

The data presented here show PAR availability at 70°S for one complete revolution of earth around the sun. The data may find applications in plant science, oceanography and marine biology in Antarctic region.

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ACKNOWLEDGEMENTS We are grateful to the Department of Ocean Development and the Defence Research and Development Organization for sponsoring the work in Antarctica.

Received 27 May 1993; accepted 13 July 1993

Reversal of interferon-induced lymphokine-activated killer resistance in two murine cell lines by exposure to acid pH

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Interferon is known to augment the expression of MHC I antigens on a variety of tumour cell lines. In most cases, a simultaneous decline in the susceptibility of these tumour cells to natural killer (NK) cells and lymphokine-activated killer (LAK) cells is also observed. In the present communication, we have studied the LAK susceptibility and MHC I levels on two NK-resistant murine cell lines P815 and SP₂O. Treatment with interferon resulted in an increased MHC I expression as well as a decreased LAK susceptibility in both cell lines. A brief exposure of the interferon-treated tumour cells to citrate buffer (pH 3) resulted in a marked decline in the levels of MHC I and restoration of LAK susceptibility of the target cells. A direct role of MHC I antigens in determining the LAK

susceptibility of target cells is suggested by these results.

LYMPHOKINE-activated killer (LAK) cells are derived primarily from natural killer (NK) cells by interleukin-2 (IL-2) activation. There are qualitative differences between LAK cells and NK cells as the former are more efficient killers and lyse a wider range of target cells, including NK-resistant target cells. Quantitative expression of MHC I antigens on a tumour cell may be an important factor in determining its susceptibility to NK cells. In many systems, an inverse correlation between the quantitative MHC antigen expression on target cells and susceptibility to NK lysis has been demonstrated (reviewed by Ljunggren and Karre¹). Some recent studies have sought to investigate the role of target cell MHC class I antigen expression on LAK susceptibility of target cells. Wiebke *et al.*² have reported that a clear-cut correlation between enhanced MHC antigen expression and decreased LAK susceptibility was not observed in human tumour cell lines. Similar results were also reported by De Fries and Golub³, who observed that LAK susceptibility of certain human tumour cell lines following interferon treatment is not dependent on increased class I antigen expression. However, by depleting class I antigen expression by exposure to acid pH, Miyatake *et al.*⁴ reported that interferon-induced resistance to LAK lysis in cultured human gliosarcoma cells is, at least in part, due to enhanced levels of class I antigen expression.

In the present report, we have investigated the LAK susceptibility of two NK-resistant cell lines of murine origin, in which MHC I expression was initially boosted by interferon treatment and reduced thereafter by acid pH treatment: P815 (mastocytoma) and SP₂O (myeloma) cell lines used in this study were propagated in culture in RPMI-1640 supplemented with 10% FCS, 2×10^{-5} 2-mercaptoethanol, 300 µg/ml glutamine and 60 µg/ml gentamicin (complete medium). In order to generate LAK cells, spleen cells from C57B1/6 mice were cultured at 5×10^6 cells/ml with 200 U/ml of interleukin 2 (IL-2, a kind gift from Hoffmann La Roche, Nutley, NJ) in complete medium. After two days, cultures were split into two and supplemented with equal volumes of fresh medium and 200 U/ml IL-2. Cells harvested on day 5 from initiation of culture were washed and used as LAK effector cells.

Tumour cells were cultured (5×10^4 cells/ml in complete medium) with or without 200 U/ml recombinant murine interferon gamma for 48 h. After culture, the cells were harvested and washed. Portions of these IFN-treated tumour cells were subjected to a brief pH 3 treatment as described by Sugawara *et al.*⁵. Briefly, cell pellets were suspended in 0.5 ml of cold 0.2 M citric-acid-Na₂PO₄ buffer (pH 3.0), containing 1 g/100 ml of bovine serum albumin. After 2 min,

Table 1. Effect of acid pH exposure on class I MHC antigen expression of interferon-treated tumour cells

Target cell	Exp.	MHC antigen expression ^a		
		Control	Interferon	Interferon and pH 3.0 ^b
P815	1	0.64	1.02	0.60
	2	0.72	1.58	0.62
SP ₂ O	1	0.43	1.21	0.08
	2	0.39	0.79	0.17

^a Tumour cells were cultured with interferon (200 U/ml of mouse recombinant interferon for 48 h) in duplicate sets. On the day of the assay, one set was exposed to acid pH as described in the text.

^b Relative quantitation of MHC I expression was done by using an ELISA-based method⁶

10 ml of cold complete medium was added to neutralize the pH and the cells were washed twice with fresh medium. Control tumour cells as well as IFN-treated tumour cells with or without pH 3 treatment were used as targets for murine LAK cells. Relative MHC I antigen expression on these cell preparations was also assessed by an ELISA-based procedure developed by us⁶. Briefly, pelleted tumour cells were suspended in 0.1 ml of hybridoma supernatant (ATCC hybridoma HB 102 secreting monoclonal antibody to H-2D^d antigen) and incubated at 4°C for 30 min. After incubation, cells were washed and suspended in 0.1 ml of 1:200 diluted rabbit anti-mouse IgG preparation coupled with horse radish peroxidase enzyme, and incubated for 30 min at 4°C. OPD was used as a substrate and the colour developed at 37°C for 10 min was read on an ELISA reader. Labelling of tumour cells and chromium release assay of cytotoxicity was performed as described before⁷. Acid-pH-treated tumour cells did not show a loss of viability, and the spontaneous release of chromium was also comparable in control and pH-3-treated tumour cells. All experiments were repeated at least four times, but only the results of representative experiments are reported here.

Table 1 shows that there was a significant increase in the expression of MHC I antigen on both tumour cell lines treated with interferon. Subsequent treatment with pH 3 buffer brought down the expression of MHC I antigens to normal or subnormal levels (Table 1). LAK susceptibility of these various tumour cell preparations is shown in Figure 1. In P815 as well as SP₂O cell lines, interferon treatment reduced the susceptibility to LAK cells. Furthermore, pH 3 treatment restored the LAK cell susceptibility in both cell lines.

According to Sugawara *et al.*⁵, tumour cells exposed to acidic buffer (pH 3.0) for a short duration lacked detectable MHC I antigens by using anti-MHC I Mab reagents. It was not clear whether there was an actual shedding of the MHC I antigens as a result of pH 3 treatment of the cells, or the acid pH denatured the MHC I antigens, rendering them unreactive to anti-MHC I Mabs. The acid-pH-treated cells lacked MHC

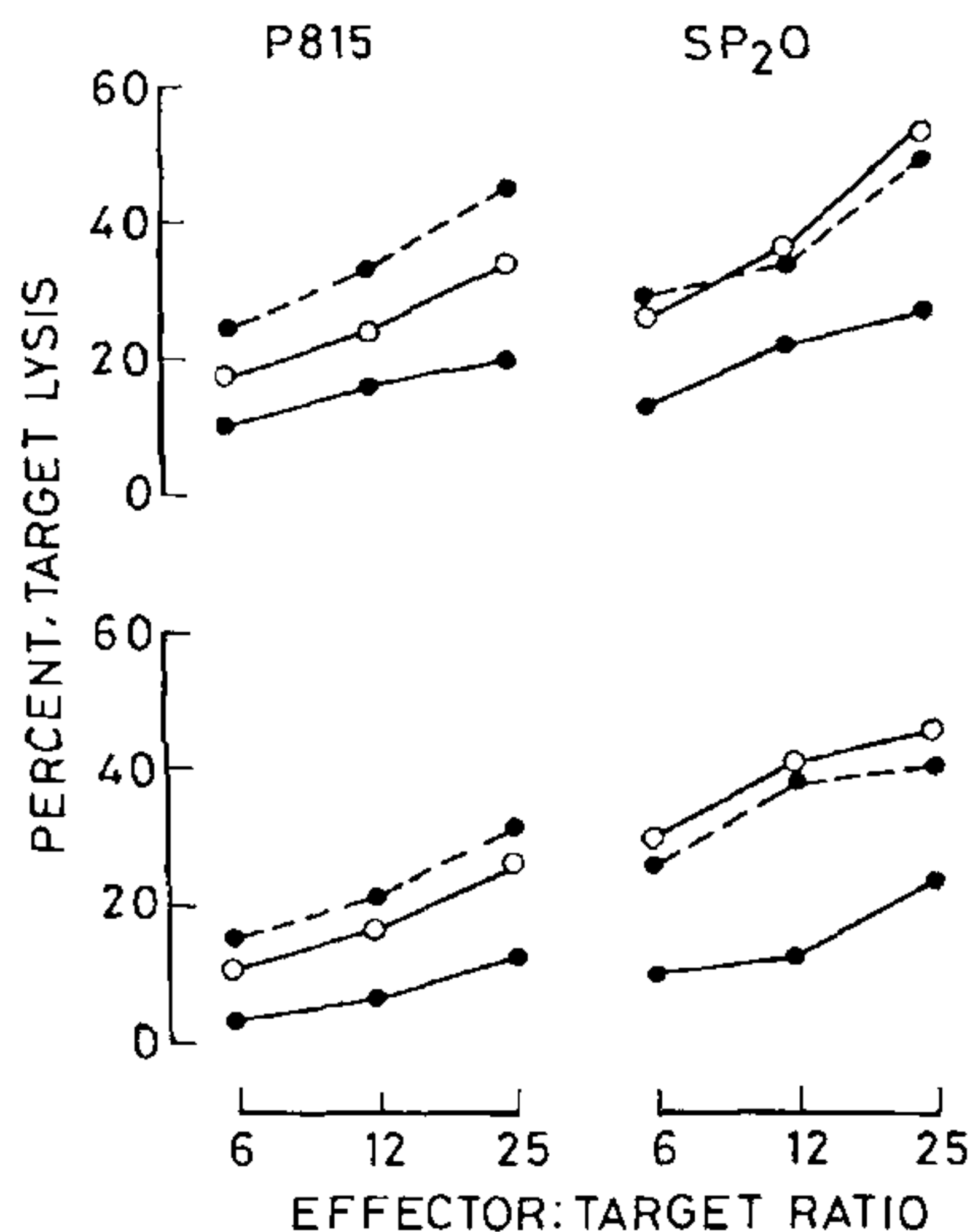


Figure 1. Lysis of various control, interferon and pH-3-treated tumour targets by murine LAK cells. LAK sensitivity of control (O) and interferon-treated cells before (●) and after (---●---) exposure to acid pH was assessed. Different targets were used in a four-hour chromium release assay of cytotoxicity, at different given E/T ratios. Results of two separate experiments using P815 (panel A) and SP₂O (panel B) target cells have been shown. Each point is a mean of three replicate assay wells. SD was always less than 2% lysis.

class I and β 2 microglobulin antigen expression, whereas the expression of other molecules like class II MHC antigens, T, B, NK and some myeloid markers was not affected⁵. Our own results show only an incomplete loss of MHC I antigens on pH-3-treated tumour cells. Selectivity of the loss of these antigens, however, appears to be confirmed by our finding that no changes occur in the expression of thy-1, LCA or class II MHC antigen expression on tumour cell lines exposed to acid pH (data not shown).

In the murine system investigated by us, an inverse correlation between the quantity of MHC I antigens present on the tumour cells and their susceptibility to lysis by LAK cells is clearly supported by our data. It is, however, not clear whether MHC I antigens influence directly the lytic process or the two phenomena are associative only.

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ACKNOWLEDGEMENT. This work was supported by a Young Scientist research grant from the Department of Science and Technology, New Delhi, to A. S.

Received 1 March 1993, accepted 2 July 1993

Pharmacologically active fatty acids of tiger prawn *Penaeus monodon* (Fabricius)

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The fatty acid compositions of the hepatopancreas and body flesh of the exportable prawn *Penaeus monodon* (Fabricius) have been studied by gas liquid chromatography and other chromatographic techniques. Altogether 24 components were detected and estimated. Major fatty acids recorded were palmitic (16:0), stearic (18:0), oleic (18:1 ω 9) and linoleic (18:2 ω 6). Of the polyunsaturated fatty acids, considerably high levels of pharmacologically active

fatty acids, viz. arachidonic pentaenoic (23:4 ω 6) eicosapentaenoic (20:5 ω 3) and docosahexaenoic (22:6 ω 3) were recorded. Thus, the species studied is also a potential source of pharmacologically active fatty acids, particularly belonging to the $-\omega$ 3 series.

BIOCHEMICAL studies on prawn is necessary for evaluating its nutritional value as well as its possibility of future usage as natural sources for biologically active components. In the present study, efforts have been made to determine various lipid components in the body of the prawn *Penaeus monodon* with special reference to the fatty acid profile of the lipid.

Fresh samples of wild *P. monodon*, collected from fishermen of Sagar Island, West Bengal, were divided into two parts, body flesh and hepatopancreas, weighed and separately extracted as follows. Body flesh and hepatopancreas were homogenized separately with MeOH-CHCl₃ (2:1), centrifuged and the residue was again homogenized with MeOH-CHCl₃-H₂O (2:1:0.8). After centrifugation the residue was again homogenized with MeOH-CHCl₃ (2:1) and recentrifuged. The supernatants were pooled and diluted with CHCl₃-H₂O (1:1). The lower CHCl₃ layer containing the total lipid was dried over anhydrous Na₂SO₄ (ref. 1). The total lipid was saponified using methanolic KOH in an atmosphere of nitrogen gas, according to Christie². Nonsaponifiables were separated with diethyl ether, after which, acidifying the aqueous layer, fatty acids were obtained by diethylether extraction, dried and methylated using diazomethane³. An aliquot of the sample was hydrogenated catalytically to confirm the unsaturated fatty acids⁴. The major components of nonsaponifiables, namely hydrocarbon and sterols were separated by thin layer chromatography (TLC) using 50% diethylether in hexane as the solvent system⁵. Sterol, thus obtained, was estimated colorimetrically using ferric chloride reagent, as described by Kates⁶. The hydrocarbons were estimated by direct weighing. Gas liquid chromatography (GLC) was done on a Hewlett-Packard instrument Model 5890, series II, equipped with a glass column (1.8 m \times 2 mm i.d.), packed with 10% diethylene glycol succinate polyester liquid phase supported on 80-100 mesh Chromosorb-W (HP) and a flame ionization detector (FID) was used. Peaks of fatty acid methyl esters were identified according to the methods of Ackman and Burgher⁷ and Ackman *et al.*⁸.

The present investigation shows that the body flesh of *P. monodon* is a rich source of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The occurrence of considerably higher levels of EPA, DHA and nonsaponifiables (Table 1) in the body flesh is common in the detritivorous benthic animals of Sunderbans estuarine complex⁹⁻¹².

About 24 fatty acids are reported from the hepatopancreas and body flesh of *P. monodon* (Table 2) Of

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