Short Communication

Decreased natural killer cell activity and interferon production by leucocytes in patients with adenocarcinoma of the pancreas

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Natural killer (NK) cells may play an important role in immune surveillance against tumours (Herberman, 1982) and interferons (IFN) may serve as modulators of their cytolytic activity (Bloom, 1980). Diminished NK activities of peripheral blood leucocytes (PBL) have been reported in patients with advanced cancers (Pross & Baines, 1976; Takasugi et al., 1977; Kadish et al., 1981; Steinhauer et al., 1982). This may be due to reduced sensitivity of NK cells to IFN or to impaired IFN production. It has been reported that in vitro preincubation of PBL with IFN largely restores NK activity in some, but not all cancer patients, and that the in vitro production of IFN by PBL is normal (Kadish et al., 1981). On the other hand, we found that PBL from patients with mid-gut carcinoids were selectively deficient with respect to production of pH2 labile IFN-α after stimulation by Staphylococcus aureus Cowan I (SACoI), while basal and IFN-enhanced NK activities did not differ from those of the controls (Funa et al., 1983). To further explore cancer-associated deficiencies in the NK–IFN system, we have investigated a group of patients with adenocarcinoma of the pancreas.

We studied seven patients aged 53 to 76 years (median age 62 years) with pancreatic adenocarcinoma (3 with verified liver metastases and 4 with local extra-pancreatic tumour infiltration), before surgery. As a control group were used 13 healthy blood donors aged 20 to 70 years (median age 61 years). PBL were prepared from heparinized venous blood by centrifugation on Ficoll–Hypaque as described elsewhere (Funa et al., 1983). The PBL were resuspended in RPMI 1640 medium, supplemented with 5% heat inactivated foetal calf serum (FCS; Flow Laboratories), 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin and 2 mM L-glutamine. NK sensitive human erythroleukaemia K 562 cells were used as targets in short term (3 h) ⁵¹Cr release assays, performed as described previously (Funa et al., 1983). Percent specific lysis was calculated with the formula:

\[
\text{Specific lysis} = \frac{\text{Exp. release} - \text{Spont. release}}{\text{Max. release} - \text{Spont. release}} \times 100.
\]

Cytotoxicity was expressed as lytic units (LU), which were calculated by multiplying by 1000 the inverted ratio of effector to target cells at which 20% specific lysis occurs.

For in vitro activation by IFN, PBL were incubated for 1 h with equal volumes of two-fold dilutions of partially purified Sendai virus-induced leucocyte IFN-α (Interferon Laboratory, University Hospital, Uppsala, Sweden), starting with 1600 U ml⁻¹ before addition of labelled K 562 target cells. Effector:target cell ratio was 50:1. The cytotoxicity was determined as described above. The percentage of specific lysis was plotted against log₁₀ IFN concentration (latter on x axis). Regression analyses were performed for each assay using the equation: \( y = a + b \log_{10} x \). The slopes of the regression lines (the b coefficients in the regression equation) indicate sensitivities of NK cells to IFN (Funa et al., 1983).

The IFN-producing capacity was determined by incubating PBL with the inducers SACoI (1/1000 v/v), Concanavalin A (ConA; 20 μg ml⁻¹), Lens culinaris lectin (LCL; 100 μg ml), and Sendai virus (5000 haemagglutinin U ml⁻¹ chorioallantoic fluid). PBL were cultured at a density of 4 × 10⁴ cells in 0.2 ml per well in flat-bottomed microtitre-plates (Nunc, Roskilde, Denmark) at 37°C in 5% CO₂ in air. After 48 h, supernatants were collected from each well for IFN assay. The supernatants with Sendai virus were dialysed against pH2 buffer before IFN assay to inactivate the virus. A conventional cytopathic effect inhibition assay on human amnion WISH cells was used to measure...
the antiviral activity in culture supernatants as previously described (Funa et al., 1983), with vesicular stomatitis virus as challenging virus. All antiviral activities were expressed as IFN-α units per ml using a standard IFN-α (G-023-901-527; NIH, Bethesda, MD) as reference.

The in vitro IFN production by PBL is shown in Figure 1. A diminished IFN production was found in patients when SACoI was used as inducer (median test with Fisher exact probability: \( P = 0.024 \)). No significant differences were seen between PBL of patients and controls with the IFN-γ inducers ConA (Fisher \( P = 0.31 \)) and LCL (\( P = 0.073 \)), and the IFN-α inducer Sendai virus (\( P = 0.48 \)). Cytotoxicity against K 562 cells in patients (median: 10 LU, \( n = 7 \)) was significantly lower than that of the control subjects (median: 42 LU, \( n = 13 \); median test with Fisher \( P = 0.027 \)), as shown in Figure 1. The sensitivity of NK cells to in vitro IFN as measured by the slopes of regression lines (b coefficients) was significantly higher in control PBL than in patients' PBL (t-test with equal variances: \( t = 3.30, df = 18, P = 0.008 \)) (shown in Figure 2).

The present study shows that patients with pancreatic adenocarcinoma have both a decreased basal NK activity and a decreased in vitro response of NK cells to IFN-α. There are several reports of reduced basal NK levels in PBL of patients with disseminated cancers (Pross & Baines, 1976; Kadish et al., 1981), and evidence exists that the defect resides in the reduced ability of NK cells to recycle the cytolytic process, while the number of NK cells may be normal (Steinhauer et al., 1982). Suppressor cells for NK activity have also been found in some tumour-bearing individuals (Eremin, 1980; Gerson, 1980). Suppressor activity can be mediated by prostaglandins produced by monocytes-macrophages (Droller et al., 1978; Koren et al., 1981).

Our patients showed an impaired ability to produce IFN upon stimulation with SACoI while normal productions were demonstrated for the

![Figure 1](image-url)
other IFN inducers, i.e., two T-cell mitogens that induce IFN-γ and Sendai virus that induce acid-stable IFN-α. A similar selective deficiency of IFN production by SACoI was seen in patients with another type of gastrointestinal cancer, mid-gut carcinoid (Funa et al., 1983). This SACoI-induced IFN was first assumed to be acid-labile IFN-α (Funa et al., 1983), but further studies added the information that this IFN is neutralized not only by anti-IFN-α antibodies, but also frequently by anti-IFN-γ antibodies. This IFN appears to be produced by null lymphocytes that in certain respects resemble typical NK cells but do not carry same spectrum of antigenic markers (Funa et al., to be published). The nature and significance of the observed deficiency in the SACoI-induced IFN production remains to be established.

In conclusion, patients with pancreatic adenocarcinomas, even with relatively localized tumour burdens, showed deficiencies in the NK-IFN system at at least three levels: (1) diminished basal NK activities, (2) a decreased sensitivity of such cells to IFN in vitro, and (3) a decreased atypical IFN production by SACoI. In the in vivo situation, these defects may be additive or even synergistic, and, assuming a role for NK cells and IFN in tumour resistance, may contribute to the rapidly invasive and metastatic growth of pancreatic adenocarcinomas.

This study was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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