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Defective immune interferon production and natural killer activity associated with poor neutrophil mobility and delayed umbilical cord separation

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SUMMARY

Two infants with recurrent infections and a history of delay in separation of the umbilical cord (1 month and 17 days) had severely impaired neutrophil mobility. In addition very poor natural killer cell (NK) activity of blood lymphocytes against a leukaemia cell line (Molt 4F) was found. Incubation of lymphocytes with lymphoblastoid interferon increased NK activity in the one case tested. No immune (γ) interferon production was detected in Raji cell and phytohaemagglutinin (PHA) stimulated cultures from the other case. Apart from an abnormal dose–response curve in thymidine uptake after PHA stimulation of blood lymphocytes, no other abnormalities were found in a range of immunological tests. Ascorbic acid improved neutrophil mobility but had no effect, on NK activity. Both children have subsequently died from septicaemic illnesses.

INTRODUCTION

Natural killer (NK) cells are mononuclear cells capable, *in vitro*, of spontaneously (i.e. without prior sensitization) killing a variety of targets particularly tumour cells and virus infected cells (Herberman & Ortaldo, 1981). Their activity may be greatly enhanced by treatment with interferon (IFN) or IFN inducers. In man, venous blood is usually a rich source of such cells and it has been suggested that they may be important as an immunological surveillance mechanism against tumour cells and in the early stages of viral infections (Herberman & Ortaldo, 1981; Roder & Haliotis, 1980). Because of the association, in the Chediak–Higashi syndrome, of low NK activity and impaired neutrophil function (Roder *et al.*, 1980) we have looked at NK activity in patients with other neutrophil defects.

The syndrome of delayed separation of the umbilical cord associated with widespread infections and impaired neutrophil mobility was first reported in five infants in 1979 (Hayward *et al.*, 1979). In spite of treatment with ascorbic acid, which improved neutrophil mobility in some of the cases, all those children subsequently died from infective complications.

We describe here two further cases with this syndrome in whom the additional features of very low NK activity, and evidence of defective immune (γ) IFN production were found.

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CASE REPORTS

Case 1—Male, birthweight 3·9 kg

This Arab child was seen when he was 11 months of age. Parents were not related. He had a history of recurrent diarrhoea, multiple recurrent skin abscesses (from which *Staphylococcus aureus* had been cultured), repeated chest and ear infections and persistent oral candidiasis. He was failing to thrive with poor weight gain. The umbilical cord had been thick and fleshy, had failed to contract down and had not separated by the 17th day, when it was removed. There was marked hepatosplenomegaly and scars, corresponding to the sites of previous abscess drainage, were found in both inguinal regions and on the scalp. Following diagnosis of the neutrophil mobility defect he was treated with ascorbic acid and prophylactic septrin. He initially improved on this regime but shortly after returning to his home country developed a septicaemic illness from which he died. No post mortem was performed.

Case 2—Female, full term delivery, birthweight 3·9 kg

She developed septic spots (due to *Staphylococcus aureus*) on her neck at the age of 4 days and required systemic antibiotics. The umbilical cord failed to separate at the normal time and she developed a large periumbilical abscess, which discharged pus yielding a mixed growth of Gram negative organisms on culture. The cord eventually separated at 1 month of age. Hepatosplenomegaly was noted at this time. Following this she had recurrent chest infections and skin sepsis and required almost continuous treatment with antibiotics. The neutrophil mobility defect was detected at 8 months of age, and from that time she was treated with ascorbic acid and prophylactic septrin, which reduced but did not eliminate the recurrent infections. She subsequently had a severe pneumonia when she required assisted ventilation, and a candida oesophagitis which left her with a partial stricture. Despite these problems she achieved normal developmental milestones and growth along normal percentiles. Her hepatosplenomegaly persisted. At the age of 20 months she developed a pseudomonas infection in the perineal region which progressed to septicaemia and death. Permission for post mortem was not granted.

METHODS

Routine immunity function tests were carried out by methods previously described (Hayward, 1977; Trompeter, Layward & Hayward, 1978).

Neutrophil mobility. Neutrophil mobility measurement has been described elsewhere (Aggett *et al.*, 1979). Briefly, neutrophils are allowed to pass through millipore membranes with or without a chemotatic stimulus and the distance migrated by the leading front is measured (μm). The effect of ascorbic acid (final concentration 2 mg/ml) is estimated by adding this to the incubation medium.

NK activity. Natural killer cell activity was measured on blood mononuclear cells which had been separated from heparinized blood by Ficoll–Hypaque (Lymphoprep, Flow Laboratories) density centrifugation and depleted of adherent cells by incubating for 1 hr on a plastic surface (Falcon tissue culture flasks) in the presence of 10% fetal calf serum. Cells from the leukaemic cell line Molt 4F were used as targets. They were labelled with chromium 51 (50 μCi ^{51}Cr per 10^6 cells for 2 hr at 37°C) washed three times and added to the effector cells in round bottomed microtitre plates in a range of effector to target cell (ET) ratios. As controls for spontaneous ^{51}Cr release, target cells were added to medium alone. After centrifugation at 200 ***g*** for 2 min the plates were incubated at 37°C for 4 hr and the supernatants harvested (Titertek Harvester system) and counted in an LKB gamma counter. NK activity was expressed as percentage specific cytotoxicity and was calculated by the formula:

$$\% \text{ Specific cytotoxicity} = \frac{\text{Counts in test supernatant} - \text{counts in control supernatant}}{\text{Total counts added to each well}} \times 100$$

For each ET ratio, the mean value of three replicates was calculated.

To look at the effect of interferon on NK activity, effector cells were incubated for an additional 2 hr at 37°C with human lymphoblastoid interferon (Wellcome), 200 or 1,000 units/ml, before being added to the target cells. The effect of ascorbic acid was measured by adding it (final concentration 2 mg/ml) to the effector cells just prior to mixing with the targets.

Interferon assay. γ IFN production was measured by stimulating 10^5 blood mononuclear cells for 6 days with either 10^5 irradiated Raji cells or PHA (Wellcome Labs.) at 1 μg/ml concentration. Supernatants were frozen at −70°C before assay. In the assay, inhibition of Semliki forest virus RNA synthesis as assessed by incorporation of tritiated uridine, was measured (Allen & Giron, 1979; Townell *et al.*, 1981). The cells used were WISH human amnion cells (Flow Laboratories) which are particularly sensitive to γ IFN. A laboratory standard of γ IFN, with activity of 1,000 units/ml on WISH cells (one unit being the quantity of interferon giving 50% inhibition of RNA synthesis) was measured in parallel. Serial dilutions of the reference interferon or of the samples were used. Acid precipitated tritium was counted on a liquid scintillation counter (LKB Wallac 81000). Counts were plotted against the log of the dilution and IFN was considered to be present when counts were less than 50% of the control infected cells (medium only added). A titre for γ IFN activity was derived from comparison with the curve obtained for the reference preparation.

RESULTS

Both children had normal numbers of blood neutrophils, lymphocytes and monocytes and mounted neutrophil leucocytosis responses to infection. Immunoglobulin levels were normal and circulating numbers of B cells, T cells and T cell subpopulations (as defined by monoclonal antisera) were within the normal ranges. Transformation responses to phytohaemagglutinin (PHA) were present but occurred at an unusually high concentration of PHA (Fig. 1). We have not seen such dose–response curves in normal subjects. A delayed cutaneous hypersensitivity response to candida antigen was positive in the one case (Case I) tested. In both children, neutrophils showed normal reduction of nitroblue tetrazolium and killing of ingested *S. aureus*.

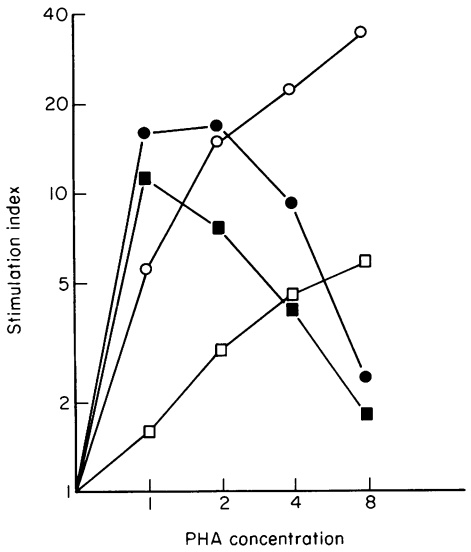

Fig. 1. Dose–response curves of PHA responses in the two cases and in controls. Each point represents the mean of duplicate values. PHA concentration is in μg/ml. O——O = Case 1; □——□ = Case 2; ●——● = control 1; ■——■ = control 2.

Table 1. Neutrophil mobility studies

	Mobility (μm)		
	−C.F.*	+C.F.	+C.F.+ascorbic acid
Patient 1†	25·9	40·5	45
Patient 2†	23·0	27·8	39·8
Patient 2 after treatment with ascorbic acid	29·9	50·3	56·0
Normal range	35–80	>90	>90‡

* C.F. = chemotactic factor.
† Means of values obtained on two separate occasions.
‡ Normals show no increment with ascorbic acid.

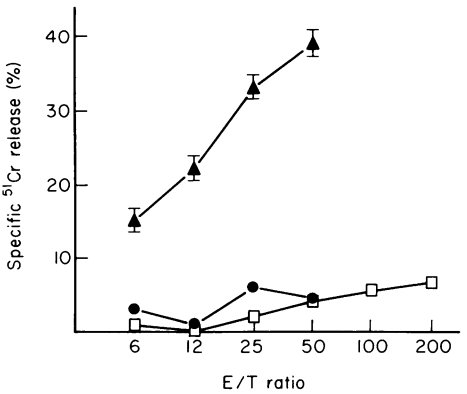

Fig. 2. Specific chromium 51 (^{51}Cr) release (NK activity) in the two Cases (●——● = Case 1; □——□ = Case 2). Mean values for normal subjects (▲——▲), adults and children, ±standard error of the mean are also shown (number studied = 44).

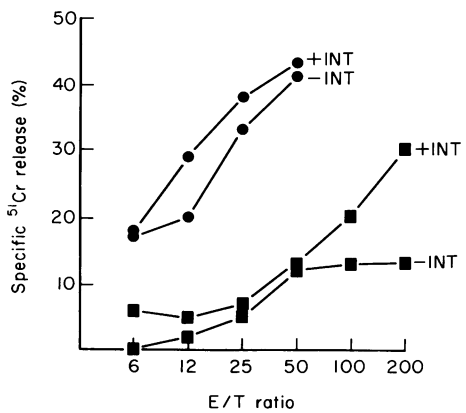

Fig. 3. (i) Effect of IFN (= INT in fig.) on NK activity of a normal subject ●——●; and on Case 1 ■——■ Peripheral blood mononuclear cells were incubated for 2 hr with or without IFN at a concentration of 200 units/ml prior to assay of NK activity. Use of 1,000 units/ml prior to assay of NK activity. Use of 1,000 units/ml of IFN produced results very similar to the lower dose (data not shown).

Mobility studies (Table 1)

The neutrophils moved very poorly in both cases, especially in the presence of a chemotactic stimulus, (each case studied twice) but some improvement was seen when ascorbic acid was added to the incubation medium. Both children were treated with 1 g of ascorbic acid per day. In Case 2 the effect of this treatment on the mobility is shown, but it was not possible to obtain follow-up data on the first case.

NK activities and interferon production

In both cases, specific chromium release was virtually zero at the usual effector to target ratios and only a very minor effect was seen when the ratios were increased to 200:1 (Fig. 2). NK activity was not affected by *in vitro* treatment of the cells from Case 2 with ascorbic acid (data not shown) but that of Case 1 increased after treatment of cells with IFN, particularly at high ET ratios (Fig. 3). This test was not performed on Case 2.

Because of this effect of IFN, an attempt was made to measure γ IFN production. Cells from the second patient (Table 2) did not produce any IFN in response to stimulation with either Raji cells or PHA (the study of Case 1 failed for technical reasons).

Table 2. Interferon production by blood mononuclear cells in culture

	Interferon (units/ml)	
Stimulus	Raji cells	PHA
Control*	> 2,000	> 2,000
Patient*	not detected	not detected

* Triplicate cultures.

DISCUSSION

We describe two children with the syndrome of impaired neutrophil mobility, frequent infections and delayed umbilical cord separation. Although we have heard that some children with this syndrome can do reasonably well (P. G. Quie and R. Wedgewood, personal communications), the fact that the five children originally described (Hayward *et al.*, 1979) have all subsequently died of infective complications (J. F. Soothill, personal communication) and the death of our two cases emphasize the severity of the immunodeficiency. This contrasts with the outlook in other children with impaired neutrophil mobility without delayed cord separation who tend to have recurrent infections but of a more minor nature (Farhoudi, Harvey & Soothill, 1978). We have measured NK activity in some of these other children and found that it falls well within the normal range. The findings here of defects in the natural killer system and in γ IFN production may account for the marked differences in clinical course between these two groups of children.

It has been reported that some patients with delayed umbilical cord separation and abnormal neutrophil chemotaxis also have abnormal leucocyte adherence (Bowen, Ochs & Wedgewood, 1979). Since we use a plastic adherence step in preparing effector cells for our NK assay, the possibility exists that failure of adherence of certain cells was responsible for the low NK activity. However, in other studies on NK activity of cells before and after a plastic adherence step we have found only minor differences which would be insufficient to account for the profound defect seen in these patients.

NK activity is also defective in other primary immunodeficiencies; these include some cases of severe combined immunodeficiency (Koren, Amos & Buckley, 1978; Sirianni *et al.*, 1981; Lipinksi *et al.*, 1980; our unpublished observations), Chediak–Higashi syndrome (Roder & Haliotis, 1980); Virelizier & Griscelli, 1980) and a syndrome of defective immune (γ) IFN production described in

children by Virelizier and colleagues (Virelizier & Griscelli, 1980; Virelizier *et al.*, 1979) in which NK activity is extremely low and can be corrected either *in vitro* or *in vivo* by administration of IFN. These children presented with recurrent viral and bacterial infections, but were not reported as having a history of delayed umbilical cord separation or of abnormal neutrophil chemotaxis. Their defective NK activity could be fully restored by treatment with leucocyte (α) IFN and it was suggested that the primary defect was lack of γ IFN production. In contrast, the defect of NK activity in Chediak–Higashi syndrome is either not affected (Virelizier & Griscelli, 1980) or only marginally improved (Haliotis *et al.*, 1980) by IFN suggesting that the primary defect lies in the NK cell itself.

The evidence in our patients suggests that defective γ IFN production was the cause of the very low NK activity. The absence of the neutrophil chemotaxis defect in the other cases described (Virelizier & Griscelli, 1980) would suggest that this was an associated finding in our cases rather than an effect of IFN deficiency. However, the IFN system is a complex one having effects on virtually all branches of the immune system (Stewart, 1979). It is conceivable therefore that several defects of γ IFN production exist, some affecting neutrophil function and others not. Clarification of this point could be obtained by studying the effects of IFN on neutrophil mobility in these patients.

IFN has, until recently, mainly been associated with anti-viral and anti-tumour immunity. However, both our cases and those of Virelizier had severe problems with infections of all kinds. This would be consistent with more recent ideas that γ IFN is a lymphokine with important and widespread immunoregulatory effects on immune responses (Stewart, 1979). On the other hand α (or anti-viral) IFN probably has a more specific anti-viral action. In fact, the patients described with defects of γ IFN production generate normal amounts of α IFN (Virelizier & Griscelli, 1980), and conversely, patients with defective α IFN production have normal γ IFN production (Isaacs *et al.*, 1981). The former observation poses a problem. Why should a child (Virelizier & Griscelli, 1980) with absent γ IFN but normal α IFN production, when treated with leucocyte (predominantly α) IFN, show a restoration of NK activity and clinical improvement? It has been postulated that the α IFN system, being easily exhaustible, is unable to maintain NK levels but that continuous administration of exogenous α interferon can do this (Virelizier & Griscelli, 1980).

γ IFN is produced by many types of lymphocytes (Epstein & Gupta, 1981), but, one study suggests that T cells, especially those lacking Fc receptors for IgG, are mainly responsible (Perussia *et al.*, 1980). Macrophages seem to enhance this production but do not produce it themselves (Epstein & Gupta, 1981). Deficiency of production therefore implies a subtle defect of T cells or macrophages. While circulating T cell and monocyte numbers were within normal limits in our patients we observed an abnormal dose–response curve to PHA stimulation. It would be interesting to see whether exogenous interferon modified this response.

Our experience with these patients illustrates how multiple immunodeficiencies may co-exist in the same patients, and shows the importance of γ IFN and/or NK activity in maintaining health.

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