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Short communication

The RNA binding protein tristetraprolin influences the activation state of murine dendritic cells

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ABSTRACT

Dendritic cells (DCs) serve to maintain peripheral tolerance under steady state conditions. Upon triggering by activation signals they initiate strong immune responses. The activation of DCs is accompanied by a rapid upregulation of proinflammatory cytokines, which were shown in other cell types to be regulated by mechanisms at the transcriptional and posttranscriptional level. Tristetraprolin (TTP), an important RNA binding protein, is involved in the regulation of mRNA stability of such cytokines. In this study we analyzed the significance of TTP for mouse DCs, which were derived from $TTP^{-/-}$ and WT bone marrow progenitor cells (BM-DCs). Unstimulated BM-DCs of $TTP^{-/-}$ mice expressed lower levels of mRNAs encoding the costimulatory molecules CD40 and CD86 and surprisingly also the canonical TTP targets TNF-α and IL-10 as compared with WT DCs. On the protein level, both DC populations expressed comparable amounts of CD80 and CD86 and of either cytokine, but $TTP^{-/-}$ DCs expressed less MHCII than WT DCs. On the other hand, $TTP^{-/-}$ DCs displayed elevated expression of other TTP target mRNAs like IL-1β, c-fos and Mkp-1. Stimulation of BM-DCs of either genotype with lipopolysaccharide resulted in a rapid upregulation to a comparable extent of all molecules monitored so far, except for c-fos mRNA. Subsequent mRNA decay analysis revealed gene-specific differences in mRNA stability, which was influenced by the presence of TTP and the activation state of the DCs. Unstimulated $TTP^{-/-}$ DCs exerted a markedly lower allogeneic T cell stimulatory potential than WT DCs. Moreover, $TTP^{-/-}$ DCs induced an altered cytokine pattern in cocultures of DCs and T cells. However, allogeneic T cells primed by unstimulated DCs of either genotype were equally refractory to restimulation and suppressed the proliferation of naive T cells to the same extent. Thus, the findings of this study lend support to the interpretation that without external stimulation antigen presenting activity in DCs in the presence of TTP is more pronounced than in its absence and that posttranscriptional regulation contributes to the control of gene expression in DCs.

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1. Introduction

RNA binding proteins (RNA-BPs) were shown to control the expression of numerous proteins by binding to the respective mRNA species encoding proto-oncogenes, growth factors, cytokines, transcription factors and others in various cell types (Eberhardt et al., 2007). Several RNA-BPs recognize AU-rich elements (AREs) which are found to be located in the 3′-untranslated region (UTR) of their target mRNA, thus exerting posttranscriptional control of expression of the respective gene. This mode of control may be crucial for limiting immune responses as was described for tristetraprolin knockout ($TTP^{-/-}$) mice (Carballo et al., 1997). Such mice suffer from slow growth, underweight and the development of polyarticular arthritis due to excessive production of the proinflammatory cytokine TNF-α by macrophages. In wild type (WT) mice, activation-induced TNF-α production is under negative control of the likewise induced RNA-BP tristetraprolin (TTP). TTP binds to several TNF-α mRNA ARE sites, thus mediating mRNA destabilization (Carballo et al., 1998). The capacity of TTP to bind mRNA is negatively regulated by p38 MAPK-dependent phosphorylation (Mahtani et al., 2001). JNK activation has been shown recently to modify the translation of the TTP mRNA (Korhonen et al., 2007). Moreover, via binding to TTP mRNA ARE sequences, TTP down-regulates its own expression (Tchen et al., 2004).

Dendritic cells (DCs) act in concert with regulatory T cells (Tregs) to maintain peripheral tolerance under steady state conditions (Tang and Bluestone, 2008). On the other hand, upon activation DCs constitute the most potent antigen presenting cells (APCs) known being capable of activating naive T cells. The APC activity of the DCs correlates with high level expression of surface bound costim-

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ulatory molecules and of secreted immunomodulatory mediators, with the latter shaping the character of the T cell response (Adams et al., 2005).

In activated macrophages several of the immunomodulatory factors, including TNF-α (Carballo et al., 1997) and IL-1β (Chen et al., 2006), were shown to be regulated posttranscriptionally by TTP as well as by other RNA-BPs (Eberhardt et al., 2007), while in DCs posttranscriptional control of gene expression has yet been scarcely addressed. Emmons et al. (2008) show in a study published recently that TTP binds numerous mRNA species in human monocyte-derived DCs.

To elucidate the role of TTP in mouse DCs, we used myeloid DCs differentiated in tissue culture from bone marrow-derived progenitor cells (BM-DCs) of $TTP^{-/-}$ (Carballo et al., 1997) and WT mice and compared their gene expression signature as well as APC capacity. Our findings indicate that unstimulated BM-DCs from $TTP^{-/-}$ as compared with WT mice showed an altered pattern of expression and stability of various mRNA species. In line with these findings the APC capacity of DCs derived from $TTP^{-/-}$ progenitors was less potent than that of WT DCs. However, stimulation of the DCs of either genotype with lipopolysaccharide (LPS) altered the stability of various mRNA species and largely abrogated the differences observed between $TTP^{-/-}$ and WT DCs in terms of mRNA expression and APC function. These results suggest that in mouse DCs regulation of gene expression at the posttranscriptional level plays an important role, with TTP being crucially involved in that regulation in unstimulated BM-DCs.

2. Materials and methods

2.1. Mice and cells

Female C57BL/6 and BALB/c mice as well as $TTP^{-/-}$ mice on the C57BL/6 background (Carballo et al., 1997, a kind gift by Dr. Blackshear, NIEHS, National Institutes of Health, Research Triangle Park, NC, USA), were bred and maintained in the Central Animal Facilities of the University of Mainz under specific pathogen-free conditions on a standard diet. The "Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised 1985) were followed. Bone marrow-derived DCs (BM-DCs) were generated as reported by Scheicher et al. (1992) with modifications as described (Gisch et al., 2007) except that DC culture supernatants were replenished on days 3 and 6, and part of the DC cultures was stimulated with LPS (1 μg/ml) on day 7. BM-DC medium (IMDM with 10% FCS; [PAA, Cölbe, Germany], 2 mM L-glutamine [Biochrom AG, Berlin, Germany], 100 U/ml penicillin, 100 μg/ml streptomycin [Gibco, Paisly, UK]) was supplemented with 5% of GM-CSF containing cell culture supernatant (Zal et al., 1994, a kind gift by Dr. Stockinger, MRC National Institute for Medical Research, Mill Hill, London). Immature BM-DCs harvested on day 8 of culture for experiments contained >85% $CD11c^+$ cells as assessed by FACS analysis. To monitor alterations in mRNA expression levels in time course studies of stimulation as well as in decay experiments employing actinomycin D (Act D), BM-DCs (10^6) were reseeded in wells of 6-well tissue culture plates (Greiner, Frickenhausen, Germany) containing 5 ml of BM-DC medium supplemented with GM-CSF, and aliquots of these cultures were stimulated with LPS (1 μg/ml) in the absence or presence of Act D (5 μg/ml) as indicated.

2.2. T cell proliferation assays

Nylon–wool-enriched BALB/c T cells (Bros et al., 2007) (3×10^5) were cocultured with graded numbers of irradiated (30 Gy) BM-DCs for 4 days on flat-bottom 96-well tissue culture plates in 200 μl of BM-DC medium. To assess the restimulatory and suppressive function of primed T cells, BM-DCs (10^6/well) were cocultured with nylon–wool-enriched BALB/c T cells (6×10^6/well) on 6-well tissue culture plates in a volume of 4 ml for 7 days, and prestimulated T cells were harvested. Graded numbers of these prestimulated T cells were cocultured with C57BL/6 splenocytes, depleted of erythrocytes and γ-irradiated (30 Gy), without (restimulation assay) or with (suppression assay) freshly isolated BALB/c T cells (3×10^5 each) for 6 days. In all coculture assays T cell proliferation was assessed by the uptake of [^{3}H] thymidine (0.25 μCi/well) during the last 16 h of culture. Cells were harvested onto glass fiber filters and retained radioactivity was measured in a liquid scintillation counter (1205 Betaplate, LKB Wallac, Turcu, Finland).

2.3. PCR and real time PCR analysis

Total RNA was isolated from at least 5×10^5 BM-DCs by using the RNeasy Plus Mini kit (Qiagen, Hilden, Germany) as recommended. RNA was reverse-transcribed applying a 1:1 mix of oligo-dT and random hexamer primers using iScript (Bio-Rad, Munich, Germany) as recommended. Primer sequences have been described (Bros et al., 2007). Additional primers were used to detect c-fos (5′-GGGGCAAAGTAGAGCAGCTA-3′, 5′-GGCTGCCAAAATAAACTCCA-3′), Cox-2 (5′-TCCTCCTGGAACATGGACTC-3′, 5′-TTCTGCAGCCATTTCCTTCT-3′), Gapdh (5′-CCATCACCATCTTCCAGGAG-3′, 5′-TTTCTCGTGGTTCACACCC-3′), and Mkp-1 (5′-GAGCTGTGCAGCAAACAGTC-3′, 5′-CTTCCGAGAAGCGTGATAGG-3′). PCR-mediated detection of TTP as well as TTP/neomycin mRNA which is apparent in $TTP^{-/-}$ cells due to the insertion of the neomycin cassette has been described (Carballo et al., 2000). All primers were purchased from Operon (Cologne, Germany). The house-keeping gene ubiquitin C served as internal control. For normalization of mRNA expression in mRNA decay analysis Gapdh was used instead. Real time PCR reactions included 200 ng of cDNA and SYBRGreen mastermix (ABgene, Hamburg, Germany) and were performed and analyzed as described (Bros et al., 2007).

2.4. Flow cytometry

BM-DCs (5×10^5) were washed in staining buffer (PBS/2% FCS). To block Fc receptor-mediated staining, cells were incubated with rat anti-mouse CD16/CD32 (2.4.G2) (Dianova, Hamburg, Germany) for 15 min on ice. Afterwards, cells were incubated with FITC-conjugated rat monoclonal antibodies (Abs) recognizing MHC class II I-A/I-E (2G9), CD80 (1G10), and CD86 (GL1) (BD Pharmingen, San Diego, CA) for 20 min on ice. Appropriate isotype controls were used. Flow cytometric analysis was performed using a FACScan flow cytometer (BD Biosciences) equipped with CellQuest Software.

2.5. Cytokine assays

ELISA capture Abs binding to murine IFN-γ (clone R4-6A2), IL-5 (TRFK5), and TNF-α (G281-2626) were purchased from BD Pharmingen and Abs to murine IL-10 (JES052A5) was obtained from R&D Systems. Biotinylated detection Abs to murine IFN-γ (AN18.17.24), IL-5 (TRFK4), and TNF-α (MP6-XT3) were obtained from BD Pharmingen and Abs to murine IL-10 (BAF417) from R&D Systems. Abs were used as recommended by the manufacturer. Recombinant murine (rm) cytokines IFN-γ, IL-5, and TNF-α used for ELISA standards were purchased from BD Pharmingen, and rm IL-10 from R&D Systems.

2.6. Statistical analysis

Data were analyzed for statistically significant differences by applying Student's *t*-test.

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