Clin. exp. Immunol. (1986) **65**, 381–386.

Modulation of cellular immunity in malnutrition: effect of interleukin 1 on suppressor T cell activity

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(Accepted for publication 25 February 1986)

SUMMARY

T-lymphocyte mitogenesis is impaired in protein-energy malnutrition. The effect of interleukin 1 (IL-1) on the augmentation of suppressor cell activity during concanavalin A-induced lymphocyte proliferation was measured using IL-1 and lymphocytes from protein malnourished and control rabbits. Addition of IL-1 to lymphocyte cultures from control donors enhanced the suppression of fresh lymphocytes to phytohaemagglutinin (PHA). Addition of IL-1 to lymphocyte cultures from malnourished donors abrograted the suppressor cell activity. Isoelectric focusing showed the presence of a protein band with a pI of about 7·0 in the IL-1 supernatants from protein malnourished and control donors. The results suggest that severe protein malnutrition alters the ability of T-lymphocytes to respond appropriately to IL-1 rather than simply affecting synthesis of this monokine.

Keywords protein malnutrition interleukin 1 suppressor T cell isoelectric focusing

INTRODUCTION

Protein-energy malnutrition (PEM) in children (Chandra & Newberne, 1977; McMurray, 1984) and animals (Hoffman-Goetz, Bell & Keir, 1985; Watson, Chien & Chung, 1983) is associated with impaired *in vitro* T-lymphocyte transformation. Although the exact molecular mechanisms underlying lymphocyte hyporesponsiveness are unknown, several investigators have described the presence of inhibitory or suppressive factors in sera and cell cultures from malnourished subjects (Beatty & Dowdle, 1979; Salimonu *et al.*, 1982). Changes in the absolute and/or proportional number of T-lymphocyte subsets may also play a role in this hyporesponsiveness. Chandra (1983) demonstrated in children with PEM reduced numbers of total T-lymphocytes (T3) which resulted from reduced numbers of helper/inducer (T4) and suppressor (T8) subpopulations. The proportional impact on the helper subset was the largest, with a resultant marked depression in the helper/suppressor (T4/T7) ratio. Similar results have been reported by Chandra, Gupta and Singh (1982) and Joffe, Kew and Rabson (1983).

Regulation of the immune response involves a consortium of positive and negative signals, some of which are of monocyte origin. For example, prostaglandin E_2 (PGE_2), produced by low density adherent cells, inhibits lymphocyte mitogenesis by elevating intracellular cyclic AMP levels in inducer/helper T-cells (Oppenheim *et al.*, 1980), by activating suppressor T cells (Stobo, 1977), and by inhibiting interleukin 1 (IL-1) synthesis by monocytes (Kunkel & Chensue, 1985). IL-1, on the other hand, appears to be a necessary amplifying signal for lymphoproliferative responses by enhancing interleukin 2 (IL-2) synthesis by T4 lymphocytes (Dinarello, 1984). Moreover, IL-1 has

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also been shown to enhance the generation of suppressor T cell activity during concanavalin A (Con A)- and histamine-induced activation (Beer et al., 1982a,b).

The aim of this study was to compare Con A-induced suppressor activity of lymphocytes from severely protein malnourished and well-nourished donors with respect to the role of IL-1 in augmentation of this response. We provide here evidence that suppressor activity generated in response to IL-1 differs between malnourished and well-nourished donors, in part as a function of IL-1 donor source. Moreover, we present preliminary data to show that alterations in lymphocyte responsiveness to IL-1 contribute to deficits in cellular immune function in protein malnutrition.

MATERIALS AND METHODS

Animals. Male New Zealand White rabbits, maintained at 21 ± 1°C, 40–50% relative humidity, on a 12 h light/12 h dark cycle, were fed a 0% casein diet (protein deprived, PD; Ralston Purina No. 5765) or pair fed a 21% casein diet (control, C; Ralston Purina No. 5657) for 8 weeks. The rationale, procedure for induction of malnutrition, and diagnostic indices of malnutrition have been described elsewhere (Bell, Hoffman-Goetz & Keir, 1986).

Interleukin 1. IL-1 containing supernatants were prepared from heat-killed, *S. epidermidis* activated blood mononuclear cells (5×10^6 cells/ml; 21% monocytes) of PD and C rabbit donors (Hoffman-Goetz *et al.*, 1985). Briefly, crude mononuclear cell supernatants were collected after an 18 h incubation at 39°C, sterile filtered (0·2 μm), concentrated 3·8-fold on ultrafiltration membranes (Amicon Canada Ltd, Oakville, Ontario YM5), and gel filtered on Sephadex G-50 packed 10 × 100 cm columns (phosphate buffered saline (PBS), pH 7·2, 4°C). Samples eluting between mol. wts 12,000 and 18,000 were pooled and dialysed for 48 h at 4°C against saline to remove sodium azide, and subsequently tested for endotoxin contamination (to 3 ng/ml) by limulus amoebocyte lysate (LAL) assays (Sigma Chemical Co., St Louis, MO, USA). Endotoxin-free partially purified IL-1 supernatants were stored at 4°C until used. IL-1 samples from control donors were tested for pyrogenicity in rabbits as described previously (Hoffman-Goetz *et al.*, 1985).

Analytical isoelectric focusing of IL-1. Analytical isoelectric focusing (IEF) was performed on 5% T, 3% C polyacrylamide gels having a thickness of 2 mm and containing carrier ampholytes with a resultant pH range of 3·5–9·5 (UltroPag; LKB). The anolyte was 1 M H_3PO_4 and the catholyte was 1 M NaOH. The gels were run at constant power (15 W) until a maximum of 1100 V was reached, at which point they were run at a constant voltage. The runs lasted 4 h and were performed at approximately 4°C (surface gel temperature) on a LKB 2117 Multiphor flatbed electrophoresis apparatus. Afterwards, the gels were fixed in a solution containing 15% trichloroacetic acid (TCA) (30 min) and 50% methanol/10% acetic overnight. The gels were silver stained using the method of Dunbar (1984). Calibration of the gels was performed with the following pI standards: phycocyanin: 4.50; β-lactoglobulin B: 5.10; bovine carbonic anhydrase: 6.00; human carbonic anhydrase: 6.50; horse myoglobin: 7.00; whalemyoglobin: 8.05; α-chymotrypsin: 8.80; and cytochrome C: 9.60.

Samples of gel filtered rabbit IL-1 were applied to prefocused gels in 50 μl aliquots using filter paper applicators; the standard mixture (1 : 10 dilution) was similarly applied in 20 μl aliquots to the surface of the gel.

Peripheral blood mononuclear cells (PBMC) PBMC were obtained from rabbits using standard venapuncture technique by centrifugation on Ficoll-Hypaque (Sigma Chemical Co., St Louis, MO, USA) to remove erythrocytes and granulocytes. PBMC were washed twice with PBS, resuspended at 5×10^6 cells/ml in RPMI-1640 medium supplemented with 10% heat inactivated, fetal bovine serum, 2mM L-glutamine, and 100 μg/ml streptomycin. Cell viability was checked by trypan blue staining and monocyte numbers were estimated histochemically by nonspecific esterase staining.

Suppressor T cell (T_s) activity. T_s were induced and their activity measured as described by Shou, Schwartz and Good (1976). Briefly, PBMC of rabbits on the various diets were incubated with 1·5 μg/ml of Con A, Con A + rabbit IL-1 supernatants (1% final volume in culture) or medium alone, for 48 h at 39°C in 5% CO_2–95% air. Con A-exposed or medium-exposed cells were washed and treated with 50 μg/ml of mitomycin C (Sigma Chemical Co., St Louis, MO, USA) for 30 min to

inhibit further cell division. Cell suspensions were washed three times in PBS to remove excess mitomycin C and resuspended in RPMI-1640 at 1×10^6 cells/ml. The T_s activity was measured by incubating 100 μl of each cell population with 100 μl of fresh allogeneic lymphocytes (1×10^6 cells/ml) in triplicate in 96-well flat bottomed microculture plates (Linbro, Flow Labs, Toronto, Ontario, Canada) for 72 h at 39°C in 5% CO_2. Co-cultures received either 1 μg/ml of PHA (Sigma Chemical Co., St Louis, MO, USA) or an equal volume of medium vehicle. Cultures were pulsed with 0·5 μCi ^{3}H-thymidine—specific activity 2 Ci/mmol (New England Nuclear, Boston, MA, USA)—for the final 6 h of incubation. The ability of the mitomycin-treated, Con A-stimulated lymphocytes to inhibit the fresh response of responding lymphocytes to PHA was used as a measure of suppressor T cell activity. Data were expressed as ct/min using standard liquid scintillation technique.

Statistical analysis. T_s activity data were analysed by analysis of variance (ANOVA) for ct/min data using a square root transformation to stabilize the variance of the subgroups. Although data were analysed on a transformed scale, the results are presented on the original ct/min scale.

RESULTS

A representative gel following IEF of gel-filtered monocyte supernatants from protein malnourished and control rabbits is shown in Fig. 1. Samples showed numerous protein bands characteristic of the partial purification procedure. Nevertheless, a band having a pI of about 7·0 was observed in

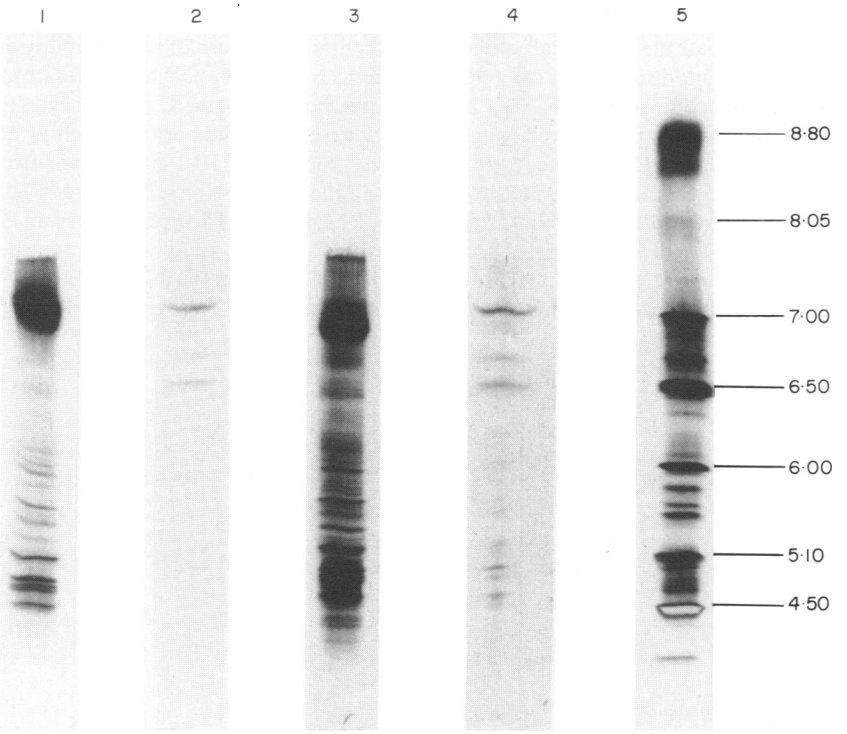

Fig. 1. Analytical IEF of partially purified rabbit monocyte supernatants. Samples from protein malnourished and control rabbits were applied to two adjacent lanes of an IEF gel (pH 3·5–9·5) by means of filter paper applicators (see Materials and Methods). Proteins of known pI were applied to a separate lane (5) in order to calibrate the pH gradient of the gel. (Lane 1) Crude monocyte supernatant sample from the control donors; (Lane 2) Gel filtered sample from the control donors; (Lane 3) Crude monocyte supernatant sample from the malnourished donors; (Lane 4) Gel filtered sample from the protein malnourished donors; (Lane 5) Standard mixture.

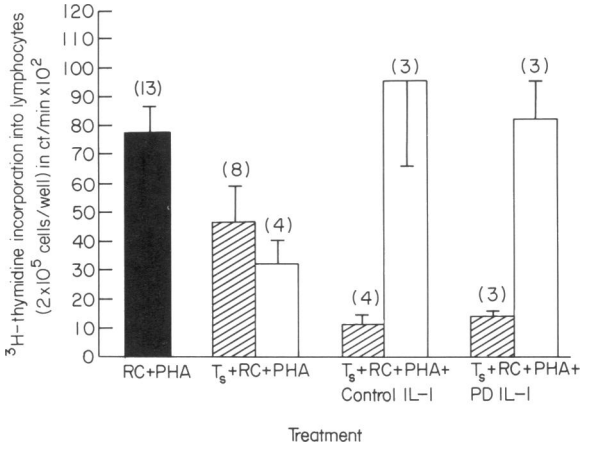

Fig. 2. The effect of partially purified IL-1 supernatants added at first culture on suppressor cell activity generated during Con A-induced proliferation. (RC) Fresh allogeneic responding lymphocytes; (T_s) suppressor cells of control and protein deprived (PD) donors. Values are group means ± s.e.; (*n*) sample size (number of rabbits tested per group). (□) Control T_s donor; (□) protein derived T_s donor.

both samples from protein deprived and control donors which is in agreement with the reported pI for purified rabbit IL-1 (Murphy, Simon & Willoughby, 1980).

In order to evaluate the functional activity of IL-1 containing supernatants, the ability of IL-1 to augment Con A-induced suppressor cell activity was measured in lymphocyte cultures from protein malnourished and control donors. Fig. 2 shows that both PD and C lymphocytes generate suppressor activity on Con A-induced lymphocyte proliferation. T_s activity was slightly, albeit not significantly, greater in PD compared with C cultures. Addition of IL-1 containing supernatants during control T_s generation (1st culture) enhanced the suppression of the fresh, allogeneic lymphocyte response to PHA. In contrast, IL-1 supplementation of lymphocytes from malnourished donors at 1st culture abrogated this suppressive activity. Analysis of variance showed significant IL-1 source ($P=0{\cdot}02$) and T_s source by IL-1 interaction ($P=0{\cdot}03$) effects.

In order to better characterize this phenomenon, suppressor cells were generated in response to

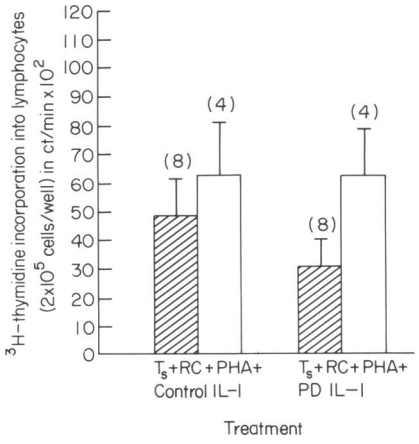

Fig. 3. The effect of partially purified IL-1 supernatants added at second culture on suppressor cell activity generated during Con A-induced proliferation. (RC) Fresh allogeneic responding lymphocytes, (T_s) suppressor cells of control and protein deprived (PD) donors. Values are group means ±s.e.; (*n*) sample size (number of rabbits tested per group). (□) Control T_s donor; (□) protein derived T_s donor.

Con A and co-cultured with allogeneic target lymphocytes and IL-1 containing supernatants from the two donor sources (2nd culture). There was a significant IL-1 source main effect ($P=0{\cdot}01$) and a significant T_s by IL-1 source interaction ($P=0{\cdot}004$) as observed with the 1st culture experiments. Fig. 3 shows that addition of IL-1 reduced, but did not eliminate, the capacity of control lymphocytes to suppress the fresh response to PHA. Addition of IL-1 to mixed lymphocyte cultures containing T_s from malnourished donors resulted in proliferative responses that were not different from baseline values (responding cells + PHA).

DISCUSSION

The results presented in this report suggest defects in the capacity of T-lymphocytes from protein malnourished rabbits to respond appropriately to exogenous IL-1. Thus, in addition to our earlier observations (Bell *et al.*, 1986) of enhanced immunosuppressive PGE_2 production in malnutrition, these present results point to functional defects in the T-lymphocyte.

Suppressor cell number and activity have been reported to be increased in protein-energy malnutrition in human and experimental animals (Chandra, 1983; Chandra *et al.*, 1982; Petro, 1985). Our data show slight, but not significant, increases in suppressor cell activity following Con A stimulation in malnourished compared with control donors. The reason for this lack of dietary effect on suppressor cell activity may be due to the small sample and large variance. Addition of partially purified monocyte supernatants to control T_s cultures enhanced the suppression of fresh, allogeneic lymphocyte responses to PHA. This finding is in agreement with studies on human T-lymphocytes showing that IL-1 or IL-1 containing monocyte supernatants augment the generation of suppressor T cells during Con A- or histamine-induced proliferation (Beer *et al.*, 1982a,b). It is interesting to note that the effect of IL-1 supernatants on the generation of control donor suppressor cells was of equivalent magnitude irrespective of whether the supernatants were from malnourished or control animals. This finding suggests that monocyte supernatants obtained from malnourished donors contain active IL-1. Addition of IL-1-containing supernatants during generation of suppressor cells in lymphocyte cultures obtained from the malnourished animals abolished the suppressive activity to fresh lymphocytes. Moreover, in the presence of IL-1-treated suppressor cells the proliferative response of fresh, responding lymphocytes to PHA was virtually unchanged compared with baseline proliferation. This finding implies a defective response of lymphocytes from protein malnourished donors to IL-1 rather than an inadequate availability of IL-1 in malnutrition. If defects in IL-1 synthesis by monocytes in protein malnutrition were present, then it would be reasonable to predict that Con A-induced suppressor activity generated by control lymphocytes would also be compromised.

Preliminary characterization of gel-filtered, partially purified monocyte supernatants showed the presence of a protein band with an isoelectric point of about 7·0 to 7·3, in agreement with values obtained by others (Murphy *et al.*, 1980; 1981; 1985). The molecular weight of this material, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis was 14,000–14,300 (L. Benjamin, personal communication), also in agreement with previously reported values for rabbit IL-1 (Dinarello, 1984). Samples obtained from both protein malnourished and control donors were observed to contain this protein band. Clearly though, these data do not present formal proof that malnourished rabbits produce IL-1 since these supernatants contain other biologically active materials which may comigrate with IL-1. Studies are in progress to further characterize this mol. wt 14,000, pI 7·0 protein by immunoblot techniques. Nevertheless, these data reinforce the hypothesis of altered T-lymphocyte responses to or handling of IL-1 in protein malnutrition.

No single mechanism underlies deficits in T-lymphocyte mediated immune responses observed in protein-energy malnutrition. The balance of immunoregulatory mediators and the capacity of T-lymphocytes to respond to these mediators are important elements in the complex process of T cell activation and clonal expansion. The results presented in this paper suggest that severe dietary protein deficiency in rabbits is associated with changes in the responsiveness of T-lymphocytes to interleukin-1.

The authors gratefully acknowledge the technical assistance of B. L. Kearns and L. Benjamin, and the secretarial support of E. Bruce. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

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