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Immunobiology 210 (2005) 97–107
Immunobiology
www.elsevier.de/imbio

LPS regulates a set of genes in primary murine macrophages by antagonising CSF-1 action

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Received 11 March 2005; accepted 10 May 2005

Abstract

We previously reported that bacterial products such as LPS and CpG DNA down-modulated cell surface levels of the Colony Stimulating Factor (CSF)-1 receptor (CSF-1R) on primary murine macrophages in an all-or-nothing manner. Here we show that the ability of bacterial products to down-modulate the CSF-1R rendered bone marrow-derived macrophages (BMM) unresponsive to CSF-1 as assessed by Akt and ERK1/2 phosphorylation. Using toll-like receptor (*tlr*)*9* as a model CSF-1-repressed gene, we show that LPS induced *tlr9* expression in BMM only when CSF-1 was present, suggesting that LPS relieves CSF-1-mediated inhibition to induce gene expression. Using cDNA microarrays, we identified a cluster of similarly CSF-1 repressed genes in BMM. By real time PCR we confirmed that the expression of a selection of these genes, including integral membrane protein 2B (*itm2b*), receptor activity-modifying protein 2 (*ramp2*) and macrophage-specific gene 1 (*mpg-1*), were repressed by CSF-1 and were induced by LPS only in the presence of CSF-1. This pattern of gene regulation was also apparent in thioglycollate-elicited peritoneal macrophages (TEPM). LPS also counteracted CSF-1 action to induce mRNA expression of a number of transcription factors including interferon consensus sequence binding protein 1 (Icsbp1), suggesting that this mechanism leads to transcriptional reprogramming in macrophages. Since the majority of in vitro studies on macrophage biology do not include CSF-1, these genes represent a set of previously uncharacterised LPS-inducible genes. This study identifies a

Abbreviations: AO-1, activating oligonucleotide-1; BMM, bone marrow-derived macrophage; CSF-1, Colony stimulating Factor-1/Macrophage Colony Stimulating Factor; CT DNA, calf thymus genomic DNA; EC DNA, *Escherichia coli* genomic DNA; ERK1/2, extracellular signal-regulated kinase 1/2; *hprt*, hypoxanthine-guanine phosphoribosyl transferase; Icsbp1, interferon consensus sequence binding protein 1; *itm2b*, integral membrane protein 2B; MAPK, mitogen-activated protein kinase; *mpg-1*, macrophage-specific gene 1; NAO-1, non-activating oligonucleotide-1; *ramp2*, receptor activity-modifying protein 2; RPM, resident peritoneal macrophage; TACE, proTNF-α-converting enzyme; TEPM, thioglycollate-elicited peritoneal macrophage; TLR, toll-like receptor

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doi:10.1016/j.imbio.2005.05.004

new mechanism of macrophage activation, in which LPS (and other toll-like receptor agonists) regulate gene expression by switching off the CSF-1R signal. This finding also provides a biological relevance to the well-documented ability of macrophage activators to down-modulate surface expression of the CSF-1R.

Keywords: CSF-1; Gene regulation; Inflammation; Lipopolysaccharide; Micro-array; Monocytes/macrophages; TLR9

Introduction

Colony stimulating Factor (CSF)-1, also termed M-CSF, is the major growth factor for cells of the monocyte/macrophage lineage (Hamilton, 1997). CSF-1 elicits its effects through a single high affinity receptor, the CSF-1 receptor (CSF-1R), which is the product of the *c-fms* proto-oncogene (Dai et al., 2002; Sherr et al., 1985). CSF-1-dependent dimerisation and activation of the CSF-1R promotes the survival, proliferation and differentiation of the mononuclear phagocyte lineage (reviewed in Roth and Stanley, 1992; Sherr, 1991). Signalling through the CSF-1R also mediates the stable induction of inducible genes such as the scavenger receptor (Guidez et al., 1998) and urokinase plasminogen activator (Stacey et al., 1995).

A major function of mononuclear phagocytes is to detect and respond to pathogens by triggering antimicrobial responses and by releasing mediators that coordinate the ensuing immune response. Activation of such innate immune cells is facilitated by germline-encoded pattern recognition receptors, which recognise invariant pathogen components, and include the toll-like receptor (TLR) family (Aderem and Ulevitch, 2000). TLR4 is required for activation by bacterial LPS, TLR2/6 mediates responses to bacterial lipoproteins and TLR9 is necessary for responses to CpG DNA (Takeda et al., 2003). Activation by conserved pathogen components result in large scale alterations of the macrophage transcriptome which facilitates the execution of specific effector functions following activation (Wells et al., 2003a). There is considerable cross-talk between TLR and CSF-1 signalling pathways in macrophages. For example, CSF-1 can enhance or inhibit responses to different TLR agonists through multiple mechanisms (Asakura et al., 1996; Chapoval et al., 1998; Evans et al., 1992; Sweet et al., 2002; Szperl et al., 1995), whilst LPS and other bacterial products down-modulate cell surface CSF-1R in murine macrophages (Guilbert and Stanley, 1984; Hume et al., 1987; Hume and Denkins, 1989; Sester et al., 1999a).

The mechanisms mediating CSF-1R down-modulation by TLR agonists have been extensively studied. We have previously shown that down-modulation in response to bacterial products such as LPS and bacterial/CpG DNA occurs rapidly in an all-or-nothing manner at the single cell level (Sester et al., 1999a, b). Using LPS or PMA as stimuli, Rovida et al. (2001) identified the proTNF-α-converting enzyme (TACE) as the protease responsible for cleavage of the CSF-1R resulting in ecto-domain shedding. Ecto-domain shedding is followed by regulated intramembrane proteolysis of the residual receptor by the γ-secretase, resulting in the release of the cytoplasmic domain from the plasma membrane to the cytosol and nucleus (Wilhelmsen and van der Geer, 2004). Despite such mechanistic knowledge of the processes involved in CSF-1R down-modulation by pro-inflammatory activators, the physiological relevance of this event has remained unknown. In this study, we assessed the consequences of TLR-mediated CSF-1R down-modulation. We identify a previously uncharacterised mechanism of LPS-mediated gene regulation in macrophages that provides a biological relevance to the known ability of pro-inflammatory activators to down-modulate CSF-1 receptor expression and is likely to be relevant to sustained pathogen challenge and chronic inflammatory states.

Materials and methods

Cell culture and reagents

RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% FCS (JRH Biosciences Inc, Lenexa, Ks), 20 U/ml penicillin (Invitrogen, Carlsbad, CA), 20 μg/ml streptomycin (Invitrogen, Carlsbad, CA) and 2 mM L-glutamine (Invitrogen, Carlsbad, CA) (complete medium) was used for culture of thioglycollate-elicited peritoneal macrophages (TEPM), resident peritoneal macrophages (RPM) and bone marrow-derived macrophages (BMM). All primary cells were maintained in a 37 °C incubator containing 5% CO_2. TEPM were obtained by i.p. injection of 1 ml 10% thioglycollate broth into CD1 outbred mice or BALB/c inbred mice followed by peritoneal lavage with 10 ml PBS 5 days later. RPM, harvested by peritoneal lavage, were from CD1 mice. BMM were prepared from femurs of CD1, C57BL/6 or BALB/c mice. Briefly, bone marrow cells were cultured for 7 days in complete medium in the presence of 10 000 U/ml CSF-1 (a gift from Chiron, Emeryville, CA) on bacteriological plastic plates. Re595 LPS from *Salmonella minnesota*