Differential regulation of the expression of transporters associated with antigen processing, TAP1 and TAP2, by cytokines and lipopolysaccharide in primary human macrophages

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Abstract. Objective: Using microarray technique we analysed global changes in gene expression of interferon-γ treated primary macrophages. Among the differential expressed genes identified we focussed on the expression of the transporters associated with antigen processing, TAP1 and TAP2, which are involved in the antigen presentation via MHC class I. Patients suffering from TAP deficiency syndrome have clinical manifestations including recurrent bacterial infections of the respiratory tract and chronic necrotizing granulomatous skin lesions. This is one reason why the regulation of TAP gene expression in antigen presenting cells such as macrophages might provide important general insights into the generation of cellular immune response to multiple pathogens. Additionally IFN-α is important in adjuvant tumourtherapie although the working mechanisms are unknown. Because of the possibility of the TAPs to be involved in these mechanisms we studied the expression of these transporters in human macrophages after stimulation with pro-inflammatory mediators.

Material and treatment: Monocyte derived macrophages were treated for 24 h with either interferon-γ, interferon-α, interleukin-1β (each 100 U/ml) or lipopolysaccharide (1 µg/ml).

Methods: IFN-γ induced gene expression was analysed using microarray technique. TAP expression was investigated by RT-PCR, northern blot- and western blot analysis.

Results: TAP1 and TAP2 were constitutively expressed at a low level. IFN-γ upregulated the expression of both transporters. LPS caused an increase similar to the effect of IFN-γ. Treatment with IFN-α stimulated also the expression, however, less than IFN-γ. In contrast, IL-1β stimulation had no effect.

Conclusion: Our data show that the transporters associated with antigen presentation are differentially regulated by pro-inflammatory mediators in human macrophages. The finding that IFN-α stimulates the expression of proteins involved in cytotoxic effector functions of macrophages contributes to the understanding of the immunoregulatory role of type I interferons and may help to explain the efficacy of IFN-α in the treatment of tumors.

Key words: Primary human macrophages – Microarray technology – TAP – Cytokines – Immune response

Introduction

The microarray gene technology facilitates the study of gene expression profiles of a given cell population or tissue [1]. Moreover, its application for investigating differential gene expression might present a powerful tool to discover genes which have not been considered to be involved in a specific biological response so far.

Activated macrophages play an essential role in inflammatory processes. They exhibit a wide range of activities, including the presentation of antigens, the recruitment of inflammatory cells, the production of a variety of immunoregulatory mediators and the destruction of pathogens and tumor cells [2]. Cytokines play a central role in macrophage physiology, both as macrophage activators and mediators of macrophage activities [3]. The best characterised macrophage-activating cytokine is interferon-γ (IFN-γ) [4]. It induces the expression of major histocompatibility complex (MHC) class II and stimulates the effector functions of macrophages mentioned above [5].

It is also well known that macrophages are able to process and present exogenous antigens via MHC class I molecules in order to activate CD8+ T cells [6]. The majority of the peptides presented by MHC class I molecules are derived from endogenous proteins [7, 8]. These proteins are degraded by a cytosolic proteolytic complex, the proteosome [9]. The peptides are subsequently imported into the endoplasmic
Materials and methods

**Materials**

Recombinant human IFN-γ and IL1-β was purchased from Promega (Mannheim, Germany). IFN-α (IFN-α2b) was from ESSEX Pharma (München, Germany) and LPS from Sigma (Deisenhofen, Germany). Oligonucleotides were obtained from MWG Biotech (Ebersberg, Germany). RPMI 1640 was from Biowhittaker (Eversiers, Belgium); S-MEM spinner medium from Life Technologies (Eggenstein, Germany). Lymphoflot was from Biotest (Dreieich, Germany) and Percoll from Sigma (Deisenhofen, Germany).

**Isolation of monocytes from human blood**

Monocytes were obtained from buffy coats, kindly provided by the local blood bank (Transfusionsmedizin, RWTH Aachen, Germany) by two step density gradient centrifugation using Lymphoflot and Percoll as described [13]. The cells were incubated at a density of 2 × 10⁶ cells/ml in RPMI 1640 medium supplemented with 5% human serum pooled from a minimum of four donors and 1% L-glutamine (Biowhittaker, Belgium) for 30 min on tissue culture dishes. During this period monocytes became adherent. They were washed three times with S-MEM spinner medium to remove contaminating lymphocytes.

**Cultivation and stimulation of cells**

Experiments were performed after 5 days of cultivation at a density of 2 × 10⁶ cells/ml in RPMI 1640 medium supplemented with 5% human serum and 1% L-glutamine (Biowhittaker, Belgium). Cells grown in a 10 mm dish were stimulated with 100 U/ml recombinant human IFN-γ, IFN-α and IL1-β and 1 µg/ml LPS for periods of time indicated in the figure legends.

**Microarray analysis**

Total RNA was isolated using peqGOLD RNA-Pure® (peqlab, Germany) as described by the manufacturer. Generation of ³²P labeled cDNA probe was done by reverse transcription of 10 µg total RNA with ³²P dCTP for 90 min at 37°C following the Mamalian GeneFilters® (Research Genetics) protocol. To remove any unincorporated nucleotides the probe was passed through Bio-Spin 6 chromatography columns (BioRad). Purified radiolabeled probe was applied to the array for hybridization at 42°C overnight. After hybridization the filters were washed twice with 2 × SSC, 0.1% SDS at 50°C for 1 h and once with 0.5 × SSC, 0.1% SDS for 30 min at 55°C. Afterwards filters were exposed to a Kodak X-OMAT AR-5 film at −70°C with intensifying screens. We examined 4400 sequence-validated cDNAs and ESTs (expressed sequence tags) arrayed on the GeneFilter® microarray (D1001, Research Genetics) and analyzed suitably exposed autoradiograms with the Pathway™ analysis software (Research Genetics).

**RT-PCR**

Total RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the standard protocol including efficient on-column digestion of RNA with RNase-free DNase I. Reverse transcription and polymerase chain reaction (RT-PCR) were performed using the GeneAmp RNA PCR kit (Perkin Elmer, Überlingen, Germany). The cDNA was synthesized from total RNA by reverse transcription at 42°C for 30 min using random hexamers. Detection of transcripts for monokine induced by gamma interferon (5′ to 3′: forward: CCT AGG CTT CCA CGT ACT GC 3′; reverse: GAA TGA AGC AAA GGG GAA CA), transporter associated with antigen processing, member 2 (5′ to 3′: forward: ACG TTC ACC CTG AGT GAT GC; reverse: GGT CCA GGA GTT GAC TGC AT), transporter associated with antigen processing, member 1 (5′ to 3′; forward: GAG ACA TCT TGG AAG TGG AC, reverse: CTC TGA GTG AGA AGT TCA GC) and β-actin (5′ to 3′: forward: ATC TGG CAC CAA CTT CTA CA, reverse: GGT TCG TGG ATG CCA CAG GA) was performed by using the indicated specific primers. PCR amplification of cDNA was performed with 35 cycles of denaturation for 45 sec at 94°C, annealing for 45 sec at 60°C, extension for 1 min at 72°C. Amplification was terminated with an extension step of 7 min at 72°C after the last cycle. PCR products were subjected to electrophoresis in an 1.8% agarose gel and stained with ethidium bromide.

**Northern blot analysis**

Total RNA was isolated using peqGOLD RNA-Pure® (peqlab, Germany) as described by the manufacturer. 20 µg of total RNA were separated on 1.5% denaturing agarose gels and transferred to positively charged nylon membranes (Roche, Germany). The membranes were pre-hybridized at 45°C for 2 h in ULTRAhyb hybridization buffer (Ambion, USA) and hybridized over night in the same solution with α-³²P-cDNA fragments labeled with the DECAprime II DNA labeling kit (Ambion, USA). Blots were washed twice with 2 × SSC, 0.1% SDS for 1 h at 50°C, followed by two washes for 30 min with 0.1x SSC, 0.1% SDS at 50°C. Afterwards blots were exposed to Kodak X-OMAT AR-5 film at −70°C with intensifying screens.

**Immunoblotting and immunodetection**

Cells were solubilized in lysis buffer (PharMingen, Germany) containing a protease inhibitor cocktail for 30 min at 4°C. Cell debris were removed by centrifugation. The samples were boiled in gel electrophoresis sample buffer and the proteins were separated on a SDS-polyacrylamid (7.5%) gel. The electrophoretically separated proteins were transferred onto PVDF membranes by the semidry western blotting method. Nonspecific binding was blocked with 10% bovine serum albumin in TBS-T (20 mM Tris/HCl, pH 7.4, 137 mM NaCl, and 0.1% Tween) for 15 min. Immunodetection was performed with the enhanced chemiluminescence system (ECL; Amersham Pharmacia Biotech, Braunschweig, Germany) using a primary polyclonal rabbit anti-human

reticulum by a heterodimer formed by the transporters associated with antigen processing, TAP1 and TAP2 [8, 10, 11] which belong to the ATP binding cassette (ABC) superfamily of transporters [10, 11]. After loading of the peptide onto the MHC-I molecules the trimeric complex of peptide, MHC class I heavy chain and β2 microglobulin is transported to the cell surface via the constitutive secretory pathway for CD8⁺ T cell recognition [12].

In this study we used microarray technology to examine the global changes in gene expression of primary human macrophages stimulated by IFN-γ. Differential expression of selected genes detected by microarray analysis was confirmed in independent experiments. Since most of the genes found to be upregulated by IFN-γ have been characterized intensively we focussed on the expression of the transporters associated with antigen processing, TAP1 and TAP2, and examined their expression upon stimulation with IFN-α, IL1-β and LPS. We found that TAP1 and TAP2 were constitutively expressed at a very low level. LPS caused an increase similar to the effect of IFN-γ. Treatment with IFN-α stimulated also the expression of TAP1 and TAP2, however, less than IFN-γ. In contrast, IL1-β stimulation had no effect on the expression of the transporters.