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8. Altered Natural Killer Cell response in Patients with Malignant Lymphoma

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Phil. Trans. R. Soc. Lond. B 1982 **299**, 141-144
doi: 10.1098/rstb.1982.0120

of the total of extractable fatty acid from the cell, leads to an increase in membrane rigidity. The capacity of IFN to increase the saturation of C_{18} fatty acids could be the effector mechanism for the following effects of IFN:

(1) increased cell membrane buoyant density (Chang *et al.* 1978) owing to higher density of stearic acid (18:0, m.p. 69 °C) over the unsaturated C_{18} fatty acids (for example, oleic acid, 18:1, m.p. 14 °C);

(2) inhibition of maturation and release by budding of retroviruses (Chang *et al.* 1977) due to reduced membrane mobility;

(3) inhibition of syncytia formation by Sendai virus-infected cells (Tomita & Kuwata 1981) (same reason as for (1) and (2));

(4) inhibition of cell division, for the same reasons as (1) and (2) but also possibly due to inhibition of cell metabolism by inhibition of lipid (membrane)-dependent enzymes (Sandermann 1978);

(5) inhibition of virus replication, mainly for reasons (1), (2) and (4);

(6) the decrease of the effects of IFN after repeated exposure of the host to IFN (refractoriness to IFN (Borden *et al.* 1975)) due to induction of desaturation promotion factor and increased membrane fluidity.

References

Apostolov, K. 1980 The effects of iodine on the biological activities of myxoviruses. *J. Hyg.*, Camb. **84**, 381–388.

Apostolov, K. & Barker, W. 1981 The effects of interferon on the fatty acids in uninfected cells. *FEBS Lett.* **126**, 261–264.

Borden, E. C., Prochownick, V. & Carter, W. A. 1975 The interferon refractory state. II. Biological characterization of a refractoriness-inducing protein. *J. Immunol.* **144**, 752–756.

Chang, E. H., Mims, S. J., Triche, T. J. & Friedman, R. M. 1977 Interferon inhibits mouse leukaemia virus release: an electron microscope study. *J. gen. Virol.* **34**, 363–367.

Chang, E. H., Jay, F. T. & Friedman, R. 1978 Physical, morphological and biochemical alterations in the membrane of AKR cells after interferon treatment. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1859.

Lampson, G. P., Tytell, A. A., Nemes, M. M. & Hilleman, M. R. 1963 Purification and characterization of chick embryo interferon. *Proc. Soc. exp. Biol. Med.* **112**, 468–478.

Sandermann, H. Jr 1978 Regulations of membrane enzymes by lipids. *Biochim. biophys. Acta* **515**, 209–237.

Tomita, Y. & Kuwata, T. 1981 Suppressive effects of interferon on cell fusion by Sendai virus. *J. gen. Virol.* **55**, 289–295.

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8. Altered Natural Killer cell response in patients with malignant lymphoma

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Natural Killer (NK) cells may be an important anti-tumour mechanism (Herberman & Holden 1978). Furthermore, interferon and interferon inducers potentiate NK-mediated cytotoxicity (Einhorn *et al.* 1978) and may constitute a mechanism whereby interferon mediates its anti-tumour effect. Patients with malignant lymphomas have long been acknowledged to have marked defects in immunity, particularly cell-mediated immunity (Hancock *et al.* 1977). NK cell function in such patients has not, however, yet been assessed.

In the present study peripheral blood lymphocytes isolated from patients with Hodgkin's and non-Hodgkin's lymphoma have been assessed for NK function, and for their response *in vitro* to interferon.

Heparinized blood was taken from a total of 41 patients with malignant lymphoma; 24 with Hodgkin's disease and 17 with non-Hodgkin's lymphoma. Thirty-five patients were assessed

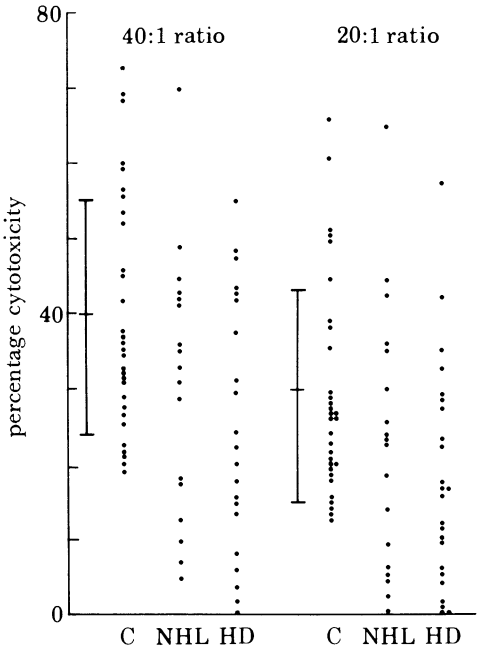

FIGURE 1. NK reactivity of peripheral blood lymphocytes from patients with Hodgkin's (HD) and non-Hodgkin's lymphoma (NHL) and control subjects (C). (Bar lines indicate 1 s.d. of controls.)

before treatment and 6 in relapse. Full histological typing and clinical staging was undertaken and natural cytotoxicity was measured using a 4 h ^{51}Cr-release assay with K562 leukaemia target cells. Blood was separated on Ficoll/Hypaque, and the lymphocyte-rich mononuclear cells (effector cells) added to target cells at an effector:target (E:T) ratio of 40:1 and 20:1. The percentage cytotoxicity was calculated by the formula

$$100\,\frac{(\text{percentage } ^{51}\text{Cr release in test}) - (\text{spontaneous } ^{51}\text{Cr release})}{100 - (\text{spontaneous } ^{51}\text{Cr release})}.$$

The NK activity of controls showed a high degree of natural variation (figure 1), but a significant number of lymphoma patients gave abnormally low levels of cytotoxicity. A total of 15 patients with lymphoma demonstrated cytotoxicity levels below any individual value shown by the controls at a 40:1 E:T ratio, and depressed NK cytotoxicity was more marked in those patients with Hodgkin's disease than those with non-Hodgkin's lymphoma. There was no correlation of cytotoxicity with age, histological type, stage of disease or with peripheral blood lymphocyte and monocyte counts.

In the interferon stimulation experiments, peripheral blood lymphocytes were incubated overnight with or without human lymphoblastoid (Namalwa) IFN-α (HuIFN-α) at a concentration of 2000 units ml^{-1},† and then assayed as above for cytotoxicity towards K562 cells;

† Units with reference to international standard.

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the cytotoxicity values obtained after pretreatment with HuIFN-α were compared with those in the spontaneous cytotoxicity assay (figure 2). All normal lymphocyte preparations showed significant enhancement of NK activity after treatment with HuIFN-α. In contrast a high proportion of patients with malignant lymphoma failed to show increased cytotoxicity after preincubation with HuIFN-α. In 5 of 11 patients with Hodgkin's and 5 of 8 with non-Hodgkin's lymphoma a significant increase in cytotoxicity was not demonstrated. The inability of patients'

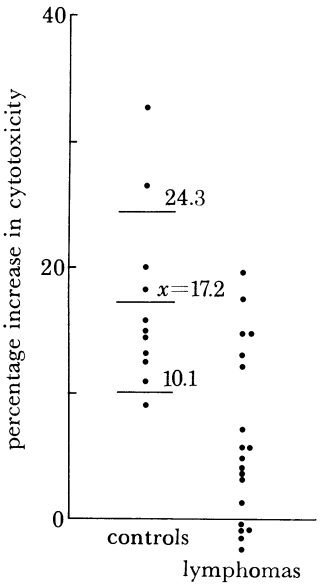

FIGURE 2. Percentage increase in cytotoxicity after treatment with interferon (E:T ratio of 20:1). (Bar lines indicate 1 s.d. of controls.)

cells to respond to HuIFN-α was not confined to those with low NK levels, and surprisingly some with seemingly normal NK reactivity failed to respond to treatment with HuIFN-α.

Cytotoxicity towards the NK-sensitive cell line K562 has therefore been shown to be significantly impaired in a proportion of patients with malignant lymphoma. This suggests a possible defect in the quantity or quality of NK cell reactivity, or alternatively that there might be suppressor cells present, for example T suppressor cells or monocytes. The failure of augmentation of NK activity by interferon is more difficult to understand, particularly since prior knowledge of a patient's spontaneous level of NK cytotoxicity did not predict subsequent potentiation. One reason for this might be that NK cells in lymphoma may already be in an activated state; another explanation is that the NK cells are defective and unable to respond to the interferon. Our findings agree with those of Platsoucas *et al.* (1980), who found impaired NK cell activity and diminished responsiveness to interferon in patients which chronic lymphocytic leukaemia (a disease related to the lymphomas).

Our studies confirm that patients with lymphoid neoplasms have defective natural resistance mechanisms and that the natural cytotoxicity of some patients is not influenced by the presence of interferon. This regulatory role is of particular importance in view of the current interest in using interferon in the therapy of malignant disease.

We acknowledge that this work was supported by The Yorkshire Cancer Research Campaign.

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References

Einhorn, S., Blomgren, H. & Strander, S. 1978 Interferon and spontaneous cytotoxicity in man. II. Studies in patients receiving exogenous leucocyte interferon. *Acta med. scand.* **204**, 477–483.

Hancock, B. W., Bruce, L., Sugden, P., Ward, A. M. & Richmond, J. 1977 Immune status in untreated patients with malignant lymphoma – a multifactorial study. *Clin. Oncol.* **3**, 57–63.

Herberman, B. B. & Holden, H. T. 1978 Natural cell-mediated immunity. *Adv. Cancer Res.* **27**, 305–377.

Platsoucas, C. D., Fernandes, G., Gupta, S. L., Kempin, S., Clarkson, B., Good, R. A. & Gupta, S. 1980 Defective spontaneous and antibody-dependent cytotoxicity mediated by E-rosette positive and E-rosette negative cells in untreated patients with chronic lymphocytic leukaemia: augmentation by *in vitro* treatment with interferon. *J. Immunol.* **125**, 1216–1223.

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