Modulation of Monocyte Type I Transforming Growth Factor-β Receptors by Inflammatory Stimuli*

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The regulatory mechanisms which control the wide array of cellular responses to transforming growth factor β (TGFB) are not understood. This report presents evidence that down-regulation of TGFB receptors on human monocytes may be one mechanism by which the effects of TGFB are regulated. Treatment of monocytes with interferon γ (IFNγ) and lipopolysaccharide for 18 h reduced monocyte receptor number (approximately 400/cell) in a dose-dependent fashion by 89% and 78%, respectively, as determined by 125I-TGFB binding. Incubation with other cytokines (granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor-1, interleukin-1, tumor necrosis factor a) did not alter the amount of TGFB bound. The decrease in 125I-TGFB binding could not be attributed to competition for receptor sites by secreted TGFB. Instead, the decline in binding was due to a loss of type I TGFB receptors, the subtype primarily expressed by monocytes, with no decrease in receptor affinity. Lipopolysaccharide-induced receptor loss was rapid (1–4 h), in contrast to the prolonged (12 h) decline induced by IFNγ. Loss of receptors was accompanied by a diminished ability of the cells to respond to TGFB with an induction of TNFα mRNA. Thus, this monocyte system is the first example of a heterologous agent causing the down-regulation of TGFB receptors with a concomitant decline in a TGFB-stimulated function.

Transforming growth factor β (TGFB) is a ubiquitous cytokine which appears to be involved in the initiation, augmentation, and resolution of numerous physiologic and pathologic processes (1). Recent evidence demonstrating that TGFB is a product of lymphoid cells has implicated a role for this peptide in host immune defense (2). Both monocytes and lymphocytes generate TGFB (3–7), and these leukocytes are among the most susceptible to functional regulation by TGFB. At picomolar concentrations, TGFB inhibits lymphocyte proliferation (7, 8). Conversely, circulating monocytes chemotax to femtomolar concentrations of TGFB (9, 10) and are further stimulated by picomolar concentrations to up-regulate FcγIII receptor (CD16), important to immunophagocytic activity (11). TGFB also induces monocytes to synthesize multifunctional cytokines involved in promoting inflammation, such as interleukin-1 (IL-1), tumor necrosis factor α (TNFα), and platelet-derived growth factor (5, 6, 9, 10, 12). In addition, TGFB stimulates monocytes to express additional TGFB, thus establishing an autocrine loop which maintains the inflammatory state (5, 6).

The multiple proinflammatory effects of TGFB on monocytes suggest the existence of regulatory mechanisms to antagonize or diminish the effects of TGFB, thus leading to the resolution of the inflammatory state. The release of TGFB in a latent form is an important regulatory mechanism (1); however, inflammatory macrophages have been shown to activate TGFB, probably through the release of a specific sialidase (13) or protease (14), resulting in active TGFB at the site of inflammation (15). Another possible regulatory mechanism is suggested by observations that sustained exposure to TGFB leads to suppression of some monocyte proinflammatory activities. For example, the generation of reactive oxygen intermediates is diminished (16), and the production of cytokines in response to a secondary stimulus is inhibited (17).

Modulation of monocyte expression of the TGFB receptor is an additional mechanism by which the proinflammatory effects of TGFB could be regulated. Preliminary evidence from this laboratory has suggested that activated monocytes exhibit a reduction in the specific binding of 125I-TGFB to monocytes (9). In this study, observation is expanded to show that interferon γ (IFNγ) and lipopolysaccharide (LPS), two substances potentially present within the inflammatory site, reduce the number of cell surface receptors to <25% of the number of receptors expressed on control cells. In addition, receptor loss is shown to correspond to a loss in capacity to be stimulated by TGFB. Thus, the proinflammatory recruitment and stimulatory influence of TGFB on blood monocytes may be moderated once the cells have become activated, thereby favoring resolution of the inflammatory response.

MATERIALS AND METHODS

Reagents—TGFB, (porcine) was obtained from R&D Systems (Minneapolis, MN) and labeled with 125I purchased from Amerham Corp. (Arlington Heights, IL). Granulocyte-macrophage colony-stimulating factor (GM-CSF, human, recombinant), macrophage colony-stimulating factor (M-CSF, human, recombinant), interleukin-1β (IL-1β, human, recombinant), tumor necrosis factor α (TNFα, human, recombinant) and IFNγ (human, recombinant) were obtained from Genzyme (Boston, MA). Lipopolysaccharide (Escherichia coli, 055:B5) was obtained from Difco Laboratories (Detroit, MI). recom-

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‡ The abbreviations used are: TGFB, transforming growth factor β; IL, interleukin; TNFα, tumor necrosis factor α; IFNγ, interferon γ; LPS, lipopolysaccharide; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
binant latent TGFβ was prepared and purified as previously described (18).

Monocyte Isolation—Human monocytes were isolated from heparinized blood of healthy normal volunteers undergoing leukapheresis at the NIH Blood Bank. The leukocyte-rich preparation was separated fromuffy coat and subjected to centrifugal elutriation as previously described (19). Monocytes obtained by this procedure have been shown to be >90% pure as judged by dual fluorescence microfluoroscopy using specific cell surface markers. The monocytes were maintained in suspension in Dulbecco's modified Eagle's medium (Mediatech, Herndon, VA) containing 20 mM glutamine, 50 μg/ml of gentamicin (Whittaker, Walkersville, MD), and 0.1% fetal bovine serum (GIBCO, Grand Island, NY) at a concentration of 3 × 10^6 cells/ml. The cells were incubated with or without the addition of various cytokines at 37 °C in a humidified atmosphere of 95% air, 5% CO2.

**TGFβ Assay—**TGFβ was assayed by inhibition of IL-1 dependent thymocyte proliferation as previously described (20). A portion of each sample was heated to 80 °C for 5 min to activate any latent TGFβ (18). The concentration of TGFβ present in heat-treated samples was equivalent to the total TGFβ concentration, active and latent; the concentration present in the non-heated samples was the amount of TGFβ activated endogenously. TGFβ concentrations were determined from a standard curve prepared with TGFβ obtained from Collaborative Research (Bedford, MA).

For some experiments, the amount of TGFβ in the cell supernatant was also quantified by a modification of the A549 competitive binding assay as described (21). "^251-TGFβ (100 pm) was incubated with A549 cultures at 4 °C for 3 h in the presence of either unlabeled TGFβ (10 pm to 2 nM/well for standard curve generation, or 10 nM for nonspecific binding determination) or cell culture supernatant. Nonspecific binding was determined in the presence of a 400-fold excess of unlabeled TGFβ. Following the binding incubation, the cells were washed three times with phosphate-buffered saline and then solubilized with a solution of Tris-HCl (pH 6.8) and EDTA to final concentrations of 1 M SDS and 0.002 M disuccinimidyl suberate (Pierce Chemical Co.) in phosphate-buffered saline. The TGFβ receptor was cross-linked to its receptor by incubation with various agents at 4 °C for 4 h. The cells were washed twice with ice-cold binding buffer (above) to remove the stimuli. For the assay, cells were suspended in binding buffer (Dulbecco's modified Eagle medium with 0.1% bovine serum albumin) at a concentration of 1 × 10^5 cells/ml. Portions of these cell suspensions were then incubated with various concentrations of labeled TGFβ for 18 h in supplemented Dulbecco's modified Eagle's medium with or without GM-CSF, TNFα, or LPS, then for an additional 3 h in the presence of absence of TGFβ. Total RNA was extracted by the acid guanidium thiocyanate/phenol/chloroform extraction procedure (25). A portion of the total RNA from each sample (5 μg) was fractionated by electrophoresis on a 1% agarose gel containing formaldehyde and transferred to a nitrocellulose filter. Blots were prehybridized for 4 h at 42 °C, and then hybridized overnight at 42 °C with a "^32P labeled cDNA probe for TNFα (26). Filters hybridized with the TNFα probe were washed with 2 × SSC, 0.1% SDS at room temperature, then once at 42 °C with 0.1 × SSC, 0.1% SDS.

**RESULTS**

Radiolabeled TGFβ Binding to Monocytes—Human monocytes were assayed to quantify TGFβ receptors immediately after isolation or after overnight culture in the presence or absence of specific cytokines or LPS. As detailed in Table I, freshly isolated human monocytes bind 0.59 ± 0.06 fmol of TGFβ/10^6 cells (average ± S.E., n = 4); this corresponds to about 360 receptors/cell, assuming a 1:1 binding stoichiometry. Because monocytes cultured overnight without serum or growth factors lose viability (27) and exhibit increased nonspecific binding (28), binding GM-CSF or M-CSF, cytokines which promote cell survival, were added to the cultures. These greatly reduced the loss of monocyte viability and thus the increase in nonspecific binding. Cells cultured for 18 h with GM-CSF (300 units/ml) bound approximately the same amount of TGFβ as freshly isolated monocytes, 0.57 ± 0.05 fmol/10^6 cells (average ± S.E., n = 9) corresponding to 340 receptors/cell. Equivalent amounts of TGFβ were bound at other GM-CSF concentrations (200, 500 units/ml); however, concentrations ≤100 units/ml were insufficient to maintain monocyte viability and the nonspecific binding increased to approximately 70% of the total. The amount of TGFβ bound by monocytes cultured with 100 units/ml of M-CSF (0.55 ± 0.07 fmol/10^6 cells, 330 receptors) was similar to that bound by the GM-CSF-treated monocytes. Since the addition of colony-stimulating factors aided in maintaining cell viability without altering the number of TGFβ receptors, monocytes incubated with GM-CSF (300 units/ml) served as the 18-h control.

**Modulation of TGFβ Binding—**To investigate if inflammatory mediators could influence expression of monocyte TGFβ receptors, monocytes were incubated for 18 h with IFN-γ (200 units/ml), a known monocyte activator, before assaying for TGFβ receptors. This resulted in a substantial decrease in the amount of specifically bound "^32P-TGFβ, from 0.57 ± 0.05 to 0.14 ± 0.04 fmol/10^6 cells (average ± S.E., n = 9) or 24% of the amount bound in fresh cells or 18-h GM-CSF control cells (Table I). Other inflammatory cytokines known to modulate monocyte function, TNFα and IL-1, were

| Treatment | TGFβ bound FMOL/10^6 Cells | n | Receptor number FMOL/10^6 Cells |
|-----------|----------------------------|---|--------------------------------|
| Fresh control | 0.59 ± 0.06               | 4 | 360                            |
| GM-CSF     | 0.55 ± 0.05               | 2 | 340                            |
| M-CSF      | 0.54 ± 0.08               | 3 | 320                            |
| IFN-γ      | 0.14 ± 0.07               | 9 | 80                             |
| TNFα       | 0.51 ± 0.06               | 3 | 310                            |
| IL-1       | 0.50 ± 0.09               | 2 | 300                            |
| LPS        | 0.53 ± 0.04               | 3 | 320                            |

**Table I**

Modulation of TGFβ receptors by cytokines and lipopolysaccharide

Binding of "^32P-TGFβ was quantified for freshly isolated monocytes or monocytes cultured in the presence of the indicated cytokines for 18 h as detailed under "Materials and Methods." The data are expressed as average ± S.E. for n separate determinations.
evaluated next. As shown in Table I, neither of these cytokines changed the amount of TGFβ bound during an 18-h incubation relative to the control cells incubated with GM-CSF or the freshly isolated monocytes. At the indicated concentrations, these cytokines also maintained cell viability. Simultaneous addition of an equivalent amount of GM-CSF, also did not change TGFβ binding. LPS, an inflammatory mediator and macrophage activator present at sites of infection, was next evaluated for its effects on TGFβ binding. Overnight incubation of monocytes with 1 μg/ml of LPS dramatically reduced the amount of TGFβ specifically bound to 0.065 ± 0.010 fmol/10⁶ cells (average ± S.E., n = 9) or 11% of the amount bound by fresh cells and 18-h control cells. Both of these activators, LPS and IFNγ, effectively maintained monocyte viability, and the simultaneous addition of GM-CSF (300 units/ml) did not prevent receptor loss, nor change its magnitude. Although the TGFβ assay is preceded by a washing step to remove the activating agents, residual IFNγ or LPS may remain which may compete with or bind the 125I-TGFβ, thus preventing its interaction with the receptor. To address this possibility, LPS (1 μg/ml) and IFNγ (200 units/ml) were directly added to freshly isolated monocytes suspended in binding buffer with 100 pm 125I-TGFβ at the onset of the 125I-TGFβ binding incubation. Even at these concentrations, neither LPS nor IFNγ reduced the binding of 125I-TGFβ to its receptor (data not shown).

Additional studies examined the concentration range over which IFNγ and LPS were effective in decreasing the amount of labeled TGFβ specifically bound. As shown in Fig. 1, LPS was extremely potent; concentrations as low as 1 ng/ml lowered 125I-TGFβ binding within 18 h. A concentration of 100 ng of LPS/ml yielded a maximal decrease in the amount of TGFβ bound. Concentrations of IFNγ ≥50 units/ml caused a maximal decline in 125I-TGFβ binding, with concentrations as low as 1 unit/ml resulting in some decrease. The simultaneous addition of GM-CSF (300 units/ml) with the various concentrations of LPS and IFNγ did not alter the results.

Further studies examined the rate at which LPS and IFNγ caused the decline in 125I-TGFβ binding (Fig. 2). Monocytes were incubated with LPS (1 μg/ml), IFNγ (200 units/ml), or GM-CSF (300 units/ml) for various periods of time up to 18 h, after which they were assayed for specific TGFβ binding. Incubation of the cells with LPS caused an almost immediate drop in TGFβ binding. Binding declined by 60% after only a 1-h incubation. By 4 h, the loss of binding was maximal with only 10% of the total binding remaining. The kinetics of IFNγ-induced decline in TGFβ binding were more protracted, with maximal reduction in 125I-TGFβ binding occurring after a 12-h incubation with the IFNγ.

Affinity Labeling of Monocyte TGFβ Receptors with 125I-TGFβ—Monocyte cell surface TGFβ-binding proteins were affinity labeled by first equilibrating equal numbers of fresh or 18-h cultured cells with 125I-TGFβ at 4 °C, then cross-linking the 125I-TGFβ to its receptor with the bifunctional cross-linker, disuccinimidyl suberate. Following separation of the solubilized cell proteins by SDS-PAGE, the labeled ligand/receptor protein bands were visualized by autoradiography. Freshly isolated human monocytes were found to express primarily the type I TGFβ receptor (for review, see Ref. 1). This appears as the 65-kDa protein band representing the receptor-ligand complex (Fig. 3, lane 1). The specificity of the interaction between 125I-TGFβ and the receptor protein is shown by the ability of a 400-fold excess of unlabeled TGFβ to completely eliminate labeled TGFβ binding (lane 2). Control monocytes incubated with GM-CSF for 18 h at 4 °C (lane 3) and at 37 °C (lane 5) also specifically bound a similar amount of 125I-TGFβ in a 65-kDa protein band corresponding to the type I receptor-ligand complex. As before, for both the 4 and 37 °C incubations, the addition of a 400-fold excess of unlabeled TGFβ eliminated the 65-kDa band, indicating the specificity of the 125I-TGFβ/protein interaction (lanes 4 and 6, respectively). Fresh and GM-CSF-treated cells from some experiments also occasionally yielded a faint band at approximately 100 kDa which was not readily discernable without overexposure of the autoradiograph, and could not be readily distinguished by densitometric scan. When identified, this band was only present in the absence of excess of unlabeled TGFβ. This protein band may represent the type II TGFβ receptor-ligand complex (1). When monocytes were incubated with LPS for 18 h at 37 °C, specific binding of radiolabeled TGFβ to the 65-kDa complex was virtually eliminated (lane 9). Nonspecific binding (background) was also greatly reduced (lane 10). When the 18-h LPS incubation was carried out at 4 °C (lane 7) rather than 37 °C, monocytes were found to specifically bind approximately the same amount of 125I-TGFβ in the 65-kDa protein band as did the freshly isolated (lane 1) and GM-CSF-treated cells (lanes 3 and 5). This suggests that LPS does not directly interfere or compete with TGFβ.

![Fig. 1. Alterations in the amount of TGFβ bound following treatment with LPS and IFNγ.](image1)

![Fig. 2. Determination of the rate of loss of TGFβ binding.](image2)

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binding to its receptor since such an interaction would take place at 4°C, as well as 37°C. Instead, LPS appears to reduce 125I-TGFβ binding following energy-requiring cellular events. In a similar manner, incubation of monocytes for 18 h at 37°C with IFNγ also resulted in a decrease in the amount of specifically bound ligand (Fig. 3, lane 13). By scanning densitometry it was determined that the IFNγ-treated cells exhibited a 75–85% decrease in labeled 65-kDa TGFβ receptor relative to that expressed by freshly isolated or 18-h GM-CSF-treated cells. As with the LPS treatment, IFNγ did not decrease 125I-TGFβ binding if the 18-h incubation proceeded at 4°C (lane 11). As before, this suggests that IFNγ does not directly inhibit or compete with TGFβ binding to its receptor.

Analysis of the Inducible Decline in Specific 125I-TGFβ Binding—To determine if the decline in specific binding of 125I-TGFβ was due to a loss of TGFβ receptors from the cell surface or a decrease in the affinity of the receptors, Scatchard analysis was performed. Freshly isolated monocytes or monocytes treated for 18 h with GM-CSF, IFNγ, or LPS were equilibrated in binding buffer containing concentrations of 125I-TGFβ ranging from 0.3 to 100 pM. The amount of TGFβ bound per 10⁶ cells was plotted against the ratio of bound over free TGFβ (Fig. 4). Fresh monocytes and cells treated for 18 h with GM-CSF and IFNγ, or LPS were equilibrated in binding buffer containing concentrations of 125I-TGFβ ranging from 0.3 to 100 pM. The amount of TGFβ bound per 10⁶ cells was plotted against the ratio of bound over free TGFβ (Fig. 4). The Scatchard plot represents the average of triplicate determinations that differed by <5%.

is an apparent loss of cell surface receptors rather than a decline in affinity. An apparent decrease in the number of cell surface receptors as determined by radiolabeled ligand-binding studies cannot distinguish between a true decrease in receptor number and an increase in receptor sites occupied by cold ligand. Monocyte activators have been shown to induce the production of TGFβ (3, 5, 6), although the protein is often released in a latent form (1) which does not bind to the receptor (28).

Superantigens from the same 18-h suspension cultures of cytokine-treated monocytes used for receptor measurements were assayed for active and latent TGFβ. This was done to determine if the decrease in 125I-TGFβ binding observed with LPS and IFNγ-treated cells was due to the release of active TGFβ. As shown in Fig. 5, LPS and IFNγ do stimulate the release of TGFβ; some of the TGFβ is found in the latent form, measurable only after heat activation. The concentration of TGFβ already in the active form in the culture medium (0.15 and 0.06 ng/ml or 6.0 and 2.4 PM, respectively) is fairly low relative to the concentration of the 125I-TGFβ present during the receptor-binding assay (2.5 ng/ml or 100 PM). This concentration of TGFβ is much lower than is required to
saturate receptor binding and therefore, the potential error introduced by the presence of the unlabeled TGFβ is not more than 5%. Incubation with GM-CSF also resulted in the release of some active TGFβ which could potentially compete with the 125I-TGFβ for receptor binding. However, as before, the concentration of the active TGFβ was low (0.11 ng/ml or 4.4 pm) and therefore would introduce only small error into the determination of TGFβ receptor number. In fact, the receptor number determined for GM-CSF-treated cells was not different from that of freshly isolated monocytes. The concentration of TGFβ in monocyte supernatants derived from cultures stimulated with M-CSF was also determined and found to be similar to that of GM-CSF.

To further demonstrate that monocytes incubated with LPS, IFNγ, or GM-CSF do not significantly activate latent TGFβ, exogenous latent TGFβ (400 pm) was added to the monocyte cultures at the onset of the 18-h incubation. The concentration of active and latent TGFβ in the resultant culture supernatant was then determined by two TGFβ assays: thyminocyte growth inhibition and displacement of 125I-TGFβ binding to A549 cultures as described under "Materials and Methods." The concentration of active TGFβ in the supernatant of the LPS, IFNγ, and GM-CSF cultures was only 2–5 pm (data not shown). As before, this suggests that these monocyte cultures do not appreciably convert latent TGFβ to active TGFβ. Separate controls showed that the presence of LPS, IFNγ, or GM-CSF in a cell-free system also does not activate latent TGFβ. Heat treatment of the various supernatants confirmed that activation of the exogenous latent TGFβ does result in a biologically active protein which displays normal TGFβ receptor binding kinetics.

Since an assay of the concentration of active TGFβ in the supernatant cannot measure TGFβ already bound to the monocytes, a neutralizing antibody to TGFβ was added to the monocyte cultures immediately prior to GM-CSF, LPS, and IFNγ addition. After an 18-h incubation, receptor number was determined for the various cultures. The presence of the TGFβ neutralizing antibody was found not to prevent the loss of monocyte TGFβ receptors following LPS and IFNγ treatments (data not shown). Separate control experiments confirmed that the antibody could neutralize the biological effects of active exogenous TGFβ. Thus, these data suggest that the release of active TGFβ by monocytes and its binding to the monocyte TGFβ receptors is not the mechanism by which TGFβ receptors are reduced.

Effect of TGFβ Receptor Down-regulation on Monocyte Responsiveness to TGFβ—Decreasing the number of TGFβ receptors on monocytes may provide a mechanism to modulate the response of the cells to TGFβ. Addition of TGFβ to freshly isolated monocytes in vitro has been shown to cause the induction of mRNA for various cytokines including TNFα (5, 6, 9, 10). Monocytes were incubated with GM-CSF, LPS, or IFNγ for 18 h, and then challenged with exogenous TGFβ (10 ng/ml) for an additional 3 h. After the second incubation, total cellular RNA was isolated and analyzed to determine if the cells could respond to the TGFβ with an increase in the expression of TNFα mRNA. As shown by Northern blot analysis, TGFβ induced TNFα mRNA expression by cells incubated with GM-CSF for 18 h (Fig. 6), as it does for freshly isolated monocytes (5, 6, 9, 10). In contrast, when LPS was added to the monocyte cultures, it resulted in the complete elimination of TGFβ-induced TNFα mRNA expression detectable by Northern blot. Examination of the 28 S and 18 S rRNA bands shows that this change in TNFα mRNA expression is not due to unequal quantities of RNA in the lanes (Fig. 6, lower panel). Receptor measurements done in parallel indicated that the TGFβ receptor level on the LPS-treated cells was only 5% of that of the GM-CSF control cells. In a similar manner, 18 h incubation with IFNγ caused a 47% decrease in the TGFβ-stimulated expression of TNFα mRNA, as quantified by densitometric scan (Fig. 6). Concurrent measurements of TGFβ receptors on IFNγ-treated cells revealed a 71% loss of TGFβ receptors. This experiment was repeated three times with identical results for the LPS treatment, and somewhat more variability seen with the IFNγ treatment: 30–50% decrease in mRNA expression. Thus, the data demonstrate reduced functional responses to TGFβ in cells exhibiting decreased receptor expression.

**DISCUSSION**

The cellular response to cytokines and hormones is often regulated at the level of receptor expression; exposure to homologous and heterologous agents causes a rapid alteration in receptor affinity or number. Evidence for the existence of this regulatory mechanism has been noticeably limited with respect to TGFβ. One cell type which does modulate TGFβ receptor expression is the T lymphocyte, a cell which is growth inhibited by TGFβ. T cells have been shown to increase the expression of type I, II, and III TGFβ receptors following stimulation with mitogens for 48 h (29). Similarly, B lymphocytes exhibit a 5–6-fold increase in TGFβ receptor expression following in vitro activation (7, 30). Adrenal cortical cells have also been reported to increase the expression of the type III TGFβ receptor subtype following stimulation with adrenocorticotropic hormone (31). Whether the increased expression of TGFβ receptors by these cells results in a greater sensitivity to TGFβ has not yet been established. Some cell types which are known to alter their responsiveness to TGFβ in conjunction with developmental changes do not simultaneously exhibit a significant change in TGFβ receptor expression (32). The monocyte system described here represents a model in which, for the first time, TGFβ receptors are shown to be down-regulated by exposure to heterologous agents. In addition, these studies demonstrate that receptor loss may affect cell function since it results in a diminished ability of the monocytes to produce cytokines in response to TGFβ.

Both LPS and IFNγ were found to decrease monocyte TGFβ receptors and this receptor modulation could not be attributed to occupancy of the binding sites with TGFβ released by the cells. The rate of receptor loss initiated by these two agents varied considerably, suggesting the process was
occurring by different mechanisms. Incubation with IFNγ resulted in a steady decline in receptor number over 12 h; this may be due to an inhibition of steady state TGFβ receptor synthesis. In contrast, the rapid reduction in receptor number induced by LPS suggests an alternative mechanism. Although the decrease in nonspecific, as well as specific, binding following LPS treatment (Fig. 3, lanes 9 and 10) might indicate an increase in fluid phase pinocytosis or membrane turnover, numerous studies have shown that this interpretation is unlikely since LPS (and IFNγ) treatment increase, as well as decrease, many plasma membrane proteins, yet have no effect on other proteins. For example, stimulation of monocytes with LPS and IFNγ has been shown to down-regulate C5a receptors, but receptors for another chemotaxis factor, formyl-methionyl-leucyl-phenylalanine, are not altered (33). In addition, treatment with LPS, IFNγ, or GM-CSF has been shown not to alter the monocyte marker protein CD14, nor do they change the expression of CD16 (FcγRIII) (11). Ding et al. (34) directly addressed the question of the selectivity of the LPS effect in conjunction with their observation that TNFα receptors were rapidly down-regulated by LPS. These researchers showed that the LPS effect was not a generalized internalization of the plasma membrane receptors because the surface expression of complement receptor type 3 did not change during the length of the incubation. In contrast to LPS, other researchers have found that IFNγ increases the number of TNFα receptors (35, 36). LPS and IFNγ have also been shown to increase the expression of the IL-8 receptor and major histocompatibility complex class II antigen (HLA-DR) (37). Thus, these studies indicate that the down-regulation of TGFβ receptors by LPS and IFNγ is a selective effect on a specific population of membrane proteins and not the result of generalized membrane internalization.

The decline in number of cell surface receptors was coupled with a diminished ability of the cells to respond to TGFβ as evaluated by induction of cytokine mRNA. Pre-treatment with LPS yielded cells which did not express TNFα mRNA in response to TGFβ stimulation; this corresponded to an almost complete loss of TGFβ receptors on these cells. IFNγ-treated cells also down-regulated TGFβ receptors, although to a lesser extent. These cells displayed a partial reduction of TNFα mRNA expression following TGFβ stimulation. This would suggest that certain functional responses of the monocyte to TGFβ are limited by receptor number. Possibly, other cell types which express a greater number of TGFβ receptors (21) would not be restricted in this manner.

Covalently linking radiolabeled TGFβ to its high affinity cell surface-binding proteins followed by electrophoretic analysis, revealed that monocytes primarily express the 65-kDa type I receptor. Thus, the analysis and characterization of [125I]-TGFβ binding can be attributed to this single receptor subtype. Freshly isolated human monocytes and monocytes maintained in GM-CSF-supplemented suspension culture were found to express approximately 360 receptors with an affinity of 7 pM. These numbers fall within the lower end of the range reported for type I TGFβ receptors on other cells: 300–4000 binding sites/cell with an affinity in the range of 5–50 pM (32). Incubation with LPS or IFNγ reduced receptor number to approximately 40 and 80 receptors/cell, respectively. Treatment with LPS also resulted in a measurable increase in TGFβ receptor affinity (2 pM); however, the limited magnitude of this change suggests that it may not be functionally significant. Cross-linking [125I]-TGFβ to monocyte receptors also revealed that the cells may express a small population of 100-kDa type II receptors; however, the sporadic appearance of the band suggests it may instead be due to changes in the percent of contaminating T lymphocytes. T cells do express the type II TGFβ receptor (29) and are the main contaminant of monocyte preparations (1-8%). Others have identified both type I and type II TGFβ receptors on human monocytes (38, 39); however, no details were given as to the relative proportion of the receptor subtypes. Analysis of receptor subtypes expressed by the U937 human monocytic leukemia cell line showed no evidence of type II receptors (32), however, these cells express the 250–350-kDa type III receptor along with the type I receptor, possibly indicating that expression of the type II receptor also varies from that of normal cells.

The absence of type III receptor expression on monocytes may have facilitated our ability to measure changes in receptor number. The number of type III receptors on other cell populations is typically much greater than type I or type II receptors, up to 10^6 sites/cell (32) and is generally not considered to fluctuate. A current hypothesis holds that type I, and possibly type II receptors are involved in signal transduction (32). If this is the case, then the type III receptor may not be subjected to up- and down-regulation in response to environmental stimuli. Thus any changes in number of type I or II receptors may not be distinguishable against a high background of a constant number of type III receptors. It is also possible that cells which express the type III receptors do not undergo down-regulation of TGFβ receptors. The type III receptor is believed to function as a reservoir or clearance system for bioactive TGFβ (32); thus, binding and storage of TGFβ by type III receptors may take the place of receptor down-regulation.

Recent advances in our understanding of the central role of TGFβ in mediating the inflammatory response supplies insight into the physiological significance of monocyte TGFβ receptor modulation by inflammatory mediators. Fiemtomular concentrations of TGFβ initiate a motility response by monocytes. Local release of TGFβ by platelets and other inflammatory cells at a site of inflammation is important in recruiting monocytes to the area. Once the cells have arrived at the site, they are thought to be exposed to higher concentrations of TGFβ and other regulatory mediators (2). In this regard, TGFβ is required at picomolar concentrations to stimulate additional monocyte functions which promote and augment the inflammatory process. Furthermore, TGFβ up-regulates its own synthesis and secretion by monocytes (5, 6), contributing to the build-up of TGFβ within an inflammatory site. These observations suggest that TGFβ is an extremely potent proinflammatory cytokine, a conclusion consistent with recent studies investigating the effects of in vivo administration of TGFβ within the synovial space (40). Thus, the continued secretion of TGFβ and the resultant monocyte activation could result in prolonged inflammatory sequelae. One mechanism which could possibly control these effects may be the loss of sensitivity of monocytes to TGFβ stimulation by the down-regulation of their receptors.

As mentioned above, modulation of chemotactic factor receptors is not limited to the TGFβ receptor. Stimulation of monocytes with IFNγ or LPS selectively down-regulates the cell surface receptor for another monocyte chemotactic peptide, C5a, but not for formyl-methionyl-leucyl-phenylalanine (33). The purpose of down-regulation of receptors for monocyte chemotactic factors after activation is of interest; under physiologic conditions, C5a or TGFβ-directed chemotaxis would no longer be required once the monocytes have been recruited and activated at an inflammatory site. In support of this hypothesis, macrophages isolated from inflammatory lesions (33) or IFNγ-treated animals (41, 42) exhibit signifi-
cantly decreased levels of C5a receptor. TGFβ receptors on inflammatory monocyte-macrophages have yet to be examined.

Another example of TGFβ receptor modulation relevant to the inflammatory response is the enhanced TGFβ receptor expression on T lymphocytes following exposure to mitogenic stimuli (29). Although activation of monocytes with LPS or IFNγ clearly has the opposite effect on TGFβ receptor expression, the physiological response to these two seemingly contradictory effects may, in fact, be complementary. In this regard, activated lymphocytes with enhanced binding capacity for TGFβ are profoundly inhibited in their growth responses by TGFβ (4, 8). The release of this potent growth inhibitor during inflammation and immune responses may represent an important negative feedback mechanism to limit proliferation and thus aid in the resolution of the inflammatory response (2, 43), consistent with recent reports documenting suppression of inflammation following systemic administration of TGFβ (44). Furthermore, monocytes which demonstrate reduced TGFβ receptors upon activation become refractory to the stimulatory potential of TGFβ. The net effect of this receptor modulation and altered capacity for TGFβ binding would be to reverse the proinflammatory sequence of events and favor resolution of the host response. In a chronic lesion, continued recruitment of circulating monocytes could promote persistent inflammation and its potential pathogenic sequelae. Thus, understanding the mechanism of TGFβ action, particularly on monocytes which are pivotal in these events, may provide insight into modulation of inflammation and repair.

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