BBA - Gene Regulatory Mechanisms 1861 (2018) 191–199

Contents lists available at ScienceDirect

BBA - Gene Regulatory Mechanisms

journal homepage: www.elsevier.com/locate/bbagrm

Epigenetic regulation by CpG methylation splits strong from retarded IFNγ-induced IL-18BP in epithelial *versus* monocytic cells☆

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ARTICLE INFO

Keywords:
IL-18 binding protein
IL-18
IFNγ
Inflammation
Monocytes
Epigenetics
CpG methylation

ABSTRACT

Interferon (IFN)-γ-inducing interleukin (IL)-18 is a crucial inflammatory cytokine systemically provided by monocytes. It is counteracted by IL-18 binding protein (IL-18BP), a decoy receptor that displays IFNγ-inducibility thus curbing inflammation by negative feedback. Since *IL18BP* inducibility is pronounced in human epithelial cells but diminished in monocytes, differential *IL18BP* regulation was investigated herein in both types of cells. Interestingly, DNA-demethylating 5-aza-2′-deoxycytidine enhanced IFNγ-induced IL-18BP only in monocytic but not in epithelial cells. Subsequent promoter analysis brought into focus a specific CpG (coined CpG2) neighboring a γ-activated site responsible for *IL18BP* induction. CpG2 was consistently methylated in monocytic but unmethylated in epithelial cells. Notably, demethylation by 5-aza-2′-deoxycytidine treatment of monocytic cells impeded methyl-CpG-binding protein-2 (MeCP2) interaction with CpG2, increased adjacent histone H3K9-acetylation, and enhanced RNA-polymerase-II recruitment to the nearby *IL18BP* transcriptional start. Both latter observations are indicative of a gene locus displaying augmented transcriptional activity. Data suggest that epigenetic silencing by single CpG methylation determines differential *IL18BP* inducibility in monocytic *versus* epithelial cells. This regulatory principle should serve and control pivotal IL-18-related cell type-specific (patho)-physiological functions. Whereas epithelial IL-18BP evidently counteracts pathological inflammation at biological barriers, retarded *IL18BP* inducibility in monocytes may be key to combat blood-borne infections in IL-18-dependent manner.

1. Introduction

Interleukin (IL)-18 [1] is a member of the IL-1 cytokine family activating corner stones of inflammatory signaling, among others nuclear factor (NF)-κB and p38 mitogen-activated protein kinase. Striking similarities exist between IL-1β and IL-18 including processing by caspase-1, an atypical mode of release, and a frequently pathogenic role in autoimmunity [2] and autoinflammation [3]. Despite widely shared characteristics with IL-1β, IL-18 also shows distinctive properties. Above all, IL-18 is crucial for interferon (IFN)-γ production by natural killer (NK) [4] and T cells where it interacts with IL-12 to back Th1-like functions [1,2]. Derived from innate immune cells, IL-18 is thus an essential bridge to adaptive immunity. IFNγ, in turn, neither directly upregulates IL-18 expression [5] nor is part of signaling pathways directly triggering IL-18 release [6]. Notably, IL-18 is constitutively expressed not only in monocytic [7] but also in epithelial cells [8,9] indicating a vital role at biological barriers. Finally, IL-18 promotes FasL-induced cytotoxicity which may particularly affect liver pathology [10].

A secreted IL-18 binding protein was identified with two splice variants (human IL-18BPa/c) serving as decoy receptors for mature (but not pro-) IL-18. IL-18BPa is by far the most prevalent human variant and is referred to herein as IL-18BP. Although to some degree constitutive in murine spleen and human peripheral blood mononuclear cells (PBMC) [11–13], IL-18BP, precisely human IL-18BPa, is strongly upregulated by IFNγ [13–15] thus enabling negative feedback regulation of the IL-18/IFNγ-axis [16]. In fact, administration of IL-18 to humans increases IFNγ as well as IL-18BP production [17] and IL-18BP is elevated in clinical inflammation, among others in inflammatory bowel diseases [18]. Interestingly, intestinal epithelial cells are a major source of IL-18BP during experimental colitis [19], an observation concurring with its induction by IFNγ in human colon carcinoma cells [14,15]. *IL18BP* promoter analysis in human HepG2 [13] and DLD1 [20] cells identified a γ-activated site (GAS) near the transcriptional start site (TSS) as crucial for gene induction by IFNγ. Notably, either a complex consisting of interferon regulatory factor-1 and CCAAT/enhancer-binding-protein-β [13] or signal transducer and activator of transcription (STAT)-1 [20] drive *IL18BP* transcription by binding to this site in cell type-specific manner. IL-18BP is also increased in

☆ Research article

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https://doi.org/10.1016/j.bbagrm.2018.01.020
Received 6 November 2017; Received in revised form 19 January 2018; Accepted 26 January 2018
Available online 31 January 2018
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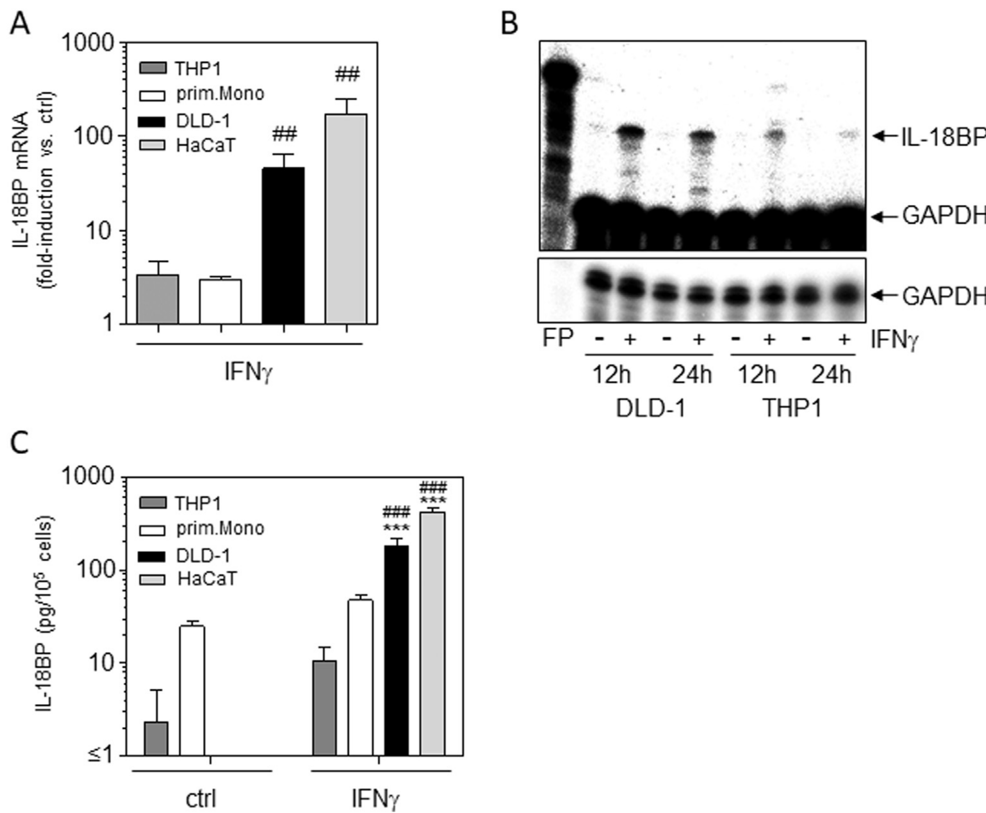

Fig. 1. Monocytic cells display retarded IFNγ-induced IL-18BP when compared to epithelial (-like) DLD1 colon carcinoma cells and HaCaT keratinocytes. (A) Indicated cell types were kept as unstimulated control or stimulated with IFNγ (20 ng/ml) for 12 h. IL-18BP mRNA was determined by realtime PCR. IL-18BP mRNA was normalized to that of GAPDH and is shown as mean fold-induction compared to unstimulated control of the same cell type ± SD (DLD1, n = 10; THP1, n = 14; HaCaT, n = 4) or ± SEM (primary monocytes, n = 10); $^{##}P < 0.01$ *versus* THP1 and primary monocytes; fold-inductions were analyzed by one-way ANOVA with *post hoc* Bonferroni correction. (B) DLD1 or THP1 cells were kept as unstimulated control or stimulated with IFNγ (20 ng/ml). After 12 h or 24 h, IL-18BP mRNA was analyzed by RNase protection assay. One of two independently performed experiments is shown. FP denotes free probe. (C) Indicated cell types were kept as unstimulated control or stimulated with IFNγ (20 ng/ml) for 12 h. Secreted IL-18BP was determined by ELISA and is depicted as pg/10^5 cells. Shown are means ± SD (n = 3 per cell line) or ± SEM (primary monocytes, n = 5). $^{***}P < 0.001$ compared to unstimulated control of the same cell type, $^{###}P < 0.001$ compared to IFNγ-stimulated THP1 cells and primary monocytes; raw data were analyzed by one-way ANOVA with *post hoc* Bonferroni correction.

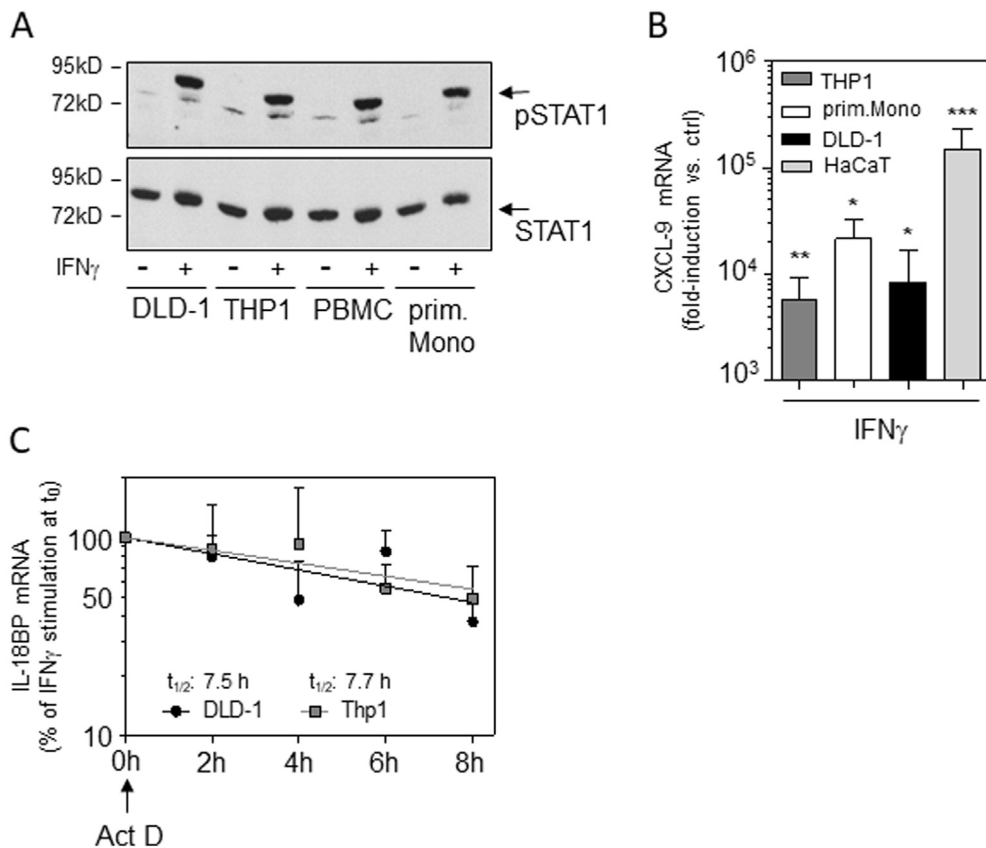

Fig. 2. Lack of obvious differences between epithelial and monocytic cells concerning IL-18BP mRNA-half-life as well as IFNγ-induced STAT1 activation and CXCL9 expression.

(A) Indicated cell types were kept as unstimulated control or stimulated with IFNγ (20 ng/ml). After 30 min cellular content of pSTAT1 and total STAT1 was determined by immunoblot analysis. One representative of three independently performed experiments is shown. (B) Indicated cell types were kept as unstimulated control or stimulated with IFNγ (20 ng/ml) for 12 h. CXCL9 mRNA determined by realtime PCR. CXCL9 mRNA was normalized to that of GAPDH and is shown as fold-induction compared to unstimulated control of the same cell type ± SD (DLD1 and HaCaT, n = 4; THP1, n = 5) or ± SEM (primary monocytes, n = 7). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to unstimulated control of the same cell type; raw data were analyzed by Student's *t*-test. (C) DLD1 and THP1 cells were stimulated with IFNγ (20 ng/ml). After 12 h actinomycin D (ActD, 10 μg/ml) was added and IL-18BP mRNA was determined by realtime PCR at indicated time points. All cultures were adjusted to a final concentration of 0.05% DMSO (vehicle for Act D). IL-18BP mRNA was normalized to that of GAPDH. Means ± SD are depicted as (% of IL-18BP mRNA at Act D addition (t_0), n = 3 per cell line); IL-18BP mRNA half-lives were calculated by nonlinear regression analysis (GraphPad 5.0).

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