

Interleukin-1 in malnutrition

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SUMMARY The effect of malnutrition on the *in vitro* production of interleukin-1 by lipopolysaccharide stimulated circulating monocytes has been investigated in children suffering from kwashiorkor and marasmus. The interleukin-1 activity was significantly lower in children with severe malnutrition. Furthermore, macrophages from children with kwashiorkor produced factors that suppressed mouse thymocyte proliferation. These observations show a significant impairment of macrophage function and provide a mechanism for the suppression of cellular immunity in malnutrition.

Recent advances in immunology have highlighted the role of macrophages in resistance to infection. *In vitro* studies have shown that macrophage derived factors can substitute the parent cell in supporting T lymphocyte functions.¹ The antigen specific activation of T cells is dependent upon two distinct signals from macrophages. The Ia⁺ macrophages present antigen to T cells in an immunogenic form that constitutes the first signal for T cell activation. Lymphocyte activation factor, which has been designated interleukin-1,² provides a maturational signal to antigen or mitogen primed T lymphocytes,³ and thus leads to the elaboration of interleukin-2, which is necessary for the amplification of the cell mediated immune response.

Several clinical studies have shown that severe protein energy malnutrition adversely effects cell mediated immune responses.^{4,5} The effect of severe malnutrition on the functional state of macrophages, however, has not been established. Therefore, we have tested *in vitro* interleukin-1 activity of macrophages in children suffering from severe protein energy malnutrition.

Subjects and methods

Eleven children aged between 2 and 5 years suffering from severe protein energy malnutrition were investigated. These children were admitted to the children's nutrition ward of Niloufer Hospital for treatment of kwashiorkor or marasmus. Five out of the 11 children investigated had kwashiorkor with severe growth retardation, oedema, and mental changes. Six children had marasmus with wasting and severe growth retardation. Children with overt infections were excluded from the study. Age

matched, well nourished children were selected as controls, based on their weight/age.⁶

The general procedures for isolation of macrophages and assay of interleukin-1 have been described by Blyden and Handschumacher.⁷ Ten ml heparinised (4 U/ml) blood was collected from each child. Parental consent was obtained.

Mononuclear cells were isolated on Ficoll Paque.⁸ The mononuclear cell layer was washed twice with phosphate buffered saline. The cells were aliquoted into sterile petri dishes containing 1 ml Rosewell Park Memorial Institute medium (RPMI) with 10% fetal calf serum (FCS). After incubation for one and a half hours at 37°C the supernatant fluid containing non-adherent cells was discarded, and the adherent cells were washed twice with the same medium.

Production of interleukin-1. Twenty µg lipopolysaccharide/ml (Difco) was added to each petri dish containing adherent cells in 1 ml RPMI containing 1% FCS. To establish the inhibitory effect of macrophage derived prostaglandins on production of interleukin-1,⁹ indomethacin (Sigma) was added at a final concentration of 7 µg/ml to one of the petri dishes and incubated at 37°C for 24 hours. The supernatant was collected and was centrifuged at 1000 rpm for five minutes to remove any particulate material and then frozen in a volume of 1 ml each. The adherent cells were dislodged from the plastic surface using 1% trypsin and were suspended in a volume of 1 ml RPMI containing 1% FCS. The cell count was determined in a haemocytometer. The cells were washed twice using the same medium and then disrupted by repeated freezing and thawing in a methanol bath, followed by treatment with a polytron homogeniser for three minutes at maximum

speed. The cell lysate thus obtained was centrifuged at 1000 rpm for five minutes to remove the particulate material and was frozen in a 1 ml volume for storage. Preliminary experiments determining interleukin-1 activity from this material indicated that cell disruption was complete.

Assay of interleukin-1. Thymocytes obtained from 4–6 week old albino Swiss inbred mice were used as the target cells. Animals were killed by cervical dislocation and the thymus gland removed. A thymocyte suspension was prepared by gently teasing the thymus gland in cold Hanks's balanced salt solution. Thymocytes were suspended in RPMI medium with 10% FCS layered in petri dishes and incubated at 37°C for one and a half hours to remove adherent cells. The supernatant fluid was centrifuged at 10 000 rpm for five minutes and the cells resuspended in RPMI containing 1% FCS. The cell count was adjusted to 2×10^6 cells/0.1 ml. Interleukin-1 samples were assayed in triplicate by the addition of 0.1 ml of the sample obtained from the crude supernatant lysate, prepared in serial dilutions ranging from 1:10 to 1:320. Interleukin-1 activity was separately tested in supernatant and lysate obtained from macrophages cultured with and without indomethacin. In all these assays, the ability of interleukin-1 to potentiate the thymocyte responsiveness to phytohaemagglutinin was measured. The

test material was added in a volume of 0.1 ml with 0.03% phytohaemagglutinin (Burroughs-Wellcome) to the thymocyte culture containing 2×10^6 cells in 3 ml RPMI with 1% FCS. Thymocyte cultures stimulated with phytohaemagglutinin alone served as controls. ^3H Thymidine incorporation into DNA was measured by the addition of 74 kBq (2 μCi) of tritiated thymidine at 72 hours. Twenty four hours later the cultures were harvested and placed in toluene based scintillation fluid and the radioactivity measured in a liquid scintillation counter. Interleukin-1 activity was expressed as the difference of mean (SE) counts per minute between the test culture and the control cultures in appropriate dilutions. A representative dose response curve is given in Figure 1 to indicate the log linear relation between the decrease in thymidine incorporation with increasing dilution of the test material.

Results

The numbers of macrophages recovered from the peripheral blood ranged from $4\text{--}6 \times 10^6$ in both controls and malnourished children. The total interleukin-1 activity obtained by pooling the activity in supernatant (extracellular activity) and cell lysate (intracellular activity) in corresponding dilutions was significantly higher in culture systems with indomethacin compared with those without (Fig. 2).

The interleukin-1 activity was found to be distributed almost equally in the extracellular and intracellular fractions in normal children (Fig. 3). The total interleukin-1 activity in normal children was found to be significantly higher than in children with marasmus or kwashiorkor (Fig. 4). In children with marasmus the intracellular and extracellular activities were found to be significantly lower than the

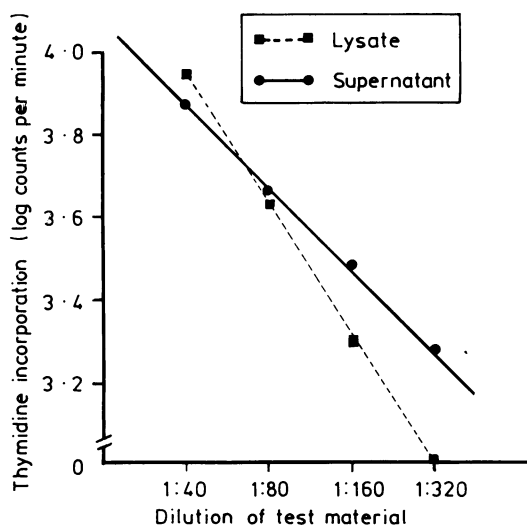


Fig. 1 Dose response curve indicating the relation between decreasing thymidine incorporation and increasing dilution of the test material.

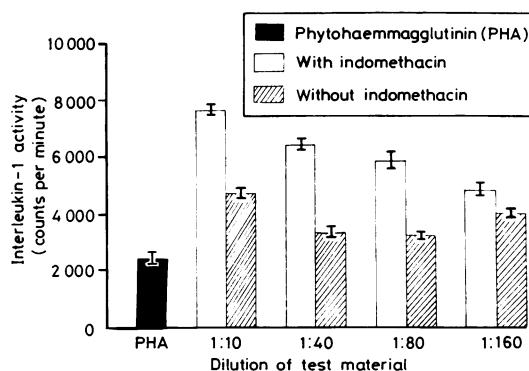


Fig. 2 Effect of indomethacin on interleukin-1 activity.

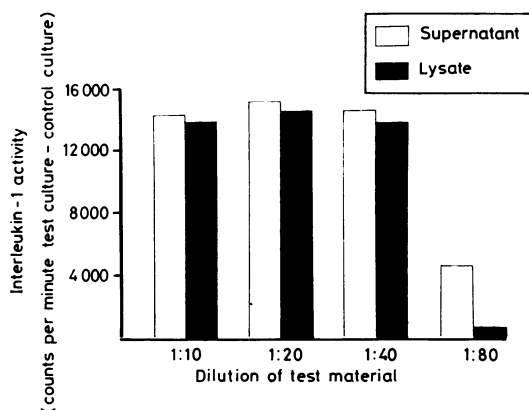


Fig. 3 Intracellular and extracellular interleukin-1 activity in normal children.

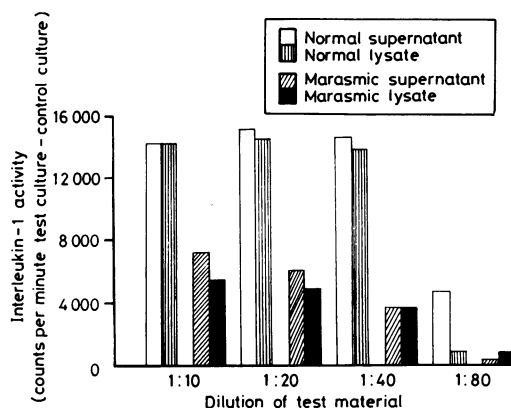


Fig. 5 Intracellular and extracellular interleukin-1 activity in children with marasmus.

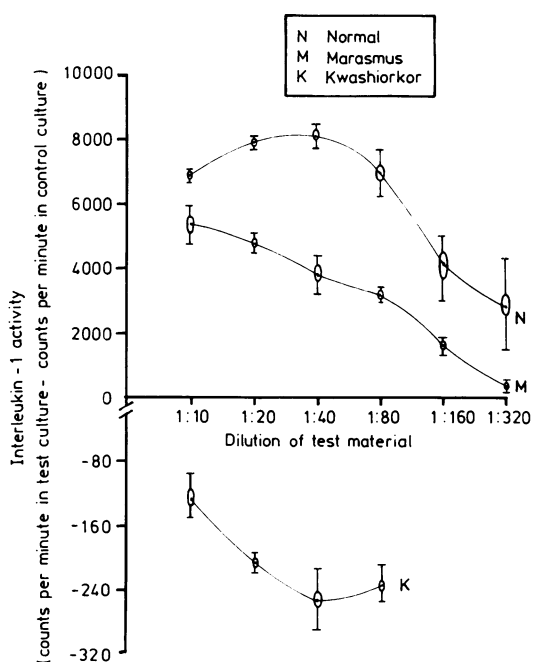


Fig. 4 Total interleukin-1 activity in malnutrition.

corresponding activities in normal children (Fig. 5). In children suffering from kwashiorkor, however, the thymidine incorporation in cultures with macrophage material was less than the incorporation observed with phytohaemagglutinin alone; this accounts for the negative figures (Fig. 4).

Discussion

It is well established that macrophages play a crucial though non-specific role in cellular immune responses. Ia⁺ macrophages are involved in the processing and presentation of antigen to specific T lymphocytes.³ Further, they are involved in the elaboration of interleukin-1, which provides a maturational signal to the antigen primed T lymphocyte.

The adverse effects of malnutrition on cell mediated immunity are thought to be due to defective T cell function. Experimental studies determining the effect of malnutrition on macrophage function are few and controversial.^{10 11} Recent clinical studies from this laboratory have shown that mobilisation of monocytes *in vivo* to a site of injury is impaired in malnourished children.¹² The decreased opsonic activity of the serum due to lowered complement concentrations in such children might impair the phagocytic capacity *in vivo*.¹³

The present study confirms that macrophage derived interleukin-1 activity is present in the cell lysate as well as in the supernatant.¹⁴ This finding strengthens the importance of estimating intracellular and extracellular interleukin-1 activity separately. The activity of interleukin-1 was found to be almost equal in the intracellular and extracellular compartments in normals. Gery, however, reported higher intracellular interleukin-1 activity from lipopolysaccharide stimulated macrophage;¹⁴ the explanation for this difference is not clear.

The suppression of interleukin-1 in culture systems without indomethacin and restoration of interleukin-1 activity with indomethacin confirms earlier observations that macrophage derived pro-

taglandins are involved in suppression of interleukin-1 activity.⁹

In the present study decreased interleukin-1 activity is shown in patients with severe protein energy malnutrition. As both intracellular and extracellular activity is lowered in children with marasmus, the intracellular production of interleukin-1 is probably compromised. In addition, the significant suppression of phytohaemagglutinin induced thymocyte responses in patients with kwashiorkor suggests the presence of a macrophage derived suppressor factor(s).

The reduced interleukin-1 activity and the possible production of a suppressor factor provide a mechanism for the defect in cell mediated immune responses in severely malnourished children.

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