*Clin. exp. Immunol.* (1982) **48**, 205–212.

# Deficient natural killer cell activity in a patient with Fanconi's anaemia and squamous cell carcinoma. Association with defect in interferon release

P. HERSEY, ANNE EDWARDS, R. LEWIS, A. KEMP* & J. MCINNES† *Medical Research Department, Kanematsu Memorial Institute, Sydney Hospital, *Department of Medicine, Royal Alexandra Hospital for Children, Sydney, Australia and †School of Pharmacy, South Australian Institute of Technology, Adelaide, South Australia*

*(Accepted for publication 9 October 1981)*

## SUMMARY

A child with Fanconi's anaemia diagnosed at 7 years of age presented in adult life with lymphopenia, recurrent warts and Bowen's disease. The latter resulted in the development of multiple cutaneous squamous cell carcinomas which metastasized to the skeleton. Investigation of her immune function revealed selective defects in natural killer (NK) cell activity. Humoral immunity and several tests of cell-mediated responses were within normal or became normal after treatment with levamisole or transfer factor. Analysis of the defect in NK activity revealed that low levels could be induced *in vitro* by fibroblast interferon. Stimulation of blood lymphocytes from the patient with the interferon inducer poly (I)–poly (C) resulted in an increase in NK activity but incubation of her lymphocytes on tumour cells did not result in an increase in NK activity or the release of interferon. This contrasted with the marked increase in NK activity and interferon release observed when lymphocytes from normal controls were incubated on tumour cells. These findings suggested the absence of NK activity in this patient was secondary to a defect in interferon release from lymphocytes on exposure to tumour antigens. It is considered that these defects may have been an important predisposing factor in the development of malignancy in this patient and possibly other patients with Fanconi's anaemia.

## INTRODUCTION

Fanconi's anaemia is a syndrome in which affected individuals have growth retardation, skin pigmentation, various congenital malformations and progressive pancytopenia (Beard *et al.*, 1973; Evans, 1979). Chromosomal abnormalities are detectable in lymphoid and fibroblast cultures and there is an increased incidence of malignancy such as acute leukaemia (Evans, 1979; Meisner, Taher & Shahidi, 1978), hepatoma and squamous cell carcinomas (SCC) (Swift, Zimmerman & McDonough, 1971) in affected individuals.

The basis for the high incidence of malignant neoplasms in this condition is unknown. Fibroblasts from affected individuals were shown to undergo transformation in culture when exposed to the simian *SV40* virus (Beard *et al.*, 1973) and this may indicate that similar transformation may occur *in vivo* during virus infections. Defects in immune surveillance may also contribute to the increased incidence of malignancy but there have been few reports on the immune

Correspondence: Dr P. Hersey, Medical Research Department, Kanematsu Memorial Institute, Sydney Hospital, Sydney, N.S.W. 2000, Australia.

0009-9104/82/0400-0205$02.00 © 1982 Blackwell Scientific Publications

status of patients with this disorder. Impairment of *in vitro* T cell function was reported in two children with Fanconi's anaemia but studies on five other cases revealed no evidence of immunodeficiency (Zaizov, Matoth & Mamon, 1978; Pederson *et al.*, 1977).

A number of studies in experimental animals have drawn attention to the importance of natural killer (NK) cells in surveillance against tumours (Kiessling & Haller, 1978). Studies in patients with melanoma suggested the recurrence-free period after removal of melanoma may be related to NK activity (Hersey *et al.*, 1978). Of particular interest were recent reports that patients with the Chediak Higashi syndrome had selective defects in NK activity to tumour cells (analogous to that reported in strains of 'beige' mice) and it was postulated that this defect may underlie the high incidence of malignancy in both mice and humans with the condition (Roder, 1980; Roder *et al.*, 1980). We describe a patient with Fanconi's anaemia who survived into adult life. This patient developed lymphopenia and suffered from Bowen's disease and recurrent vulval warts for a number of years. Overt development of squamous cell carcinomas occurred in the perianal and vulval areas and metastasized to regional lymph nodes and skeletal areas. Immunological investigations revealed markedly reduced NK activity in peripheral blood leucocytes (PBL).

## CASE REPORT

S.I., a female child, presented at age 7 years with symptoms of anaemia. At that time she was noted to be in the 10th centile for height and the 3rd centile for weight. Other features consistent with Fanconi's anaemia were areas of pigmentation on her trunk, absent radial pulses and evidence of pancytopenia on haematological investigations. Nerve deafness had been diagnosed at 6 months of age and a patent ductus arteriosus repaired at 4 years. The patient had recovered normally from rubella, measles and mumps at the age of 5–6 years. There was no family history of pancytopenia or congenital defects. Chromosomal studies revealed breaks and endoreduplication consistent with Fanconi's anaemia.

She was treated with androgens and responded well. These were continued until age 22. Her subsequent course was relatively uneventful until age 24 when Bowen's disease and warts in the vulvo-vaginal and cervical areas were noted. A cone biopsy of the cervix-uteri revealed dysplasia and an area of carcinoma *in situ*. During her 26th year she was re-admitted to hospital on two occasions for surgical resection of squamous cell carcinomata (SCC) in areas of Bowen's disease around the anal and vulval regions. Lymph node metastases to left inguinal lymph nodes were identified at the second operation. In her 27th year, SCC metastases to skeletal areas were confirmed by biopsy of the right iliac crest. There was rapid progression of the tumour and she died shortly after.

Immunological investigations conducted during management of the SCC were as follows; (1) The percentage of E rosettes fluctuated between 50–59% (N = 52–75%). There was a marked increase to 80% after initiation of transfer factor (TF) therapy and high normal values (70–75%) persisted throughout the period of therapy with TF. The percentage of B lymphocytes was within normal in all assays; (2) Delayed skin test responses to PPD, Candida and SKSD were negative but became positive to PPD after levamisole treatment and transiently positive to Candida after therapy with TF. Initial application of dinitrochlorobenzine failed to induce delayed hypersensitivity but repeated applications did so; (3) Mixed lymphocyte responses were detected in cultures with patient PBL against mitomycin C treated allogeneic lymphocytes and K562 cells. (Stimulation index 2·5 and 2·3 respectively); (4) Mitogenic responses to PHA of the patient's lymphocytes were normal; (5) Neutrophil function studies (NBT reduction, myeloperoxidase estimation, random migration, chemotaxis and live yeast particle phagocytosis and killing) were normal; (6) Serum antibody titre to tetanus 1/80 (> 1/10), diphtheria 1/40 (> 1/10), casein 1/256 (> 1/4) and lactalbumin 1/32 (> 1/4) indicated normal humoral immune responses; (7) Immunoglobulin levels IgA, IgG, IgM and IgE were within normal; (8) HLA antigens were detected on her lymphocytes.

### *Therapy*

Following the detection of SCC and the discovery of absent NK activity, attempts were made to

boost the latter activity by vaccination with *Bacillus Calmette Guerin* (BCG) (60 mg wet weight, $10^8$ viable organisms; CSL, Melbourne) intradermally by five applications of 20 needle Heaf gun. After three vaccinations at weekly intervals, no evidence of delayed skin hypersensitivity was detected. marked skin test reactivity to BCG however occurred when levamisole 2·5 mg/kg was given twice weekly for 2 weeks. Levamisole therapy was ceased at this time because of allergic reactions to the drug. Following removal of the second SCC from vulval region and left inguinal lymph nodes, therapy with TF (1–2 units each week for 5 weeks) was given. Interferon $2{\cdot}8 \times 10^8$ units from cultured fibroblasts (supplied by Dr Rentschler, in lyophilized form, $4{\cdot}3 \times 10^6$ international units (i.u.)/mg protein) was given i.m. on two occasions, 3 weeks apart.

## MATERIALS AND METHODS

*Measurement of NK activity.* This was assessed in $^{51}$Cr release assays as described in detail elsewhere (Hersey *et al.*, 1980a). The target cells used were from the MM200, MM96 melanoma cell lines, the MCF-7 breast carcinoma, Chang cell and K562 myeloid cell lines. The K562 cell line was a kind gift of Dr Shellam, Department of Bacteriology, University of Perth. The origin of the other cell lines were as described previously (Hersey *et al.*, 1980a). Target cells $3 \times 10^3$ (or $10^4$ K562) in 0·5 ml of RPMI + 10% fetal calf serum (FCS) were mixed with effector cells at ratios of 100, 30 and 10:1 in round bottomed 10 × 77 mm tubes, in triplicate and incubated overnight (except for K562) at 37° C in 7% $CO_2$. NK activity against K562 cells was carried out over 4 hr. At the end of the incubation period, the tubes were centrifuged for 5 min at 400 *g* and the supernatant removed. This, and the remaining tube, were counted in a gamma counter and percentage $^{51}$Cr release measured by the formula: % $^{51}$Cr release $= \frac{2a}{a+b} \times 100$ where a = counts in supernatant tube alone minus background counts and b = counts in remaining tube minus background counts. Results were expressed as % $^{51}$Cr release above baseline by subtraction of % $^{51}$Cr release from cultures of target cells alone.

In vitro *stimulation of NK activity on tumour cells and associated production of interferon.* These methods were adapted from those of Grohmann *et al.* (1978). MM200 were placed into 4 wells ($3 \times 10^5$/well) of a Costar 24 × 2 ml-well plate. The following day, the tumour cells were treated with mitomycin C (25 μg/ml) for 1 hr to prevent further cell division.

Lymphocytes from the patients and control normal subjects were added to 2 wells ($2 \times 10^6$/well) containing tumour cells and 2 wells without tumour cells. Following incubation for 24 hr the supernatants and lymphocytes were collected. Separation of lymphocytes from MM200 cells which had detached from the plate was effected by centrifugation on Ficoll-Hypaque. The lymphocytes from each of the various wells were then incubated with K562 and MM200 target cells in 4 and 16 hr assays respectively.

To test the supernatants from the cultures for stimulating activity, lymphocytes from the peripheral blood of a third normal control person were incubated in the undiluted supernatants from the cultures for 4 hr at 37°C. The lymphocytes were then washed once and added to the K562 and MM200 target cells in a 4 hr and 16 hr assay respectively. Additionally, supernatants from the cultures were assayed for the presence of interferon (see assay details below).

*Stimulation of lymphocytes with polyinosinic–polycytidylic acid [poly (I)–poly (C)].* Poly (I)–poly (C) (Sigma catalogue P4136) was prepared as a stock solution of 1 mg/ml in RPMI. PBL were purified by centrifugation on Ficoll-Hypaque and incubated with poly (I)–poly (C) at final concentrations of 100, 50 and 25 μg/ml for 16 hr at 37° C. They were then washed twice in Hanks' balanced salt solution (HBSS) and added to $^{51}$Cr labelled target cells in a 16 hr assay.

*Measurement of antibody dependent (K) cell-mediated cytotoxic activity (ADCC).* This was carried out in $^{51}$Cr release 16 hr assays using Chang cells sensitized with heat inactivated rabbit anti-Chang cell sera at a final dilution of $1:10^4$. Effector target cell ratios were 100 and 30:1. Further details are described elsewhere (Hersey, Edwards & Edwards, 1976).

*E rosettes, B lymphocytes.* The methods used for the estimation of these subpopulations of lymphocytes are fully described elsewhere (Hersey *et al.*, 1980b).

*Interferon assays.* Fibroblast interferon in serum samples and in the 'Fiblaferon' were assayed

for antiviral activity in aneuploid human fibroblast cells from line GM2504 by CSL (Melbourne).

Interferon produced by patient and control PBL were assayed as follows:
Linbro TC96 microtitre trays were seeded with Vero cells ($3 \times 10^4$ cells/well) in 0·2 ml modified Eagles' basal medium (Auto-pow, Flow Laboratories) containing 25 mM HEPES, pH 7·4 and 10% FCS, covered with a non-porous adhesive sealer and incubated for 1–3 days. The medium was then removed, interferon dilutions in medium A were added (0·1 ml/well; medium A, as above except FCS was 2%), the plate was then sealed and incubated for 16 hr. Encephalomyocarditis (EMC) virus was then added (5000 PFU in 0·05 ml medium A/well) and following incubation for 40–48 hr the contents were decanted and the cell layers stained for 10 min with 0·05 ml of staining solution (0·25 g NaCl, 0·75 g crystal violet, 50 ml ethanol, 5 ml of 35% formaldehyde and 100 ml of water). Finally the cell layers were carefully washed with warm 25–35° C water, air dried and visually inspected. End points were estimated as the reciprocal of the interferon dilution which gave 50% protection of the cell layer. Interferon titres were determined relative to a leucocyte international standard (G-023-901-527; source: Antiviral Substances Programme of the National Institute of Allergy and Infectious Diseases, NIH. Bethesda, Maryland, USA) or relative to a laboratory standard previously related to an international standard.

## RESULTS

*Absent NK activity and normal antibody dependent (K) cell-mediated cytotoxicity*

Peripheral blood lymphocytes (PBL), taken from the patient prior to immunotherapy, were tested against target cells known to be sensitive to NK activity and for K cell activity against Chang cells sensitized with rabbit antisera to Chang cells. Fig. 1(a) illustrates a representative result. No NK cell activity was detectable against any of the target cells (including MCF-7 and T24 cells not shown in the figure) but there was high K cell activity against sensitized Chang cells. The latter result was also repeated with melanoma cells sensitized with antisera from a human patient (data not shown). In Fig. 1(b), NK activity of a normal person against the target cells is shown. Addition of the patient's serum at final dilutions of 1/10 and 1/100 did not inhibit this activity. The latter results indicated the deficient NK activity was inherent in the PBL of the patient was not due to serum factors.

Vaccination with BCG or BCG plus levamisole did not increase NK activity despite marked skin test responses to BCG after levamisole treatment (see Case report). Administration of fibroblast interferon i.m. was not associated with an increase in NK activity at 24 hr but at 48 hr post-injection, low levels were recorded against both target cells. Interferon levels in sera before and at 24 and 48 hr after injection were 1·2, 3·2 and 2·5 units/ml respectively. The injected interferon assayed at $1{\cdot}5 \times 10^6$ units/ml and 2·5 ml was given. Poor absorption of i.m. fibroblast interferon into

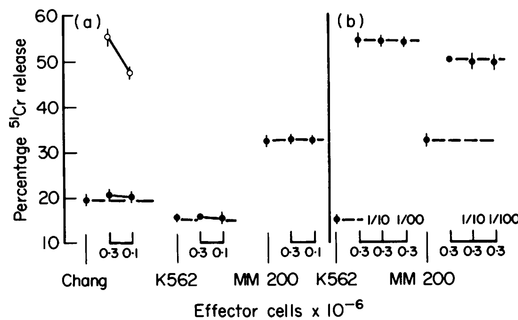


**Fig. 1.** (a) Absence of NK activity (●——●) in PBL from patient against the target cells Chang, K562 and MM200 in 16 hr assays. ADCC (K cell) activity against Chang cells sensitized with rabbit antisera (1/100 final dilution) is shown by the open circles (○——○). (b) NK activity of PBL from normal subject against K562 (effector: target cell ratio of 30: 1) and MM200 (effector: target cell ratio of 100: 1). Serum from the patient at a final dilution of 1/10 and 1/100 had no effect on NK activity of the normal PBL. Vertical bars indicate standard deviations.

the circulation has been described previously (Edy, Billian & DeSomer, 1978). Prior to the second administration of interferon, low levels of NK activity were detected against the melanoma target cell but at 14 and 36 hr after interferon NK activity was absent.

*Induction of NK activity* in vitro *by fibroblast interferon*

PBL from the patient and control were incubated in fibroblast interferon for 3 hr at 37° C, washed and then added to the K562 target cells in a 4 hr assay. The results, shown in Table 1, indicate that the maximal increase of NK activity in patient PBL (8% $^{51}Cr$ release) was noted at $10^2$ units of interferon.

The maximal increase in PBL from 2 normal controls was 11–12% noted at $10^2$ and $10^3$ units of interferon. These results were repeated using the MM200 target cells in an 18 hr assay. NK activity of PBL from patient incubated alone for 3 hr was $2 \pm 1\%$. Maximal NK activity was $10 \pm 2\%$ in PBL incubated in $10^3$ units of interferon. The control PBL incubated alone had NK activity of $5 \pm 2\%$. The maximal NK activity was $11 \pm 1\%$ after pre-incubation in $10^3$ units of interferon.

*NK activity of PBL after incubation on tumour cells*

To analyse further the nature of the defect in NK activity in the patient, patient and control lymphocytes were incubated on MM200 melanoma cells as described for 24 hr and then tested for NK activity on MM200 and K562 target cells. As shown in Table 2, PBL from normal donors showed a marked increase in NK activity against both target cells and the low cytotoxic activity against the MM200 target cell was seen in PBL cultured alone as well as in MM200 cells.

**Table 1.** Effect of interferon *in vitro* on NK activity of patient and control blood lymphocytes

| Donor of lymphocytes | Units of interferon | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | $10^2$ | $10^3$ | $10^4$ | $10^5$ |
| Patient | $1 \pm 1 \cdot 0$ | $5 \pm 0 \cdot 5$ | $8 \pm 1 \cdot 5$ | $4 \pm 0$ | $3 \pm 0 \cdot 5$ | $1 \pm 1 \cdot 1$ |
| Control 1 | $32 \pm 5 \cdot 0$ | — | $34 \pm 3 \cdot 0$ | $43 \pm 3$ | $32 \pm 3$ | — |
| Control 2 | $28 \pm 3 \cdot 0$ | — | $40 \pm 4 \cdot 0$ | $39 \pm 4$ | $23 \pm 2$ | — |

Figures indicate percentage $^{51}Cr$ release above baseline release (6·0%) from K562 target cells above. 30:1 effector to target cell ratio. 4 hr assay.

**Table 2.** NK activity of PBL cultured with and without tumour cells

| | Target cells and number of PBL $\times 10^{-5}$ | | | | | |
|---|---|---|---|---|---|---|
| | K562* ($4 \pm 0 \cdot 5$) | | | MM200† ($32 \pm 0 \cdot 5$) | | |
| | 3 | 1 | 0·3 | 3 | 1 | 0·3 |
| Patient PBL alone | $1 \pm 0 \cdot 5$ | 0 | 0 | $11 \pm 0 \cdot 5$ | $5 \pm 1$ | 0 |
| Patient PBL+MM200 | 1 | $0 \pm 0 \cdot 5$ | 0 | $10 \pm 1$ | $8 \pm 0 \cdot 5$ | $2 \pm 1$ |
| Control PBL alone | $65 \pm 1 \cdot 5$ | $40 \pm 2$ | $16 \pm 2$ | $43 \pm 1$ | $26 \pm 0 \cdot 5$ | $10 \pm 1 \cdot 5$ |
| Control PBL+MM200 | $78 \pm 1 \cdot 5$ | $61 \pm 2$ | $32 \pm 1 \cdot 5$ | $52 \pm 0 \cdot 5$ | $44 \pm 1 \cdot 0$ | $29 \pm 2 \cdot 5$ |

Figures in the table are the percentage $^{51}Cr$ release above baseline release from the target cells. The latter is indicated in brackets.

* 4 hr assay. $10^4$ target cells per culture.

† 16 hr assay. $3 \times 10^3$ target cells per culture.

*NK stimulating activity and interferon levels in supernatants from cultures of tumour and lymphoid cells*

The supernatants from the cultures indicated in Table 2 were tested for their ability to stimulate NK activity in lymphocytes from a second normal donor. The results in Table 3 indicate that supernatants from the cultures with patient's lymphocytes had no effect on NK activity against either target cell but pre-incubation of the normal lymphocytes in supernatants from cultures of the control lymphocytes with tumour cells resulted in an increase in NK activity against both target cells. Supernatants from the cultures of control lymphoid cells alone also increased NK activity. This was less than that observed from cultures containing tumour cells in assays against the MM200 target cell but was equivalent in assays against the K562. (We interpret the latter result to indicate that endogenous release of interferon in the 24 hr culture period was sufficient to result in maximal killing of the highly NK sensitive K562 target cell but not the less sensitive MM200 cells).

The interferon levels in supernatants from cultures with patient's lymphocytes incubated with or without MM200 tumour cells had less than 2·5 units whereas that from the control lymphocytes alone had 25–125 units/ml and from control lymphocytes incubated with tumour cells had 5,000 units/ml. Supernatants from MM200 cultures alone had less than 2·5 units/ml.

*Stimulation of NK activity by poly (I)–poly (C)*

PBL from the patient and a control subject were tested for NK activity against the MM200 target

**Table 3.** Comparison of NK activity induced in normal lymphocytes by pre-incubation in supernatants from co-cultures of patient or control lymphocytes with tumour cells

| | Effector cell numbers $\times 10^{-5}$ | | | | | |
|---|---|---|---|---|---|---|
| | MM200† (36±0·5) | | | K562‡ (5±0·5) | | |
| Origin of supernatants used for pre-incubation* | 3 | 1 | 0·3 | 3 | 1 | 0·3 |
| 0 | 27±0·5 | 20±1·0 | 10±0 | 62±1·5 | 38±1·5 | 18±3·0 |
| Patient PBL alone | 33±1·0 | 21±2·0 | 11·5±1·0 | 64±2·0 | 37±1·0 | 20±3·0 |
| Patient PBL+MM200 | 27±1·0 | 20±2·0 | 10±0 | 62±3·0 | 38±2·0 | 20±3·0 |
| Control PBL alone | 39±2·0 | 27±3·0 | 14±1·0 | 73±4·0 | 55±2·0 | 28±2·0 |
| Control PBL+MM200 | 51±2·0 | 41±3·0 | 30±0·5 | 72±2·0 | 52±3·0 | 26±2·0 |

* Pre-incubation was for 4 hr. Supernatants were collected after 24 hr from the cultures indicated.

† 16 hr assay. $3 \times 10^3$ target cells per culture.

‡ 4 hr assay. $10^4$ target cells per culture.

Figures in the table are the percentage $^{51}$Cr release above baseline release from the target cells. The latter are indicated in brackets.

**Table 4.** Induction of NK activity by pre-incubation of lymphocytes with Poly I–C.

| | Concentration of Poly I–C (μg/ml) used for pre-incubation (16 hr) | | | |
|---|---|---|---|---|
| Lymphocyte donor | 0 | 100 | 50 | 25 |
| Patient | 1±1·0 | 16±2 | — | — |
| Control | 11±1·0 | 20±0·5 | 15±1 | 14±1·0 |

Effector:target cell ratio, 100:1. Target cell MM200 16 hr assay. Figures refer to %$^{51}$Cr release above baseline release (30±1%)

cell after incubation in poly (I)–poly (C) for 16 hr. The data in Table 4 indicate that this resulted in a pronounced increase in NK activity in PBL from the patient, which was equivalent to that observed from the normal control. Interferon levels were not determined in the supernatants.

## DISCUSSION

The clinical features of this patient were typical of those seen in Fanconi's anaemia. The presentation with pancytopenia at the age of 7 years, the hyperpigmented skin lesions, nerve deafness, patent ductus and the subsequently discovered horseshoe kidney are all features of Fanconi's anaemia (Beard *et al.*, 1973; Evans, 1979). The natural history of Fanconi's anaemia is not clearly defined (Lipton & Nathan, 1980). The anaemia of this patient responded well to androgen therapy; however a neutropenia was frequently observed throughout childhood and early teenage life but this appeared to remit spontaneously in early adult life. From the age of 24, there was marked lymphocytopenia which was associated with development of extensive Bowen's disease and recurrent warts. These symptoms remained the dominant clinical disorder in adult life rather than that of anaemia.

Investigation of the immune system revealed normal humoral immunity and neutrophil function. T cell immunity seemed somewhat impaired with absence of skin test responses to antigens PPD, SKSD and Candida prior to levamisole and TF therapy. Furthermore, skin sensitization with DNCB required several applications. Similar findings were made in a previously described case of Fanconi's anaemia (Pederson *et al.*, 1977). The most marked cell-mediated defect in the patient was an absence of NK activity to several cultured tumour cells. This included studies against K562 cells which are known to be particularly sensitive to NK activity. The absence of NK activity *in vivo* persisted despite attempts to increase NK activity by BCG vaccination. Defects in NK activity were previously described in patients with Chediak Higashi syndrome (Roder *et al.*, 1980), a child with recurrent infections (Virelizier & Griscelli, 1980) and in mice homozygous for the beige hair colour (Roder *et al.*, 1980). In common with these reports NK activity in our patient was partially restored *in vitro* by addition of interferon. This suggests that the low NK activity in these conditions is not due to inherent defects of NK cells but is due to abnormalities in interferon release. This conclusion was supported by studies showing that incubation of the patient's lymphoid cells on tumour cells did not result in an increase in NK activity or of interferon levels in the culture supernatants, whereas similar treatment of lymphoid cells from normal subjects did so. These results were similar to those reported by Virelizier & Griscelli (1980).

These results contrasted with those obtained after stimulation of her lymphoid cells with the interferon inducer, poly (I)–poly (C) in that this resulted in an increase in NK activity comparable to that observed in lymphoid cells from normal subjects. This may indicate that her lymphocytes failed to release interferon on exposure to tumour cells because of defective recognition of antigens on the tumour cells by her lymphocytes. Alternatively, the two stimuli (poly (I)–poly (C) and tumour cells) may induce different types of interferon from the lymphoid cells, e.g. poly (I)–poly (C) may release predominantly $\alpha$ (Type I) interferon whereas incubation on tumour cells may release predominantly $\gamma$ (Type II) interferon (Trinchieri *et al.*, 1978; Santoli & Koprowski, 1979). If this is correct, the lymphoid cells in this patient may have had a selective defect in $\gamma$ interferon release.

Whether the abnormalities in NK activity were responsible for the manifestations of warts and Bowen's disease followed by SCC in this patient is speculative. It is of interest that the warts virus is related to the simian *SV40 papova* virus which is known to transform fibroblasts from patients with Fanconi's anaemia *in vitro*. The warts virus is also implicated in the formation of epidermodysplasia verruciformis which can give rise to Bowenoid changes in the skin in a distribution similar to that observed in this patient (Rook, Wilkinson & Ebling, 1978). SCC commonly develops in these lesions. It is conceivable therefore that the chromosomal defect in this patient resulted in abnormalities of interferon production which may have predisposed epithelial cells to infection with this virus and hence the development of warts, Bowen's disease and SCC. This may have been due to absence of a direct anti-viral action of interferon or absence of interferon induced NK activity against virus infected cells. Further studies are needed to examine whether NK activity is an

important effector mechanism against cells infected with this papova virus. If this proves to be so, patients with this symptom complex may benefit in the future from administration of leucocyte interferon in doses monitored to provide maximal stimulation of NK activity.

This work was supported in part under NCI Contract NO1-CB-74120. We wish to thank Professor Basten for kind provision of transfer factor used in therapy, Janssen Pharmaceuticals for supply of levamisole and Dr Rentschler, Arzneimittel GmBH and Co. for their generous provision of 'Fiblaferon' for trial of therapy in this patient. We also wish to thank Dr Cheyne, CSL, Melbourne for assay of fibroblast interferon, Sister J. Gardner for assistance in collection of specimens and Georgina Phillips for expert technical assistance.

## REFERENCES


Beard, M.E.J., Young, D.E., Bateman, C.J.T., McCarthy, G.T., Smith, M.E., Sinclair, L., Franklin, A.W. & Bodley-Scott, R. (1973) Fanconi's anaemia. *Quart. J. Med.* **XLII**, 403.

Edy, V.G., Billiau, A. & DeSomer, P. (1978) Non-appearance of injected fibroblast interferon in the circulation. *Lancet*, **i**, 451.

Evans, D.I.K. (1979) Constitutional Aplastic Anaemia. In *Aplastic Anaemia.* (ed. by C. G. Geary) pp 173. Ballière Tindall, London.

Grohmann, P., Holmes, W., Porzsoll, F., Quiri, I., Miller, R.G. & Phillips, R.A. (1978) Stimulation of human NK cell activity by cultured cells. 1. Response of normals. *Int. I. Cancer*, **22**, 528.

Hersey, P., Edwards, A. & Edwards, J. (1976) Characterization of mononuclear effector cells in human blood. *Clin. exp. Immunol.*, **23**, 104.

Hersey, P., Edwards, A., Milton, G.W. & McCarthy, W.H. (1978) Relationship of cell-mediated cytotoxicity against melanoma cells to prognosis in melanoma patients. *Br. J. Cancer*, **37**, 505.

Hersey, P., Edwards, A. & McCarthy, W.H. (1980a) Tumour-related changes in natural killer cell activity in melanoma patients. Influence of stage of disease, tumour thickness and age of patient. *Int. J. Cancer*, **25**, 187.

Hersey, P., Wotherspoon, J., Reid, G. & Gunz, F.W. (1980b) Hypogammaglobulinaemia associated with abnormalities of both B and T lymphocytes in patients with chronic lymphatic leukaemia. *Clin. exp. Immunol.*, **39**, 698.

Kiessling, R. & Haller, O. (1978) Natural killer cells in the mouse: an alternative immune surveillance mechanism? In *Contemporary Topics in Immunobiology.* (ed. by N. L. Warner). Vol. 8, pp 171. Plenum Press.

Lipton, J.M. & Nathan, D.G. (1980) Aplastic and hypoplastic anemia. *Ped. clin. N. Am.* **27**, 217.

Meisner, L.F., Taher, A. & Shahidi, N.J. (1978) Chromosome changes and leukemic transformation in Fanconi's anemia. In *Aplastic Anemia.* (ed. by S. Hibino *et al.*) pp 253. University Park Press, Baltimore.

Pederson, F.K., Hertz, H., Lundsteen, C., Platz, P. & Thomsen, M. (1977) Indications of primary immune deficiency in Fanconi's anaemia. *Acta Paediatr. Scand.*, **66**, 745.

Roder, J.C. (1980) Do NK cells play a role in anti-tumour surveillance? *Immunol. Today*, *I.* October **1**, 96.

Roder, J.C., Haliotis, T., Klein, M., Korec, S., Jetts, J.R., Ortaloo, J., Herberman, R.B., Katz, P. & Fauci, A.S. (1980) A new immunodeficiency disorder in humans involving NK cells. *Nature*, **284**, 553.

Rook, A., Wilkinson, D.S. & Ebling, F.J.G. (1978) In *Textbook of Dermatology*, 3rd Ed, pp 626. Blackwell Scientific Publications, Oxford.

Santoli, D. & Koprowski, H. (1979) Mechanisms of activation of human natural killer cells against tumour and virus-infected cells. *Immunol. Rev.*, **44**, 125.

Swift, M., Zimmerman, D. & McDonough, R. (1971) Squamous cell carcinomas in Fanconi's anemia. *J.A.M.A.*, **216**, 326.

Trinchieri, G., Santoli, D., Dee, R.R. & Knowles, B. (1978) Antiviral activity induced by culturing lymphocytes with tumour-derived or virus-transformed cells. Identification of the antiviral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. exp. Med.*, **147**, 1299.

Virelizier, J. & Griscelli, C. (1981) Selective defect of interferon secretion in a child with repeated infections and a secondary defect of NK activity. *Arch. Fr. Pediatr.*, **38**, 77.

Zaizov, R., Matoth, Y. & Mamon, Z. (1978) Long-term observations in children with Fanconi's anemia. In *Aplastic Anemia.* (ed. by S. Hibino *et al.*). pp 243. University Park Press, Baltimore.