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Activation of IL-1β and TNFα genes is mediated by the establishment of permissive chromatin structures during monopoiesis

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ABSTRACT

IL-1β and TNFα participate in a wide range of immunoregulatory activities. The overproduction of these cytokines can result in inflammatory and autoimmune diseases. Monocytes are the main producers of both cytokines. In contrast, studies with highly purified polymorphonuclear leukocytes (PMN) showed their inability to synthesize IL-1β and TNFα. Mature monocytes and PMN are derived from the same precursors. However, the reason for the differential IL-1β and TNFα expression is not elucidated. Our study investigates the epigenetic mechanisms that may explain this apparent discrepancy.

The expression and promoter accessibilities of IL-1β and TNFα genes of primary and *in vitro* differentiated monocytes and PMN and their common precursors were compared. The effects of histone deacetylase (HDAC)-inhibition by trichostatin A (TSA) on IL-1β and TNFα expression and their promoter structures were measured in promyeloid HL-60 cells. Cytokine expression was assessed by real-time PCR and ELISA. Chromatin structures were analyzed using chromatin accessibility by real-time PCR (CHART) assay.

The proximal IL-1β promoter was remodeled into an open conformation during monopoiesis, but not granulopoiesis. Although stimulation-dependent, remodeling of the TNFα promoter was again only observed in monocytes. TSA activated IL-1β and TNFα expression and supported chromatin remodeling of their promoters in HL-60 cells.

The ability to express IL-1β and TNFα is linked to a cell type specific promoter structure, which is established during monocytic but not granulocytic differentiation. The participation of acetylation in IL-1β and TNFα promoter activation shed new light on the regulation of IL-1β or TNFα expression. These data may have implications for understanding the progression from normal to disease conditions.

Introduction

IL-1β and TNFα belong to the class of immediate early genes (Falvo et al. 2010; Ramirez-Carrozzi et al. 2006). Both exert a broad spectrum of inflammatory, metabolic, hematopoietic and immunological properties in various tissues (Dinarello 1991; Lee et al. 2003; Tracey and Cerami 1994). However, their dysregulation leads to severe tissue damage and has been implicated in the etiology of inflammatory and autoimmune diseases, such as rheumatoid arthritis and diabetes, but also in aging and zinc-deficiency (Dinarello 1991, 2009; Haase and Rink 2009; Kominato et al. 1995; Lee et al. 2003; Miao et al. 2004; Tracey and Cerami 1994).

Expression of the human IL-1β gene is regulated by the proximal promoter region and an IL-1β enhancer region (Liang et al. 2006; Wessels et al. 2010a; Zhang et al. 2008), located ~3 kb upstream of the transcriptional start site (Shirakawa et al. 1993; Tsukada et al. 1996). TNFα expression is primarily controlled by a small promoter fragment spanning −120 to −1 (Sullivan et al. 2007). In contrast to IL-1β, chromatin remodeling of the proximal TNFα promoter occurs after LPS stimulation (Lee et al. 2003). Inducible transcriptional activation of both cytokines predominantly depends on binding of the myeloid transcription factors PU.1 and C/EBPβ as well as of the ubiquitous transcription factor NFκB to the proximal IL-1β or TNFα promoter (Dinarello 2009; Gabay et al. 2010; Grondin et al. 2007; Kominato et al. 1995; Liang et al. 2006; Unlu et al. 2007; Zhang et al. 2008).

Abbreviations: CHART, chromatin accessibility by real-time PCR; HDAC, histone deacetylase; HPC, hematopoietic progenitor cells; TSA, trichostatin A; VD3, 1α,25-dihydroxyvitamin D3.

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Monocytes and PMN originate from the same myeloid precursors sharing various features such as phagocytosis, surface receptors and production of cytokines (Dale et al. 2008). However, studies revealed that PMN of healthy donors, unlike monocytes, do not produce pro-inflammatory IL-1β and TNFα as formerly stated by other laboratories (Mantovani et al. 2011; Schroeder et al. 2006; Xing and Remick 2004). The mechanisms responsible for cell-type dependent IL-1β and TNFα expression appear to be epigenetically regulated. This is supported by reports indicating a developmental-dependent chromatin remodeling of the IL-1β promoter during monopoiesis and a stimulation dependent increase in TNFα promoter accessibility of monocytic cell lines (Lee et al. 2003; Liang et al. 2006; Wessels et al. 2010a, b; Zhang et al. 2008).

Histonedeacetylases (HDAC) are capable to modify chromatin structures (de Ruijter et al. 2003). Interestingly, HDAC inhibition has been shown to activate IL-1β and TNFα expression in various cell types including monocytic cell lines (Lee et al. 2003; Mahlknecht et al. 2004; Sato and Mitchell 2006). But little is known about the effects of acetylation on IL-1β and TNFα promoter accessibilities.

Most epigenetic studies on IL-1β and TNFα gene regulation used cell lines. In the present study, IL-1β and TNFα promoter structures of human primary PMN and monocytes as well as *in vitro* differentiated human CD34$^+$ hematopoietic progenitor cells (HPC) were analyzed. We could show for the first time in primary cells that the IL-1β promoter is opened into a poised structure during monopoiesis, but not during granulopoiesis. Stimulation-dependent chromatin remodeling of the TNFα promoter was also monocyte-restricted and did not occur in PMN or HPC. For both promoters chromatin remodeling was supported by inactivation of HDAC. These results may present two different paradigms for the cell-type specific activation of immediate early genes explaining monocyte-restricted IL-1β and TNFα expression, in parallel.

Materials and methods

Materials

Human macrophage-colony stimulating factor (M-CSF) and human granulocyte (G)-CSF were purchased from Peprotech (Hamburg, Germany), Percoll (1.131 ± 0.005 g/ml) from GE Healthcare (Freiburg, Germany), Ficoll (1.077 g/ml) from Biochrom (Berlin, Germany) and RPMI-1640 medium, L-glutamine, nonessential amino acids, penicillin, streptomycin, 10× and 1× phosphate-buffered saline (PBS) from Lonza (Verviers, Belgium). Fetal calf serum (FCS) was from PAA (Coelbe, Germany) and lipopolysaccharide (LPS, *Escherichia coli* serotype 0111:B4), phorbol myristate acetate (PMA), bovine serum albumin (BSA) and Trichostatin A (TSA, *Streptomyces* sp.) were all from Sigma–Aldrich (Taufkirchen, Germany). Phycoerythrin (PE)-labeled anti-CD14 (clone MΦP9) and isotype-matched control anti-IgG2bκ, fluorescein isothiocyanate (FITC)-labeled anti-CD66b (clone G10F5) and isotype matched control anti-IgM were purchased from BD PharMingen (Heidelberg, Germany). 1α,25-dihydrorhodamin D3 (VD3) was obtained from Biomol (Hamburg, Germany). Micrococcal nuclease (MNase) and proteinase K were purchased from Fermentas (St. Leon-Rot, Germany), hydroxyethyl starch from Fresenius (Bad Homburg, Germany) and May–Gruenwald–Giemsa staining from Merck (Darmstadt, Germany).

Cell isolation and cultivation

Human PMN of high purity were isolated from healthy donors, as described previously (Sato and Mitchell 2006; Schroeder et al. 2006). Briefly, the cells were separated by two density gradients of Percoll after sedimentation through hydroxyethyl starch. Any remaining erythrocytes were removed by hypotonic lysis. Peripheral blood mononuclear cells (PBMC) were prepared by Ficoll gradient centrifugation and plastic-adherent PBMC were used as enriched monocytes and incubated for 1 h in 10 ml Petri dishes (37 °C, 5% CO_2), as described previously (Schroeder et al. 2006). All cells were cultured in RPMI-1640 containing 10% low-endotoxin fetal calf serum and supplemented with 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin in a 5% CO_2 humidified atmosphere at 37 °C.

For hyperacetylation experiments, HL-60 cells were cultured in the presence of the acetyldeacetylase inhibitor TSA (100 ng/ml) for 24 h. The differentiation of HL-60 cells into monocytic cells using VD3 (100 nM) for 72 h was performed and monitored as described recently (Wessels et al. 2010a). Cells were stimulated with LPS (250 ng/ml) for 1 h, 3 h, or 24 h and used for CHART assay, quantitative PCR or ELISA, respectively.

Isolation and in vitro differentiation of CD34$^+$ hematopoietic progenitor cells

Isolation of CD34+ hematopoietic progenitor cells (HPC) from cord blood using a human CD34 Micro Bead Kit (Miltenyi, Bergisch Gladbach, Germany), and their expansion and differentiation was performed as reported (Schroeder et al. 2005). For differentiation M-CSF (25 ng/ml) or G-CSF (10 ng/ml) respectively was added to RPMI-1640 otherwise supplemented as described above. Monocytic cells were differentiated for 7 days and granulocytic cells for 14 days. HPC as well as *in vitro* differentiated monocytes and PMN were examined by May–Gruenwald–Giemsa staining. Differentiation progress was confirmed by flow cytometry with antibodies against CD14 and CD66b. Image acquisition was performed using an AxioSkop 2 FS (Zeiss, Cologne, Germany, magnification 400×).

Enzyme-linked immunosorbent assay (ELISA)

Supernatants were harvested after stimulation, stored at −20 °C until measurement, and only thawed once for cytokine detection. IL-1β and TNFα protein production were quantified using OptEIA assays from BD PharMingen according to the manufacturer's instructions.

Reverse transcription and real-time PCR

Total cellular RNA was isolated using NucleoSpin RNA II-Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. RNA was reverse-transcribed using qScript cDNA Synthesis Kit in reactions containing 50 ng/μl RNA (Quanta Bioscience, Gaithersburg, MD, USA). Primers for IL-1β (Stordeur et al. 2002), TNFα (forward primer: 5′-ATGAGCACTGAAAGCATGATCC-3′; reverse primer: 5′-GAGGGCTGATTAGAGAGAGGTC-3′) and for the housekeeping gene *glycerinaldehyde-3 phosphate* dehydrogenase (GAPDH) (Zarember and Godowski 2002) were added at a final concentration of 0.1 μM. IL-1β, TNFα and GAPDH real-time PCRs were performed with 2 μl cDNA in 25 μl reaction volumes in duplicates using Brilliant Sybr Green qPCR Master Mix (Applied Biosystems, Darmstadt, Germany) with the following parameters: one initial step at 95 °C for 15 min was followed by 40 cycles of 95 °C for 30 s and 56 °C for 30 s. For quantification, standard curves were generated using 10-fold serial dilutions of cDNA from peripheral blood mononuclear cells. The mRNA levels of the cytokines were normalized to GAPDH mRNA levels.