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Tumour necrosis factor production by IL-2-activated macrophages *in vitro* and *in vivo*

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SUMMARY

Purified human and murine monocytes can be activated *in vitro* by human recombinant IL-2 to secrete tumour necrosis factor (TNF). TNF mRNA is detected within 4 h of addition of IL-2. Signal transduction does not appear to require expression of the Tac peptide of the IL-2 receptor. IL-2 does, however, induce Tac mRNA and Tac peptide expression on peripheral blood monocytes (PBM). In addition to these *in vitro* studies, PBM retrieved from IL-2-treated cancer patients and mice have been shown to have a greatly increased capacity for TNF production. These studies identify a pathway for macrophage activation by IL-2 both *in vitro* and *in vivo*. They also suggest a mechanism for some of the immunoregulatory and toxic effects of IL-2 based immunotherapy regimens.

INTRODUCTION

Many of the regulatory and effector functions of macrophages are mediated by secreted polypeptides (Unanue & Allen, 1987). TNF is one such monokine with a broad range of immunoregulatory and effector functions (Old, 1985; Dinarello *et al.*, 1986; Ruddle, 1987, Beutler & Cerami, 1986–1987; Pennica *et al.*, 1987). Originally described as a factor that causes *in vivo* haemorrhagic necrosis of the meth A sarcoma in mice, TNF is now known to play a major role in monocyte-mediated cytotoxicity and is directly cytotoxic *in vitro* for a variety of transformed cell lines. In addition to its cytotoxic action, TNF is an important immunoregulatory molecule and a mediator of endotoxic shock. TNF can also activate neutrophils, endothelial cells, vascular smooth muscle cells and fibroblasts (Ruddle, 1987; Pober *et al.*, 1986a; Vogel *et al.*, 1987).

There is emerging evidence that certain macrophage functions may be modulated by IL-2. Macrophages treated *in vitro* with IL-2 have enhanced tumouricidal, microbicidal and respiratory burst activity (Malkovsky *et al.*, 1987; Wahl *et al.*, 1987). Monocytes can express IL-2 receptors (Tac) following *in vitro* activation with lipopolysaccharide and interferon-gamma; IL-2 interacting with these receptors may play a regulatory role in modifying monocyte function (Wahl *et al.*, 1987; Holter *et al.*, 1987). Addition of IL-2 directly to monocytes has been reported not to lead to Tac expression (Wahl *et al.*, 1987), and it is unclear what the role of this molecule is in IL-2-induced macrophage activation. It is also not clear whether some of the IL-2-induced functional changes in monocyte behaviour are due to TNF production. Nedwin *et al.*, (1985) have shown that addition of IL-2 to PBM cells *in vitro* leads to TNF synthesis, but a role for lymphocytes in the phenomenon was not excluded.

In this study, we demonstrate that human and murine macrophages can be activated both *in vitro* and *in vivo* to produce TNF. IL-2, acting directly on monocytes, was found to induce IL-2 receptor mRNA and peptide, but this receptor peptide does not appear to play an initiating role in TNF synthesis. As a result of our *in vitro* and *in vivo* studies we propose that some of the *in vivo* effects of IL-2 may in fact be mediated by TNF produced by macrophages, including some of the toxicity and/or anti-tumour activity associated with IL-2-based clinical immunotherapy regimens.

Abbreviations: PBM, peripheral blood monocytes: PMF, peritoneal macrophages.

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MATERIALS AND METHODS

Reagents

RPMI-1640 medium and Hanks' balanced salt solution (HBSS) were purchased from Flow Laboratories (MaClean, VA). Fetal calf serum (FCS) (screened for low endotoxin content) was purchased from Sigma Chemical Company (St Louis, MO) and used as a 20% supplement. Nutridoma, a defined additive for serum-free culture medium, was purchased from Boehringer-Mannheim (Indianapolis, IN) and used as a $\times 1$ concentrate in RPMI-1640. Penicillin, streptomycin and fungizone were added to both serum-containing and serum-free media. Recombinant human IL-2 and TNF were the generous gifts from the Cetus Corporation, Emeryville, CA. This IL-2 is pharmaceutical

grade and has a specific activity of 3×10^6 U/mg protein and contains no more than 0·05 ng endotoxin/mg protein as determined in the Limulus assay. Rabbit-anti-IL-2 (Amgen, Thousand Oaks, CA) was used at 1:100 dilution. Rabbit anti-TNF and rabbit anti-lymphotoxin (LT) (Endogen, Boston, MA) were both used at 1:400 dilutions. Polymixin B and *Escherichia coli* lipopolysaccharide (LPS) (No. 0111:84) were purchased from Sigma.

Isolation of human monocytes

Human peripheral monocytes were separated from heparinized whole blood by centrifugation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ). Washed cells were plated onto 24-well Costar plates at a density of $2{\cdot}5 \times 10^6$ cells/ml/well in RPMI-1640 + 20% FCS. The use of 20% FCS minimized lymphocyte adherence. After incubation at 37° for 45 min, non-adherent cells were vigorously washed off the monocyte monolayers with several changes of warm HBSS. The remaining adherent cells (averaging 2×10^5/well) were >98% pure monocytes as determined by immunoperoxidase staining using the monocyte/macrophage-specific anti-Leu-M3 monoclonal antibody (Becton-Dickinson, Sunnyvale, CA). Monocyte monolayers were then cultured in serum-free medium. Polymixin B (10 μg/ml, which binds to LPS) was added to cultures to offset the effects of any possible contaminating endotoxin in these cultures. PBM were incubated in some experiments with a murine monoclonal antibody to the IL-2 (Tac) receptor (gift of Dr T. A. Waldman, NIH, Bethesda, MD) at a final dilution of 1:500.

Large numbers of purified monocytes were required for RNA studies and were obtained from Red Cross buffy coat packs using centrifugal counterflow elutriation. From 50 to 80×10^6 PBM with purity >98% were routinely obtained from two packs.

TNF assay

TNF concentrations were measured in an 18-hr cytotoxicity assay employing ^{51}Chromium-labelled, actinomycin D-treated murine L929 (American Type Culture Collection, Bethesda, MD) cells in a modification of previously described procedures (Flick & Gifford, 1984). Twenty million murine L929 fibroblasts in log growth phase were retrieved by trypsinization, washed three times in culture medium, and chromated with 0·25 mCi Na $^{51}CrO_4$. The murine L929 fibroblasts were then washed three times in medium and plated in U-bottomed 96-well microtitre plates at a final concentration of 5×10^4 cells/well/0·2 ml in the presence of actinomycin D at 5 μg/ml in RPMI-1640 + 10% FCS. After 18 h in a humidified 5% CO_2 incubator, the plates were centrifuged on platforms at 700 ***g*** for 5 min to firmly pellet the L929 cells, and 0·1 ml of supernatant was retrieved and counted in a gamma counter. The percentage of specific cytotoxicity was calculated using the following equation:

$$\frac{\text{experimental counts} - \text{spontaneous counts}}{\text{total counts} - \text{spontaneous counts}} \times 100.$$

A TNF dose–response curve using recombinant TNF (Cetus, Emeryville, CA) was run with each assay. Rabbit polyclonal antisera to human recombinant TNF and human recombinant lymphotoxin (LT) (Endogen, Boston, MA) were used at a final dilution of 1:400. The neutralizing titre of this antiserum was 5–10×10^6 U/ml.

Murine studies

Normal C3Hf/Sed/Kam (bred at UCLA) received i.p. injections of IL-2 (5×10^4 U twice daily (BID) for 3 days). Mice were killed 16 hr after the last injection. Murine PBM were isolated as described for PBM. Peritoneal macrophages (PMF) were isolated by peritoneal lavage with HBSS and purified by adherence under conditions that were identical to those for human PBM. These cells were cultured for 24 hr with no additional LPS (1 μg/ml) or IL-2 (10^3 U/ml). Cell-free supernatants were assayed for TNF.

Clinical studies

Six cancer patients were treated with 15,000 U/kg IL-2 (Cetus) as bolus infusions three times a day for two consecutive days as part of a UCLA Human Subject Protection Committee-approved protocol. These patients had the following diseases: Patients 1 and 5 primary lung adenocarcinoma; Patients 2, 4 and 6, primary renal cell carcinoma; and Patient 3, metastatic synovial cell sarcoma to the lung. PBM were isolated at various intervals and cultured for 24 hr in the presence or absence of LPS (10 μg/ml) and cell-free supernatants assayed for TNF.

Flow cytometry

For studies of IL-2 (Tac) receptor expression, PBM were isolated by centrifugal counterflow elutriation (Beckman, Palo Alto, CA) and cultured in polypropylene tubes (2×10^5/ml) in RPMI supplemented with 1% Nutridoma and polymyxin B (10 μg/ml). These measures substantially prevented adherence and largely reduced possible exposure to low levels of contaminating endotoxin. Cells were stained with fluoresceinated anti-Tac (Becton-Dickinson) monoclonal antibody in the presence of 5% heat-inactivated normal rabbit serum to inhibit Fc receptor binding, and analysed using an Ortho Cytofluorograph (Westwood, MA).

Slot blot hybridization

RNA hybridization were conducted essentially as described by Maniatis *et al.* (1983). PBM were isolated from buffy coat packs (American Red Cross, Los Angeles, CA) using centrifugal counterflow elutriation. PBM were >98% pure by immunperoxidase staining, as described. RNA was extracted using the guanidinium–CsCl method and 5 μg and 1 μg whole cellular RNA slotted onto Biotrans nylon membranes using a slot-blot apparatus (Bethesda Research Labs, Bethesda, MD). After baking *in vacuo* at 80° for 2 hr, membranes were prehybridized at 42° for 6–12 hr in a prehybridization buffer (50% formamide, 5 × SSC, 5 × Denhardt's, 0·6 mg/ml sheared DNA, 5% Dextran SO_4, 0·5% SDS). Hybridization (50% formamide, 5 × SSC, 5 × Denhardt's, 0·1% SDS, 1 mM EDTA, 10% dextran sulphate, 0·2 mg/ml sheared DNA) with ^{32}P-cDNA probes was conducted for 12–16 hr at 42° in a shaking water bath. Posthybridization washes consist of 2 × SSC/0·1% w/v SDS for 3 min at room temperature twice and 0·2 × SSC/0·1 w/v SDS for 15 min at 65° twice. Autoradiography was carried out at −70° for 1–3 days using Kodak XAR-5 film and Cronex intensification screens.

The human TNF cDNA probe (Pennica *et al.*, 1984) is an 800 bp EcoR1 fragment full-length coding region on the Sp64 cloning vector, which was the generous gift of the Genentech Corporation, So. San Francisco, CA. The human IL-2 (Tac) receptor cDNA probe (Nikaido *et al.*, 1984) is a 400 bp Pst 1 fragment carried in pBR322 and was the gift of Dr T. Honjo,

Kyoto University. The beta actin cDNA probe (Ponte *et al.*, 1983) is a 700 bp fragment (Bam H1/Eco R1) obtained from the plasmid pHFBA-3ut and was the gift of Dr P. Koeffler, UCLA. Plasmids were isolated chromatographically and inserts purified after complete restriction endonuclease digestion by electroelution and nicktranslated using a kit (Bethesda Research Lab.) and ^{32}P-CTP (Amersham, Arlington Heights, IL). The specific activity was $> 10^8$ c.p.m/μg DNA. At least 10^6 c.p.m./ml boiled probe in hybridization solution was used.

RESULTS

Human studies

Highly purified human PBM were cultured *in vitro* for 24 h in the presence of recombinant IL-2. IL-2-activated monocytes produce a cytotoxin in a dose-dependent fashion (Fig 1). These results are representative of over 20 experiments. This cytotoxin was completely neutralized by a rabbit xenoantiserum specific for TNF (Table 1). Murine L929 cells are also sensitive to lymphotoxin (LT), but an antiserum to this lymphokine had no effect on the cytotoxic activity of any of these monocytes

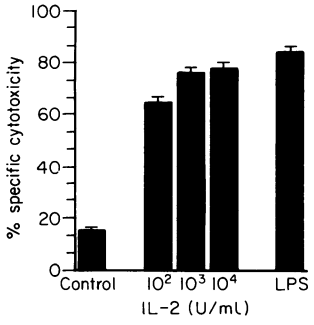

Figure 1. Production of TNF by IL-2-activated PBM. Purified PBM were cultured *in vitro* for 24 hr in the presence of IL-2, LPS or no addition (control). Cell-free supernatants were assayed in the L929 assay.

Table 1. Serological identity of cytotoxin produced by IL-2-activated human PBM

Addition to monocyte cultures*	Antiserum addition to supernatants tested in L929 assay	% cytotoxicity
None	—	4·0±0·2
IL-2 10^3 U/ml	—	11·9±1·9
10^3 U/ml	Ra-a-TNF	2·8±1·7
10^3 U/ml	Ra-a-LT	11·2±5·7
LPS 10 μg/ml	—	82·0±1·9
10 μg/ml	Ra-a-TNF	3·4±2·1
10 μg/ml	Ra-a-LT	66·4±5·2

*Monocytes were incubated *in vitro* for 24 hr with IL-2, LPS or no addition. Cell-free supernatant were assayed in the L929 cytotoxicity assay. Some supernatants aliquots were assayed in the presence of neutralizing rabbit antisera to either TNF (Ra-a-TNT) or lymphotoxin (Ra-a-LT).

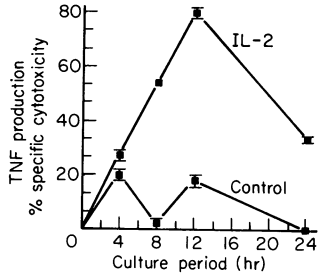

Figure 2. Kinetics of TNF production of IL-2-activated human PBM. PBM were cultured in the presence of 10^4 U/ml IL-2 for indicated time periods.

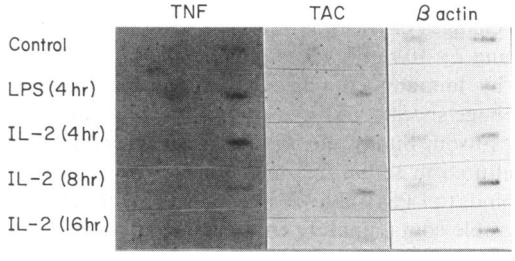

Figure 3. TNF and TAC mRNA expression by IL-2-activated PBM. PBM were incubated in suspension cultures with no addition, IL-2 (10^4 U/ml) or LPS (1 μg/ml) for the indicated time periods. Whole cellular RNA was extracted and 1 μg (left side) and 5 μg (right side) slotted onto nylon membranes. Membranes were hybridized with ^{32}P-cDNA probes for TNF, TAC or beta actin as indicated. Membranes were developed on film for 24 (beta actin) or 72 (TNF, TAC) hr.

supernatants. The kinetics of IL-2-induced TNF production was examined; TNF was detected as early as 8 hr and was declining by 24 hr (Fig. 2). The activating effect of IL-2 was 87%, inhibited by a rabbit xenoantiserum to IL-2 (used at a 1:100 dilution and incubated with IL-2 for 24 hr at 4° prior to addition to monocyte cultures). This observation, coupled with our use of polymyxin B in these serum-free cultures, argues against contaminating endotoxin as being responsible for these effects.

We next examined the kinetics of TNF mRNA induction by IL-2-activated PBM using RNA slot-blot hybridization. Normal human PBM were isolated from buffy coat packs by centrifugal counterflow elutriation and incubated for various time periods in polypropylene tubes using serum-free, polymyxin-containing culture medium. These measures retarded monocyte adherence, which is known to induce TNF mRNA (but not TNF protein secretion) (Haskill *et al.*, 1988). PBM were incubated with LPS (10 μg/ml or IL-2) (10^4 U/ml) for various intervals and whole cellular RNA extracted using the guanidinium–CsCl method. Five and one microgams of RNA were slotted on to nylon membranes and hybridized with ^{32}P-cDNA probes for TNF and beta actin, respectively, the latter acting as an additional control for RNA content. A representative autoradiograph is presented in Fig. 3. As expected, LPS was a potent inducer of TNF mRNA. Likewise, with IL-2 there was a strong signal for TNF with peak mRNA expression at 4 hr and a reduction by 8 and 16 hr. These results coincide nicely with the kinetics of TNF protein production.

Table 2. Expression of the IL-2 (Tac) receptor by human PBM*

Addition to monocyte cultures	% cells expressing the IL-2 (Tac) receptor
Control	5·3
IL-2 (10^4 U/ml)	28·4
LPS (10 μg/ml)	47·1

*Highly purified PBM were cultured in polypropylene tubes and expression of the IL-2 (Tac) receptor was assessed by cytofluorometry, as described in the Materials and Methods, after 24 hr of culture.

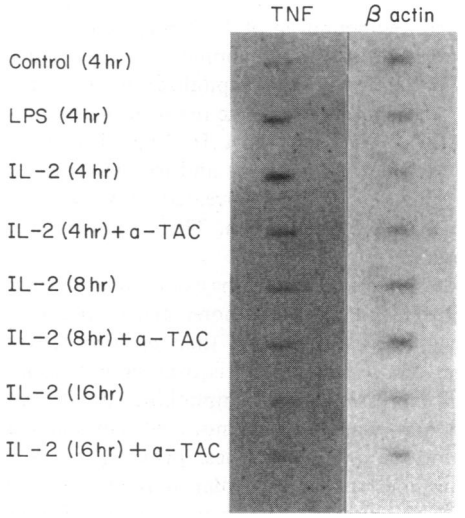

Figure 4. TNF mRNA expression by IL-2-activated PBM in the presence of monoclonal anti-TAC antibody. PBM were incubated in suspension culture for indicated time periods with LPS (10 μg/ml), IL-2 (10^4 U/ml) and/or monoclonal-a-Tac antibody (1:500 dilution). Five micrograms whole cellular RNA were slotted onto nylon membranes and hybridized with ^{32}P-cDNA probes for TNF and beta actin. Membranes were developed on Kodak film for 24 hr.

IL-2 interacts with cells through a membrane-associated IL-2 receptor (IL-2R), which is now known to be represented by Tac and p70 subunits (Bich-Thuy *et al*; 1987). We were interested in defining the role of the IL-2R in monocyte activation. Freshly isolated PBM do not express detectable Tac peptide; unfortunately, no antisera are currently available to detect the p70 peptide. PBM incubated for 24 hr in suspension culture with IL-2 were induced to express the Tac peptide, as assessed by flow cytometry (Table 2). Almost 1/3 of PBM stained for Tac after 24 h of culture in the presence of IL-2. As previously reported by Holter *et al.* (1987), LPS was a strong induction signal. As shown in Fig. 3, IL-2 induced Tac mRNA as early as 4 hr after culture, with peak expression at about 8 hr. RNA studies also showed that TNF mRNA expression was not influenced by the presence of a monoclonal anti-Tac antibody (Fig. 4). This anti-Tac antibody at this dilution effectively

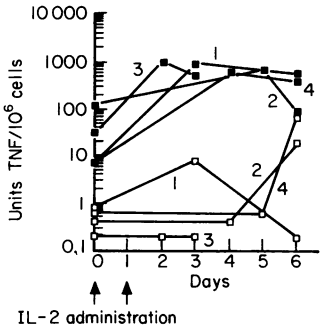

Figure 5. TNF production by PBM from IL-2-treated cancer patients. Four cancer patients (nos 1–4) received i.v. bolus IL-2 (Cetus) on Days 0 and 1 (15,000 U/kg BID for 2 days). PBM were retrieved and cultured *in vitro* in the presence (solid box) or absence (open box) of LPS (1 μg/ml). After 24 hr of culture, cell-free supernatants were assayed for TNF.

Table 3. IL-2 activation of murine PBM and PMF *in vitro* and *in vivo*

Cell type	Retreatment regimen*	Units TNF/ml *In vitro* addition† Control	IL-2	LPS
PBM	Saline	4·0±0·49	8·0±1·2	32·1±9·5
PBM	IL-2	6·2±0·98	16·0±1·04	>160
PMF	Saline	3·4±0·25	3·5±1·02	6·1±1·22
PMF	IL-2	2·7±0·40	3·2±0·46	>160

*Groups of five normal C3Hf/Sed/Kam mice received 5×10^4 U IL-2 (Cetus) i.p. twice daily for 3 days.

†PBM and PMF were retrieved and cultured *in vitro* for 24 hr in the presence of IL-2 (10^3 U/ml), LPS (1 μg/ml) or no addition (control). Cell-free supernatants were assayed for TNF.

inhibited IL-2-driven proliferation of peripheral leucocytes and inhibited the induction of LAK cells (data not shown). These results suggest that TNF mRNA induction by IL-2 does not require Tac expression by human PBM.

In addition to these *in vitro* studies, we had the opportunity to study monocyte function in several cancer patients receiving IL-2 therapy. Six patients with lung or kidney tumours received a course of IL-2 given by systemic bolus infusion (15,000 U/kg TID for 2 days). PBM were obtained at intervals before, during and after IL-2 infusion and their ability to elaborate TNF in subsequent 24-hr cultures (with or without LPS) was measured. The results of Patients 1–4 are presented in Fig. 5. Cells from these four patients showed a marked augmentation (4–315-fold) of LPS-stimulated TNF production (closed boxes) following IL-2 infusion, with peak titres around Days 2–4. In three patients, enhanced basal TNF production by PBM (not exposed to LPS) was noted (open boxes). This peak seemed to be somewhat delayed compared to PBM activated with LPS. Patients 5 and 6 did not exhibit enhanced TNF production in the presence or absence of LPS.

Murine studies

Parallel studies performed in mice confirmed that IL-2 given *in vitro* or *in vivo* could stimulate macropahges towards TNF production. Normal C3Hf/Sed Kam mice were injected i.p. with 5×10^4 U IL-2 twice daily for 3 days. Their PBM and peritoneal macrophages (PMF) were isolated and cultured for 24 hr in the presence of LPS (1 μg/ml) or IL-2 (10^3 U/ml). Cell-free supernatants were assayed for TNF. The results of one such experiment are shown in Table 3. Both PBM and PMF from IL-2-treated mice were more responsive to *in vitro* stimulation with IL-2 and LPS than controls. *In vitro* addition of IL-2 to PBM from normal or IL-2-treated mice enhanced TNF production, an effect that was not seen with PMF.

DISCUSSION

Macrophages play a central regulatory and effector role in immunity and inflammation. Through the phenomenon of 'macrophage activation', these cells acquire a broad range of functions—enhanced phagocytic activity, production of free radicals, expression of class II major histocompatibility antigens, expression of Fc receptors and the synthesis and release of monokines such as TNF and IL-1. With the acquisition of these properties, macrophages can greatly influence immunological networks (Unanue & Allen, 1987).

TNF is an important macrophage-derived protein having a broad range of regulatory and inflammatory properties. TNF is not produced constitutively and the gene may be activated by a variety of macrophage activation signals such as interferon-gamma (Beutler *et al.*, 1986) or bacterial LPS. It is now becoming clear that macrophage function is also influenced by IL-2 (Malkovsky *et al.*, 1987; Nedwin *et al.*, 1985; Holter *et al.*, 1987; Numerof *et al.*, 1987).

A necessary requirement for IL-2 activation of macrophages would presumably be the expression of an IL-2 receptor (IL-2R) on the cell surface. It is now known that the high affinity IL-2R on T cells is a bimolecular complex composed of the Tac and p70 subunits (Bich-Thuy *et al.*, 1987). The p70 subunit has a large intracytoplasmic domain and several lines of evidence suggest that signal transduction is directed through this peptide. Unfortunately, no antisera or molecular probes are currently available for this subunit. We elected to examine the possible role of the Tac peptide on monocyte activation. Human PBM do not normally express the Tac receptor but Holter *et al.* (1987) have demonstrated that it can be induced *in vitro* by LPS, interferon-gamma or adherence to plastic. They could not demonstrate that IL-2 induced this peptide, which is at variance with our findings. This discrepancy is most likely due to differences in culture conditions. The PBM used in our studies were cultured in polypropylene tubs (to prevent adherence) and in a serum-free, endotoxin-free culture medium. These measures kept Tac expression by control PBM at 24 hr at about 5% in the presence of IL-2; 28% of PBM expressed Tac. In contrast, almost 25% of the control PBM cultured for 24 hr in the Holters, *et al.* (1987) study expressed Tac, perhaps masking any IL-2 effect.

Our studies clearly indicate the IL-2 can induce TNF mRNA very rapidly. This induction is not inhibited by anti-Tac antiserum and occurs concurrently with Tac mRNA production. We suspect that signal transduction is, in fact, being mediated by another receptor, possibly the p70 subunit. This is certainly plausible since the p70 subunit of the IL-2 receptor seems to be responsible for signal transduction in other cell types. Future availability of antisera to this subunit will permit a more precise dissection of the mechanism of activation.

Several studies have shown that other macrophage properties can be modulated by IL-2. Malkovsky *et al.* (1987) demonstrated that IL-2-treated PBM have increased non-specific tumouricidal activity against a cultured tumour cell line. Holter *et al.* (1987) demonstrated that IL-2-activated PBM have augmented production of H_2O. Our studies support the fact that IL-2 can directly activate monocytes, and extend these findings to TNF production. In addition, they provide some mechanistic insight into the triggering of monocyte activation by IL-2.

These *in vitro* observations with IL-2 may have *in vivo* correlates as well. Our studies of peripheral monocyte function in IL-2-treated cancer patients demonstrated a dramatically augmented ability of PBM to be activated to TNF production. Not only was spontaneous TNF production enhanced in three of six patients, but LPS-triggered monocytes displayed an even more marked augmentation of TNF production in four of six patients. This IL-2 dosage regimine is not particularly toxic (only one patient required hospitalization for capillary-leak syndrome) and employs a dosage regimen that is substantially less than those given in most IL-2-based immunotherapy protocols. Peripheral monocytes and to a lesser extent peritoneal macrophages, from IL-2-treated mice were also more responsive for TNF production. These findings support our clinical observations.

We have been impressed by the extent of *in vivo* macrophage activation by IL-2. TNF production is at least an order or two in magnitude greater after *in vivo* than *in vitro* activation. We suspect that our *in vitro* model is over-simplified; it does not permit a full expression of the 'lymphokine cascade' mechanism. This cascade, in which one or more cytokines activate target cells to produce other cytokines, provides for a very real amplification mechanism in cellular immunity. Such a cascade may explain the discrepancies in the kinetics of *in vitro* IL-2-induced TNF production between Nedwin *et al.* (1985), who used unseparated peripheral mononuclear cells, and our results with purified macrophages.

In addition to clarifying an important immunoregulatory pathway, these studies may explain some of the toxic side-effects or perhaps even one mechanism of the anti-tumour effect of IL-2-based immunotherapy regimens (Lotze *et al.*, 1986, 1987; LaFreniere & Rosenberg, 1985; Mule *et al.*, 1986). IL-2 treatment in humans of sufficient dose and duration is associated with toxic side-effects that include renal, hepatic and pulmonary dysfunction due to a capillary-leak syndrome. These side-effects are reminiscent of the systemic effects of TNF. Accordingly, TNF may be responsible for some of the toxic side-effects of IL-2 administration. Although the anti-tumour effects of IL-2 administration are thought to be due to lymphokine-activated killer cells, IL-2-activated macrophages might also make some contributions. Additional studies are obviously needed to further clarify the role of IL-2-activated macrophages in patients receiving this form of therapy.

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