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Expression and regulation of Schlafen (SLFN) family members in primary human monocytes, monocyte-derived dendritic cells and T cells

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ABSTRACT

Schlafen (SLFN/Slfn) family members have been investigated for their involvement in fundamental cellular processes including growth regulation, differentiation and control of viral replication. However, most research has been focused on the characterization of Slfns within the murine system or in human cell lines. Since little is known about SLFNs in primary human immune cells, we set out to analyze the expression and regulation of the six human *SLFN* genes in monocytes, monocyte-derived dendritic cells (moDCs) and T cells. Comparison of *SLFN* gene expression across these three cell types showed high mRNA expression of *SLFN11* in monocytes and moDCs and high *SLFN5* expression in T cells, indicating functional importance within these cell types. Differentiation of monocytes to moDCs leads to the gradual upregulation of *SLFN12L* and *SLFN13* while *SLFN12* levels were decreased by differentiation stimuli. Stimulation of moDCs via human rhinovirus, lipopolysaccharide, or IFN-α lead to strong upregulation of *SLFN* gene expression, while peptidoglycan poorly stimulated regulation of both *SLFNs* and the classical interferon-stimulated gene *MxA*. T cell activation was found to downregulate the expression of *SLFN5*, *SLFN12* and *SLFN12L*, which was reversible upon addition of exogenous IFN-α. In conclusion, we demonstrate, that *SLFN* gene upregulation is mainly dependent on autocrine type I interferon signaling in primary human immune cells. Rapid decrease of *SLFN* expression levels following T cell receptor stimulation indicates a role of SLFNs in the regulation of human T cell quiescence.

1. Introduction

The *Schlafen* (*Slfn, SLFN*) family was initially reported as a family of growth-regulatory genes in mice that is differentially regulated during thymocyte development and T cell activation [1]. The name „Schlafen“ is derived from a German word, meaning “to sleep” and refers to the G_0/G_1 cell cycle arrest that was observed upon ectopic expression of prototypic Slfn1 in NIH-3T3 fibroblasts [1]. *Slfn* genes have been evolutionarily conserved across species with gene duplications giving rise to ten murine (*Slfn*) and six human (*SLFN*) family members [2]. All Slfn proteins share a core region harboring an divergent AAA domain, which is presumed to confer ATPase activity [2]. Adjacent to the N-terminal part of this domain lies the so-called “Slfn-box”, which is a domain of unknown function showing partial homology to the conserved domain signature COG2865 found in putative transcriptional regulators and helicases [3–5]. Slfns are typically divided into three subgroups depending on size and domain composition. Subgroup I comprises short Slfn isoforms in the range of 37–42 kDa, while Subgroup II Slfns are 58–68 kDa in size. Subgroup III contains the largest Slfn family members (100–104 kDa), which are also characterized by the presence of signature motifs homologous to the helicase superfamily I and to the UvrD DNA helicase superfamily [3,6]. While murine Slfns are distributed across all three subgroups, humans only possess SLFNs classified as subgroup II (SLFN12, SLFN12L) and subgroup III (SLFN5, SLFN11, SLFN13, SLFN14) [6]. Slfn5/SLFN5 and Slfn14/SLFN14 are the only one-to-one orthologs shared by mice and men, which is in line with the rapid evolution of this gene family, especially in the mouse [2,7,8].

Several studies have implicated at least some Slfn family members as regulators of cellular growth processes and/or

Abbreviations: SLFN, Schlafen (family member); moDC, monocyte-derived dendritic cell; HRV, human rhinovirus; IFN, interferon (e.g. IFN-α); ISG, interferon-stimulated gene; LPS, lipopolysaccharide; PGN, peptidoglycan; TLR, Toll-like receptor.

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differentiation [1,3,9–15]. However, it is still poorly understood how these Slfns interfere with the cell cycle machinery. While one study demonstrated that Slfn1 mediates growth-inhibitory effects via inhibition of cyclin D1 promoter activity [9], another one was unable to reproduce these findings [16]. Other observations point to a general role of Slfns in immune processes, since Slfns are preferentially expressed in lymphoid tissues [1], are inducible by type I interferons [10,13,17], TLR ligands [7,18] or bacterial infection [3] and have been demonstrated to interfere with viral replication [17,19]. At present, most research performed on Slfn family members has been focused on murine Slfns. With the exception of SLFN11, human SLFNs have not been extensively studied. SLFN11 was recently identified as an HIV-1 restriction factor, which limits retroviral replication by inhibiting the production of HIV-1 proteins by binding of cellular tRNAs [17]. Consistent with this finding, SLFN11 expression is elevated in HIV-1 infected patients that are capable of suppressing viral replication [20]. A role for SLFN11 has also been suggested in cancer therapy, since its expression was positively correlated with the growth-inhibitory action of topoisomerase inhibitors on human cancer cells and shows a positive correlation with tumor-free survival in Ewing's sarcoma patients [21–25]. Further evidence regarding a function of Slfns in immune responses is also provided by the existence of Slfn-like genes in orthopoxviruses, where they have been suggested to serve as viral virulence factors [2,26]. *Berger* et al. have also reported severe susceptibility to bacterial or viral infection in mice with a loss-of-function mutation in Slfn2 due to a loss of cellular quiescence in monocytes and T cells [27].

Since little is known about the expression and regulation of *SLFN* genes in primary human immune cells, we sought to characterize the expression of the six human *SLFN* genes in cell types at the interface between the innate and adaptive immune system. Thus, we selected primary $CD14^+$ monocytes, which give rise to monocyte-derived dendritic cells (moDCs) *in vivo* under inflammatory conditions [28] and T lymphocytes that are activated by these cells upon encounter with their cognate peptide-MHC complex. In this study, we show that human *SLFNs* are regulated during the differentiation of monocytes to moDCs and are inducible in these cells mainly via type I interferon signaling. Furthermore, we report moderate downregulation of several *SLFN* family members during the activation of primary human T cells.

2. Materials and methods

2.1. Media, reagents and chemicals

Cells were maintained in RPMI 1640, supplemented with 2 mM L-glutamine, (both Gibco Ltd., Paisley, Scotland), 100 U/mL penicillin, 100 μg/mL streptomycin (PAA Laboratories, Austria) and 10% FCS (Gibco, Paisley, Scotland). Recombinant human GM-CSF and IL-4 were kindly provided by Novo Nordisk A/S (Bagsværd, Denmark). IFN-α2b was obtained from PBL Biomedical Laboratories (Piscataway, NJ). Lipopolysaccharide (LPS) from *E. coli* 0127:B8 and peptidoglycan (PGN) derived from *S. aureus* were purchased from Sigma-Aldrich (St. Louis, MO). Human rhinovirus serotype 14 (HRV14) was prepared as described [29]. CD3 (clone OKT3) and CD28 (clone 15E8) antibodies were from Janssen-Cilag (Vienna, Austria) and Caltag Laboratories (Burlingame, CA), respectively.

2.2. Primary cell isolation

Buffy coats from healthy donors were obtained from either the University Clinic for Blood Group Serology and Transfusion Medicine, Medical University of Vienna or the Austrian Red Cross (both, Vienna, Austria). Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized buffy coats via standard density gradient centrifugation using Ficoll-Paque™ Plus (GE Healthcare, Chalfont St. Giles, UK). T cells and monocytes were purified from PBMCs using the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously [30]. Briefly, T cells (total $CD3^+$ cells) were obtained via depletion of CD11b, CD14, CD16, CD19, CD33, and MHC class II-positive cells. Enrichment of monocytes was achieved via positive selection using biotinylated CD14 mAb [30].

2.3. Generation and stimulation of moDCs

Generation of moDCs was performed by culturing purified monocytes for 6 days with a combination of GM-CSF (50 ng/ml) and IL-4 (35 ng/ml). On day 6 of differentiation, moDCs were stimulated using 1 $TCID_{50}$/cell of HRV14, 100 U/ml IFN-α2b, 1 μg/ml LPS or 10 μg/ml PGN.

2.4. T CELl proliferation

Purified T lymphocytes were activated at 2×10^5 cells/well in MAXISORP Nunc-Immuno plates (Thermo Scientific, Waltham, MA) using plate-bound anti-CD3 or a combination of anti-CD3 and anti-CD28 in the presence or absence of 100 U/ml IFN-α2b. Antibodies were coated overnight at 4 °C, using a concentration of 2 μg/ml for each antibody. T cell proliferation was monitored via [methyl-3H] thymidine (PerkinElmer/New England Nuclear Corporation, Wellesley, MA) incorporation. On day 3 of activation, cells were pulsed with 0.05 mCi/well of [methyl-3H] thymidine 18 h prior harvesting. Detection was performed using a microplate scintillation counter (Topcount; Packard, Meriden, CT) as counts per minute (CPM). All assays were performed in triplicates.

2.5. Quantitative real-time PCR (qPCR)

$1–2 \times 10^6$ cells per sample were lysed in peqGOLD TriFast (Peqlab, Erlangen, Germany). Subsequently, total RNA was isolated via chloroform extraction according to the manufacturer's instructions. Reverse transcription of mRNA was performed using RevertAid H Minus Reverse Transcriptase (Thermo Scientific) and oligo $(dT)_{18}$ primers. cDNA was stored at −20 °C until use. qPCR was performed using SYBR Green qPCR master mix (Quanta Biosciences, Gaithersburg, MD) on a CFX96 Real-Time PCR detection system (Bio-Rad, Hercules, CA). For amplification, a standard program was applied (10 min at 95 °C, 40 cycles of each 15 s at 95 °C, 15 s at 60 °C and 45 s at 72 °C). Primers used in this study are depicted in Table 1. Primer sequences for *CD3E* and *HPRT* have been described before [7,31]. All primers were synthesized by Sigma-Aldrich (Steinheim, DE).

2.6. Data analysis

Data analysis for qPCR was performed using Bio-Rad CFX Manager Software (Bio-Rad, Hercules, CA). Amplification specificity was determined via melting curve analysis and/or agarose gel electrophoresis. Amplicons yielding Ct values of 31 or higher were defined to be undetectable (UD). Relative expression was calculated in Microsoft Office Excel (Microsoft, Redmond, WA) using the $2^{-\Delta\Delta Ct}$ method [32]. Data was plotted and statistically analyzed using GraphPad Prism software (La Jolla, CA). Unpaired, two-tailed Student's *t*-test was performed, considering p-values < 0.05 as statistically significant.

Table 1
Primers used in this study.

Target mRNA	Forward primer 5′-3′	Reverse primer 5′-3′
CD3E [31]	TGAGGGCAAGAGTGTGTGAG	TCCTTGTTTTGTCCCCTTTG
GAPDH	CGACCACTTTGTCAAGCTCA	AGGGGAGATTCAGTGTGGTG
HPRT [7]	TCAGGCAGTATAATCCAAAGATGGT	AGTCTGGCTTATATCCAACACTTCG
KLF2	CTACACCAAGAGTTCGCATCTG	AGTTGCAGTGGTAGGGCTTC
MxA	ACCTGATGGCCTATCACCAG	TTCAGGAGCCAGCTGTAGGT
SLFN5	AATTGCCCACAAGAGAATGG	AGCGTTTCTGCTGCTCTTTC
SLFN11	CCTCCCCTTAGCAGACCAGT	TTCCCCGAAAGAAAGGTTG
SLFN12	CATTACCTGCTCCCCACAGT	GCCCTTTCTGACAGAGTCCA
SLFN12L	TTGACCGAGAAGGAATGGAT	GCAGAAGGTTTTTGGAGCAC
SLFN13	GACGCAGATCCAGAGTTTCC	AAATGTCCTGGTGGAACTGG
SLFN14	TCAGTCAGCTCCTCCCAGTT	CAAGGATGTATCAGGGTCTTCA

3. Results

3.1. Basal expression levels of SLFNs in primary human monocytes, moDCs and T cells

Since little is known about the expression of SLFNs in primary human immune cells, we analyzed basal mRNA expression levels of the six human *SLFN* family members in unstimulated monocytes, moDCs and T lymphocytes. While *SLFN5* and *SLFN11* showed the most robust expression across all three cell types, *SLFN14* was only detectable at low levels in T cells, and was undetectable in monocytes and moDCs (Fig. 1). *SLFN5*, *SLFN12L* and *SLFN13* expression was highest in T cells, whereas *SLFN11* were more prominently expressed in monocytes and moDCs, although these differences did not reach statistical significance due to high inter-donor variability for *SLFN11* expression especially in monocytes (Fig. 1). Basal levels of *SLFN12L* and *SLFN13* expression were relatively low in monocytes, but appeared to be upregulated during differentiation into moDCs. Conversely, the levels of *SLFN12* mRNA were decreased in moDCs vs. monocytes (Fig. 1). To determine the kinetics of this regulation, we analyzed the expression of *SLFN* mRNA in monocytes stimulated with IL-4/GM-CSF over a six days time course. In line with our previous results, *SLFN5* and *SLFN11* expression was only slightly altered during moDC differentiation (Fig. 2). *SLFN12* expression was markedly reduced, starting from day one of differentiation and was maintained at this level during the entire observation period (Fig. 2). Conversely, expression of *SLFN12L* and *SLFN13* mRNA was induced during differentiation, with induction starting at day 2 and day 4, respectively (Fig. 2).

3.2. SLFNs are induced in moDCs upon autocrine type I interferon signaling

SLFN genes have been investigated in the context of viral infection before [17,19]. DCs are sentinel cells, that are among the first cells to sense viral infection at anatomical interfaces with the external environment [33]. Thus, we hypothesized, that viral infection of moDCs would induce transcriptional activation of *SLFNs*. We chose for our studies HRV14, which is a human-specific pathogen, that is able to enter moDCs, but does not replicate in these cells [34]. We inoculated moDC cultures with HRV14 and analyzed the mRNA levels of all known human *SLFN* genes in untreated cells and at 4 and 24 h (hours) after the inoculation with the virus. Most *SLFNs* displayed induction kinetics similar to the classical interferon-stimulated gene (ISG) *MxA* [35], showing only slight induction after 4 h and a marked increase after 24 h (Fig. 3A), indicating that their induction is mainly dependent on autocrine type I interferon signaling. To further explore this concept, we treated moDCs with IFN-α over the same time course. However, unlike infection with HRV14, direct addition of this type I interferon induced strong induction of *SLFNs* already at the early time point and was found to be decreased by 24 h (Fig. 3B). Again, *SLFN* gene expression closely mirrored the expression pattern of *MxA*, although the strength of the induction is not comparable to the latter in terms of fold induction (Fig. 3B).

We then investigated the effect of the classical pathogen associated molecular patterns LPS and PGN on *SLFN* gene expression, which are potent and poor inducers of IFN-β, respectively [36–38]. As expected, TLR4 stimulation of moDCs via LPS strongly induced *MxA* transcription, which was maximal after 4 h and already declined at the 24 h time point (Fig. 4A). Interestingly, this pattern of induction was not shared by all *SLFNs*, since *SLFN12L* and *SLFN13* mRNAs were found to be further upregulated at 24 h, while *SLFN12* transcripts appeared to stabilize at the levels observed after 4 h (Fig. 4A).

Conversely, addition of PGN to cultured moDCs only modestly enhanced transcription of the *MxA* gene, which returned to basal levels by 24 h (Fig. 4B). Most *SLFNs* shared this relatively weak transcriptional induction, whereas *SLFN12* was found to be consistently downregulated at both of the two time points observed (Fig. 4B).

3.3. SLFN genes are downregulated during T cell activation

Murine and rat *Slfns* are regulated during thymocyte development and T cell activation [1,3,39]. The exit of T lymphocytes out of a quiescent state during activation is an active process, that not only requires expression of growth-promoting factors, but also suppression of quiescence-enforcing genes [40]. Hypothesizing, that *SLFNs* might be involved in the exit or maintenance of human T cell quiescence, we activated primary T lymphocytes with plate-bound anti-CD3 or a combination of CD3 and CD28 antibodies and analyzed *SLFN* gene expression via qPCR. We could detect expression of all human *SLFNs* in isolated T cells, albeit *SLFN14* transcripts were found to be expressed at low levels and could not be reproducibly quantified upon culture of the cells (Fig. 1 and data not shown). Most *SLFN* genes were downregulated after activation, independent of the presence of costimulation (Fig. 5A). While *SLFN5* appeared to be gradually downregulated during the 24 h time course, *SLFN12* as well as *SLFN12L* were downregulated by roughly 50% already 6 h after activation and were maintained at these levels for the entire observation period (Fig. 5A). *SLFN11* and *SLFN13* expression was not consistently altered during human T cell activation. Comparing the regulation of *SLFNs* to the classical T cell quiescence factor *KLF2*[41,42], we found that activation mediated down-regulation of *SLFNs* was less pronounced, since *KLF2* mRNA levels were reduced by 80% already 6 h after activation and were further reduced by 24 h post stimulation (Fig. 5A)

Since type I interferon signals strongly induced *SLFN* gene expression in moDCs, we hypothesized that these signals would also revert activation-induced downregulation in T cells. Indeed, we found that addition of exogenous IFN-α induced transcript

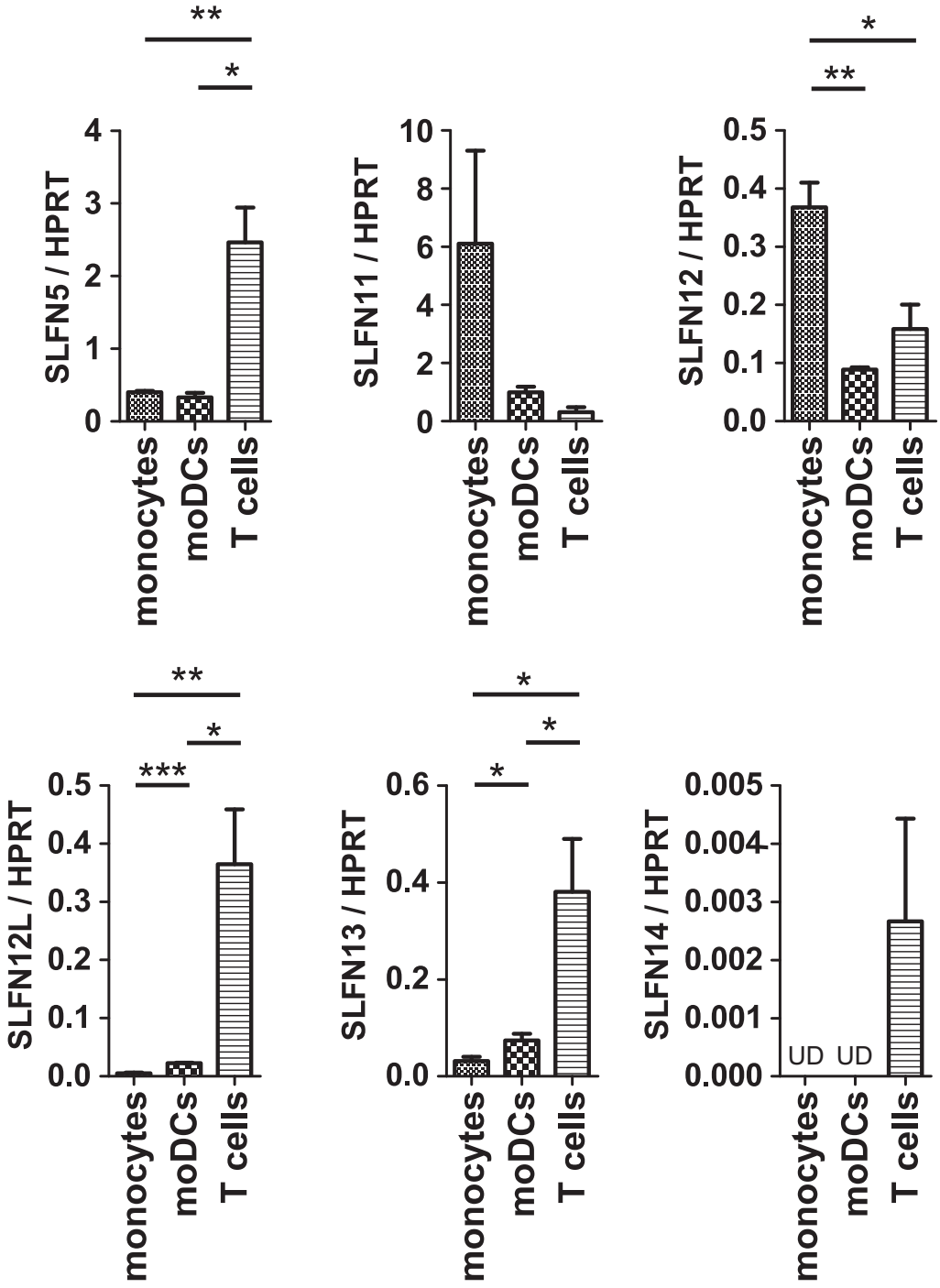

Fig. 1. *Basal SLFN gene expression in human monocytes, moDCs and T cells.* Levels of the indicated SLFN mRNAs were quantified via qPCR in isolated CD14$^+$ monocytes ($n=4$), moDCs on day 6 of differentiation ($n=3$) and CD3$^+$ T cells ($n=3$). Data are expressed relative to the reference gene *HPRT*. UD, undetectable. Mean ± SEM are given. $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ (unpaired *t*-test).

levels of most *SLFNs* to or above those of unstimulated cells (Fig. 5B) indicating a dominance of type I interferon signaling over T cell activating stimuli at least in this setting. Interestingly, the expression of *SLFN11* and *SLFN13* was not much altered by IFN-α in activated T cells suggesting different induction thresholds in these cells (Fig. 5B). However, SLFN gene induction did not correlate with reduced T cell growth, since we found no differences in the proliferation of T cells activated in the presence or absence of IFN-α (Fig. 5C).

4. Discussion

Since their discovery in the late 1990s, Slfn/SLFN family members have been investigated for their involvement in fundamental cellular processes including growth regulation, differentiation and control of viral replication. While Slfns have been studied also in non-hematopoietic cells, several studies have highlighted their role in cells of the immune system [1,3,7,18,27,39]. However, most of this work has been performed with rodent cells or human tumor cell lines, while little is still known about the role of SLFNs in primary human cells.

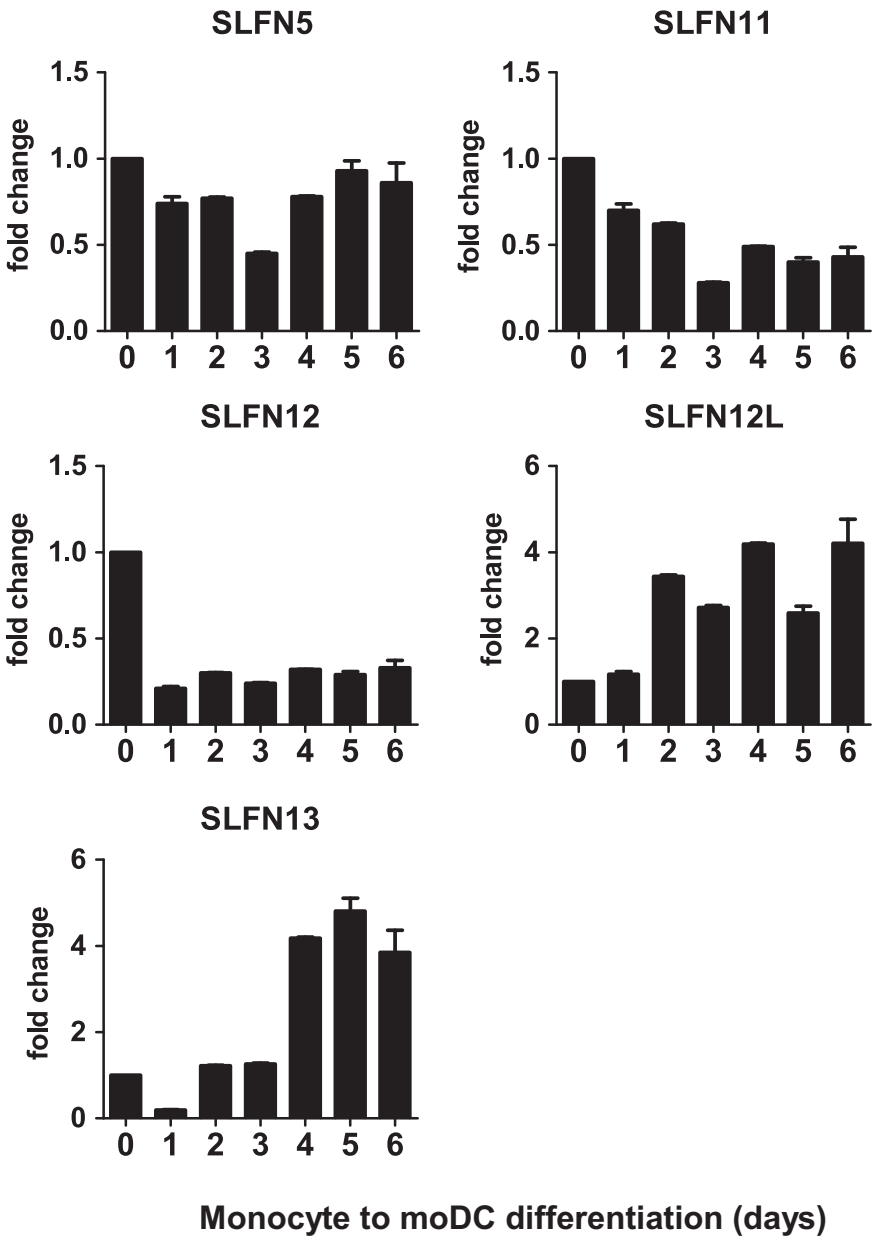

Fig. 2. *Regulation of SLFNs during monocyte to dendritic cell differentiation*. Monocytes were differentiated to moDCs using 50 ng/ml GM-CSF and 35 ng/ml IL-4. Gene expression was monitored over a 6 days time course. Cells were harvested at the indicated days and mRNA expression of *SLFN* genes was determined via qPCR. Expression values were calculated relative to *HPRT* as a reference gene and normalized to unstimulated cells. Data is representative of two different donors. Error bars indicate mean ± SD of technical replicates. Note, that for some bars SD is too small to be discernible.

Differentiation of monocytes to moDCs via IL-4 and GM-CSF leads to the differential regulation of several hundred genes including transcription factors, cytokines and cell surface receptors [43], indicating that a multitude of factors is involved in the functional specialization of these cells. Thus, downregulation of *SLFN12* as well as upregulation of *SLFN12L* and *SLFN13* point to different requirements of these molecules in moDC function. Murine *Slfn4* was previously reported as a modulator of myelopoiesis, which is downregulated upon CSF-1 mediated bone marrow-derived macrophage differentiation [7]. Since human SLFN12 shares the highest sequence identity with murine Slfn4, our data suggest similar roles for this molecule in the differentiation of human myeloid cells, which is consistent with previous observations [7].

Dendritic cells are key players in both, innate and adaptive immune responses against viruses [44]. However, many viruses have developed mechanisms to counteract DC-mediated immunity [44–48]. Similarly, infection of moDCs by HRV14 has been demonstrated to lead to impaired moDC function resulting in reduced T cell stimulatory capacity [49,50]. HRV14 ssRNA has been shown to induce IFN-α secretion in these cells, while failing to induce NF-κB-dependent expression of pro-inflammatory cytokines [34]. In line with these findings, our data indicate, that *SLFN* gene expression induced upon HRV14 infection of moDCs is mainly attributable to type I interferon production. Sensing of HRV14 ssRNA presumably occurs through the cytoplasmic pattern recognition receptor RIG-I [34], since TLR7 is not expressed in moDCs [51,52], whereas TLR8 stimulation is rather associated with the induction of a pro-inflammatory cytokine milieu via MyD88-dependent signaling [53]. PGN-mediated stimulation of TLR2 further underlines the strong dependency of SLFN gene expression on

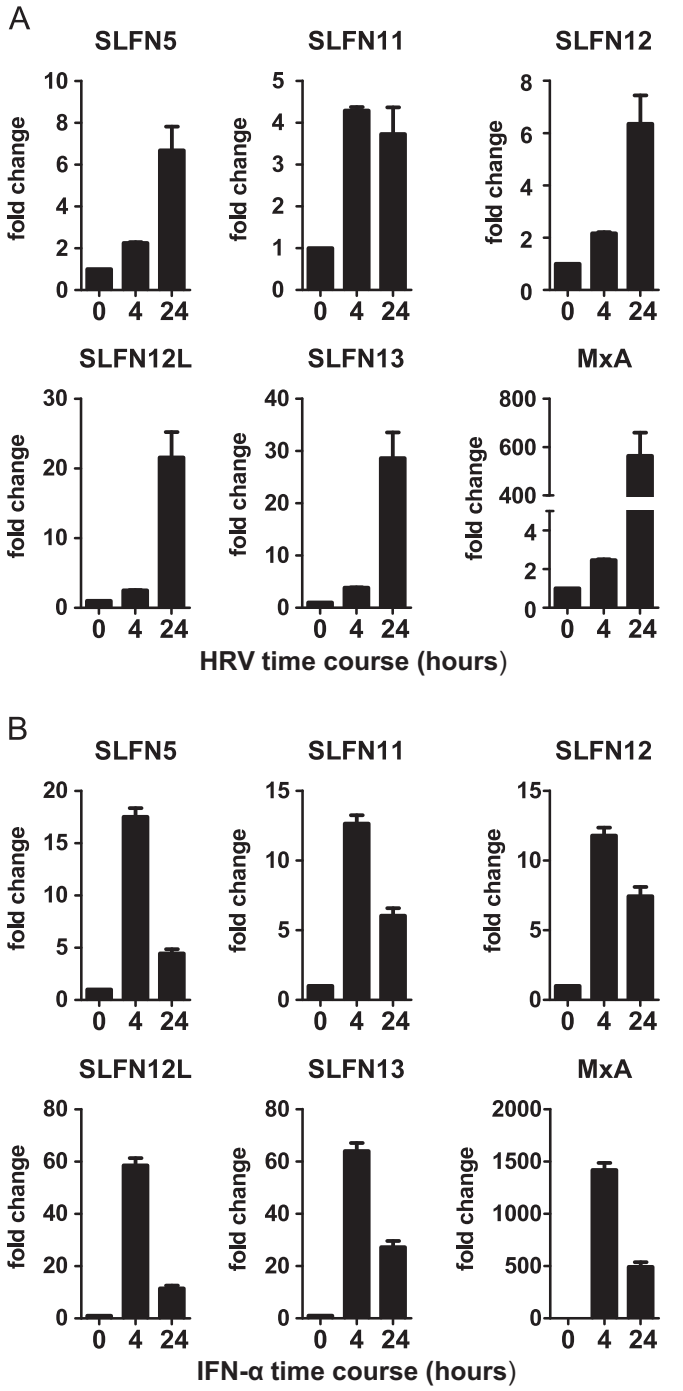

Fig. 3. *SLFN family members are induced by HRV14 and IFN-α in moDCs.* moDCs were stimulated over a 24 h time course with (A) 1 $TCID_{50}$/cell of HRV14 and (B) 100 U/ml of IFN-α. Cells were lysed at the given time points and total RNA was isolated, reverse transcribed and mRNA expression of the indicated genes was determined via qPCR. Expression values were calculated relative to *GAPDH* as a reference gene and normalized to unstimulated cells. Data is representative of two independent experiments or three different donors. Error bars indicate mean ± SD of technical replicates. In some cases, SD is too small to be visible.

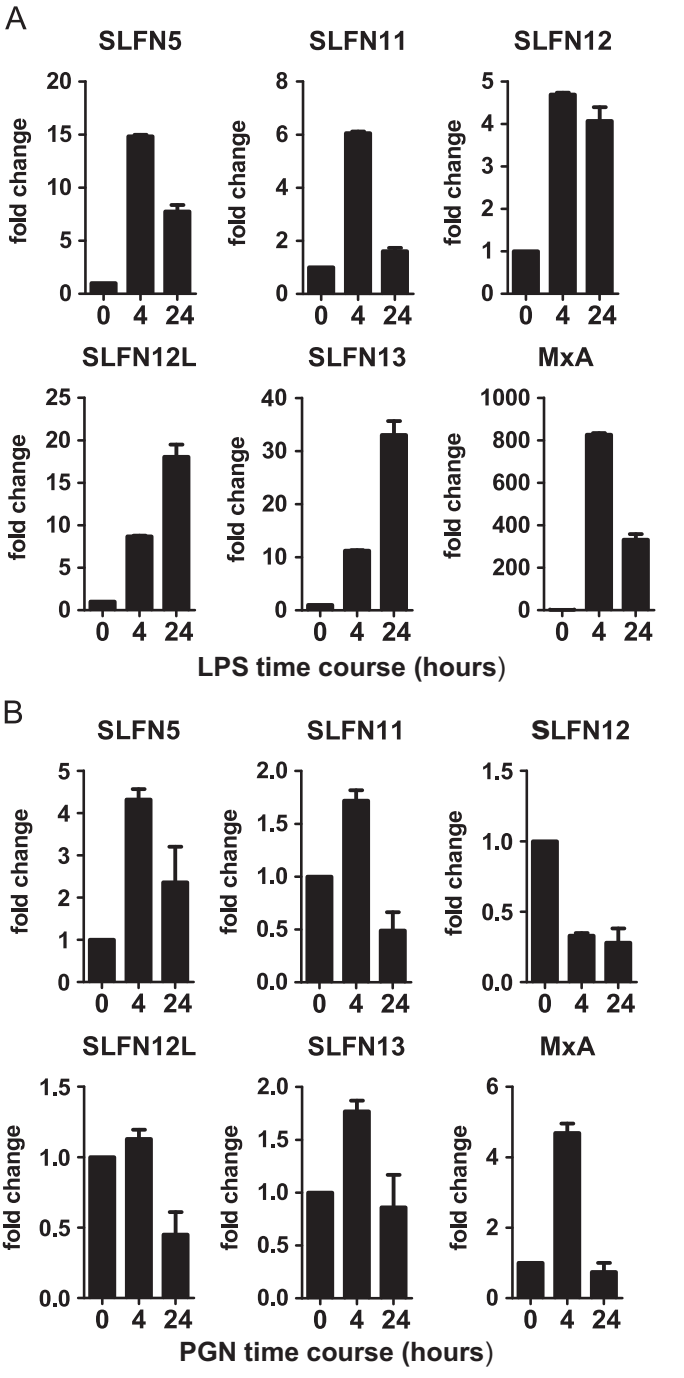

Fig. 4. *SLFN family members are induced by LPS but only poorly by PGN stimulation.* moDCs were stimulated over a 24 h time course with (A) 1 μg/ml LPS and (B) 10 μg/ml PGN. Cells were harvested at the given time points and mRNA expression of the indicated genes was determined via qPCR. Expression values were calculated relative to *GAPDH* as a reference gene and normalized to unstimulated cells. Data is representative of two independent experiments or three different donors. Error bars indicate mean ± SD of technical replicates. Note, that for some bars SD is too small to be discernible.

type I interferon, since it poorly stimulated both *SLFN* and *MxA* expression.

The induction of ISGs via type I interferons depends on the presence of interferon-stimulated response elements (ISREs) in the promoter region of the ISG, enabling transcriptional activation through binding of the ISGF3 transcription factor, which is a complex of phospho-STAT1/STAT2 heterodimers and IRF-9 [35]. Bioinformatic analysis via MatInspector [54] revealed less than 2 canonical ISRE sites for most human *SLFN* genes, which is considerably lower than the 6 sites predicted for *MxA*. While this numerical difference could explain the lower inducibility of *SLFNs* compared to *MxA* via type I interferon, it is unclear, if these canonical ISREs are both, necessary and sufficient to induce *SLFN* gene transcription, or whether other elements are involved in interferon-dependent and -independent regulation of *SLFN* genes. Further studies would be needed to clarify these open questions.

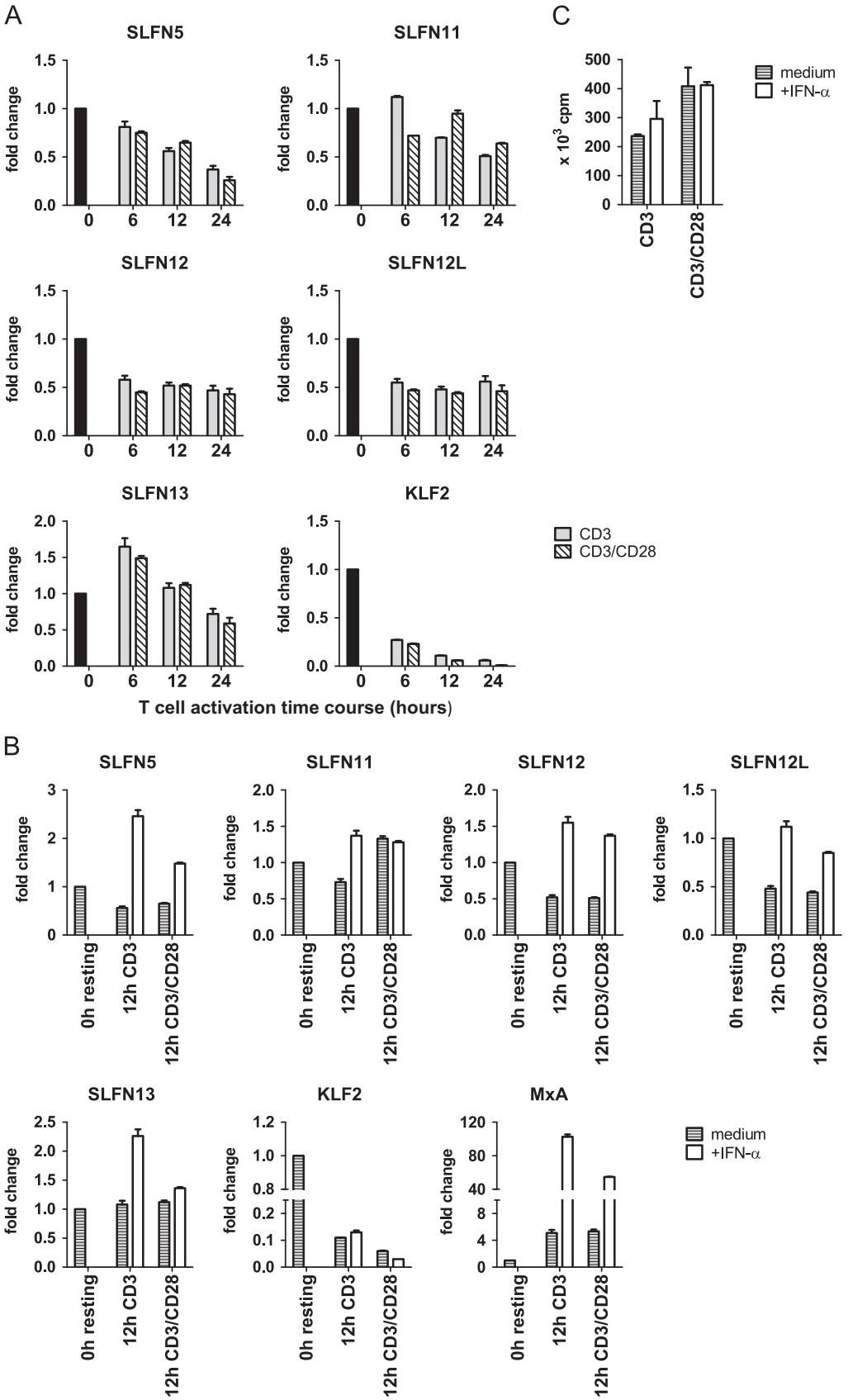

Fig. 5. *Regulation of SLFNs during the activation of primary human T cells.* T cells were activated with plate bound anti-CD3 (coated at 2 μg/ml) or a combination of anti-CD3 and anti-CD28 (2 μg/ml each) for (A) 6 h, 12 h and 24 h. (B) Cells were activated as above for 12 h in the presence or absence of 100 U/ml of IFN-α. *SLFN* gene expression was determined via qPCR. Data is displayed relative to *CD3E* and is normalized to unstimulated controls. (C) T cells were activated in the presence or absence of IFN-α as indicated above. T cell proliferation was measured on day 3 via [methyl-3H] thymidine incorporation. Error bars indicate mean ± SD of technical replicates. Note, that in some cases SD is too small to be discernible.

SLFN11 has been recently described as a potent inhibitor of HIV-1 by interfering with viral protein synthesis [17]. Our data indicate a typical type I interferon-dependent expression pattern for this factor in human immune cells since mRNA levels were inducible by HRV, LPS and IFN-α, but were rather insensitive to PGN, IL-4/GM-CSF-mediated moDC differentiation or T cell activating stimuli. However, we found, that basal *SLFN11* mRNA levels were highest among all SLFNs in monocytes and moDCs (Fig. 1) suggesting functional importance. High SLFN11 levels in $CD4^+$ T lymphocytes have been described to correlate with the control of viral replication in HIV-infected individuals [20]. Although $CD4^+$ T cells are seen as the main viral reservoir during HIV infection, latency may also be established in cells of the monocyte/macrophage lineage [55]. Thus, high SLFN11 expression in monocytes and macrophages might be of similar importance in controlling HIV infection.

Maturation of moDCs via LPS revealed differential mRNA induction kinetics for *SLFN5*, *SLFN11* and *SLFN12* compared to *SLFN12L* and *SLFN13*, indicating that transcriptional regulation of *SLFNs*, although sharing many features with the expression pattern of classical ISGs, might not be solely dependent on type I interferons. In fact, the further increase of *SLFN12L* and *SLFN13* mRNA levels observed at the late time point of LPS stimulation of moDCs, is reminiscent of the concept of "inflammation suppressor genes" [56], which suggests upregulation of negative-feedback regulators following the initial rapid induction and decline of pro-inflammatory mediators. It is tempting to speculate, that SLFN12L and SLFN13 might interfere with a potentially pro-inflammatory role of other SLFNs, by direct or indirect inhibition of their function. Similar expression kinetics for murine *Slfns* have been reported in LPS-stimulated bone marrow-derived macrophages [7], although our data do not indicate any correlation of this differential expression pattern with the presence or absence of the helicase domain, since it is present in genes with early (SLFN5, SLFN11) as well as with late (SLFN13) expression peaks.

Maturation of moDCs via the TLR4 stimulus LPS or the TLR2 stimuli PGN/Pam3cys not only differs in its potential to induce type I interferon but also in regard to T cell polarization via differential IL-12p70 expression [57,58]. As a result, moDCs, which are matured via TLR4 or TLR2 signals, promote Th1 and Th2 cytokine secretion, respectively, in co-cultured T cells [57]. This divergent functional outcome is mirrored by the differential regulation of *SLFN12* in LPS and PGN stimulated moDCs, suggesting that high *SLFN12* levels could be a marker for moDCs with high Th1 polarizing capacity, while low levels would be indicative of the capability to induce Th2 cells. If so, SLFN12 could be functionally involved in polarizing moDC stimulatory capacity.

Murine *Slfn1*, *Slfn2*, *Slfn5* and *Slfn8* [1,3] have been described to be downregulated during T cell activation, while *Slfn3*, *Slfn4* and *Slfn9* [1,3,39] are induced by T cell activating stimuli. However, direct effects on the growth of thymocytes and peripheral T cells, respectively, have only been described for *Slfn1* and *Slfn8* transgenic mice, which both display reduced thymic cellularity [1,3]. Our own results obtained in human T cells suggest that most human *SLFNs* are downregulated within 24 hours after activation indicating that high expression levels might interfere with the process of T cell activation or proliferation. Type I interferons have been associated with the inhibition of cell growth and the induction of apoptosis in various cancer cell lines [59–62]. Their precise role on T cell activation is less clear and might be context-dependent, since type I interferons have been reported to promote [63] as well as inhibit T cell expansion [64] or differentiation [65]. In our setting, the observed upregulation of SLFNs during IFN-α stimulation of activated T cells could not be directly associated with the inhibition of cell growth, since we did not observe growth-inhibition of interferon-stimulated human T cells in vitro. Nevertheless, high basal expression levels of SLFN5 in resting T cells followed by downregulation after activation-similar to the T cell quiescence factor KLF2 [41,42]-suggest that SLFN5 might be part of a complex array of factors involved in actively maintaining T cell quiescence [40,66]. However, overexpression and RNAi approaches would be necessary to clarify, whether SLFN5 or other members of this protein family may indeed possess growth regulatory functions in human T cells.

In summary, we describe here for the first time the expression and regulation of SLFN family members in primary human monocytes, moDCs and T lymphocytes. Our data indicates functional roles of SLFN11, SLFN12L and SLFN13 in monocytes/moDCs, whereas SLFN5 could be a regulator of human T cell activation. Rapid downregulation of SLFN12 by various activation or differentiation stimuli in different cell types suggests a generally inhibitory role of this factor. Thus, we envisage that our study will provide incentives for further work aiming to characterize the functional role of SLFNs in primary human immune cells.

Conflicts of interest

The authors declare that there are no conflicts of interests.

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