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Role of ascorbate in the activation of NF-κB by tumour necrosis factor-α in T-cells

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The first product of ascorbate oxidation, the ascorbate free radical (AFR), acts in biological systems mainly as an oxidant, and through its role in the plasma membrane redox system exerts different effects on the cell. We have investigated the role of ascorbate, AFR and dehydroascorbate (DHA) in the activation of the NF-κB transcription factor in Jurkat T-cells stimulated by tumour necrosis factor-α (TNF-α). Here we show, by electrophoretic mobility shift assays, that ascorbate increases the binding of NF-κB to DNA in TNF-α-stimulated Jurkat cells. The ability of ascorbate to enhance cytoplasmic inhibitory IkBα protein degradation correlates completely with its capacity to induce NF-κB binding to DNA and to potentiate NF-κB-mediated transactivation of the HIV-1 long terminal repeat promoter in TNF-α-stimulated Jurkat cells but not in cells stimulated with PMA plus ionomycin. AFR behaves like ascorbate, while DHA and ascorbate phosphate do not affect TNF-α-mediated NF-κB activation. These results provide new evidence for a possible relationship between the activation of the electron-transport system at the plasma membrane by ascorbate or its free radical and redox-dependent gene transcription in T-cells.

INTRODUCTION

Cell plasma membrane contains an electron-transport chain which is essential in the control of cell growth and differentiation [1]. Ascorbate is a redox active compound that by two one-electron reduction steps acts as an antioxidant to prevent lipid peroxidation by quenching oxygen-derived radicals or regenerating the lipophilic antioxidant α-tocopherol [2]. The first product of ascorbate oxidation, the ascorbate free radical (AFR), acts mainly as an oxidant in the cell system and is reduced to ascorbate at the plasma membrane by the coenzyme Q-dependent NADH:AFR reductase [3], leading to the regeneration of ascorbate at the cell surface [4,5]. This cycle of ascorbate/AFR is maintained by cells *in vivo* and *in vitro* [6,7] and participates in the potentiation of plasma-membrane-dependent mechanisms to control cell growth [8].

In recent years, emerging evidence suggests an important role for this vitamin in cell growth [8,9]. Ascorbate increases the mitogen response of human peripheral lymphocytes to lectins [10] and human promyelocytic cell growth in serum-limiting media [11], and also induces programmed cell death in neuroectodermal and melanoma cells [12].

Although the mechanisms used by external oxidants in order to regulate cell growth are largely unknown, it is possible that they modulate cell signalling and/or the activity of some transcription factors. Among those transcription factors, NF-κB is one of the more extensively studied. This factor, formerly described as a kappa enhancer binding transcription factor constitutively present in the nuclei of mature B-cells [13], is part of a pleiotropic family of transcription factors involved in the regulation of many cellular genes whose products participate in the mechanisms of cell growth and also in the transcriptional regulation of determinated viral genes [14,15]. In most cell types, NF-κB is found in an inactive cytosolic form, retained by association with its inhibitor, IκB [14,15]. Upon cell activation by phorbol esters, mitogens, cytokines, UV and viral proteins, IκB undergoes rapid phosphorylation and degradation, then releasing the active NF-κB complex to the nucleus [16,17]. It is assumed that redox reactions may be mediators of different activating signals [18]. NF-κB can be activated by exogenous hydrogen peroxide in some types of cells [19], and protein phosphorylation is known to be increased after the action of extracellular oxidants in T-cells [20]. Also, NF-κB activation by different stimuli can be inhibited by antioxidant thiols or iron chelators by preventing IκBα phosphorylation [21], a process that is absolutely required for the IκBα ubiquitination and degradation by the 26S proteasome [22,23].

We show here the role of ascorbate in the mechanisms activated by tumour necrosis factor-α (TNF-α) in Jurkat cells. Ascorbate and its free radical potentiate IκBα degradation and NF-κB activation, and consequently HIV long terminal repeat (HIV-LTR) gene transactivation. Moreover, this activation leads to the inhibition of cell growth. We discuss our data on the basis of the action of ascorbate through its free radical which, as an oxidant, activates the plasma membrane electron transport and leads to the redox-dependent regulation of gene transcription by NF-κB.

MATERIALS AND METHODS

Cell lines and reagents

The Jurkat T-cell line was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The 5.1 (obtained from Dr. N. Israël, Institut Pasteur, Paris, France) and J-170 (obtained from Dr. A. Corbí, C.S.I.C., Granada, Spain) cell lines

Abbreviations used: AFR, ascorbate free radical; DHA, dehydroascorbate; LTR, long terminal repeat; TNF-α, tumour necrosis factor-α; EMSA, electrophoretic mobility shift assay.

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are Jurkat-derived clones stably transfected with a plasmid containing the HIV-LTR or the CD11a promoter-derived luciferase gene respectively. Cells were maintained in exponential growth in RPMI 1640 (Sigma Chemical Co.) supplemented with 10% (v/v) fetal-calf serum, 2 mM L-glutamine and penicillin/streptomycin (100 units/ml) in a humidified atmosphere of air/CO_2 (19:1) at 37 °C. In addition, 5.1 and J-170 cells were always maintained in the presence of geneticin (100 μg/ml). PMA, ionomycin, ascorbate, dehydroascorbate (DHA) and superoxide dismutase were all from Sigma Chemical Co. (Deisenhofen, Germany). Ascorbate phosphate was obtained from Dr. D. J. Morré (Purdue University, West Lafayette, IN, U.S.A.). [*methyl*-^{3}H]Thymidine (68 Ci/mmol) was from ICN (Irvine, CA, U.S.A.). AFR was prepared as a mixture of ascorbate and DHA at equimolar concentration in distilled water, adjusted to pH 6.5 with 1 M imidazole, as described elsewhere [11].

Cell extracts and mobility shift assays

Jurkat cells were cultured at 2×10^5 cells/ml and stimulated with TNF-α, or PMA plus ionomycin, in the presence or absence of 0.2 mM ascorbate or its derived products for the indicated times, and then the cells were washed twice with cold PBS. Cellular extracts were prepared as described previously [24]. For the electrophoretic mobility shift assay (EMSA), a double-stranded oligonucleotide containing the κB binding site located in the enhancer region of the H-2^b promoter was used (KBF). The binding reaction mixture contained 10 μg of protein extracts, 1 μg of poly(dI-dC), 20 mM Hepes (pH 7), 50 mM NaCl, 2 mM dithiothreitol, 0.01% (w/v) Nonidet P40, 100 μg/ml BSA, 4% (w/v) Ficoll and 100000 c.p.m. of end-labelled DNA fragments in a total volume of 20 μl. After 30 min incubation at room temperature, the mixture was electrophoresed through a 6% (w/v) polyacrylamide gel under non-denaturing conditions and subsequently dried and exposed at −80 °C to XAR film. When indicated, preimmune serum or specific antiserum anti-p50 (0.5 μl) was included in the binding reaction before the addition of the radiolabelled probe.

Western blots

Proteins (30 μg) were boiled in Laemmli buffer and electrophoresed in denaturing SDS/polyacrylamide (12%) gels. Separated proteins were transferred to nitrocellulose membranes and immunodetection of IκBα protein was carried out with the anti-(MAD-3) monoclonal antibody 10B, given by Drs. K. Wood and R. T. Hay (St. Andrews, Fife, U.K.). Immunodetection of the p50 subunit of NF-κB was performed with a specific anti-p50 antiserum kindly provided by Dr. A. Israël (Institute Pasteur). Proteins detected by the primary antibody were visualized with the use of an enhanced chemiluminescence system (Amersham, Amersham, Bucks., U.K.).

Transient transfections and luciferase activity

Jurkat cells (2×10^7) were transfected by electroporation (Bio-Rad Gene Pulser apparatus) at 150 V/960 μF a with 20 μg of the following plasmids: (1) the (κB)x3-conaluc plasmid that was constructed by inserting three copies of the MHC enhancer κB site upstream of the conalbumin promoter followed by the luciferase gene (KBF-Luc); (2) the HIV-LTR plasmid followed by the luciferase gene; and (3) the ΔHIV-LTR mutated in the NF-κB binding site linked to the luciferase gene (given by Dr. A. García, Institut Pasteur). Transfected Jurkat, J-170 or 5.1 cells were stimulated for 6 h and then lysed in 25 mM Tris/phosphate buffer (pH 7.8), 8 mM $MgCl_2$, 1 mM dithiothreitol, 1% (v/v) Triton X-100 and 15% (v/v) glycerol. Luciferase activity was measured in a luminometer (Berthold). The background obtained with the lysis buffer was subtracted from each experimental value. Experiments were performed in duplicate and the mean RLU (relative light unit) values were calculated. All the experiments were repeated at least three times. Statistical analysis was performed using analysis of variance followed by the Student–Newman–Keuls method with values of $P < 0.05$ considered to be significant.

Proliferation assays

Jurkat cells (2.5×10^5) were incubated in a 96-well microtitre plate (200 μl/well) in complete RPMI-1640 medium and then stimulated with different doses of TNF-α with or without 0.2 mM ascorbate. Proliferation was measured by [^{3}H]thymidine incorporation (0.5 μCi/well) for the last 6 h of a 48-h culture period. Radioactivity incorporated into DNA was measured by liquid-scintillation counting and specific activity was expressed as d.p.m./10^6 cells.

RESULTS AND DISCUSSION

To study the role of ascorbate in the activation of NF-κB we stimulated Jurkat cells, a T lymphoblastoid cell line, with TNF-α, or PMA plus ionomycin, for 60 min in the presence or absence of 0.2 mM ascorbate, a concentration that has been shown to be optimal to stimulate growth and differentiation induced by hapten stimuli [25]. Nuclear extracts were obtained and analysed by EMSA using an NF-κB-specific ^{32}P-labelled oligonucleotide. As shown in Figure 1 (left), ascorbate mediated a clear increase in the NF-κB binding induced by TNF-α. In contrast, when the cells were stimulated with PMA plus ionomycin the ascorbate effect was less evident. The specificity of NF-κB binding was

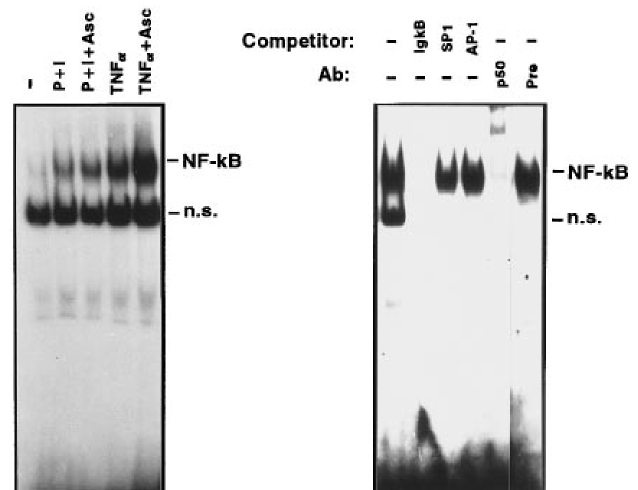

Figure 1 Ascorbate increases TNF-α-mediated NF-κB activation

Left: Jurkat T-cells were stimulated with PMA (P; 50 ng/ml) plus ionomycin (I; 0.5 μg/ml) or with TNF-α (TNF$_\alpha$; 50 ng/ml) for 60 min in the presence or absence of ascorbate (Asc; 0.2 mM). Nucleoproteins were extracted and subjected to EMSA using an end-labelled κB probe. Right: KBF binding of proteins from TNF-α-plus-ascorbate-stimulated cells was competed with a 50-fold molar excess of cold oligonucleotide IgκB which contains the NF-κB motif of the Ig promoter, and the unrelated oligonucleotides SP1 and AP-1. The same protein extract was preincubated either with anti-p50 antiserum or with preimmune serum (Pre) before the addition of the radiolabelled KBF probe. The NF-κB/DNA complex is indicated (n.s., non-specific).

Table 1 Effect of ascorbate on NF-κB transcription in TNF-α-stimulated Jurkat cells

Jurkat cells were transfected with either the NF-κB-dependent luciferase reporter constructs KBF-Luc and HIV-LTR-Luc or the NF-κB mutant of HIV-LTR (mHIV-LTR-Luc). After 12 h cells were stimulated with TNF-α (50 ng/ml) with or without ascorbate (0.2 mM) for 6 h. Luciferase activity was measured as described in the Materials and methods section and transcriptional activity expressed as x-fold transactivation over the values obtained with non-stimulated cells. Values are means $\pm$ S.D. of three independent experiments. $^{*}P < 0.01$ compared with TNF-α-stimulated cells.

		Luciferase activity (fold-induction)		
Treatment	Plasmid transfected ...	KBF-Luc	HIV-LTR-Luc	mHIV-LTR-Luc
None		1	1	1
TNF-α		15.3 $\pm$ 2	17 $\pm$ 2.5	1
TNF-α + ascorbate		27 $\pm$ 3*	32 $\pm$ 3.1*	1
Ascorbate		1	1	1

Table 2 Effect of ascorbate on TNF-α- or PMA-plus-ionomycin-stimulated 5.1 and J-170 cells

5.1 and J-170 cells were stimulated with either TNF-α or PMA plus ionomycin (I) in the presence or absence of ascorbate (0.2 mM) for 6 h. Results are means $\pm$ S.D. of five independent experiments. $^{*}P < 0.001$ compared with TNF-α-stimulated 5.1 cells; $^{**}P < 0.25$ (not significant) compared with PMA + I-stimulated 5.1 cells.

	Fold-induction	
Treatment	5.1 cells	J-170 cells
None	1	1
TNF-α	16.3 $\pm$ 2	1
TNF-α + ascorbate	29.3 $\pm$ 4*	1
PMA + I	6.2 $\pm$ 3	1
PMA + I + ascorbate	4.1 $\pm$ 2**	1
Ascorbate	1	1

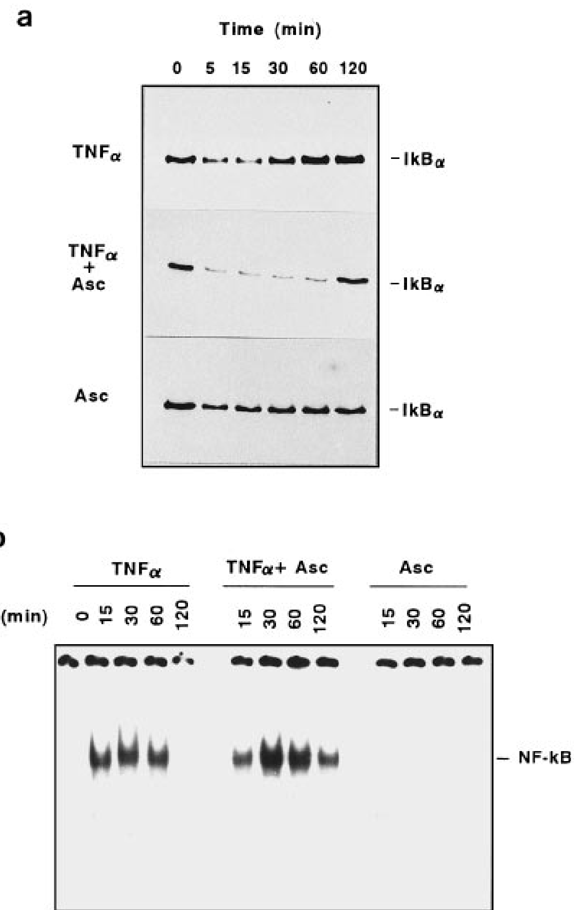

Figure 2 Effect of ascorbate on the kinetics of TNF-α-mediated degradation of IkBα and NF-κB binding to DNA

Jurkat cells were stimulated with TNF-α (TNF_{α}; 50 ng/ml), TNF-α plus ascorbate (Asc) (0.2 mM) or ascorbate alone. At the times indicated cells were collected for the preparation of cellular extracts and analysed for IκBα expression by Western blot analysis (**a**) and for DNA binding of NF-κB by EMSA analysis (**b**).

demonstrated by cold competition and supershift experiments. In Figure 1 (right) it is shown that an excess (50 $\times$) of cold NF-κB consensus site (IgκB), but not of SP1 or AP-1 cold oligonucleotides, competed with the upper complex. The lower complex was not specifically competed by the three cold oligonucleotides. Moreover, the anti-p50 antiserum but not the preimmune antiserum prevented the binding of NF-κB to DNA. Again, both antisera were able to inhibit the binding of the lower complex to DNA, indicating that the binding of this complex was not specific.

To demonstrate that increased NF-κB binding activity was also reflected by increased NF-κB-mediated transcriptional activation, we transiently transfected Jurkat cells with a reporter gene construct carrying three copies of the NF-κB motif in front of the luciferase gene, or with a plasmid containing the HIV-1-LTR followed by the luciferase gene, or with an mHIV-LTR plasmid in which the NF-κB site was mutated. Transfected cells were stimulated with TNF-α, TNF-α plus ascorbate, or ascorbate alone for 6 h and luciferase activity was measured and used as an index of transactivation. Ascorbate itself was not able to induce either NF-κB-dependent, or HIV-LTR, transactivation (Table 1). Notwithstanding, ascorbate clearly increased NF-κB-mediated HIV-LTR transactivation induced by TNF-α in Jurkat cells. Results obtained with the NF-κB-Luc plasmid and with the NF-κB mutant of HIV-LTR demonstrated that the activity of ascorbate in TNF-α-induced NF-κB activation was specific for this factor. We then used 5.1 cells, a Jurkat-derived clone stably transfected with a plasmid containing the HIV-LTR gene in front of the luciferase gene, as another approach to study the role of ascorbate on the HIV-LTR transcriptional regulation. As expected, ascorbate potentiated TNF-α-induced HIV-LTR transactivation, and did not affect the activation mediated by PMA and ionomycin (Table 2). To control the effect of ascorbate on luciferase activity, a Jurkat cell (J-170) line stably transfected with a plasmid containing the luciferase gene reported by the CD11a promoter gene was stimulated with the same stimulus that was used for 5.1 cells. CD11a gene promoter was not transactivated by either TNF-α, or PMA plus ionomycin, and ascorbate did not affect the luciferase activity measured in this cell line (Table 2). As was described above, binding activity detected in stimulated cells by both TNF-α and PMA plus ionomycin correlated with the transcriptional activity, but ascorbate potentiation was specific for TNF-α-mediated stimulation. Thus the action of ascorbate in the Jurkat cell line is dependent on the appropriate stimulus. This is in agreement with

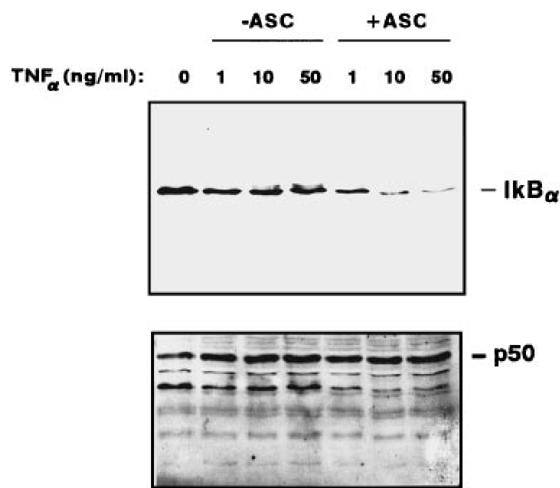

Figure 3 Dose is dependent on TNF-α-induced IκBα degradation in Jurkat cells

Jurkat cells (0.5×10^6/ml) were plated in 35 mm Petri dishes and stimulated with the indicated doses of TNF-α (TNF$_\alpha$) in the presence or absence of ascorbate (ASC; 0.2 mM). After 1 h of incubation the cells were collected for the preparation of cellular extracts, and analysed for IκBα (IκB$_\alpha$) and p50 expression by Western blot.

Table 3 Effect of TNF-α on ascorbate-treated 5.1 cells

5.1 cells were incubated with ascorbate (0.2 mM) and stimulated with different concentrations of TNF-α for 6 h. Results are means $\pm$ S.D. of three independent experiments. Index of transactivation was calculated as the ratio between luciferase activity detected in stimulated versus non-stimulated cells. $^*P < 0.05$ compared with TNF-α-stimulated 5.1 cells; ns, not significant.

Treatment	Index of transactivation
None	1
TNF-α (1 ng/ml)	10.2 ± 2
TNF-α (5 ng/ml)	12.3 ± 3
TNF-α (10 ng/ml)	15 ± 5
TNF-α (1 ng/ml) + ascorbate	14.4 ± 4 (ns)*
TNF-α (5 ng/ml) + ascorbate	24.3 ± 2*
TNF-α (10 ng/ml) + ascorbate	29.2 ± 2*

the previous concept that ascorbate functions at the plasma membrane may be regulated by growth factors [26,27].

NF-κB activation is always preceded by proteolytic degradation of the inhibitory κB proteins [14–16,24]. Several IκB proteins have been identified at the molecular level, and two major species, IκBα and IκBβ, were found to be degraded in response to external stimuli [24,28], but only IκBα is degraded in response to TNF-α [28]. Thus we investigated the effect of ascorbate on IkBα degradation in TNF-α-stimulated Jurkat cells. This cytokine induced a rapid degradation of the IκBα protein followed by its recovery which was completed 30–60 min after stimulation (Figure 2a). When ascorbate was present, the degradation of IκBα was found to be more intense and its recovery delayed, and protein levels similar to that of non-stimulated cells were found after 2 h of activation. The more persistent degradation of IκBα in TNF-α plus ascorbate-activated cells parallelled with an increased NF-κB binding to DNA through time (Figure 2b). Ascorbate alone did not induce IκBα degradation (Figure 2a), or NF-κB binding to DNA (Figure 2b), or NF-κB transactivation (Tables 1 and 2).

The effect of ascorbate on Jurkat cells stimulated with decreasing doses of TNF-α was also studied. Western blots with proteins from total cell extracts showed that ascorbate increased the degradation of IκBα mediated by TNF-α, even at the lower doses tested. When the blot was reprobed with an anti-p50 antiserum it was found that the steady-state levels for p50 protein remained unchanged (Figure 3). Furthermore, the transcriptional activity of HIV-LTR induced by TNF-α in 5.1 cells was also increased by the presence of ascorbate (Table 3). Physiologically, this may be relevant since the concentration of TNF-α may vary in the different sites of the organism, and the effects of a small amount of this cytokine may be greatly enhanced by the presence of ascorbate.

To shed light on the mechanism of action of ascorbate on NF-κB activation in Jurkat cells, we stimulated Jurkat cells with TNF-α in the presence of ascorbate, AFR or DHA for 60 min. We found that AFR behaved similarly to ascorbate in increasing IκBα degradation and DNA binding of NF-κB in TNF-α-stimulated Jurkat cells (Figure 4). In contrast, DHA (the fully oxidized form of ascorbate) did not affect the activation of NF-κB by TNF-α (Figure 4). Similar results were found with

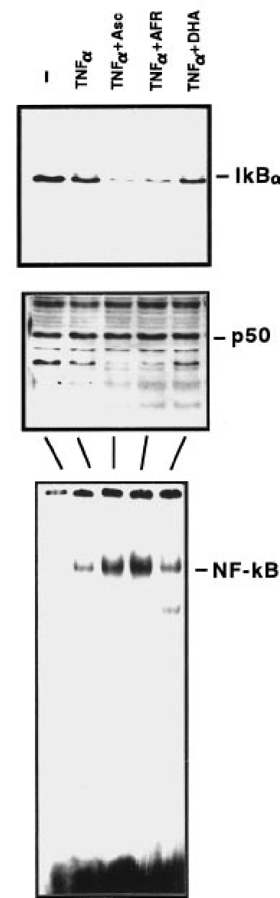

Figure 4 AFR but not DHA increases both IkBα degradation and NF-κB binding in TNF-α-stimulated Jurkat cells

Cells were stimulated with TNF-α (TNF$_\alpha$; 50 ng/ml) in the presence of either ascorbate (Asc), AFR or DHA (0.2 mM) as indicated. After 1 h, total cellular extracts were analysed for IkBα (IκB$_\alpha$) and p50 protein expression, and NF-κB binding to DNA as indicated in Figure 2.

Table 4 Effect of ascorbate, ascorbate phosphate (ASC-P), AFR, DHA and imidazole (I) on HIV-LTR transcription in TNF-α-stimulated 5.1 cells

5.1 cells were incubated with ascorbate, ASC-P, AFR, DHA and I at the indicated concentrations, stimulated with TNF-α for 6 h and luciferase activities were measured. Index of transactivation was calculated as the ratio between luciferase activity detected in stimulated and non-stimulated cells. Results are means ± S.D. of three independent experiments. *Compared with TNF-α- or TNF-α-plus-ASC-P-stimulated cells; **compared with TNF-α-stimulated cells (ns, not significant).

Treatment	Index of transactivation
None	1
TNF-α	14.1 ± 3
TNF-α + ascorbate (0.025 mM)	20.3 ± 3 $P < 0.05$*
TNF-α + ascorbate (0.05 mM)	22.6 ± 2 $P < 0.05$*
TNF-α + ascorbate (0.1 mM)	25.7 ± 2 $P < 0.05$*
TNF-α + ascorbate (0.2 mM)	28.5 ± 5 $P < 0.05$*
TNF-α + ASC-P (0.025 mM)	15.5 ± 4 ns**
TNF-α + ASC-P (0.05 mM)	14.1 ± 3 ns**
TNF-α + ASC-P (0.1 mM)	15.2 ± 2 ns**
TNF-α + ASC-P (0.2 mM)	17 ± 4 ns**
TNF-α + AFR (0.2 mM)	31.5 ± 3 $P < 0.05$*
TNF-α + DHA (0.2 mM)	15.2 ± 3 ns**
TNF-α + I (1 mM)	14.3 ± 3 ns**

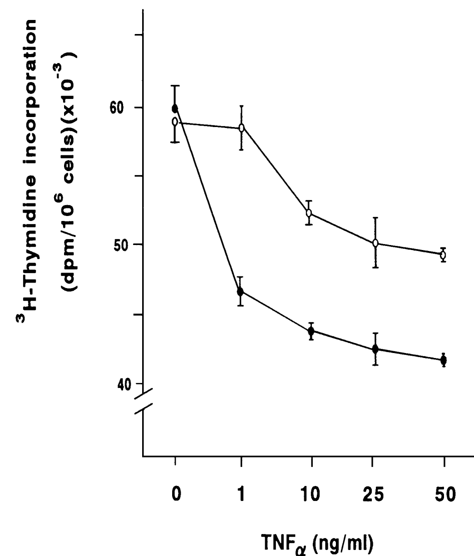

Figure 5 Effect of ascorbate on TNF-α-induced growth inhibition

Jurkat T-cells were incubated with different doses of TNF-α (TNF$_\alpha$) in the presence (●) or absence (○) of ascorbate (0.2 mM). DNA synthesis was measured by [^{3}H]thymidine incorporation for the last 6 h of the 48-h culture period. Mean values and standard deviations of triplicate samples are given. $P < 0.05$ for all concentrations of TNF-α when compared with TNF-α plus ascorbate-stimulated cells.

transactivating experiments in 5.1 cells (Table 4), where TNF-α-mediated HIV-LTR transcription was significantly increased by ascorbate and AFR. This increase was not observed in the presence of DHA or 1 mM imidazole (maximal concentration contained in the AFR preparation). Interestingly, the HIV-LTR transactivation in TNF-α-stimulated 5.1 cells was not significantly affected by ascorbate phosphate, a form of ascorbate that cannot be oxidized (Table 4) [29]. Thus it is likely that the apparent activity of ascorbate is mediated by the generation of AFR, the first product of its oxidation [2], and it is not due to the production of superoxide since exogenously added superoxide dismutase did not affect the action of ascorbate or AFR on HIV-LTR transcription in TNF-α-activated 5.1 cells (results not shown). It has been shown that ascorbate action on the stimulation of growth of different cells *in vitro* is also carried out by its free radical but not by superoxide generation [11,27].

The current concept is that IκBα is phosphorylated at serine residues 32 and 36 by an ubiquitinated IκB kinase. After phosphorylation, IκBα is ubiquitinated on lysine residues and then degraded by the ubiquitin proteasome pathway [23,30,31]. Several possibilities exist to explain the role of ascorbate and its free radical on the IκBα degradation mediated by TNF-α in Jurkat cells. Ascorbate and AFR could be involved in the ubiquitination of either the IκB kinase or the IκBα protein mediated by TNF-α. Mild oxidation of RNase A protein renders it more susceptible to ubiquitination [32]. However, these compounds could increase the catalytic activity of the IκB kinase or could inhibit some serine phosphatase involved in the NF-κB signalling pathway. It has been suggested that IκBα phosphorylation is regulated by a balance between kinase and phosphatase activities [21,33]. The putative phosphatase(s) involved in IκBα phosphorylation has not yet been identified and whether it may be inhibited by ascorbate remains to be studied.

AFR is the substrate for the NADH-dependent trans-plasma membrane electron transport of mammalian cells [3,5], and its activation at the cell surface induces changes to a more oxidative state of cytosol by increasing oxidized pyridine nucleotides [11]. The activation of this redox system may modulate the cytoplasmic redox equilibrium [34] and induce changes in the conformation of transcription factors [35]. Then, it is possible that ascorbate action at the plasma membrane involves changes in the signalling pathways used by TNF-α in Jurkat cells, probably starting at the receptor itself. For instance, it has recently been shown that ascorbate can mediate the activation of latent transforming growth factor-β1 receptor [36].

TNF-α is cytotoxic for a variety of cell lines by increasing the intracellular oxidative status. In Jurkat cells as well as in other T-cells, TNF-α is not cytotoxic but inhibits cell growth [37]. To investigate the role of ascorbate in TNF-α-mediated arrested growth of Jurkat cells, we studied the incorporation of [^{3}H]thymidine in the presence or absence of ascorbate (0.2 mM) at different concentrations of TNF-α. Figure 5 shows that ascorbate itself does not significantly affect the growth of Jurkat cells, but increases growth inhibition of this cell line in the presence of all the doses of TNF-α. This enhanced inhibition of cell growth correlated with the changes observed in the NF-κB activation in Jurkat cells.

Finally, our results demonstrate that in TNF-α-stimulated Jurkat cells, ascorbate and its free radical potentiate NF-κB activation and this effect is mediated by an accelerated rate of IκBα degradation. Redox modulation of cell nutrients due to plasma-membrane-acting factors or stress-induced responses may induce changes in cell signalling or protein conformation that could count as an important mechanism in the regulation of transcription factors and gene expression.

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