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Regulation of interleukin-6 receptor expression by interleukin-6 in human monocytes – a re-examination

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Abstract

We have studied the expression and regulation of the interleukin-6 receptor (gp80) and its signal transducer gp130 in primary human blood monocytes. Here, we show that freshly isolated human monocytes express mRNAs for gp80 and gp130. In contrast to a previous report [(1989) FEBS Lett. 249, 27–30] we find that neither lipopolysaccharide nor interleukin-6 (IL-6) lead to a down-regulation of IL-6 receptor mRNA in monocytes. Also in the human monocytic cell line Mono Mac 6 no effect of IL-6 on receptor mRNA levels was observed. For signal transducer gp130 mRNA in monocytes a small and transient up-regulation by IL-6 was found.

Key words: Interleukin-6; Cytokine receptor; Lipopolysaccharide; Monocyte

1. Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine which is synthesized by many different cells after appropriate stimulation. It acts on a wide spectrum of target cells and exerts multiple functions during the immune response, hematopoiesis, neural differentiation and the acute phase reaction [1–5]. IL-6 acts via a cell surface receptor complex composed of two subunits: an 80 kDa IL-6 binding protein (IL-6 receptor) and a 130 kDa signal transducing protein, gp130 [6–8]. The regulation of the expression of the IL-6 receptor and of gp130 was studied in a number of different cells [9–19]. In human hepatoma cells HepG2 and Hep3B, in rat hepatocytes and in human epithelial cells UAC it was found that the synthetic glucocorticoid dexamethasone but not IL-6 stimulates the expression of the IL-6-R [9,10,16], whereas the expression of gp130 was up-regalated by IL-6 and a combination of IL-6 and dexamethasone [14,15]. For human monocytes, it was previously reported that lipopolysaccharide leads to a down-regulation of IL-6-R mRNA expression and that this down-regulation is mediated by interleukin-6 [20,21]. In the present study we have analyzed the expression and regulation of the IL-6-R (gp80) and its signal transducer (gp130) in freshly isolated human monocytes. In contrast to the report of Bauer et al. [20,21] we find that neither LPS nor IL-6 do significantly alter the expression of the IL-6-R whereas for gp130 a weak stimulatory effect of IL-6 was found.

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Abbreviations: IL-6, interleukin-6; IL-6-R, interleukin-6-receptor; LPS, lipopolysaccharide; TGF-β1, transforming growth factor-β1.

2. Materials and methods

2.1. Chemicals

TFG-β1 (1×10^6 U/mg) was purchased from Genzyme (Cambridge, MA). Human recombinant interleukin-6 (5×10^6 B-cell stimulatory factor-2 units/mg protein) was kindly provided by Drs. T. Hirano and T. Hishimoto (Osaka University, Japan). The biological activity of the IL-6 used was checked with the B9 assay (see section 2.4). Fetal calf serum (FCS) and RPMI were from Gibco (Eggenstein, Germany). Restriction enzymes and random primed DNA labeling kit were obtained from Boehringer (Mannheim, Germany).

2.2. Isolation of human monocytes

Human monocytes were isolated from buffy coats (Institut für Transfusionsmedizin, RWTH Aachen, Germany) with a Ficoll-Paque gradient followed by hypotonic density centrifugation in Percoll [22]. The cells were cultivated in Teflon bags in RPMI 1640 medium plus 5% heat-inactivated autologous serum at a cell density of 2×10^6/ml in 7.5% CO_2 humidified atmosphere. All media and sera used were checked for low endotoxin concentration (< 0.05 ng/ml) by using the Limulus amelocyte lysate assay (Sigma, München, Germany). Morphological differentiation of cells was studied by staining with Mayer-Hämalaun (Merck, Darmstadt, Germany). Purity of monocytes was greater than 85% in all experiments.

2.3. MonoMac 6 cell line

The monoblastic leukemia cell line Mono Mac 6 was kindly provided by Dr. H.W.L. Ziegler-Heitbrock (Institut für Immunology, München, Germany). The cells were cultured in RPMI 1640 medium containing 2× non-essential amino acids, 9 μg/ml insulin, 1 mM oxalacetate, 1 mM sodium pyruvate and 10% FCS.

2.4. IL-6-assay

The IL-6 assay was performed using the murine plasmacytoma cell line B9 kindly provided by Dr. L. Aarden (Amsterdam, The Netherlands) [23]. Cells were cultured with dilutions of supernatants from human monocytes for 68 h on microculture plates (5×10^3 cells/well). The cell number was determined using the Celltiter 96 proliferation assay (Promega, Madison, IL) as described by the manufacturer. The absorbance at 550 nm was recorded using an ELISA reader. One B9 unit is the amount of IL-6 that resulted in half-maximal growth of the B9 cells. Approximately 120 B9 units correspond to 1 B-cell stimulatory factor-2 (BSF-2) unit.

2.5. RNA isolation

RNA from monocytes was isolated using the method of Chirgwin et al. [24]. Human monocytes were dissolved in 2 ml of 4 M guanidinium thiocyanate containing 25 mM sodium citrate, pH 7, 0.5% sodium

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N-laurylsarcosine, 0.1 M 2-mercaptoethanol and 0.1% Antifoam A (Sigma). The solution was sheared by passage through a needle (26-G × 23 mm) 5–6 times and centrifugation through a 5.7 M cesium chloride cushion for 20 h at 36,000 rpm and 20°C in a Beckman ultracentrifuge. The RNA pellet was dissolved in water (containing 0.07% diethyl pyrocarbonate) and precipitated for 30 min at −80°C after adding two volumes of 96% ethanol. This precipitation was repeated once. The pellet was washed twice with 70% ethanol, dried and dissolved in water. The amount of RNA was quantified by absorbance measurements at 260 nm.

2.6. Northern blot analysis

5 μg of total RNA were separated on 1% denaturing agarose gels and transfered to GeneScreen Plus membranes (Dupont-New England Nuclear, Dreieich, Germany). The filters were prehybridized at 68°C for 2 h in 10% dextran sulfate, 1 M sodium chloride, 1% SDS, and hybridized in the same solution with cDNA fragments labeled by random priming [25]. Hybridisations were performed with the a 1.2 kb *PstI*/*XhoI* IL-6-R-cDNA, a 1.1 kb *EcoRI* IL-6-cDNA, a 3.0 kb *AccII*/*BamHI* gp130-cDNA and a 1.3 kb *PstI* GAPDH-cDNA fragment, respectively.

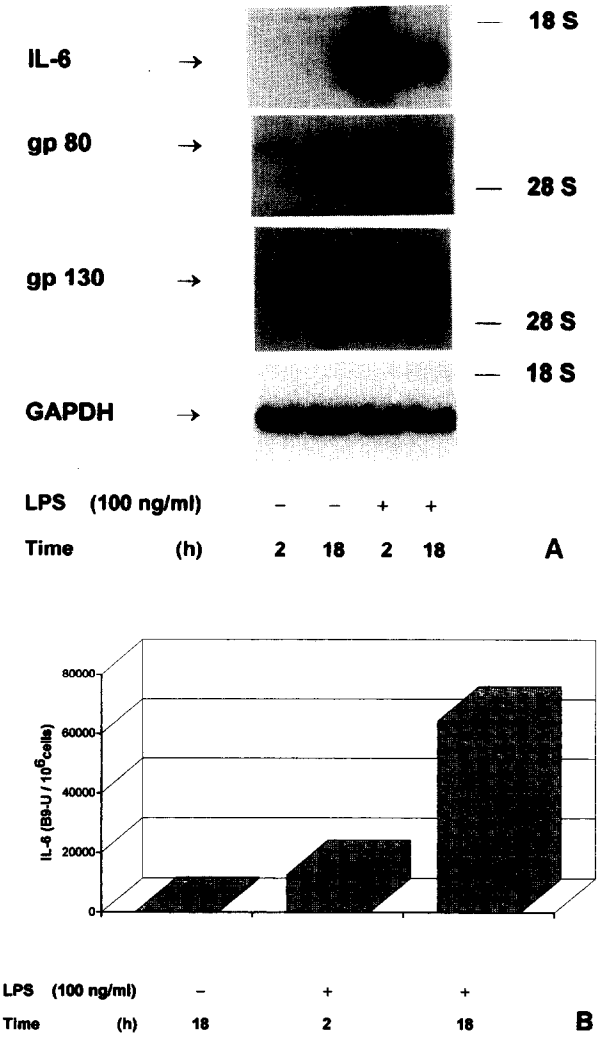

Fig. 1. (A) Messenger-RNA levels for IL-6, gp80 and gp130 in human monocytes treated with LPS. Human monocytes were incubated with 100 ng/ml LPS for 2 and 18 h. RNA was isolated and 5 μg were subjected to Northern blot analysis. GAPDH was used as a control for equal loading of the gels. (B) IL-6 secretion by LPS-stimulated monocytes as measured in the B9-cell proliferation assay.

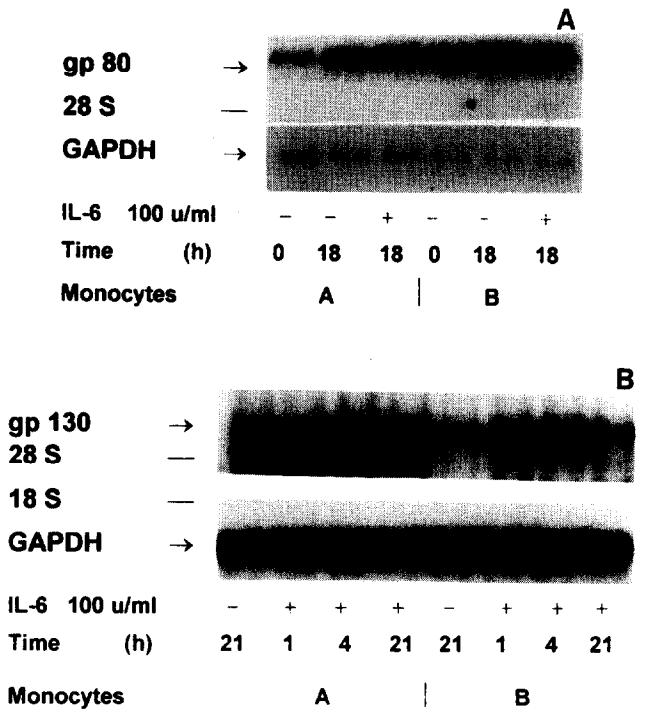

Fig. 2. Messenger-RNA levels for gp80 (A) and gp130 (B) in human monocytes upon IL-6 stimulation. Human monocytes were incubated with IL-6 (100 BSF-2 units/ml) for the times indicated in the figure. RNA was isolated and 5 μg were subjected to Northern blot analysis. Two different experiments are documented.

3. Results

Human blood monocytes were prepared from buffy coats using Ficoll-Paque and Percoll gradients. Cultivation was performed in Teflon bags with 5% autologous serum which prevented the adherence of monocytes to a substratum known to activate these cells [26,27]. Monocytes were incubated with LPS, a well-known inducer of cytokine synthesis in these cells [28–30]. Two and 18 h after stimulation, cells were harvested and subjected to a Northern blot analysis. A strong induction of IL-6 mRNA expression was detected 2 h after LPS addition (Fig. 1A, upper panel). This induction was accompanied by an increase in bioactive IL-6 protein in the medium (Fig. 1B). When the expression of the IL-6-R (gp80) and gp130 was analyzed in these cells, mRNAs for both proteins were detected. However, no regulation by LPS was observed (Fig. 1, middle panels).

When human monocytes were stimulated with IL-6 for 18 h, again no significant change in IL-6-R (gp80) mRNA levels were seen (Fig. 2A). This result was reproduced in several independent experiments with monocytes from more than ten different donors. An increase of expression of gp80 between 0 and 18 h of incubation was regularly observed in non-stimulated cells and is probably due to the 'stress' on the cells during the purification procedure (Fig. 2A).

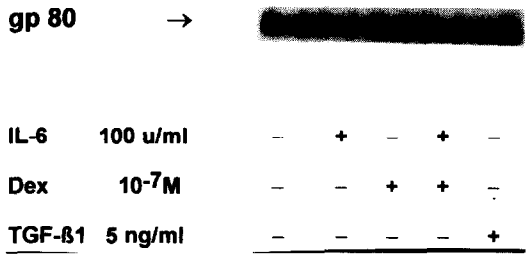

Fig. 3. Gp80 mRNA expression in Mono Mac 6 cells treated with IL-6, dexamethasone and TGF-β1. Mono Mac 6 cells were stimulated for 18 h with IL-6 (100 BSF-2 units/ml) and/or dexamethasone (10^{-7} M), and TGF-β1 (5 ng/ml). RNA was isolated and detected by Northern blot analysis.

When expression of gp130 mRNA was studied, a transient increase was seen 4 h after stimulation with IL-6 (Fig. 2B). However, at 21 hours basal levels were reached again. Dexamethasone had no effect on the expression of IL-6-R and gp130 mRNAs (data not shown).

When mRNA levels for the IL-6-R were analyzed in the monoblastic leukemia cells Mono Mac 6, which display many functional and phenotypic characteristics of mature monocytes [31], again no regulation by IL-6 and/or dexamethasone was found. Also TGF-β1 had no effect (Fig. 3).

4. Discussion

From our data we conclude that IL-6 does not impair the mRNA expression of its receptor (gp80) in primary human blood monocytes. This finding contradicts a previous report, which described the down-regulation of IL-6-R mRNA by LPS and IL-6 [20,21]. This discrepancy might be due to the different procedures used for the isolation and culture of monocytes. Bauer and coworkers used an additional adherence step to further increase the purity of their monocyte preparation and they cultured their cells on plastic dishes [21]. However, it is known that adherence of monocytes to a substratum leads to their activation [26,27]. Therefore, it is possible that the IL-6 effect observed by these authors is due to a co-activation of the cells by adherence.

For signal transducer gp130 a small and transient up-regulation by IL-6 was found. A similar regulation of gp130 by IL-6 was observed in other cell types [14,15].

In striking contrast to the inability of IL-6 to impair the IL-6-R mRNA expression is its ability to down-regulate IL-6-R surface protein. We have recently demonstrated that after binding of IL-6 to its surface receptor in HepG2 cells, ligand and receptor are endocytosed thereby leading to a loss of IL-6 surface binding sites [32]. This process is mediated by the signal transducer gp130 (Dittrich et al., submitted). Although this endocytosis has not been analyzed in monocytes yet, it is reasonable to assume that a similar down-regulation of surface receptor protein might take place in these cells upon IL-6 incubation.

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