Selective Regulation of Cytokine Induction by Adenoviral Gene Transfer of IκBα into Human Macrophages: Lipopolysaccharide-Induced, But Not Zymosan-Induced, Proinflammatory Cytokines Are Inhibited, But IL-10 Is Nuclear Factor-κB Independent

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Macrophages are the major cytokine producers in chronic inflammatory diseases, but the biochemical pathways regulating cytokine production are poorly understood. This is because genetic tools to dissect signaling pathways cannot be used in macrophages because of difficulties in transfection. We have developed an adenoviral technique to achieve high efficiency gene delivery into macrophages and recently showed that spontaneous TNF-α production in rheumatoid arthritis joint cells, chiefly from macrophages, is 75% blocked by adenoviral transfer of IκBα. In this report we use the same adenovirus to investigate whether the production of a number of proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6, and IL-8) from human macrophages depends on NF-κB. While the cytokine response to certain inducers, such as LPS, PMA, and UV light, is blocked by overexpression of IκBα, the response to zymosan is not. In contrast, anti-inflammatory mediators (IL-10 and IL-1 receptor antagonist) induced by LPS are only marginally inhibited by IκBα excess. These studies demonstrate several new points about macrophage cytokine production. First, there is heterogeneity of mechanisms regulating both the proinflammatory and anti-inflammatory cytokines within populations of a single cell type. In addition, the results confirm the utility of the adenoviral technique for functional analysis of cytokine induction. The results also confirm that there are autocrine and paracrine interactions regulating cytokine synthesis within a single cell type. The selectivity of NF-κB blockade for proinflammatory but not anti-inflammatory mediators indicates that in macrophages, NF-κB may be a good target for the treatment of chronic inflammatory diseases. The Journal of Immunology, 1999, 162: 2939–2945.

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The successful clinical trials of anti-TNF-α Ab in rheumatoid arthritis (3, 5), reviewed in Reference 2, and Crohn’s disease (6) have prompted considerable interest in alternative strategies to inhibit the production of TNF-α. Because macrophages are the major producers of TNF-α and other proinflammatory cytokines in the rheumatoid joint (1), as well as of the anti-inflammatory cytokines, understanding the signaling pathways involved in the induction of these mediators is of major importance for developing novel therapeutic strategies in chronic inflammatory diseases.

In a previous paper (7), we described a novel technique to achieve efficient, virtually 100%, adenoviral gene transfer into human macrophages, subsequent to up-regulation of integrins. This technique was used to effect adenoviral transfer of the IκBα molecule into human macrophages, as previously reported in endothelial cells (8). Massive overexpression of IκBα was achieved, with consequent inhibition of NF-κB activity. It was observed that the LPS-induced expression of TNF-α in human macrophages was potently inhibited by the blocking NF-κB. This was in contrast to previous studies in human cells, which did not indicate a role for NF-κB in TNF expression (47, 48). However, unlike our studies, these had been performed in transformed cell lines mainly of lymphoid origin, which may not reflect the situation in normal macrophages.

In this paper, the effect of NF-κB down-regulation on a spectrum of proinflammatory cytokines, namely IL-1β, IL-6, and IL-8, and anti-inflammatory molecules IL-10, IL-1ra, and the p55 and p75 soluble TNF-receptors is studied. The aim was to resolve some
of the conflicting evidence concerning macrophage production of cytokines. While there is reasonably good evidence that TNF-α (9, 10), IL-1β (11, 12), and IL-6 (13) can be regulated by NF-κB in various cell types, there are few data concerning the other cytokines of interest. The lack of κB binding sites in the human IL-10 promoter made it unlikely that IL-10 was under direct NF-κB control (14).

Another aim of this study was to use the IκB adenovirus as a tool to determine whether different macrophage activators, e.g., LPS, PMA, UV light, and zymosan, were dependent on NF-κB. In the RAW 264.7 macrophage-like cell line, the UV-induced TNF-α response has been proposed not to involve NF-κB activation, since it was unaffected by mutation of all four κB sites within the TNF-α promoter (15). With regard to the activation of monocyteic cells induced by PMA, results disagree: some studies (16, 17) suggest that NF-κB is involved, and others (18) do not. Recently, in a model of TH-1 cells modified through stable retroviral gene transfer of IκB, PMA-induced IL-1, IL-6, and IL-8 were unaffected (19). Zymosan particles are yeast cell derivatives that induce cytokines and are used to simulate receptor-mediated cell stimulation, probably in the β-glucan receptor (20). Both in mouse bone marrow-derived macrophages (21) and in human monocytes (22), binding of zymosan induces rapid tyrosine phosphorylation of a number of protein substrates. In human monocytes, zymosan induces association of the p53–56kD tyrosine kinases and the cytoskeleton (23). The tyrosine kinase inhibitor genistein proved to be a potent inhibitor of zymosan-induced eicosanoid formation in mouse peritoneal macrophages (24). There is some doubt, however, as to whether zymosan acts via NF-κB. In rat liver macrophages, zymosan is incapable of activating NFκB (18), although it does activate the transcription factor activator protein-1 both in rat liver macrophages (18) and in the U937 human monocytic cell line (25).

The adenoviral infection technique (7) has enabled us to address many of these issues regarding the NF-κB dependence or independence of the signal transduction pathways utilized by these various stimuli in human macrophages.

## Materials and Methods

### Isolation of peripheral blood monocytes

Single-donor plateletphoresis residues were purchased from North London Blood Transfusion Service (Colindale, U.K.). Mononuclear cells were isolated by Ficoll-Hypaque centrifugation preceding monocyte separation in a Beckman Instruments (Torrence, CA) JEL elutriator. Monocyte purity was assessed by flow cytometry and was routinely >90%.

### Adenoviral vectors

Recombinant, replication-deficient adenoviral vectors encoding *Escherichia coli* β-galactosidase or having no insert (Adv0) were generously provided by Drs. A. Byrnes and M. Wood (Oxford, U.K.). An adenovirus encoding porcine IκBα (AdvIκBα) with a CMV promoter and a nuclear localization sequence was generously provided by Dr. R. de Martin (Vienna, Austria). Viruses were propagated in the 293 human embryonic kidney cell line and purified by ultracentrifugation through two cesium chloride gradients. The titers of viral stocks were determined through a plaque assay on 293 cells, as described (26).

### Infection techniques

The elutriated human monocytes were incubated at ~2 × 10^6/ml in RPMI 1640 with 25 mM HEPES and 2 mM l-glutamine supplemented with 5% (v/v) heat-inactivated FCS and 10 U/ml penicillin/streptomycin. To optimize infection, purified human monocytes were pretreated with macrophage CSF (100 ng/ml; obtained from Genetics Institute, Boston, MA) for 4 h to allow up-regulation of integrin α5β1, which has previously been shown to be essential for adenovirus infection of monocytes (27). The cells were then replated on 100-mm petri dishes and infected for 2 h with a multiplicity of infection (MOI) of between 10:1 and 120:1 (in most experiments, 20:1, 40:1, or 80:1 was used) of either AdvIκBα or Adv0, in serum-free RPMI 1640. Cells were then incubated in RPMI 1640 supplemented as above for 48 h to allow for significant overexpression of IκBα, as assessed (7). During the changes of medium involved, nonadherent cells were discarded, resulting in a further purification of monocyte-derived macrophages.

### Cytokine analysis

For Northern blot analysis experiments, cells were replated at 5–10 × 10^6 cells per 100-mm petri dish and stimulated with LPS (10 ng/ml), PMA (10 nM), zymosan (30 μg/ml), ionomycin (1 μM), or UV irradiation (200 J). After 4 h, cells were harvested, and mRNA was extracted and subjected to Northern blot analysis as in Reference 28.

For the assays for cytokine production, cells were replated at 5 × 10^4 cells per well on a 96-well dish and stimulated as above for 4 or 16 h. Supernatants were analyzed for TNF-α (29), IL-1β, IL-6, and IL-8 (30), IL-10 (31, 32), IL-1ra, and the p55 and p75 soluble TNF receptors (33) by ELISA. The proteosome inhibitor benzoxycarbonyl-Ile-Glu(O-tosyl-buty)-Ala-leucin (PSTI) was obtained from Calbiochem (Nottingham, U.K.).

### Electrophoretic mobility shift assay

Nuclear extracts were prepared and 20 μg of protein was analyzed for NF-κB activity as previously described (34).

### Statistical methods

All statistical testing was performed using a paired comparison, one-sided Student’s t test, except when a Scheffe test of multiple comparisons was used as indicated (35).

### Results

#### Cytokine production in response to various stimuli

LPS is capable of inducing all the cytokines (TNF-α, IL-1β, IL-6, and IL-8) and inhibitors (IL-10, IL-1ra, and the soluble TNF receptors) assayed in this study (Table I; results are means of 4–11 experiments) each using blood from different donors. The induction of TNF-α mRNA by zymosan has previously been reported to be far weaker than LPS-induced TNF-α mRNA in mouse peritoneal macrophages (36), but the TNF-α response to zymosan in macrophage CSF-treated human monocytes was equal to that of LPS (Table I). LPS was a much stronger inducer of IL-1β than any other stimulus used, in agreement with observations in mouse macrophages (36). IL-6 was induced equally well by LPS and zymosan. IL-8

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Proinflammatory Cytokines (pg/ml)</th>
<th>Antiinflammatory Cytokines (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α</td>
<td>IL-1</td>
</tr>
<tr>
<td>LPS</td>
<td>9331 ± 740</td>
<td>3777 ± 311</td>
</tr>
<tr>
<td>Zymosan</td>
<td>7811 ± 711</td>
<td>241 ± 111</td>
</tr>
<tr>
<td>PMA</td>
<td>5844 ± 555</td>
<td>444 ± 201</td>
</tr>
<tr>
<td>UV light</td>
<td>1966 ± 366</td>
<td>249 ± 120</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>X ± 0</td>
<td>204 ± 89</td>
</tr>
</tbody>
</table>

Table I. Cytokine production induced by various stimuli
could be induced by the entire array of stimuli. Zymosan and LPS were the only stimuli to induce IL-10 and the p55 soluble TNF receptor (Table I), whereas PMA could induce IL-1ra and p75 soluble TNF receptor.

**Does IκBα overexpression inhibit LPS-induced proinflammatory cytokines?**

We previously observed that infection at an MOI of 20–80:1 with the IκBα adenovirus produced high levels of IκBα expression. This resulted in a potent inhibition of LPS-driven TNF-α induction in human macrophages by blocking NFκB function (7). This was not due to loss of cells through apoptosis or other causes of cell death (7). The induction of IL-1β and IL-8 by LPS also appears to be strongly NF-κB dependent (Fig. 1, A and B), and there is potent inhibition of both of these cytokines already at 20:1 of AdvIκBα. Results using supernatants harvested after 4 h of incubation were similar to those after 16 h of incubation (not shown). In contrast to the other cytokines studied here, there was a potentiation of IL-6 production of ∼20–30% in Adv0-infected cells, with LPS as well as with other stimuli (Fig. 1C), although adenovirus infection alone had no effect. Nevertheless, IL-6 expression was strongly inhibited by infection with AdvIκBα in human macrophages (Fig. 1C).

Since TNF-α can induce the synthesis of other cytokines, e.g., IL-1β, IL-6, and IL-8, the potent inhibition by AdvIκBα might be secondary to inhibition of TNF-α. Culturing with exogenous TNF-α partially abrogated the effect of IκBα overexpression on IL-1β production (Fig. 2A), indicating at least a partial dependence on TNF-α (there was just a 20% inhibition in the presence of 100 ng/ml TNF-α, compared with 62% inhibition with no cytokine; difference was significant (p < 0.005) between these two comparisons using a Scheffé multiple-comparisons test). However, similar experiments showed no major role for TNF-α in LPS-induced expression of IL-6 and IL-8 (results not shown).

To further investigate the signaling mechanisms involved, we sought to characterize whether the TNF-α-driven IL-1β response might be less NF-κB dependent than the IL-1β response induced by LPS. One difficulty in doing this was the fact that TNF-α is a weaker inducer of IL-1β in our system than LPS (Table I). However, the IκBα-induced inhibition of TNF-α-induced IL-1β was relatively less potent than that seen with TNF-α-induced IL-6 (Fig. 2, B and C), as expected from the restoration of IL-1β synthesis by TNF-α in cells infected with AdvIκBα.

**Does IκBα overexpression affect LPS-induced anti-inflammatory cytokines?**

The major anti-inflammatory cytokine produced by macrophages is IL-10 (37, 38). In LPS-stimulated cells, there was gradual inhibition with increasing virus titers, but statistically significant inhibition (p < 0.05) was only noted at 60:1 or 80:1 of AdvIκBα (Fig. 3A). This inhibition was still quite modest (30% at most), and since the human IL-10 promoter lacks κB sites (14), we investigated whether the inhibitory effects of AdvIκBα infection were indirect, occurring via its effects on proinflammatory cytokines that are known to influence IL-10 expression (32, 39, 40). A combination of TNF-α and LPS considerably potentiated the IL-10 response and partially abrogated the inhibition by AdvIκBα (Fig. 3B).
FIGURE 3. Inhibition of NF-κB has only minor effects on IL-10 production. Effect of infection of human monocyte-derived macrophages with various titers of AdvIkBα or Adv0 on IL-10 production induced by LPS (10 ng/ml), expressed as a percentage of IL-10 production induced by the same stimulus in uninfected cells (A). In B, pretreatment with 20 ng/ml TNF-α abrogates the inhibitory effect of AdvIkBα infection on LPS-induced IL-10 production (pg/ml). Error bars indicate SEM (n = 7–10).

FIGURE 4. Effect of IkBα infection on IL-1ra production. Effect of infection of human monocyte-derived macrophages with various titers of AdvIkBα or Adv0 on the LPS-induced production of IL-1ra, expressed as a percentage of the production of the same cytokine in uninfected cells challenged with the same stimulus (A). Pretreatment with IL-1 or TNF-α (20 ng/ml) alone does not affect the inhibitory effect of AdvIkBα infection on the LPS-induced production of IL-1ra, but treatment with both cytokines moderately reverses it (B). Error bars indicate SEM (n = 7–8).

contrast, IL-1 failed to have any significant effect (results not shown).

The LPS-induced production of IL-1ra was significantly (p < 0.05) inhibited (~30%) by IkBα overexpression (Fig. 4A). This response was only modestly affected by adding back IL-1 and TNF-α (Fig. 4B). In human macrophages, LPS induces the production of the p75 soluble TNF receptor and to a lesser extent the p55 soluble TNF receptor (Table I). Infection with 40:1 of AdvIkBα was observed to significantly (p < 0.001) inhibit the LPS-induced production of both of these soluble receptors (Fig. 5); this response was unaffected by adding back IL-1 or TNF-α (results not shown).

**Does IkBα overexpression inhibit proinflammatory cytokines induced by PMA or UV light?**

The induction of TNF-α by PMA is very potently (~90%) inhibited by IkBα overexpression, even more strongly than in LPS-stimulated (~80%) (7) or even UV light-stimulated cells (~80%) (Table II). Again, results using supernatants harvested after 4 h of incubation were similar to those using 16 h of incubation (data not shown). A series of Northern blot analysis experiments demonstrated that, as for LPS-induced TNF-α (7), the TNF-α mRNA expression in response to PMA and UV light was ablated by IkBα overexpression (Fig. 6, A and B).

As shown for TNF-α, the induction of IL-1β and IL-6 by PMA or UV light was NF-κB dependent (Table II). Once again, the inhibition of IL-1β and IL-6 production by the AdvIkBα infection appears to be more potent in PMA-treated cells than in cells treated with LPS or UV light.

There was also significant (p < 0.001) inhibition (50–60%) of the IL-8 response when cells infected with AdvIkBα were stimulated with PMA or UV light (Table II). Ionomycin, a stimulus that did not induce significant amounts of the other cytokines of interest, induced a discernible IL-8 response, which was also inhibited by IkBα overexpression (data not shown).

PMA did not induce detectable amounts of IL-10, but it induced a detectable p75 soluble TNF receptor response, which was significantly (p < 0.001) inhibited (75% at 40:1 of AdvIkBα) by IkBα overexpression, as was the PMA-induced production of IL-1ra (40% inhibition at 40:1 of AdvIkBα; p < 0.005).

**Does IkBα overexpression affect zymosan-induced cytokines?**

In contrast to the prior stimuli when the macrophages were activated with zymosan, infection with AdvIkBα had no effect whatsoever on TNF-α protein (Table II) or mRNA expression (Fig. 6C), even at MOI of 80:1. The induction of IL-1β and IL-8 by zymosan was also unaffected by the IkBα overexpression (Table II), but there was a small (10–15% compared with uninfected cells) and statistically nonsignificant inhibition of IL-6. Zymosan-induced IL-10, IL-1ra (Table II), and the soluble TNF receptors (not shown) were also refractory to inhibition by IkBα overexpression.

The independence from NF-κB of zymosan-induced TNF was further emphasized by studies with the proteosome inhibitor PSI. Inhibition of proteosome function inhibits IkBα degradation, thus preventing NF-κB nuclear translocation (41, 42). PSI was very
effective in blocking LPS-induced TNF production, but it did not affect the response to zymosan (Fig. 7).

To study the mechanisms involved in more detail, a series of electrophoretic mobility shift experiments was performed. While LPS (10 ng/ml) induced a rapid (20–30 min) and potent activation of NF-κB, the activation of this transcription factor by zymosan (30 μg/ml) was slower (detectable only after 60 min) and much weaker (Fig. 8). Increasing the zymosan concentration did not enhance its effect on NF-κB (not shown). The activation of NF-κB by either stimulus was inhibited by IκBα overexpression (Fig. 8).

Although a 60-min incubation with TNF-α (1–10 ng/ml) activated NF-κB weakly, lower concentrations did not, and the amount of TNF-α produced after a 60-min incubation with zymosan is <100 pg/ml (not shown), arguing against a feedback loop of this kind being involved.

Discussion

We have recently shown that adenoviral gene transfer into macrophages provides a reliable, reproducible, and convenient method of studying intracellular signaling pathways (7). Transfer of the IκBα effectively inhibits NF-κB activity in human macrophages, mainly through the overexpression of IκBα inhibiting nuclear translocation of the p65/p50 subunits of NF-κB. This blocked LPS-induced TNF-α both at the mRNA and the protein levels. Even more interesting, it was found that the endogenous production of TNF-α from rheumatoid synovial mononuclear cell cultures was also inhibited (7).

The fact that LPS-induced TNF-α, IL-1β, and IL-6 were all NF-κB dependent cytokines could be expected from the majority of data from murine cells and monocyte/macrophage cell lines (9, 10). Our results on LPS-induced cytokines also agree well with the results of Makarov et al. (19) on LPS-induced IL-1β, IL-6, and IL-8 in monoctytic THP-1 cells stably modified through retroviral

<table>
<thead>
<tr>
<th>Treatment</th>
<th>p75 % of LPS-induced production from uninfected cells</th>
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<tbody>
<tr>
<td>Adv1κBα 40:1</td>
<td>10.4 ± 1.0</td>
</tr>
<tr>
<td>Adv1κBα 20:1</td>
<td>9.7 ± 2.7</td>
</tr>
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</table>

**Table II. Effects of Adv1κBα infection on cytokine production induced by various stimuli**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-1ra</th>
<th>p75 sTNF-R</th>
<th>p44 sTNF-R</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA Adv0</td>
<td>95.8 ± 3.3</td>
<td>120.9 ± 6.7</td>
<td>120.4 ± 10.9</td>
<td>124.5 ± 4.1</td>
<td>124.5 ± 7.8</td>
<td>110.2 ± 7.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PMA Adv1κBα</td>
<td>10.4 ± 1.0</td>
<td>16.5 ± 0.8</td>
<td>8.5 ± 0.5</td>
<td>30.5 ± 2.0</td>
<td>60.8 ± 11.7</td>
<td>22.8 ± 2.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>UV light Adv0</td>
<td>98.9 ± 2.3</td>
<td>102.2 ± 6.8</td>
<td>133.7 ± 8.2</td>
<td>108.5 ± 2.3</td>
<td>94.8 ± 8.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>UV light Adv1κBα</td>
<td>21.7 ± 0.9</td>
<td>40.2 ± 3.6</td>
<td>28.3 ± 2.6</td>
<td>54.5 ± 3.6</td>
<td>68.9 ± 5.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Zymosan Adv0</td>
<td>97.5 ± 6.6</td>
<td>95.4 ± 9.8</td>
<td>143.6 ± 19.2</td>
<td>93.0 ± 4.0</td>
<td>108.0 ± 5.8</td>
<td>107.2 ± 8.4</td>
<td>123.6 ± 11.7</td>
<td>92.7 ± 7.2</td>
</tr>
<tr>
<td>Zymosan Adv1κBα</td>
<td>93.5 ± 6.4</td>
<td>92.8 ± 10.0</td>
<td>90.1 ± 12.9</td>
<td>94.5 ± 2.8</td>
<td>103.6 ± 9.1</td>
<td>90.3 ± 9.2</td>
<td>110.1 ± 6.4</td>
<td>101.4 ± 4.4</td>
</tr>
</tbody>
</table>

*Percent of cytokine production compared with untreated cells (± SEM, n = 5–9). The virus titer was 40:1 in each experiment.*
gene transfer of IκB. Among the LPS-induced, proinflammatory cytokines studied here, IL-6 was most potently inhibited (>85%) by overexpression of IκBα, whereas there was always some residual production of TNF-α or IL-1β even in LPS-stimulated cells infected with high titers (120:1) of AdvIκBα (not shown). This may reflect a certain amount of preformed cytokine mRNA, but this could not be demonstrated in unstimulated cells (data not shown), and furthermore LPS (7), PMA-induced or UV-induced (Fig. 6) TNF-α mRNA was profoundly down-regulated by IκBα. However, it could not be excluded that this residual cytokine production emanated from the few uninfected cells still present, and work is in progress to elucidate this question using intracytoplasmic staining for cytokines. Another nonexclusive hypothesis (discussed in more detail below) is that TNF-α and other proinflammatory cytokines can be induced through both NF-κB-dependent and NF-κB-independent pathways.

Our finding that UV light induces a whole spectrum of proinflammatory cytokines in a NF-κB-dependent manner is novel. It is in contrast to the earlier report that UV-induced TNF-α in RAW 264.7 cells does not involve NF-κB (15). Similarly, the finding that the PMA-induced induction of TNF-α and other proinflammatory cytokines is profoundly down-regulated by IκBα overexpression disagrees with several earlier studies in stably transformed human cell lines (19). In our hands, this stimulus was actually the one most strongly dependent on NF-κB, as judged by the percentage of inhibition, reproduced in seven separate experiments. These discrepancies between results obtained with human primary cells and those from various transformed cell lines indicate that, at least in some instances, the latter are questionable models for studying cytokine cell signaling occurring in primary cells, as is the case in vivo. In a way, this is not surprising, since there are interactions between the enzymes and transcription factors of the cell cycle machinery and the regulation of cytokine genes, e.g., Rb regulates ets, which is involved in cytokine activation (43).

With regard to macrophage signal transduction, one of the most remarkable findings in the present study was that zymosan, although a very powerful macrophage activator, does not appear to require NF-κB for the induction of either pro- or anti-inflammatory cytokines. These findings would imply that there are, in human macrophages, both NF-κB-dependent and NF-κB-independent pathways of cytokine induction involved in the induction of TNF-α and other proinflammatory cytokines. Although zymosan does activate NF-κB, it does so more slowly and much less potently than LPS (Fig. 8), and it is likely that some other transcription factor mediates the zymosan-induced cytokine production. The modest (15%) inhibition of zymosan-induced IL-6 observed in cells overexpressing IκBα is of questionable significance and may reflect the observation that this cytokine was the most potently affected by IκBα overexpression, irrespective of stimulus (see Table II).

Another finding of importance is that in human macrophages, IL-10 is under complex control, and in LPS-stimulated cells, it appears to be at least partially driven via LPS-induced TNF-α and IL-1. It is interesting to note that, even at 40:1 of AdvIκBα, when LPS-driven TNF-α is abrogated by >60% (7), IL-10 is still not significantly inhibited (Fig. 3). At higher virus titers, resulting in even stronger inhibition of TNF-α, there is some effect also on IL-10, but never, even with 80:1 of the virus, exceeding 30%. This is completely reversible by adding back TNF-α, which implies that LPS-induced IL-10 is partly driven secondarily by TNF-α. This finding agrees well with previous reports (14, 32, 40, 44) and indicates that autocrine interactions can take place, even in short-term (16-h) cultures such as these.

Another intriguing finding was that the IκBα-inhibition of the LPS-induced production of IL-1β (Fig. 2A), but not the production of IL-6, IL-8, or the soluble TNF receptors (data not shown), was also somewhat abrogated when TNF-α was restored. This indicates that TNF-induced IL-1β is mainly independent of NF-κB. This finding also suggests that IL-1β is also, although to a lesser extent than IL-10, driven partly by LPS-induced TNF-α. This result echoes the previous work in rheumatoid arthritis joint cell cocultures, in which TNF-α blockade was found to inhibit the production of IL-1 (45) and subsequently of IL-6, IL-8, IL-10 and granulocyte-macrophage CSF (30, 46), which has led to the concept of a TNF-α-dependent “cytokine cascade” in inflammatory sites such as the rheumatoid synovium (1). The current macrophage cultures are shorter term than the rheumatoid synovial cultures (16 h vs 5 days), which may explain why the “cascade” appears more marked in the latter system.

The dissection out of signaling pathways in normal primary cells is necessary, as there are differences from cell lines (see above). This is now possible within human macrophages, from
either normal or pathological specimens, using this adenosine technique. The model of human macrophages infected with Ad5ΔE3A has, from a cytokine point of view, provided results similar to those from infecting human synovial co-cultures with the same virus (7) (J. Bondeson et al., manuscript in preparation). Taken together, the data suggest that NF-κB is an important therapeutic target in chronic inflammatory diseases, allowing profound down-regulation of macrophage-produced proinflammatory cytokines while not directly affecting the most important anti-inflammatory cytokines, IL-10 and IL-1ra. This would redress the disturbed equilibrium between these mediators (1).

Acknowledgments

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References