cells were washed in medium 199 and then assayed for cytotoxicity against uninfected L929 cells. In a typical experiment the cytotoxicity of untreated spleen cells for uninfected target cells was $16{\cdot}2 \pm 2{\cdot}0$ and for Sendai virus-infected target cells $33{\cdot}0 \pm 2{\cdot}5$. After 5 min preincubation of the spleen cells with SS the cytotoxicity for uninfected target cells was $34{\cdot}5 \pm 2{\cdot}0$. Similar values, indistinguishable from cytotoxicity of untreated lymphocytes for target cells infected with Sendai virus, were found after preincubation for 10, 20, 40 and 60 min. Exposure of lymphocytes to control supernatant media from uninfected L929 cell cultures had no such effect, showing that the increase in cytotoxicity was due to activation of NK cells by some factor present only in the supernatant of virus-infected cultures.

The NK cell-enhancing effect of SS was compared with that of interferon. Purified mouse fibroblast interferon was a generous gift from Professor D. C. Burke, Department of Microbiology, Warwick University, Warwick, and had been prepared by the method of de Maeyer-Guinard *et al.* (1978). The activity of the preparation was approximately 10^8 international units of interferon per mg protein assayed by inhibition of SFU nucleic acid synthesis (Atkins *et al.* 1974). Spleen cells were incubated for 10 min with either SS or purified interferon and, after washing, were assayed for cytotoxicity on uninfected L929 cells. The results are shown in Fig. 1. Both undiluted SS and SS diluted 10^{-1} induced significant enhancement of NK cell killing of uninfected L cells. Preincubation with purified interferon induced a similar increase in NK cell cytotoxic activity, although this effect was still evident at 100-fold higher dilution of purified interferon. The interferon titres of the two preparations were assayed by their capacity to inhibit the cytopathic effect of vesicular stomatitis virus in L929 cells. The titre of the purified mouse interferon was 10^7 units/ml and that of SS 10^5 units/ml. Thus, it is clear that interferon can activate NK cells to increased cytotoxic activity and that the increase observed on preincubation of spleen cells with SS correlates well with the interferon titre of this preparation.

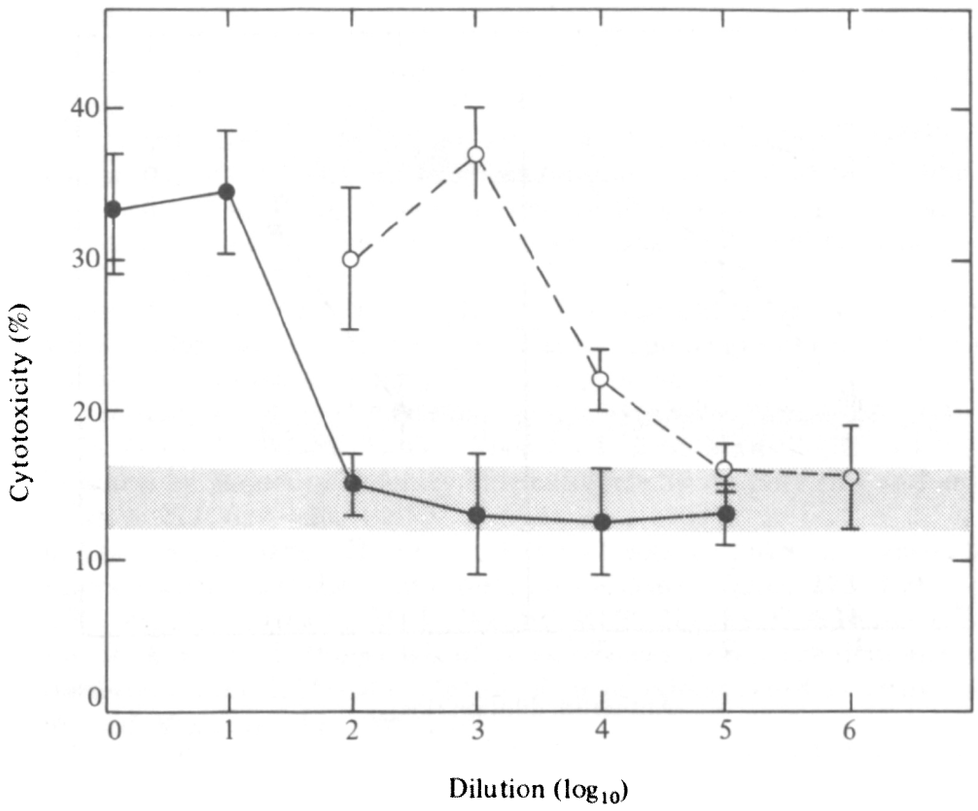

Fig. 1. Effect of purified interferon and SS upon NK cell cytotoxicity. Spleen cells were incubated for 10 min with either purified type 1 fibroblast interferon (○---○) or SS (●——●) at a concentration of $2{\cdot}5 \times 10^7$ spleen cells per ml. Splenocytes were then washed in medium 199 and assayed for cytotoxicity on uninfected L cells at a spleen cell:target cell ratio of 50:1. The shaded bar represents the cytotoxicity of control spleen cells, preincubated in medium 199 alone, for uninfected L cells (mean of eight replicates ± 95% confidence limits).

The capacity of anti-serum to mouse fibroblast type 1 interferon to neutralize the enhancing effect of interferon and SS on NK cell activity was measured. The antiserum was a generous gift from Dr Anna Inglot, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Warsaw, Poland, and had been raised in sheep by modification of the method of Gresser *et al.* (1976). Interferon and SS were incubated with 10-fold dilutions of anti-interferon serum. Spleen cells were then added and incubated for 10 min at 33 °C. After washing, the spleen cells were assayed for cytotoxicity on uninfected L929 cells (Fig. 2). At an antiserum dilution of 10^{-1}, the NK cell-activating activity of interferon was significantly reduced so that the cytotoxicity of the preincubated NK cells was indistinguishable from that of untreated lymphocytes. With increasing dilution of the antiserum, this inhibitory effect was reduced; when the antiserum was diluted 10^{-3}, no significant impairment of interferon activation of NK cells was apparent. When lymphocytes incubated in SS–anti-interferon globulin mixtures were examined a similar pattern was apparent; NK cell activation property of SS was neutralized by anti-interferon globulin diluted 10^{-1}. Increasing dilution of the antiserum again resulted in decreasing impairment of activation.

The results described above demonstrate that the apparent preferential killing of Sendai virus-infected target cells by unsensitized NK cells is due to activation

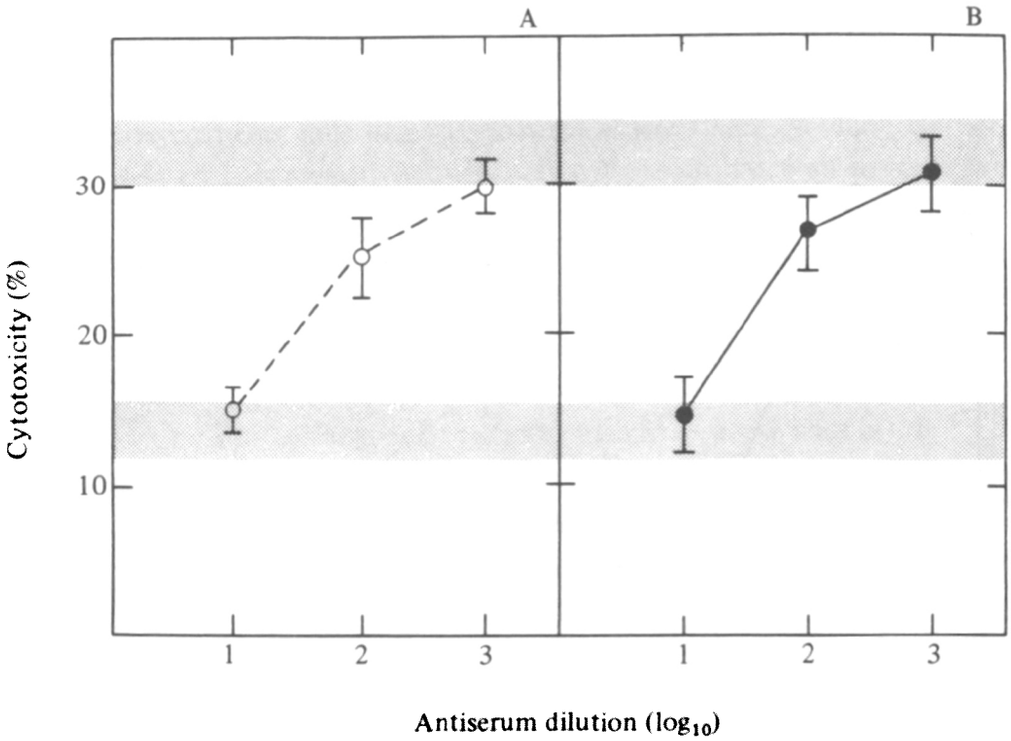

Fig. 2. Effect of anti-interferon globulin on enhancement of NK cell activity by interferon and SS. Interferon diluted 10^{-2} in medium 199 (A) and undiluted SS (B) were incubated with 10-fold dilutions of heat-inactivated anti-mouse type 1 fibroblast interferon serum for 1 h at room temperature. Spleen cells were then added at a concentration of $2{\cdot}5 \times 10^7$ cells per ml, and incubated for 10 min at 33 °C. The splenocytes were washed in medium 199 and assayed for cytotoxicity on uninfected L929 cells at a spleen cell:target cell ratio of 50:1. The lower shaded bars represent the cytoxicity of control spleen cells preincubated in medium 199. The upper bars indicate the cytotoxicity of spleen cells preincubated in (A) interferon-diluted 10^{-2} and (B) undiluted SS. All figures are the mean of eight replicates $\pm$ 95 % confidence limits.

of the NK cells by interferon released from the virus-infected cells. Once interferon is present in the system there is no requirement for Sendai virus infection of the target cells for NK cell killing. Therefore, the role of such cell killing in recovery from acute virus infections must remain in doubt. Previous work (Becker, Fenyö & Klein, 1976; Heberman *et al.* 1977) has demonstrated NK cell killing of transformed cells of lymphoid origin and L929 cells are a fibroblast-derived transformed cell line which excretes C type particles (Luftig, McMillan & Gudger, 1974). The susceptibility of untransformed cells, infected with viruses other than tumour viruses, is at present under study. If such cells prove susceptible to the action of NK cells, there exists the potential for NK cell-mediated destruction of virus-infected tissues of the body. In this event, the present demonstration that interferon can so rapidly enhance the lytic capacity of NK cells together with the fact that type 1 interferon is synthesized early in the course of acute virus infections (Robinson, Cureton & Heath, 1968) suggests that NK cells may constitute a valuable component of host resistance to acute virus infections.

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