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The expression of interleukin-6 by a rat macrophage-derived cell line

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A stable rat macrophage-derived cell line (RMSV1) was established by transformation of primary peritoneal exudate cells with the SV40 virus. The RMSV1 cell line was used as a model to study the regulation of the interleukin-6 (IL6) gene expression in rat macrophages with respect to lipopolysaccharides (LPS), interleukin-1 (IL1) and glucocorticoids. The IL6 mRNA level in RMSV1 cell lines was induced 20-fold within 4 h by LPS, whereas IL1 had no effect. The glucocorticoids were able to inhibit completely the induction of the IL6 mRNA synthesis by LPS, indicating the negative regulation of the IL6 gene expression by glucocorticoids.

Interleukin-6; Macrophage cell line; Expression; Regulation; mRNA level; Rat

1. INTRODUCTION

Interleukin-6 (IL6) is a multifunctional cytokine with a broad spectrum of biological activities on different target cells [1–5]. It is not yet completely understood how IL6 generally acts in the network with other cytokines and hormones to regulate the growth, development and activities of cells in the hematopoietic, immune and host defense system. IL6 expands the production of hemato-poietic cells by activation of progenitor cells, induces the synthesis of acute phase proteins in the hepatic system and immunoglobulins in B-cells, and increases the body temperature during inflammation. The cell types which produce high levels of IL6 are distributed throughout the body, such as macrophages, fibroblasts, monocytes, endothelial cells and keratinocytes. In these cell types IL6 is synthesized in response to different inflammatory signals, including bacterial products such as lipopolysaccharides (LPS). LPS, a major component of the outer cell wall of bacteria is a prototypic stimulant which can dramatically enhance the inflammatory potential and performance of macrophages. LPS treatment causes the rapid and transient elevation of steady state levels of numerous macrophage specific mRNAs including those coding for the inflammatory cytokines such as IL6, IL1, and TNF-α.

In the present study we generated a stable rat macrophage-derived cell line from rat peritoneal exudate cells (PECs) as a model system to study the regulation of the IL6 expression in response to LPS, IL1 and glucocorticoids.

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Abbreviations: IL1, interleukin-1; IL6, interleukin-6; LPS, lipopolysaccharide; PECs, peritoneal exuate cells; TNF-α, tumor necrosis factor α

2. EXPERIMENTAL

For the generation of a macrophage-derived cell line, Brewers thioglycollate medium was injected into rats intraperitoneally and peritoneal lavage cells were prepared after 4 days [6]. After adherence the PECs were infected with the DNA tumor virus SV40 at a multiplicity of about 0.1 plaque performing units/cell for 2 h after adherence of the PECs. Loci of growing cells developed 2 weeks after transfection were expanded into the stable rat cell line RMSV1 by multiple rounds of cloning in Dulbeccos Modified Eagles Medium supplemented with 10% fetal calf serum. Cells were cultured to 80% confluency and treated with fresh medium containing 0.5% fetal calf serum prior to the incubation with LPS, IL1 or glucocorticoids.

For the immunoprecipitation the RMSV1 cell line was incubated in presence of 10 μg/ml LPS (Sigma) and 1 mCi [^{35}S]methionine (Amersham) for 20 h. Culture supernatants were performed as described [7]. The anti-rat IL6 serum used was a polyclonal rabbit serum raised against a synthetic peptide representing amino acids 31–40 of the mature rat IL6 [6] coupled to bovine serum albumin.

For the quantitation of mRNA by dot hybridization analysis total RNA was extracted from cultured cells of 3 dishes for each time point with guanidium thiocyanate and sedimented through a cushion of cesium chloride [8]. The RNA was dotted onto nylon membranes and hybridized [9] with the rat IL6 cDNA [6]. After fluorography the dots were quantitated by densitometry using the LKB Ultrascan XL densitometer. The Northern blot hybridizations were performed as described previously [6,9].

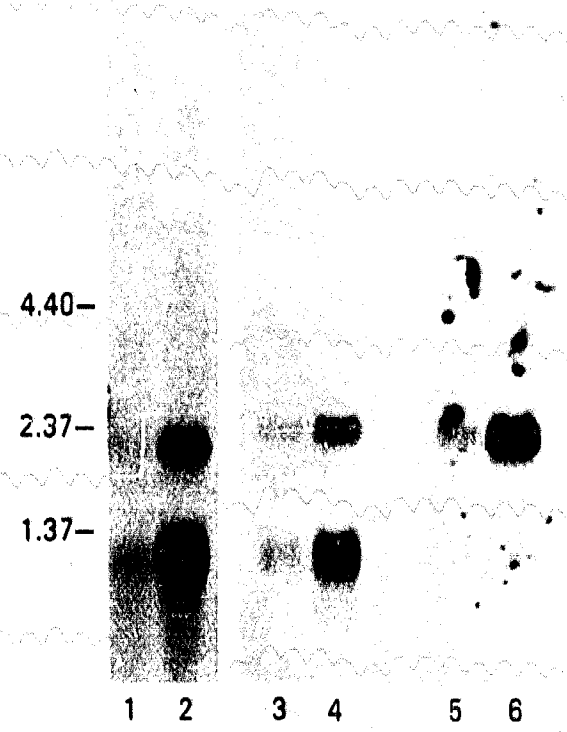

Fig. 1. Northern blot analysis of mRNA from RMSV1 cells after LPS stimulation. Total RNA was isolated from primary culture of PECs (lanes 1 and 2) and from cultures of RMSV1 cell line (lanes 3, 4, 5 and 6) without (lanes 1, 3 and 5) and after stimulation with 10 μg/ml LPS for 18 h (lanes 2, 4 and 6). 20 μg total RNA were electro-phoretically separated in an agarose formaldehyde gel, transferred to nylon membranes and hybridized either with cloned rat IL6 cDNA [6] (lanes 1, 2, 3 and 4) or with cloned murine IL1 cDNA probe, respectively (lanes 5 and 6). Size evaluation was made by comparison with the mobilities of RNA size markers.

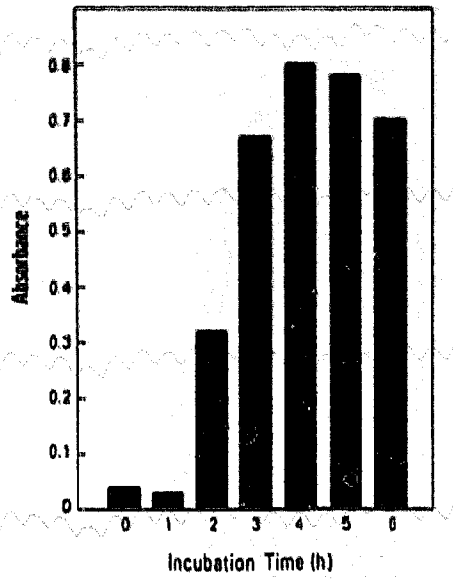

Fig. 2. Induction of IL6 mRNA by LPS in RMSV1 cells. Total RNA was isolated from 3 dishes of confluent RMSV1 cells cultured in presence of 0.5% fetal calf serum and 0.1 μg/ml LPS for 1, 2, 3, 4, 5 and 6 h. 10 μg total RNA each was dotted onto nylon membrane and hybridized with rat cDNA [6]. After exposure for fluorography the signal intensity was quantitatively analysed by scanning densitometry.

3. RESULTS AND DISCUSSION

Previously we demonstrated that primary cultures of adherent rat PECs produced two different IL6 mRNA species, a major species 1.2 kb in length and a minor 2.4 kb species [6]. After stimulation with 10 μg/ml LPS for 18 h both IL6 mRNA species were induced about 20- to 25-fold in PECs (Fig. 1, lanes 1 and 2). To obtain a reproducible cell source for studies of the IL6 gene regulation and expression, primary PECs were transformed into a stable rat macrophage-derived cell line (RMSV1) by transfection with SV40. The RMSV1 cell line could be split and plated indefinitely and displayed macrophage-like cellular morphology and other properties characteristic of highly differentiated macrophages. In particular, both IL6 mRNA species were also detectable and both were inducible by LPS (Fig. 1, lanes 3 and 4). An additional macrophage-specific character was the inducibility and synthesis of the 2.4 kb IL1 mRNA in RMSV1 cells by LPS simultanously to the IL6 mRNA (Fig. 1, lanes 5 and 6). To quantitate the time course of the IL6 mRNA induction by LPS, total RNA was extracted from RMSV1 cells after stimulation with 1.0 μg/ml LPS. The IL6 mRNA was increased 20-fold by LPS with a maximum at 4 h (Fig. 2). The largest relative increase in the IL6 mRNA concentrations was measured when the fetal calf serum was lowered to 0.5% during the LPS induction. This reduced the basal level expression of the IL6 gene induced by serum factors [10].

The metabolic labeling of the RMSV1 cells cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum without (Fig. 3, lane 1) or in presence of LPS (Fig. 3, lane 2) and following immunoprecipitation of cell supernatants using anti-rat IL6 serum revealed two IL6 bands: a main IL6 band (A) and a very weak IL6 band (B) with apparent molecular masses of 24 and 25 kDa, respectively. The used anti-rat IL6 serum was generated against a synthetic decapeptide representing N-terminal sequences of the mature rat IL6 [11]. The results clearly demonstrated that the rat IL6 is possibly secreted mainly in the unglycosylated form. The observed molecular mass of the main IL6 band was in agreement with the previously obtained amino acid sequence of the mature rat IL6 which lacks any potential *N*-glycosylation sites [6]. In contrast, both the human and murine IL6 showed, respectively, at least 5 and 8 IL6 forms with molecular masses ranging between 20–30 kDa reflecting the different *N*- and *O*-glycosylation possibilities [12–14]. Regarding the high basal level of IL6 due to the effect of serum factors, the

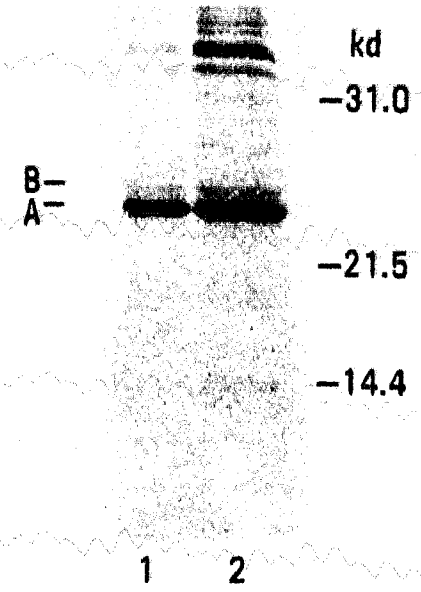

Fig. 3. RMSV1 cell line secreted immunoprecipitable IL6. Newly synthesized and secreted proteins by the RMSV1 cell line were metabolically labeled with [^{35}S]methionine without (lane 1) or in presence of 10 μg/ml LPS for 20 h (lane 2) prior to the immunoprecipitation. Size markers (ordinate in kDa); A, B: the characteristic IL6 doublet with components of 24 and 25 kDa.

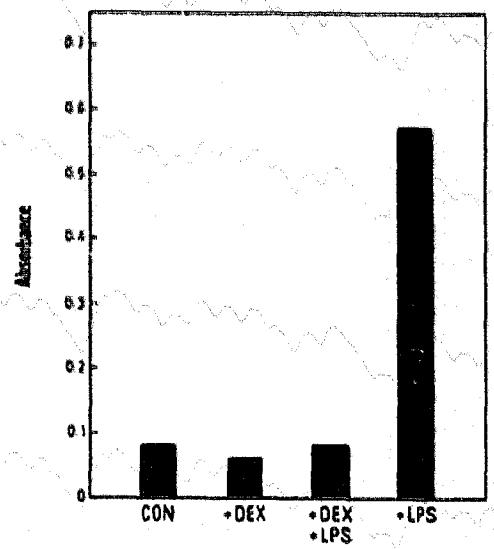

Fig. 5. Effect of glucocorticoids on the synthesis of IL6 mRNA in RMSV1 cells. Ten μg total RNA were isolated from RMSV1 cells incubated in DMEM medium containing 0.5% fetal calf serum (CON), additional with 10^{-7} M dexamethasone (DEX), with 10^{-7} M dexamethasone and 10 μg/ml LPS (DEX + LPS), or with 10 μg/ml LPS (LPS) for 6 h. The RNA dots were hybridized with rat IL6 cDNA [6] and analysed by densitometry after fluorography.

newly synthesized IL6 was apparently increased only 2-fold.

The macrophages play a central role in the host defense system as the main mediator between primary inflammatory events and the hepatic system. Macrophages activated by various inflammatory signals including LPS, transiently produce high levels of IL6, IL1 and TNF-α, the principal mediators of the liver acute phase response. IL6 mainly triggers the induction of the acute phase proteins which in turn act locally in the host defense system [4]. But a full acute phase response is also dependent on the additional action of IL1 and glucocorticoids. Therefore, it was necessary to study the interaction of IL1 and glucocorticoids on the IL6 regulation in macrophages.

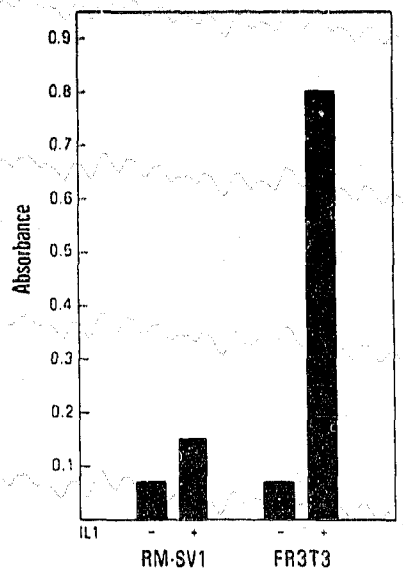

Fig. 4. Effect of interleukin-1 on the regulation of IL6 mRNA in RMSV1 cells. Total RNA was isolated from RMVS1 cells (RM-SV1) and rat fibroblast cell line FR3T3 (FR3T3) cultured without (−) or in presence of 200 units/ml recombinant murine IL1 (+) for 6 h. 10 μg total RNA of RMSV1 cells and 50 μg of FR3T3 cells were hybridized with the rat cDNA clone [6] and analysed quantitatively.

When the RMSV1 cells were cultured in presence of IL1 only a weak effect was observed. The IL6 mRNA was increased 2-fold within 6 h (Fig. 4, RM-SV1). Under the same experimental conditions IL1 increased the IL6 mRNA levels up to 16-fold in the rat fibroblast cell line FR3T3 (Fig. 4, FR3T3). This finding confirmed the RMSV1 cell line as macrophage-derived cell line and distinguishes it from the other IL6-producing cell types, especially from the monocytes. Monocytes and macrophages are normally the most abundant cell types among the adherent PECs. These data also described the strong relationship between IL6 and IL1 in the acute phase response. Activated macrophages secrete IL1 which triggers in fibroblasts the synthesis and secretion of IL6 [15] resulting in an amplified IL6 stimulus of the hepatic system during inflammation.

Previous results showed that the induction of various cytokine mRNAs such as granulocyte macrophage colony stimulating factor, TNF-α, or IL1 was prevented by glucocorticoids [16–18]. In understanding the IL6 expression in macrophages we studied the effect of

glucocorticoids on the induction of IL6 mRNA by LPS in the RMSV1 cell line. In presence of 10^{-7} M dexamethasone the RMSV1 cells failed entirely to synthesize the IL6 mRNA after treatment with LPS (Fig. 5). Dexamethasone alone had no effect, as shown in comparison to the untreated cells.

The results were emphazing the anti-inflammatory and immuno-suppressive role of glucocorticoids in the acute phase system. IL6 and IL1, both released by macrophages are able to stimulate the synthesis of the adrenocorticotropic hormone (ACTH) in the pituitary gland [19]. The ACTH then acts to increase the secretion of glucocorticoids in the cortex of adrenal gland. Glucocorticoids are essential cofactors to obtain the full acute phase response and act in synergy with the inflammatory cytokines to cause the induction of the acute phase proteins in vivo. In macrophages, glucocorticoids inhibit finally the synthesis of IL6 (Fig. 5) as well as of IL1, resulting in the deactivation of the acute phase response of the liver and restoration of the disturbed homeostatic balance.

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