DOWNREGULATION OF TUMOR NECROSIS FACTOR RECEPTORS ON MACROPHAGES AND ENDOTHELIAL CELLS BY MICROTUBULE DEPOLYMERIZING AGENTS

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TNF-α is a potent mediator of inflammation (1, 2). Its actions are believed to result from binding to a single class of receptors (TNF-αR) on the surface of a wide variety of cells (3–6). In principle, agents that affect the number or affinity of TNF-αR, such as IFN-γ (7–9), LPS (10), or activators of protein kinase C or A (11–14), may modulate some of TNF-α's biologic effects.

Among antineoplastic and nonsteroidal antiinflammatory drugs are compounds, such as colchicine, nocodazole, vincristine, vinblastine, and podophyllotoxin, which bind tubulin and inhibit its polymerization. Disassembly of cytoplasmic microtubules (MT)[1] by these agents causes mitotic arrest in dividing cells, suggesting a mechanism of action for their antineoplastic effects. It is less clear how colchicine exerts antiinflammatory actions in conditions as diverse as gout, familial Mediterranean fever, cirrhosis, pericarditis, and Behcet's disease. Possible mechanisms include inhibition of the ability of polymorphonuclear leukocytes to migrate (15), degranulate (16), or translocate 5-lipoxygenase (17). We have observed that exposure of murine and human macrophages and human umbilical vein endothelial cells to micromolar concentrations of five different MT depolymerizing agents resulted in a rapid decrease in surface TNF-αR. MT appeared to be required either for the synthesis of TNF-αR or for their translocation to the plasma membrane. This effect may contribute to the antiinflammatory actions of MT depolymerizing agents.

Materials and Methods

Materials. Human rTNF-α (4.1 $\times$ 10^7 U/mg protein), murine rINF-γ (5.2 $\times$ 10^7 U/mg protein) and antiserum 15E against murine TNF-α (6.4 $\times$ 10^5 neutralizing units/ml) were kindly provided by Genentech, Inc. (South San Francisco, CA). Na^{125}I (carrier-free) was from the Radiochemical Center, Amersham, UK. Thioglycollate broth (Brewer) was obtained from Difco Laboratories (Detroit, MI). The following were from Sigma Chemical Co. (St. Louis, MO): colchicine, β-lumicolchicine, γ-lumicolchicine, nocodazole, vinblastine, vincristine, podophyllotoxin, cytochalasin B, dihydrocytochalasin B, cytochalasin E, PGE$_1$, dibutyryl-cyclic adenosine monophosphate, indomethacin, ibuprofen, acetylsalicylic acid, and cycloheximide.

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[1] *Abbreviation used in this paper:* MT, microtubule.

Cell Culture. Murine macrophages were washed from the peritoneal cavities of female CD1 mice (Charles River Breeding Laboratories, Wilmington, MA). Where indicated, mice were injected intraperitoneally with 2 ml of thioglycollate broth (Brewer, 4%) 4 d earlier as described (18). Adherent monolayers were obtained by plating 8×10^5 cells per well in 24-well plastic trays or 2×10^5 cells per well in 96-well plastic trays in RPMI 1640 (KC Biological, Inc., Lenexa, KS) containing 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT). Monolayers were maintained at 37°C in 5% CO_2/95% air for 1–4 d before the experiments. Human macrophages were allowed to mature from peripheral blood monocytes in Teflon beakers as described (19). Second-passage human umbilical vein endothelial cells were the gift of Dr. Eric Jaffe (Cornell University Medical College, New York, NY) and were prepared as described (20). Human polymorphonuclear leukocytes (PMN) were isolated from heparinized venous blood on Neutrophil Isolation Medium (Los Alamos Diagnostics, Los Alamos, NM) as described (21).

Iodination of rTNF-α and rIFN-γ. Human rTNF-α and murine rIFN-γ were iodinated according to the method of Aggarwal et al. (22), and stored at 4°C for no longer than 6 wk. In brief, 100 μl of 0.1 M phosphate buffer (pH 7.4) was added to a glass tube coated with 5 μg of Iodogen (Pierce Chemical Co., Rockford, IL) and incubated with 1 mCi of $Na^{125}I$ for 10 min at 4°C. The mixture was then transferred to a tube containing 10 μg of rTNF-α or rIFN-γ for 10 min at 4°C. The reaction was stopped by addition of one drop of 1 M KI. Iodinated material was separated from free iodide by filtration through a PD-10 column (Pharmacia Fine Chemicals, Piscataway, NJ) that had been equilibrated with 0.1 M phosphate (for rTNF-α) or 20 mM Tris buffer (for rIFN-γ), both containing 0.1% gelatin, pH 7.4. ^{125}I-rTNF-α had a specific activity of ~300 Ci/mmol monomer, and migrated as a single band with an apparent molecular mass of ~16 kD on an 11% polyacrylamide gel under non-reducing conditions. ^{125}I-rIFN-γ had a specific activity of ~100 Ci/mmol monomer. Both cytokines retained their ability to activate resident mouse peritoneal macrophages for enhanced H_2O_2 release (18).

Receptor Binding Assay. Cell monolayers in 24-well trays were overlaid with 0.3 ml per well of binding buffer (HBSS containing 10% FCS) and incubated with indicated concentrations of ^{125}I-rTNF-α in the presence or absence of a 100-fold excess of unlabeled rTNF-α. After 3–4 h at 4°C, binding buffer was removed and the monolayers rinsed four times with ice cold 0.9% NaCl. Cells were solubilized in 0.3 ml of 0.3 N NaOH and radioactivity determined in a gamma counter (Packard Instrument Co., Downers Grove, IL). Binding to human PMN in suspension was carried out as described (10). Nonspecific binding was <15% of the total and was subtracted to calculate specific binding. When the effects of drugs were examined, the cells were incubated with the drugs for 1 h at 37°C before the binding assay.

To assay IFN-γ receptor (IFN-γR), the above method was modified to reduce sticking of radiolabeled rIFN-γ to plastic: (*a*) Binding was carried out at room temperature instead of 4°C. (*b*) Binding buffer was changed to RPMI 1640 containing 50 mM Hepes, 10 mM NaN_3, and 1% (wt/vol) BSA. (*c*) Cell-bound ^{125}I-rIFN-γ was eluted with 50 mM glycine in saline, pH 3, at 4°C for 5 min (in contrast, ^{125}I-rIFN-γ bound to plastic can be eluted with NaOH). Nonspecific binding was <10% of the total, and was subtracted to calculate specific binding.

Hydrogen Peroxide Release. H_2O_2 secretion was measured fluorometrically by the horseradish peroxidase–catalyzed oxidation of fluorescent scopoletin to a nonfluorescent product, as described in detail (23). The protein content of cells adherent at the start of the assay in each well was measured by the method of Lowry et al. (24) with BSA as a standard. Specific release was calculated after subtracting H_2O_2 and protein values from the cell-free wells.

Measurement of LPS. LPS contamination of reagents was monitored by a chromogenic limulus amebocyte lysate test (Whittaker Bioproducts, Inc., Walkersville, MD) with a sensitivity of ~10 pg/ml.

Results

Decreased ^{125}I-rTNFα Binding to Macrophages after Preincubation with MT Depolymerizing Agents. It was established previously that murine macrophages bind ^{125}I-labeled

human rTNF-α specifically and reversibly (10). Preexposure of macrophages to 3 μM nocodazole for 1 h at 37°C inhibited the subsequent binding of ^{125}I-rTNF-α at 4°C (Fig. 1). Scatchard analysis of the data indicated that nocodazole affected the numbers of receptor sites rather than their affinity (Fig. 1, *inset*). TNF-αR of human macrophages were reduced in a similar fashion in response to colchicine in two experiments (not shown). Mouse macrophages were used to characterize this effect further.

Pretreatment with three other MT depolymerizing agents, vincristine, vinblastine, and podophyllotoxin, also diminished subsequent ^{125}I-rTNF-α binding in a concentration-dependent manner (Fig. 2). In contrast, β- and γ-lumicolchicine, which do not depolymerize MT, did not suppress binding of ^{125}I-rTNF-α. The microfilament-disrupting agents cytochalasin B, dihydrocytochalasin B, and cytochalasin E had little or no effect (Table I). As a control, the effect of colchicine on IFN-γR, another cytokine receptor on the macrophage surface, was tested under the same conditions. Binding of rIFN-γ was not affected by colchicine or nocodazole (Table I). This suggested that the effects of colchicine and nocodazole on TNF-αR did not result from nonspecific toxicity.

Downregulation of TNF-αR by MT-depolymerizing agents depended on temperature. In contrast to results at 37°C, preexposure of macrophages to 10 μM colchicine or 10 μM nocodazole at 4°C caused no detectable change in ^{125}I-rTNF-α binding. Nor did colchicine or nocodazole compete with TNF-α binding sites at 4°C (not shown).

Recovery of TNF-αR after exposure to MT-depolymerizing agents correlated with the reversibility of their binding to tubulin. After a 1-h exposure to 10 μM colchicine or 3 μM nocodazole, macrophages were incubated in drug-free medium for various
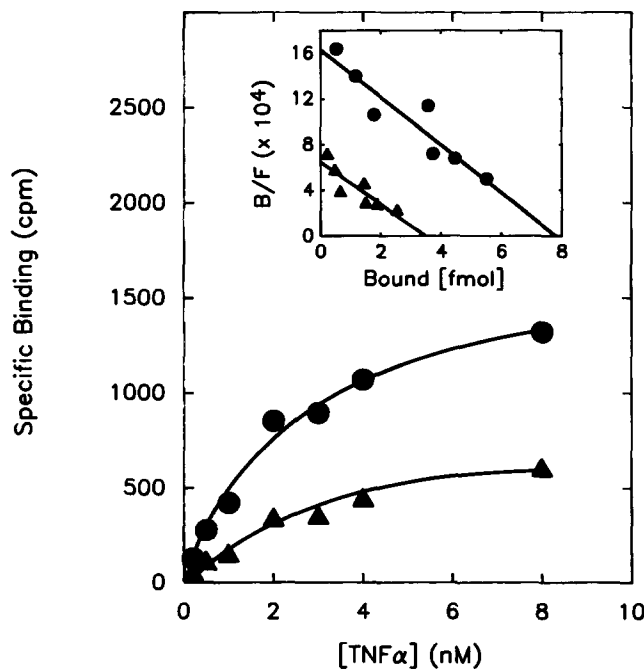

Figure 1. Decreased surface TNF-αR after treatment with nocodazole. Indicated amounts of ^{125}I-rTNF-α were added to murine peritoneal macrophages pretreated with (▲) or without (●) 3 μM nocodazole for 1 h at 37°C. Binding was assayed as described. (*Inset*) Scatchard analysis of the equilibrium binding data. The results are means for triplicates.
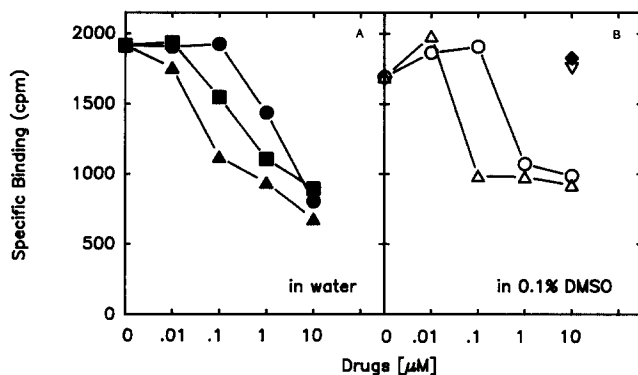
FIGURE 2. Concentration-dependent inhibition of ^{125}I-rTNF-α binding to macrophages by preincubation with different MT depolymerizing agents. Indicated concentrations of vincristine (■), vinblastine (▲), colchicine (●), nocodazole (○), podophyllotoxin (△), β-lumicolchicine (▽), or γ-lumicolchicine (◆) were incubated with macrophages for 1 h at 37°C in the absence (A) or presence (B) of 0.1% DMSO, used as a vehicle for the water-insoluble agents. The binding assay was then performed at 4°C. The results are representative of five separate experiments, and are means for triplicates (SEM <5%).

periods before the ^{125}I-rTNF-α binding assay (Fig. 3). Colchicine binds irreversibly to tubulin (25). The ability of macrophages to bind rTNF-α continued to decrease for at least 4 h after a 1-h exposure to colchicine, by which time binding capacity had decreased 75%. 18 h after the 1-h exposure to colchicine, the capacity of the cells to bind rTNF-α was still suppressed by 46%. Nocodazole binds reversibly to tubulin (26); its effect on binding of rTNF-α was 50% reversed by 1 h after its removal.

Effect of Antibody Against Murine TNF-α.   Since macrophages secrete TNF-α, it was possible that MT-depolymerizing agents downregulated TNF-αR by triggering endogenous TNF-α release, leading to internalization of ligand-receptor complexes. To test this, we made use of a neutralizing antiserum (15E) against murine TNF-α. When present during the pretreatment of the cells with colchicine or nocodazole, 15E (sufficient to neutralize 1000 U/ml TNF-α) did not prevent the subsequent decrease in ability of the cells to bind exogenous rTNFα (Table II). As a positive con-

TABLE I

Effect of Cytoskeleton Disrupting Agents on TNF-αR or IFN-γR

Reagents*	Concentration	Specific binding[†] by	
		rTNF-α	rIFN-γ
		cpm	
–	–	967 ± 22 (100)[§]	991 ± 84 (100)
Cytochalasin B	5 μg/ml	994 ± 50 (103)	ND
Dihydrocytochalasin B	5 μg/ml	946 ± 19 (98)	ND
Cytochalasin E	5 μg/ml	816 ± 57 (84)	ND
Colchicine	10 μM	470 ± 25 (49)	1,011 ± 74 (102)
Nocodazole	10 μM	399 ± 17 (41)	971 ± 97 (98)

* Each reagent was incubated with macrophages at 37°C for 1 h before the TNF-αR assay.
[†] Means ± SE for triplicates from one of three similar experiments.
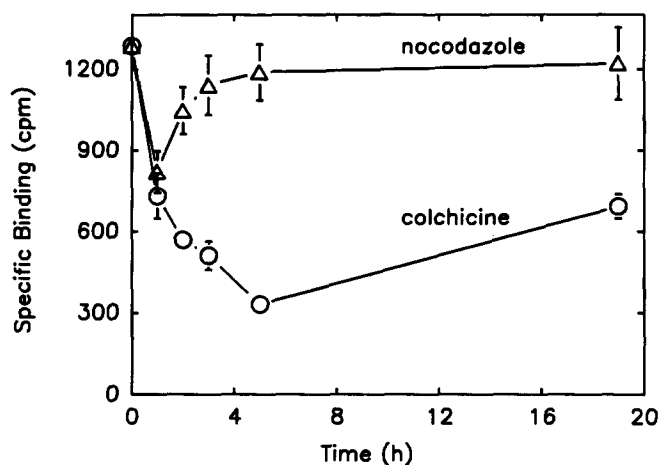[§] Numbers in parentheses represent percent of control.

FIGURE 3. Recovery of TNF-α binding sites on macrophages after pulsing for 1 h at 37°C with 10 μM colchicine (O) or 3 μM nocodazole (Δ). The time represents the duration between addition of drugs and the start of the binding assay. The results are means $\pm$ SE of triplicates. Where error bars are not seen, they fall within the symbols.

trol, in studies to be reported elsewhere (Ding, A. H., F. Porteu, E. Sanchez, and C. F. Nathan, manuscript submitted for publication), antibody 15E did block downregulation of TNF-αR in response to a drug that elicits TNF-α release (not shown).

Role of the Cyclooxygenase Pathway. Colchicine has been reported to induce the synthesis of cyclooxygenase products, leading to an increase in the intracellular level of cAMP (27, 28). To test whether this pathway was involved in the colchicine-induced downregulation of TNF-αR, macrophages were exposed to PGE$_1$ or dibutyryl-cAMP, a lipid soluble analog of cAMP, for 1 h at 37°C, and then the binding of ^{125}I-rTNF-α was assessed. Neither of these agents decreased ^{125}I-rTNF-α binding (Table III). In fact, high concentrations of PGE$_1$ or dibutyryl-cAMP had the opposite effect. Moreover, the cyclooxygenase inhibitors indomethacin (10^{-7} to 10^{-5} M), ibuprofen (10^{-7} to 10^{-5} M), and acetylsalicylic acid (10^{-5} to 10^{-3} M) did not prevent colchicine-induced downregulation of TNF-αR (data not shown).

Role of LPS. Since TNF-αR of macrophages and endothelial cells can be downregulated by traces of LPS (10), a common contaminant of commercial reagents, the LPS content of all the MT-depolymerizing agents used in this study was deter-

TABLE II

Effect of Antiserum Against TNF-α on Colchicine and Nocodazole Action

Reagents	Specific binding*	Percent inhibition
	cpm	
Preimmune serum[‡]	907 $\pm$ 20	–
+ 10 μM colchicine	397 $\pm$ 23	56.2
+ 10 μM nocodazole	323 $\pm$ 66	66.6
Antiserum 15E[‡]	994 $\pm$ 47	–
+ 10 μM colchicine	411 $\pm$ 42	58.7
+ 10 μM nocodazole	392 $\pm$ 13	60.6

* Means $\pm$ SE for triplicates from one of four similar experiments.
[‡] Diluted 1:640.

TABLE III
Effect of PGE₁ and Dibutyryl-cAMP on TNF-αR

Reagents*	Specific binding[‡]
	cpm
–	880 ± 52
PGE₁	
10⁻⁹ M	734 ± 34
10⁻⁸ M	881 ± 68
10⁻⁷ M	1,244 ± 118
Dibutyryl-cAMP	
10⁻⁶ M	874 ± 108
10⁻⁵ M	951 ± 167
10⁻⁴ M	1,045 ± 129

* Incubated with macrophages at 37°C for 1 h before the binding assay.
[‡] Means ± SE for triplicates from one of three similar experiments.

mined. At the concentrations of drugs used, the LPS content was <10 pg/ml, i.e., below the minimum effective concentration of LPS (10). Moreover, as assessed by immunofluorescence microscopy, the MT network remained intact in LPS-treated macrophages, even though MT disappeared in macrophages treated with MT-depolymerizing agents (not shown). Thus the effect of MT-depolymerizing agents on TNF-αR was not due to LPS, and the effect of LPS on TNF-αR was not due to depolymerization of MT.

Effect of Cycloheximide. With a human myosarcoma cell line, inhibition of protein synthesis with cycloheximide resulted in decreased surface expression of TNF-αR with a half-life of 2 h (29). This suggested that TNF-αR on the myosarcoma cells turned over in the absence of ligand, perhaps by internalization, and that de novo protein synthesis was necessary for replacement of TNF-αR on the cell surface. If a similar situation existed in macrophages, then MT-depolymerizing agents might reduce binding of rTNF-α by interfering with the ability of the cell to replace TNF-αR as TNF-αR were lost from the cell surface. According to this hypothesis, cycloheximide should inhibit binding of rTNF-α to a similar extent and with a similar time course as colchicine. As shown in Fig. 4, this was the case. Exposure of macrophages to 10 μg/ml of cycloheximide at 37°C resulted in 50% reduction of TNF-αR in 60 min in the absence of added ligand. The effect of colchicine on IFN-γR, a receptor known to be insensitive to cycloheximide (30), was tested under the same conditions. Binding of rIFN-γ was not affected by colchicine (Fig. 4). This suggested that the effects of colchicine and cycloheximide on TNF-αR did not result from nonspecific toxicity, and that in the absence of exogenous ligand, TNF-αR turns over on the macrophage surface more quickly than IFN-γR.

Inhibition of TNF-α-mediated Macrophage Activation by Colchicine. We next asked if downregulation of TNF-αR by colchicine or cycloheximide would affect TNF-α-mediated macrophage activation. Resident macrophages were pulsed for 1 h at 37°C with medium alone, colchicine, or cycloheximide. As an additional control, some macrophages were pulsed with β-lumicolchicine. After removal of these reagents, cells were incubated with increasing amounts of rTNF-α for 48 h at 37°C, washed, and ex-
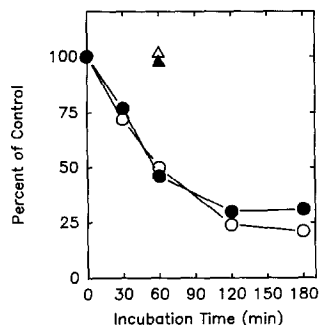
FIGURE 4. Time course of colchicine- and cycloheximide-induced TNF-αR downregulation. Cells were incubated with 10 μM colchicine (●, ▲) or 10 μg/ml cycloheximide (O, △) at 37°C for the time indicated followed by the rTNF-α (●, O) or the rIFN-γ (▲, △) binding assay. The results are expressed as a percent of the specific binding seen with cells treated with medium alone, and are means of triplicates (SEM <6%).

posed to PMA to test their capacity to release H_2O_2 (31). Fig. 5 shows that pulsing with cycloheximide or colchicine, but not β-lumicolchicine, blunted the response of macrophages to rTNF-α.

Effect of Colchicine on TNF-αR of Human Endothelial Cells and PMN. The interaction of PMN with endothelium plays a central role in the development of inflammation, and can be regulated by TNF-α (32). We therefore examined the effect of MT-depolymerizing agents on TNF-αR in human endothelial cells and PMN. As shown in Figs. 6 and 7, 1-h treatment with colchicine or nocodazole resulted in a 38-47% downregulation of TNF-αR in endothelial cells, while β-lumicolchicine had no effect. The concentration-response curves and kinetics of the effect of these agents on endothelial cells matched those for mouse macrophages, and the Scatchard plots were almost the same (Fig. 6, *inset*). Consistent with these findings, endothelial cells also responded to cycloheximide with decreased ^{125}I-rTNF-α binding (Fig. 7). However, no changes were found in TNF-αR when human PMNs in suspension were exposed for 1 h to either 10 μM colchicine or 10 μg/ml cycloheximide (not shown).

Discussion

Incubation of macrophages or endothelial cells with any one of five MT depolymerizing agents in micromolar concentrations at 37°C resulted in a concentration-dependent downregulation of surface TNF-αR. As a consequence, macrophages exposed to MT-depolymerizing agents became refractory to TNF-α, a rare example
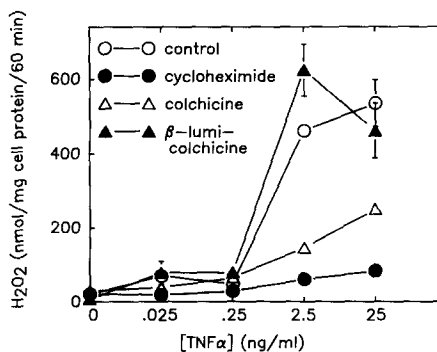

FIGURE 5. Inhibition of TNF-α induced H_2O_2 release by colchicine and cycloheximide. Resident macrophages were pulsed for 1 h at 37°C with medium (O), 10 μM colchicine (△), 10 μg/ml of cycloheximide (●), or 10 μM β-lumicolchicine (▲). After removal of drugs, indicated concentrations of rTNF-α were added, and incubated with cells for an additional 48 h at 37°C in a 5% CO_2/95% air incubator. Then H_2O_2 release was determined in the presence of 100 ng/ml of PMA. The results are means ± SE for triplicates.
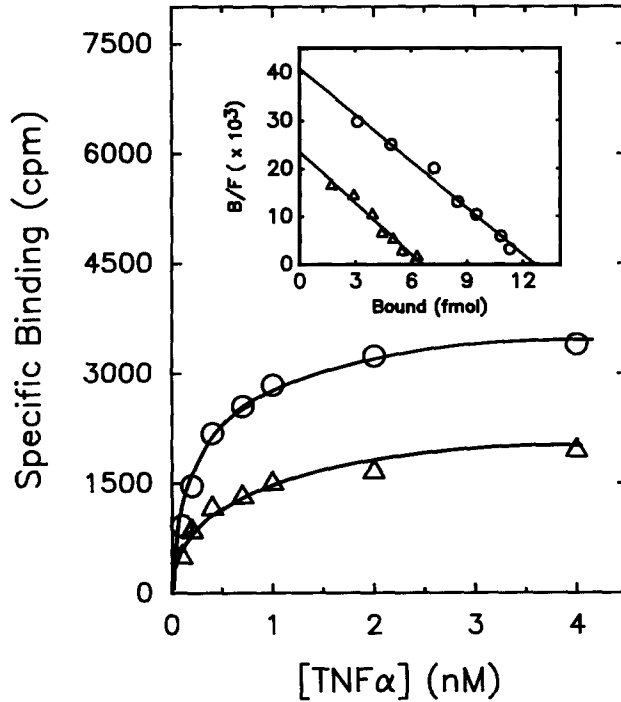
FIGURE 6. Decreased TNF-αR on human endothelial cells after treatment with colchicine. Human endothelial cells were pretreated with (△) or without (○) 10 μM colchicine for 1 h at 37°C. Other conditions were the same as the legend for Fig. 1.

of inhibition of macrophage activation in vitro by a pharmacological agent in clinical use.

The five effective agents belong to four structurally distinct classes; the only action they are known to share is the ability to depolymerize MT. Inactive isomers of colchicine were ineffective, as were microfilament-destabilizing cytochalasins. The spectra of biologic effects of the drugs, their effective concentrations, and the relative reversibility of their actions provide strong evidence that MT disassembly was responsible for the observed downregulation of TNF-αR.
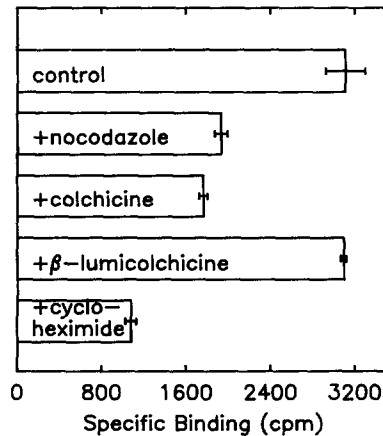

FIGURE 7. Effect of drugs on TNF-αR of human endothelial cells. Confluent monolayers of endothelial cells were incubated with medium alone, 10 μM nocodazole, 10 μM colchicine, 10 μM β-lumicolchicine, or 10 μg/ml cycloheximide for 1 h at 37°C before the binding assay. The results are means ± SE for triplicates.

Several mechanisms might have been at work: competition by the MT depolymerizing agents with ^{125}I-rTNFα for its receptor; triggering of the release of endogenous TNF-α, followed by ligand-induced receptor downregulation; activation of protein kinase A via elevation of intracellular cAMP; internalization triggered by LPS contaminating the reagents (10); decrease of TNF-αR synthesis; or impairment of transport of newly synthesized TNF-αR to the plasma membrane. Our data exclude the first four explanations but are consistent with either of the last two. We could not distinguish whether the effect of colchicine on TNF-αR was due to inhibition of TNF-αR synthesis, interference with transport of TNF-αR to the plasma membrane, or both. The question can only be answered when specific antibodies and cDNA probes for TNF-αR become available.

It has been suggested that MT can affect polyribosome stability (33) and mRNA levels (34). Macrophage and endothelial cell surface TNF-αR declined as rapidly after exposure to cycloheximide, an inhibitor of protein synthesis, as they did after exposure to colchicine. Surface receptors for another macrophage-activating cytokine, IFN-γR, were insensitive both to cycloheximide and to colchicine over the same time interval. With another phagocyte, PMN, TNF-αR were insensitive both to cycloheximide and to colchicine, consistent with the limited protein synthetic capacity of PMN. Thus, in different cell types, depolymerization of cytoplasmic MT appeared to have an impact on the turnover of TNF-αR similar to that of interrupting the synthesis of TNF-αR.

MT are required for the transport of several kinds of hormone receptors to the plasma membrane. Colchicine prevented the recycling of insulin receptors to the plasma membrane in rat hepatocytes after treatment with high concentrations of insulin (35). Likewise, colchicine inhibited the recovery of cardiac myocyte β-adrenergic receptors after their downregulation by an agonist (36). MT were required for apical surface expression of a plasma membrane protein of unknown function in intestinal epithelial cells (37). Finally, MT-depolymerizing agents interfered with the secretion of a variety of proteins and peptides, including insulin (38), very low density lipoproteins (39, 40), fibroblast growth factor (41), lysosomal enzymes (42), and thyroxine (43). In contrast to the studies cited above with insulin receptors and adrenergic receptors, downregulation of TNF-αR by colchicine was demonstrable without massive ligand-induced internalization of the receptors. This probably reflected rapid constitutive turnover of TNF-αR on macrophages and endothelial cells. In contrast to cycloheximide, none of the MT-depolymerizing agents tested appeared to downregulate TNFαR completely, suggesting that expression of a portion of nascent TNFαR does not depend on the MT network. A similar observation was made for insulin receptors (35).

Emigration of leukocytes across the endothelium is a critical aspect of inflammation. TNF-α can play an important role in this process (32), and can induce macrophages and endothelial cells in such sites to release other inflammatory mediators, such as IL-1, IL-6, neutrophil activating peptides, eicosanoids, and reactive oxygen and nitrogen intermediates (18, 44–48). It is possible that the effects of MT-depolymerizing agents seen here with macrophages and endothelial cells may also be exerted on some other types of cells on which TNF-α has potentially important actions, including synovial cells (49) and lymphocytes (50). Thus, the downregulation of TNF-αR by MT depolymerizing agents may contribute to their antiinflammatory effects.

If pharmacologic compounds that depolymerize MT can modulate TNF-αR, perhaps endogenous signals that affect MT function can also affect TNF-αR. The polymerization state of MT is sensitive to the intracellular concentration of calcium (51). It is also tightly controlled by MT-associated proteins, whose functions, in turn, are regulated by kinases (52–56). Our findings raise the possibility that certain hormones or cytokines could modulate the expression of receptors for the same or other hormones or cytokines through the interaction of second messenger systems with the MT network.

Summary

Exposure of murine and human macrophages and human umbilical vein endothelial cells to micromolar concentrations of five microtubule (MT)-depolymerizing agents (colchicine, nocodazole, podophyllotoxin, vincristine, and vinblastine) resulted in a loss of binding sites for iodinated TNF-α. The reduction amounted to 40–60% by 1 h and $\sim$75% by 2–4 h. In 1 h, specific binding was reduced 50% by 0.1–5 μM of these drugs at 37°C, but not at 4°C. Inactive isomers of colchicine were ineffective, as were microfilament-destabilizing cytochalasins. The active agents did not compete with TNF-αR for binding. Antiserum against TNF-α did not neutralize the effect of colchicine and nocodazole. PGE_1 and dibutyryl-cAMP could not mimic, and cyclooxygenase inhibitors could not prevent the drug effects. All the binding sites were regenerated within 3 h after removal of nocodazole, which binds tubulin reversibly, whereas little recovery was found even 18 h after the removal of colchicine, which binds tubulin irreversibly. These findings suggested that MT disassembly was responsible for the observed downregulation of TNF-αR. The protein synthesis inhibitor cycloheximide inhibited binding of TNF-α to a similar extent and with a similar time course as colchicine in the absence of added ligand. Neither drug affected binding of IFN-γ to macrophages, nor binding of TNF-α to human polymorphonuclear leukocytes. Thus, an intact MT network appears to be important in maintenance of the steady state of TNF-αR on those cells in which TNF-αR turns over rapidly in the absence of ligand. The antiinflammatory actions of MT-depolymerizing agents may result in part from their interference with the ability of such cells to respond to TNF-α.

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