ANTAGONISTIC CONTROL OF TUMOR NECROSIS FACTOR RECEPTORS BY PROTEIN KINASES A AND C

Enhancement of TNF Receptor Synthesis by Protein Kinase A and Transmodulation of Receptors by Protein Kinase C

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TNF is now recognized as a typical member of the cytokine family with pleiotropic cellular activities (1–3). One of the main questions regarding TNF action relates to the intracellular control mechanisms that regulate TNF response patterns in both qualitative and quantitative terms. Though expression of TNF-specific membrane receptors is given in most normal and malignant tissues (4, 5), there is increasing evidence that control mechanisms of TNF responsiveness are effective at both receptor and postreceptor levels. For example, control at a postreceptor level is suggested from recent data showing that TNF-induced modulation of transcriptional programs of U937 cells was dependent on the differentiation status of the cell (6) and associated with the presence of a cytosolic phosphoprotein (pp26), a presumed TNF-specific signal transducer (7). Moreover, dominance of resistance to TNF-mediated growth inhibition of somatic cell hybrids between TNF-sensitive and -resistant cell lines was also shown to be due to postreceptor control (8, 9). At the receptor level, there exists a quantitative relationship between receptor number and sensitivity of a given TNF-responsive cell (3). Thus, we have shown that a non–tissue-specific, protein kinase C (PKC)[1]-dependent control mechanism exists that downregulates TNF receptor (TNF-R) function by affecting receptor affinity (10, 11). This PKC-induced transmodulation of TNF-R is associated with reduction in TNF sensitivity, which can be fully recovered by de novo synthesis and membrane expression of receptor protein (10). To date, little is known about the mechanisms regulating TNF-R expression, which is apparently constitutive in most tumor cells including leukemia and lymphoma cell lines (4, 5). In contrast, in normal peripheral blood T lymphocytes, TNF-R expression is activation dependent (12). IFN-γ has been recognized as one of the exogenous stimulatory signals of TNF-R expression (5, 13). We have here identified protein kinase A (PKA) as one of the endogenous regulatory elements that control TNF-R expression by a mechanism independent of and antagonistic to the previously described PKC-mediated control of receptor affinity (10).

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[1] *Abbreviations used in this paper:* CHX, cycloheximide; DB, dibutyryl; LT, lymphotoxin; OAG, oleyl acetyl glycerol; PK, protein kinase.

Materials and Methods

Reagents. N-2-*O*-dibutyryladenosine 3'5'cyclic monophosphate (DBcAMP), 8-bromoadenosine 3'5'cyclic monophosphate (8-Br-cAMP), 3-isobutyl-1-methyl-xanthine (IBMX) and 1-oleyl-2-acetyl rac-glycerol (OAG) were from Sigma Chemical Co., Munich, FRG. *N*-[2-(methylamino) ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-8) was from Seikagaku America, Inc., St. Petersburg, FL.

Cytokines. Purified rTNF-α, lymphotoxin (LT, TNF-β), IFN-γ, and IFN-α were provided by Dr. G. Adolf, Boehringer Ingelheim, Vienna, Austria. TNF, LT, IFN-γ and IFN-α were iodinated by the lactoperoxidase method as described (5, 14), with the exception that iodination of IFN-α was performed in a sodium acetate buffer at pH 5.5. The specific radioactivity of the radioiodinated cytokines was 20–50 μCi/μg. Bioactivity of the iodinated material was determined as described and was always >60% (5).

Cells and Culture Conditions. All cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in Click's/RPMI 1640 culture medium (Biochrom, Berlin, FRG) supplemented with 5% FCS, 10 mM Hepes, pH 7.3, and 50 μM 2-ME (5, 10).

PBMC were isolated from Ficoll-separated blood leukocytes by plastic adherence for 2 h at 37°C. Purity of the preparations obtained was >95%, as controlled by direct immunofluorescence flow cytometry (EPICS C) using the Mo 2 antibody (Coulter Electronics, Krefeld, FRG). After cell culture, the adherent cells were kept for 15 min on ice and subsequently removed by vigorous pipetting.

Binding Assays. Determinations of binding capacities using iodinated cytokines were performed in triplicate essentially as described (5, 10), using saturating concentrations of the respective ligands (60 ng/ml ^{125}I-TNF, ^{125}I-LT, and ^{125}I-IFN-α; 10 ng/ml ^{125}I-IFN-γ) in PBS supplemented with 2% FCS and 0.02% sodium azide. Cells (1–2 × 10^6 in 300 μl final volume) were incubated with the radiolabeled cytokine for 1–2 h at 0°C and washed three times thereafter. In each case, nonspecific binding was determined in the presence of a 200-fold excess of unlabeled ligand (three replicates). Saturation binding studies were performed similarly in duplicates at different concentrations of radiolabeled ligand (10) with subsequent Scatchard analysis using the program "Enzfitter" (Elsevier, Biosoft, London, U.K.).

Results

Dibutyric-cAMP Reversibly Enhances TNF-R Expression in HL-60 Cells. 24-h treatment of HL-60 cells with the membrane-permeable cAMP derivative dibutyric-cAMP (DBcAMP) resulted in a drastic enhancement of ^{125}I-TNF and ^{125}I-LT binding capacities, whereas specific binding for IFN-α and -γ remained largely unaffected (Fig. 1). Scatchard analysis of binding data demonstrate an enhancement in the number of TNF-Rs with an affinity comparable to that of untreated control cells (Fig. 1, *inset*).

DBcAMP acts in a dose-dependent manner. Concentrations in the range of 40 μM already significantly enhanced TNF binding capacity; maximum stimulation was obtained with 0.5–1 mM of DBcAMP treatment for 24 h (data not shown). Kinetic analyses revealed that TNF binding starts to increase after 4–6 h of culture in the presence of 1 mM DBcAMP, with a peak in TNF-R expression after 18–24 h (Fig. 2), and a subsequent slow decline to 70% of maximal enhancement of TNF-R expression during the next 5 d of culture in the presence of DBcAMP (data not shown). However, when PKA stimulation was abrogated after 24 h of DBcAMP treatment, TNF binding capacity decreased to pretreatment values within 6 h (Fig. 2).

Upregulation of TNF-Rs Is Not the Consequence of Terminal Differentiation and Is Mediated by PKA. As cAMP is known to induce cellular differentiation in HL-60 cells into the monocyte/macrophage and/or granulocyte pathway (15), various agents inducing differentiation were investigated for their potential to modulate TNF binding ca-

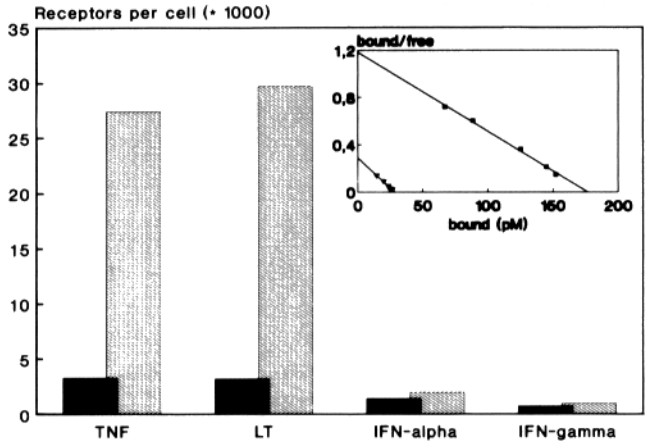

FIGURE 1. DBcAMP selectively enhances TNF and LT high affinity binding capacity. HL-60 cells were incubated for 24 h in the absence (■) or presence of 1 mM DBcAMP (▨), followed by determination of TNF, LT, IFN-α, and IFN-γ binding capacities. (*Inset*): Scatchard analysis of ^{125}I-TNF saturation binding studies performed on control HL-60 cells (▲) and DBcAMP-pretreated HL-60 cells (■).

pacity. In addition to DBcAMP, a significant upregulation of TNF-Rs could also be induced with butyrate and retinal (Table I). In contrast, DMSO, IFN-γ, granulocyte/macrophage (GM)-CSF, and TNF itself failed to induce a significant upregulation of TNF binding capacity. As shown previously (10), PMA treatment caused a reduction in specific TNF binding due to PKC-mediated transmodulation of the receptors.

To ensure the central role of PKA in upregulation of TNF binding capacity, we evaluated additional modulators of the PKA signal pathway. These included 8-bromo-cAMP, which in contrast to DBcAMP, cannot be metabolized to other potentially active second messengers such as monobutyrate-cAMP and butyrate (16). Further, the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX) was tested, which is known to raise the intracellular level of endogenously produced cAMP by blocking its degradation (17). Dibutyric-cGMP (DBcGMP) served as an additional control. As shown in Table II, the two PKA activators 8-Br-cAMP and IBMX

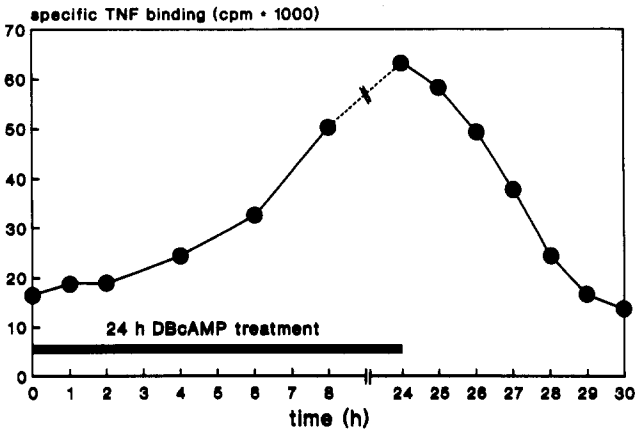

FIGURE 2. PKA-mediated enhancement of TNF-R expression is reversible. HL-60 cells were cultured for 24 h in the presence of 1 mM DBcAMP, washed twice to remove the PKA activator, and culture was continued. Before treatment and during culture, at the time points indicated, aliquots were taken to determine specific TNF binding capacity.

TABLE I

Effect of Various Agents on TNF-R Expression in HL-60 Cells

Substance (concentration)	Induced pathway of differentiation*	Specific TNF binding‡ (percent of untreated control)
DBcAMP (1 mM)	Monocyte/granulocyte	**623 ± 84**
DMSO (1%)	Granulocyte	113 ± 16
Retinal (5 μM)	Granulocyte	**223 ± 27**§
IFN-γ (10 ng/ml)	Monocyte	99 ± 13
TNF (10 ng/ml)	Monocyte	71 ± 28
Sodium butyrate (0.5 mM)	Monocyte/eosinophil	**287 ± 71**
GM-CSF (1 μg/ml)	Eosinophil	112 ± 10
PMA (10^{-10} M)	Macrophage	37 ± 18

* For references, see Collins (15).

‡ HL-60 cells were cultured for 24 h in the presence of the substances indicated. Subsequently, specific TNF binding (expressed in percent of the untreated control; 3,600 ± 1,245 TNF-R/cell) was determined as described in Materials and Methods. Mean ± SD of three independent experiments. Boldfaced numbers indicate statistically significant enhancement ($p < 0.01$).

§ Significant enhancement of TNF binding capacity by retinal treatment of HL-60 cells was only revealed upon prolonged culture (>48 h). The data shown represent maximum expression obtained after 72 h culture in the presence of retinal.

significantly enhanced the TNF-binding capacity of HL-60 cells, whereas the protein kinase G activator DBcGMP proved ineffective. At suboptimal concentrations, IBMX and DBcAMP synergistically enhanced TNF binding capacity (Table II, Exp. 1). The simultaneous addition of H-8, a potent inhibitor of protein kinases, in particular of PKA, completely abolished cAMP-mediated upregulation of TNF binding capacity (Table II, Exp. 2). Moreover, H-8 treatment also reduced TNF binding

TABLE II

DBcAMP Enhances TNF-receptor Expression Via Stimulation of Protein Kinase A

Exp.	Substance added (concentration)	Specific ^{125}I-TNF-binding capacity
		%
1*	DBcGMP (1.0 mM)	104
	8BrcAMP (5.0 mM)	262
	DBcAMP (0.3 mM)	242
	DBcAMP (1.0 mM)	602
	IBMX (0.1 mM)	115
	IBMX (1.0 mM)	280
	IBMX + DBcAMP (0.1 mM + 0.3 mM)	402
2‡	DBcAMP (1.0 mM)	411
	H-8 (30 μM)	35
	DBcAMP + H-8 (1.0 mM + 30μM)	65

* HL-60 cells were treated for 24 h; see footnote to Table I for details. Representative data from one of three experiments are shown.

‡ HL-60 cells were treated for 7 h; mean of two experiments.

capacity of unstimulated HL-60 cells, indicating a direct involvement of protein kinases in both constitutive and cAMP-enhanced TNF-R expression (Table II, Exp. 2). Sensitivity to kinase inhibitors and the selectivity of cAMP for PKA, therefore, points to a central role of PKA in control of TNF binding capacity.

PKA Activation Results in Enhanced TNF-R Synthesis. Upregulation of TNF binding capacity could be either due to a change in TNF-R synthesis or, alternatively, might reflect changes in the kinetics of receptor degradation or export into the cellular membrane. To exploit these possibilities, we compared the basic receptor turnover in both untreated and DBcAMP-treated HL-60 cells. The rate of TNF-R degradation was examined by blocking de novo protein synthesis with cycloheximide (CHX). In both untreated control cells and DBcAMP pretreated cells, the decrease in TNF binding capacity followed kinetics of first order with an identical half-life of ~2 h, as indicated by the parallel and linear slopes in a semilogarithmic plot (Fig. 3).

These data already suggested that upregulation of TNF-Rs by PKA might reflect a proportional enhancement in receptor synthesis rather than changes in turnover rate. As specific TNF-R probes for direct determination of expression at transcriptional or posttranscriptional levels are not yet available, protein synthesis-dependent recovery of specific TNF-binding capacity upon proteolytic digestion of TNF-Rs was studied to estimate the rate of TNF-R synthesis. We investigated the reexpression of TNF-Rs in HL-60 cells by determining the specific membrane TNF binding capacity upon trypsin treatment, which caused a 70–95% reduction in functional TNF-Rs. However, in contrast to U937 cells (10), HL-60 cells contain a significant intracellular pool of TNF-Rs. Thus, initial experiments revealed that upon tryptic digestion of TNF-Rs, 10–40% of pretreatment TNF-R levels can be transiently regained in CHX-pretreated (10 μg/ml) HL-60 cells within 1 h after trypsin treatment (data not shown). Therefore, in further experiments the difference in TNF-R membrane reexpression in the absence and presence of CHX was taken for evaluation of receptor de novo synthesis. Fig. 4 shows that the de novo protein synthesis–dependent TNF-R membrane expression of DBcAMP-treated cells increased eightfold as compared with that of the untreated control HL-60 cells.

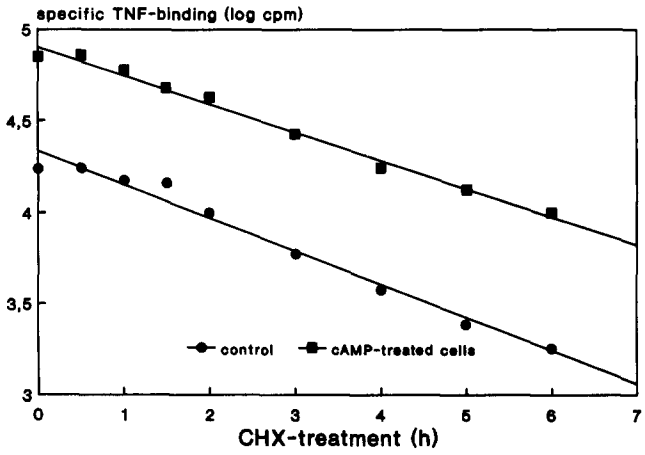

FIGURE 3. Identical half-lives of TNF-receptors in untreated and DBcAMP-treated HL-60 cells. HL-60 cells were cultured for 24 h in the presence of 1 mM DBcAMP (■) or were left untreated (●). Then 10 μg/ml of CHX was added to both groups and cell culture was continued. At the time points indicated, specific TNF binding capacity was determined from aliquots. Results are given in cpm at a semilogarithmic scale.

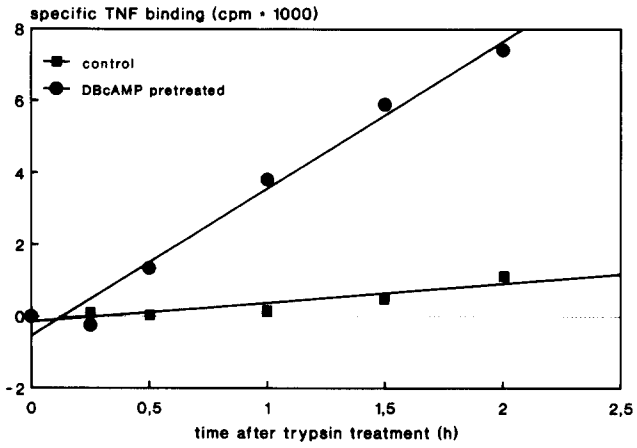

FIGURE 4. DBcAMP enhances reexpression of TNF-R in HL-60 cells after proteolytic digestion. Control HL-60 cells (■) and DBcAMP-stimulated (24 h, 1 mM DBcAMP) HL-60 cells (●) were treated at 37°C with 0.1% trypsin for 15 min and washed twice. The two groups were each split into two aliquots for further incubation in the absence and presence of 10 μg/ml of CHX, respectively. Before and after trypsin treatment and at the subsequent time points as indicated, specific TNF binding capacity was determined from aliquots of all four groups. Specific TNF binding capacity of HL-60 cells was reduced from 5,145 and 39,377 cpm to 1,515 and 2,688 cpm by trypsin treatment of control and DBcAMP-treated cells, respectively. The figure depicts the kinetics of recovery of protein synthesis- dependent TNF binding, i.e., Δ cpm = specific TNF binding of untreated and CHX-treated cells are plotted vs. time.

Stimulation of PKA Does Not Interfere with PKC-mediated Transmodulation of TNF-Binding Capacity. In light of a potential crosstalk of PKA and PKC (18), it was of interest to investigate whether TNF-R transmodulation by PKC is still effective under conditions of TNF-R upregulation by PKA. Accordingly, HL-60 cells were either left untreated or were pretreated for 24 h with DBcAMP, and subsequently, TNF binding capacity was determined in both groups before as well as 0.5 and 24 h after transient stimulation of PKC by pulse treatment with OAG. The data obtained revealed that upon OAG treatment, TNF-binding capacity was reduced to a similar extent in both cAMP-treated (6% of control) and untreated (10% of control) HL-60 cells (Table III). Moreover, after transient activation of PKC, TNF binding capacity reached the respective pretreatment values within 24 h (Table III), suggesting that PKC does

TABLE III

TNF-R Transmodulation by PKC Is Equally Effective in Untreated and DBcAMP-pretreated HL-60 Cells

Time point of binding assay*	Specific TNF binding of HL-60 cells‡	
	Control	DBcAMP-stimulated
OAG pulse treatment		
Before	9,427 (100)	51,977 (100)
Directly after	588 (6)	5,183 (10)
24 h after	10,521 (112)	50,030 (96)

* HL-60 cells, either DBcAMP-pretreated or left untreated, were incubated at room temperature for 30 min with 250 μM OAG, washed twice, and further cultured for 24 h in the presence and absence of 1 mM DBcAMP, respectively. Before, directly after OAG treatment, and at the end of the 24-h culture period, specific TNF binding capacity was determined from aliquots.

‡ Specific TNF binding capacity is given in cpm per 10^6 cells (%). Data shown are derived from one representative experiment.

TABLE IV
Cell-specific Sensitivity to Upregulation of TNF-R by PKA Stimulation

Cell	Origin	Specific TNF binding*
		%
HL-60	Promyelocyte	**688 ± 122**
U937	Histiocyte	**262 ± 82**
K562	Erythroblast	**172 ± 34**
HuT78	T cell leukemia	116 ± 18
Jurkat	T cell leukemia	105 ± 13
Molt3	T cell leukemia	117 ± 16
YT	T cell leukemia	87 ± 27
Gl134	EBV-transformed B cell line	91 ± 17
Colo205	Colon carcinoma	97 ± 10
WiDR	Colon carcinoma	90 ± 14
SW 480	Colon carcinoma	**178 ± 26**
HeLa	Cervix carcinoma	84 ± 33
WiSH	Fibroblast	94 ± 11
L929	Murine fibrosarcoma	100 ± 11
EL4	Murine thymoma	**574 ± 93**
Normal peripheral blood monocytes/macrophages		**281 ± 54**

* The cells were cultured for 24 h with 1 mM DBcAMP, and subsequently, specific TNF-binding capacity was determined (given in percent of the untreated controls; 100% = 2,600 – 14,600 cpm). Mean ± SD of at least three independent experiments.

not interfere with either normal or with PKA-enhanced TNF-receptor synthesis, but rather controls the binding affinity of membrane-expressed receptor molecules.

Cell Specificity of PKA-induced Receptor Upregulation. A number of different tumor cell lines were investigated for DBcAMP stimulation of TNF-R synthesis. 5 of 15 investigated cell lines of distinct tissue origin did respond with a significant enhancement in specific TNF binding capacity; aside from two human myeloid TNF producer cell lines, U937 and HL-60, the human erythroblastoid leukemia cell K562 as well as the murine T cell lymphoma EL4 and a human colon carcinoma cell line (SW480) were found to be responsive (Table IV), suggesting that this mechanism of receptor control is neither species nor strictly tissue specific. However, the data shown in Fig. 4 suggest that PKA-mediated upregulation of TNF-R expression is particularly effective in tumor cells derived from myeloid origin. Therefore, we investigated whether in normal peripheral blood monocytes/macrophages TNF-R expression is also controlled by PKA. On average, purified, unstimulated monocytes constitutively expressed ~1,000 TNF-R (data not shown). Similar to established cell lines, PKA stimulation was effective in normal peripheral blood monocytes, yielding, on average, a threefold (281 ± 54%) enhancement of TNF-R (Table IV). Again, affinity of TNF-R remained unchanged upon DBcAMP treatment of the cells, as indicated by the parallel slopes of the Scatchard diagram shown in Fig. 5.

Discussion

The present investigations aimed at an identification of intracellular control mechanisms of TNF-R expression, a condition requisite for TNF responsiveness. The data show that activators of PKA can selectively upregulate TNF-R expression, pre-

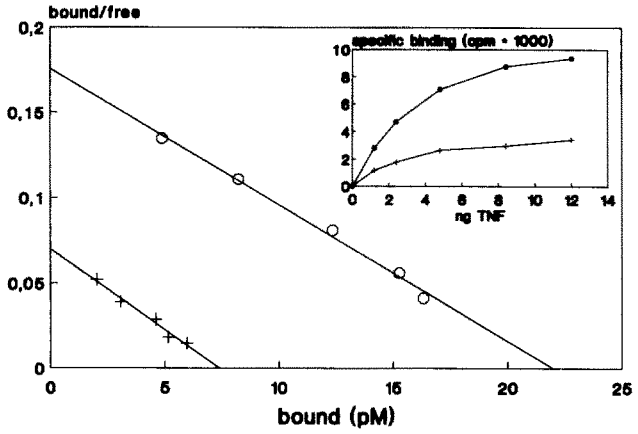

FIGURE 5. Enhancement of TNF-R in peripheral monocytes/macrophages by PKA stimulation. Plastic adherent cells were incubated in the absence (+) or presence (O) of 1 mM DBcAMP for 24 h. The diagram shows the Scatchard plot of one representative experiment out of three (unstimulated cells: 760 receptors/cell, K_d = 1.05 × 10^{-10} M; stimulated cells: 2,260 receptors/cell, K_d = 1.25 × 10^{-10} M). (*Inset*) Respective binding data.

dominantly in normal and malignant cells of the myeloid lineage, whereas protein kinase inhibitors interfere with TNF-R expression. In HL-60 cells, on average, a sevenfold stimulation was achieved after 24 h of treatment with optimal concentrations of DBcAMP (Table II), yielding ~28,000 receptors/cell. As expected, the binding capacity for ^{125}I-LT was enhanced in parallel, which is in agreement with the current view of a single cellular membrane receptor binding both cytokines (19). In contrast, expression of both types of IFN receptors (α, γ) proved to be insensitive to this stimulation regimen (Fig. 1).

Kinetics of receptor degradation after blocking of protein synthesis with CHX was found to be of first order, suggesting that TNF-R degradation might simply reflect normal cellular membrane turnover (20). Half-life of TNF-Rs of ~2 h was identical in both cAMP-treated and untreated HL-60 cells (Fig. 3), indicating that degradation of TNF-Rs increases in parallel with an increase in TNF-R number triggered by PKA stimulation. This leads to the conclusion that PKA activation most likely causes an enhancement of TNF-R synthesis. At present, due to lack of appropriate probes, TNF-R gene transcription and/or translation cannot be directly analyzed. Therefore, estimates on the rate of TNF-R synthesis have to rely on the indirect approach of quantification of protein synthesis-dependent acquisition of TNF binding capacity. The different membrane reexpression rates upon removal of TNF-R by tryptic digestion (Fig. 4) strongly argue for enhanced synthesis of receptor proteins in cAMP-treated as compared with untreated HL-60 cells. Moreover, complete recovery from PKC-mediated transmodulation of TNF-R in cAMP-treated cells (Table III) further supports this reasoning. The latter experiments also suggest that PKC-mediated transmodulation and PKA-mediated receptor upregulation are independent of each other and can be effective at the same time, resulting in a phenotypically antagonistic action.

Aside from DBcAMP, sodium butyrate and retinal were also found to upregulate TNF-Rs in HL-60 cells. While retinal is known to activate PKA (21), butyrate is thought to mainly affect histone deacetylation (22). As DBcAMP is known to become processed intracellularly, resulting in the release of monobutyric cAMP and butyrate (16), it is conceivable that DBcAMP is effective exclusively via intracellular

release of butyrate and not via activation of PKA. The latter possibility could be excluded, as 8-Br-cAMP, an additional activator of PKA, and IBMX, an inhibitor of degradation of endogenously produced cAMP (17), also enhanced TNF binding capacity, whereas DBcGMP was ineffective. Moreover, H-8, a potent inhibitor of PKA, completely abolished cAMP upregulation of TNF-R (Table II). Whether cAMP and butyrate enhance TNF-R expression *via* shared signal pathways or act completely independent of each other, is unknown at present.

Since DBcAMP, retinal, and butyric acid are known to induce differentiation in HL-60 cells (15), upregulation of TNF-R expression in these cells might represent a consequence of an altered differentiation status triggered by these various agents. However, in regard to TNF-R expression, both DBcAMP (Fig. 2) and butyrate action (data not shown) were fully reversible. Furthermore, maximum effects of DBcAMP and butyrate were observed after 24 h of treatment, at which time only ~5% of the HL-60 cells are considered to be differentiated (23). Apparently, enhancement of TNF-Rs is not linked to irreversible processes of cellular differentiation. These suggestions are supported by the finding that TNF-Rs are upregulated by activation of PKA in tumor cell lines of myeloid origin and in normal peripheral blood monocytes/macrophages representing various stages of the myelomonocytic differentiation pathway (Table IV, Fig. 5). Various activators of PKA as well as IBMX were also effective in these cells (data not shown).

Recent work from our laboratory (24, 25) and from others (26) provided evidence that production of TNF itself is also controlled by protein kinases. Thus, activation of PKC leads to induction of TNF mRNA and/or secretion of the protein (24, 25). PKA, on the other hand, has been demonstrated to act as a negative regulator on TNF production (26). These effects are exactly opposed to those of the respective protein kinase on TNF-R synthesis (PKA) and its functional status (PKC). As shown here, both mechanisms can be effective in a single cell type, that is per se TNF sensitive and a potential TNF producer, such as normal peripheral blood monocytes as well as the monocytic cell lines HL-60 and U937 (5, 6, 27, 28). Moreover, as both protein kinases apparently participate in TNF signal transduction (7, 24, 29), we suggest that a regulatory circuit could be effective in the following way: activation of PK-A results in an enhancement of TNF-R expression paralleled with inhibition of TNF-production; on the other hand, activation of PKC induces production and secretion of mature (17 kD) TNF and simultaneously inactivates expressed TNF membrane receptors (transmodulation), thereby inducing TNF resistance (10). As TNF-Rs are ubiquitously expressed (4, 5) and many different cell types are potential TNF producers (24, 30–32), such an antagonistically acting regulatory circuit may be used to control autocrine stimulation, and may enable cells to completely switch from a signal transmitter (TNF producer) to a receiver (TNF responder) status.

Summary

We have investigated control mechanisms of TNF receptor expression (TNF-R) in various human tumor cells and normal peripheral blood monocytes. Activators of protein kinase A (PKA) signal transduction pathways were found to enhance TNF-R expression up to sevenfold, whereas in the same cells, IFN-α and -γ receptors remained unaffected. Inhibitors of protein kinases downregulate both constitutive and cAMP-enhanced TNF-R expression. Binding studies revealed an increase in TNF-

R numbers without a change in receptor affinity. Both, direct activators of PKA and inhibitors of phosphodiesterase, raising intracellular levels of cAMP, were found to be effective. As activation of PKA does not slow down the degradation rate of TNF-Rs, but rather enhances protein synthesis-dependent reexpression of TNF-Rs after transient PKC-mediated transmodulation and after tryptic digestion of TNF-Rs, it is concluded that PKA stimulates TNF-R synthesis. Maximum TNF-Rs enhancement is reached after 24 h of stimulation and is reversible, suggesting that receptor upregulation is not linked to irreversible steps of cellular differentiation. PKA-mediated enhancement of TNF-R expression was predominantly observed in normal peripheral blood monocytes and tumor cell lines of myeloid origin. As in these typical TNF producer cells, the production of TNF is also controlled by PKA and PKC, a regulatory circuit is proposed, by which these two independent signal pathways antagonistically regulate TNF production and, at the receptor level, TNF sensitivity.

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