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Dendritic Cells Activated by IFN-γ/STAT1 Express IL-31 Receptor and Release Proinflammatory Mediators upon IL-31 Treatment

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IL-31 is a T cell-derived cytokine that signals via a heterodimeric receptor composed of IL-31Rα and oncostatin M receptor β. Although several studies have aimed to investigate IL-31-mediated effects, the biological functions of this cytokine are currently not well understood. IL-31 expression correlates with the expression of IL-4 and IL-13 and is associated with atopic dermatitis in humans, indicating that IL-31 is involved in Th2-mediated skin inflammation. Because dendritic cells are the main activators of Th cell responses, we posed the question of whether dendritic cells express the IL-31R complex and govern immune responses triggered by IL-31. In the current study, we report that primary human CD1c+ as well as monocyte-derived dendritic cells significantly upregulate the IL-31Rα receptor chain upon stimulation with IFN-γ. EMSAs, chromatin immunoprecipitation assays, and small interfering RNA-based silencing assays revealed that STAT1 is the main transcription factor involved in IFN-γ–dependent IL-31Rα expression. Subsequent IL-31 stimulation resulted in a dose-dependent release of proinflammatory mediators, including TNF-α, IL-6, CXCL8, CCL2, CCL5, and CCL22. Because these cytokines are crucially involved in skin inflammation, we hypothesize that IL-31–specific activation of dendritic cells may be part of a positive feedback loop driving the progression of inflammatory skin diseases. The Journal of Immunology, 2012, 188: 000–000.

Dendritic cells (DCs) are highly specialized, professional APCs equipped with a plethora of intracellular and extracellular pattern recognition receptors. Upon activation by various stimuli, DCs undergo a maturation process that endows them with distinct DC pathways downstream of the receptor demonstrated activation of p38MAPK, ERK1/2, and JNK1/2, and phosphorylation of the STAT family members STAT1, STAT3, and STAT5 (1, 5, 7, 9, 10).

At present, little is known about the biological consequences of IL-31 signaling. Transgenic mice overexpressing IL-31 develop a severe skin phenotype closely resembling the skin from patients with atopic dermatitis (AD) (1, 11). Analysis of skin biopsies from patients with different types of inflammatory skin diseases showed that IL-31 is overexpressed predominantly in pruritic forms of skin inflammation (12). Moreover, leukocytes from patients with AD or allergic contact dermatitis (ACD) show significantly enhanced IL-31 expression that is associated with elevated expression of IL-4 and IL-13 as compared with healthy volunteers (3, 12). On the basis of these findings, IL-31 has recently begun to be looked at as a possible mediator in the pathogenesis of Th2 cytokine-mediated inflammatory skin diseases like AD and ACD. In contrast, other published studies support a role for IL-31–induced signaling in limiting the severity of Th2-mediated inflammation in the lung and gut (13, 14). For example, IL-31RA–deficient mice injected with Schistosoma mansoni eggs developed a more severe pulmonary Th2 inflammation than did wild-type (WT) animals. The results of a similar study likewise suggest a regulatory role for IL-31/IL-31R interactions in the intestine following infection with the gastrointestinal helminth Trichuris muris (14). Taken together, these data indicate that IL-31/IL-31R interactions may play an important role in limiting Th2-mediated inflammatory responses in the lung and the intestine, whereas, in the skin, IL-31 action is positively correlated with inflammation.

Abbreviations used in this article: ACD, allergic contact dermatitis; AD, atopic dermatitis; ChIP, chromatin immunoprecipitation; DC, dendritic cell; GAS, γ-activation sequence; IL-31Rα, IL-31Rα; moDC, monocyte-derived DC; OSMRB, oncostatin M receptor β; qRT-PCR, quantitative real-time PCR; SEB, staphylococcal enterotoxin B; siRNA, small interfering RNA; TARC, thymus- and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; WT, wild-type.

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functions, including capture and presentation of Ag, migration, costimulation, and the release of T cell-polarizing cytokines (15).

Because DCs are the main orchestrators of T cell responses, we sought to address a potential contribution of DCs to the complex picture of IL-31–mediated effects. As none of the studies analyzing IL-31R expression has reported on its expression on DCs, IL-31–mediated effects on DCs have remained speculative to date. In this study, we show that IL-31RA surface expression on DCs is enhanced in response to IFN-γ. Moreover, we demonstrate that the transcription factor STAT1 is crucial for IFN-γ–dependent IL-31RA expression. Once DCs express the cognate receptor, they become responsive to IL-31 and secrete substantial amounts of proinflammatory cytokines and chemokines upon IL-31 stimulation. Because the mediators released by IL-31–stimulated DCs are crucially involved in skin inflammation, we speculate that activation of DCs by IL-31 is part of a positive feedback loop driving the progression of inflammatory skin diseases.

Materials and Methods

All studies involving human cells were conducted in accordance with the guidelines of the World Medical Association’s Declaration of Helsinki.

Generation of human monocyte-derived DCs and isolation of primary human blood DCs

Monocyte-derived DCs (moDCs) were generated according to standard protocols. Briefly, adherent monocytes were cultured in DC medium (RPMI 1640 [PAI, Pasching, Austria], 10% FCS [PAI], 2 mM t-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-ME [all Life Technologies Laboratories, Grand Island, NY]) supplemented with 50 ng/ml GM-CSF and 50 ng/ml IL-4 (gift of Novartis, Vienna, Austria) for 6 d. Primary CD1c+ DCs were isolated from CD19-depleted PBMCs using a BDCA1+ kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer’s instructions. Isolated cells were phenotyped by FACS analysis and cultured in RPMI 1640 (PAI), 10% FCS (PAI), 2 mM t-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all Life Technologies Laboratories).

DC stimulation

For IL-31R expression studies, 1 × 10^5 CD1c+ DCs/ml and 1.5 × 10^5 moDCs/ml, respectively, were plated in DC medium. Cells were stimulated with 10 ng/ml IFN-γ, 50 ng/ml GM-CSF, and 50 ng/ml IL-4. Briefly, 10 ng/ml IFN-γ were added to DCs. IL-31–mediated effects on DCs have remained speculative to date. In this study, we show that IL-31RA surface expression on DCs is enhanced in response to IFN-γ. Moreover, we demonstrate that the transcription factor STAT1 is crucial for IFN-γ–dependent IL-31RA expression. Once DCs express the cognate receptor, they become responsive to IL-31 and secrete substantial amounts of proinflammatory cytokines and chemokines upon IL-31 stimulation. Because the mediators released by IL-31–stimulated DCs are crucially involved in skin inflammation, we speculate that activation of DCs by IL-31 is part of a positive feedback loop driving the progression of inflammatory skin diseases.

STAT1 small interfering RNA knockdown

To avoid unintended side effects (e.g., cellular stress responses like the PKR/IFN response) in the knockdown experiments, we used a chemically modified oligonucleotide employing Steall RNAi technology (Invitrogen) with the following sequence: sense, 5′-GGAGUUGAAGAACUCCAGA-ACTCAU-3′; and antisense, 5′-AUGAAGUCUAGGAUUGCUAA-UCC-3′. Cells were transfected with Lipofectamine RNAiMax reagent (Invitrogen), according to the manufacturer’s guidelines. Briefly, 5 × 10^5 cells were plated in antibiotics-free DC medium and transfected with 100 pmol/well small interfering RNA (siRNA). For moDC experiments, cells were transfected on day 5 of the differentiation period. Transfection efficiency was routinely >90%, as assessed by flow cytometry with fluorescent control RNA oligonucleotides (BlockIT; Invitrogen). Knockdown efficacy was analyzed 4 d posttransfection by Western blotting.

Preparation of nuclear extracts and EMSAs

Nuclear extracts from IFN-γ–induced or noninduced moDCs were prepared according to the method of Andrews and Fuller (17). Generation of double-stranded oligonucleotide probes and EMSAs was carried out, as described previously (18–20). For competition assays, nonlabeled oligonucleotide was added in 50-fold molar excess to the binding reaction 30 min prior to the radiolabeled probe. Supershifting of bands was achieved by adding 500 ng/μl Ab (anti-STAT1, anti-STAT3, and anti-STAT5), all purchased from Santa Cruz Biotechnology, Heidelberg, Germany) to the binding reaction.

Sequences of the oligonucleotides are as follows (STAT consensus nucleotides underlined): site 1 sense, 5′-GTTATAAACAATTTTTT-TTCTCGGAATTAATATTAT-3′; and antisense, 5′-GCTAATTATTATA-ATTCCAGAGAAAGAAAATGTATAAT-3′; site 1 mut sense, 5′-GTTA-ATAAACATTTTCTTTTCTGGAATTAATATTAT-3′; and antisense, 5′-GCTAATTATTATA-ATTCCAGAGAAAGAAAATGTATAAT-3′; site 2 sense, 5′-GGCCACCTTCTGGAATTTCTTCTTGTTTGAAAT-3′; and antisense, 5′-GAAATTTCAATAGAGAAAGAGAAATTG-3′; site 3 sense, 5′-GGCTACACATTTTCTTTTATTT-CCTGAGGAAAT-3′; and antisense, 5′-GAGTAAACCTTCAAT-GAATAAAGAATGTGTA-3′.

Chromatin immunoprecipitation assay

moDCs were generated as described above and cultured in DC medium at a density of 1.5 × 10^5 cells/ml. In total, 5 × 10^5 moDCs were stimulated with 10 ng/ml IFN-γ for 4 h. Chromatin immunoprecipitation (ChIP) was performed using the LowCell# ChIP kit (Diagenode, Liége, Belgium), according to the manufacturer’s instructions. Chromatin was fragmented by 15 min of sonication in a Bioruptor sonicator (Diagenode). Precipitation reactions contained chromatin from 3 × 10^5 cells and 3 μg Abs (anti-STAT1, Santa Cruz Biotechnology, or rabbit negative control IgG, Diagenode). Precipitation of the STAT1-binding motif was analyzed by means of qRT-PCR using iQ SYBR Green Supermix (Bio-Rad) and the primers listed as follows: IL-31RA STAT1 site 1 sense, 5′-GCTGAGCCACA-
Treated with IFN-γ, performed time kinetic- and dose-response studies. moDCs were isolated from human blood samples. Because previous studies reported that IL-31RA expression is induced by various stimuli, including IFN-γ, LPS, and SEB (1, 21–23), DCs were either left untreated or stimulated with IFN-γ, LPS, or SEB. Additionally, 1 aliquot of cells was treated with IFN-γ and LPS simultaneously, because this combination was shown to be required to induce mRNA expression of both receptor chains (IL-31RA and OSMRB) in monocytes (1). Based on the finding that IL-31 is preferentially released by activated Th2 cells (1), we further treated DCs with IL-4, the Th2-associated cytokine TSLP (24), and anti-CD40, which mimics DC activation by T cells via CD40. Because previous studies reported that IL-31RA expression is induced by various stimuli, including IFN-γ, LPS, and SEB (1, 21–23), DCs were either left untreated or stimulated with IFN-γ, LPS, or SEB. Additionally, 1 aliquot of cells was treated with IFN-γ and LPS simultaneously, because this combination was shown to be required to induce mRNA expression of both receptor chains (IL-31RA and OSMRB) in monocytes (1). Based on the finding that IL-31 is preferentially released by activated Th2 cells (1), we further treated DCs with IL-4, the Th2-associated cytokine TSLP (24), and anti-CD40, which mimics DC activation by T cells via CD40. Nevertheless, moDCs were treated with 10 ng/ml IFN-γ for time periods ranging from 2 to 72 h. As shown in Fig. 2C (upper panel), IL-31RA mRNA peaks at 24 h, whereas IL-31RA surface expression reaches a maximum after 72 h (Fig. 2C, lower panel). Together, a maximum of IL-31RA expression was observed after treatment with ~10 ng/ml IFN-γ for 72 h. 

IFN-γ enhances mRNA expression of short and long IL-31RA isoforms

It was reported that at least five different isoforms (isoform 1, NM_139017; isoform 2, NM_001242636; isoform 3, NM_001242637; isoform 4, NM_001242638; and isoform 5, NM_001242639) of IL-31RA exist; they are identical throughout the transmembrane domain, but clearly differ from each other with respect to the intracellular domain (1, 6). Screening of several melanoma, glioblastoma, and tumor cell lines revealed that mainly two receptor isoforms are naturally expressed, as follows: the long receptor containing 745 residues and a short isoform of 560 aa (26). The shorter isoforms (isoforms 3 and 4) lack three tyrosine residues known to be critical for STAT activation (7). To analyze the expression of short and long isoforms, CD1c+ DCs from nine different human donors were cultured in the absence or presence of IFN-γ for 6 h. qRT-PCR using isoform-specific primers and primers detecting all isoforms revealed that both short and long IL-31RA isoforms were upregulated by IFN-γ. Yet, the effect of IFN-γ on the expression of short isoforms seems to be more pronounced (Fig. 3).

IFN-γ induces binding of STAT1 to specific motifs within the IL-31RA promoter

IFN-γ signals primarily through the JAK/STAT pathway. Activation of the IFN-γ receptor complex activates JAK1, which in turn phosphorylates critical tyrosine residues within the IFNGR1 chain, thereby providing docking sites mainly for the transcription factor STAT1, but also for STAT3 and STAT5 (27–29). Phosphorylated STAT1s then form homodimers and enter the nucleus, where they bind γ-activation sequences (GAS) and initiate or suppress transcription of IFN-γ-responsive genes (27–32).
To analyze STAT activation in human DCs, moDCs were generated and stimulated with 10 ng/ml IFN-γ for the indicated times, and activation of STAT1, STAT3, and STAT5 was analyzed by Western blotting. Interestingly, not only STAT1, but also STAT3 and STAT5 were phosphorylated 30 min after IFN-γ stimulation, and the activation persisted for up to 16 h (Fig. 4).

To investigate whether STATs contribute to IL-31RA expression by binding to the gene locus, we analyzed a 2050-bp fragment of the IL-31RA promoter for the presence of putative GAS motifs characterized by the sequence TTC(N)3GAA (33–35). In silico analysis revealed the presence of three putative motifs located within positions −2047/−2041 (site 1), −21794/−21788 (site 2), and −1036/−1030 (site 3) relative to the translational start site (6) (Fig. 5A). Interaction of the respective STATs with these putative GAS motifs within the IL-31RA promoter was assessed by EMSA. Incubation of nuclear extracts from IFN-γ–induced moDCs with the oligonucleotide harboring site 1 resulted in the formation of an IFN-γ–induced nucleoprotein complex (Fig. 5B). In contrast, no IFN-γ–induced complex was observed using probes containing the more proximal GAS motifs (site 2 or 3). Addition of a 50-fold molar excess of unlabeled WT oligonucleotide (competitor) resulted in a loss of the IFN-γ–induced complex formation, whereas addition of a mutated oligonucleotide competitor, in which a mutation from TTC(N)3GAA to TAT(N)3GAA was introduced into the GAS consensus motif of site 1, did not block formation of the IFN-γ–induced complex (Fig. 5C). To determine which of the IFN-γ–activated STATs forms a complex with site 1, specific Abs directed against STAT1, STAT3, or STAT5 were added prior to the addition of radiolabeled probes. Anti-STAT1 Abs specifically reduced the formation of the IFN-γ–induced nucleoprotein complex and led to the formation of a supershifted complex (Fig. 5C), whereas neither anti-STAT3 nor anti-STAT5 Abs had an effect on the IFN-γ–induced complex. However, addition of anti-STAT3 Abs diminished a second nucleoprotein complex that appears independent of IFN-γ stimulation. These results suggest that IFN-γ exclusively induces the interaction of STAT1 and specific DNA motifs within the human IL-31RA promoter. To evaluate the capacity of STAT1 to bind the GAS consensus motif of site 1 in living cells, we performed ChIP with STAT1-specific Abs. Enrichment of specifically precipitated DNA containing the motif of site 1 was analyzed by qRT-PCR using specific primer pairs. Precipitation of chromatin with normal rabbit IgG serum resulted in equal amplification of IFN-γ and uninduced samples. In contrast, immunoprecipitation from IFN-γ–induced cells with anti-STAT1 resulted in enrichment of the DNA containing the motif of site 1.
GAS-binding motif of site 1 (Fig. 5D). This indicates that IFN-γ stimulates the direct interaction of STAT1 with a specific GAS motif in the IL-31RA promoter.

**IFN-γ–induced IL-31RA expression is decreased in STAT1–deficient DCs**

To investigate the functional role of STAT1 in IL-31RA expression, RNA interference experiments were carried out in moDCs. Four days after transfection, the silencing efficiency of the STAT1 siRNA was determined. As shown by Western blot analysis, STAT1 siRNA dramatically reduced pSTAT1 protein levels in IFN-γ–treated cells compared with cells transfected with control oligonucleotide, whereas ERK levels remained nearly unaffected (Fig. 6A). qRT-PCR analysis 24 h after IFN-γ stimulation revealed a reduction in IFN-γ–dependent IL-31RA mRNA expression in STAT1–silenced cells compared with cells transfected with control oligonucleotide (Fig. 6B). Specificity of the STAT1 siRNA was controlled by transfecting STAT1 siRNA into HEK293 cells. Four days after transfection, the expression of STAT1, STAT3, and STAT5 was analyzed by Western blotting (Fig. 6C). Taken together, these data clearly show that the transcription factor STAT1 is critically involved in the IFN-γ–induced expression of IL-31RA.

**IL-31 stimulation of primary CD1c+ DCs expressing the IL-31R complex results in the release of proinflammatory cytokines**

Recent literature provides evidence that IL-31 is a potent inducer of proinflammatory mediators in various cell types, including epithelial cells, eosinophils, colonic subepithelial myofibroblasts, PBMCs, and macrophages (10, 22, 36). Whereas human bronchial epithelial cells and freshly isolated human eosinophils respond to IL-31 by secreting epidermal growth factor, vascular endothelial growth factor, MCP-1 (MCP-1/CCL2), IL-6, CXCL8/IL-8 (10), PBMCs and macrophages instead respond to IL-31 and coactivate Toll-like receptors 2 and 4 in living cells, chromatin fragments from uninduced and IFN-γ–treated moDCs were precipitated using anti-STAT1 or an equal amount of control IgG. The data represent the amplification results of one of three independent experiments.

**Discussion**

DCs are pivotal in bridging the innate and adaptive immune responses. They are the most potent type of APCs and are uniquely capable of promoting the differentiation of naïve Th cells into different types of effector Th cells, thereby initiating specific immune responses (37). DCs express a large repertoire of innate
immune receptors and sentinel the periphery, where they can recognize and respond to various microbial components. Upon exposure to microbial stimuli, DCs undergo phenotypic and functional changes, becoming immunogenic APCs and priming appropriate T cell responses (15, 38). Besides microbial components, specific chemokines and cytokines can contribute to DC activation (24, 39, 40).

To our knowledge, the present study provides the first evidence that, under certain conditions, DCs express the heterodimeric IL-31R, which allows for IL-31–dependent DC activation. Interestingly, IFN-γ acts as potent inducer of IL-31R expression, whereas LPS and SEB, two stimuli known to effectively prime DCs to induce Th cell responses (38, 41), do not enhance IL-31R expression. In contrast, macrophages and monocytes upregulate IL-31RA expression in response to SEB, and show enhanced IL-31RA mRNA expression upon IFN-γ stimulation (1, 21, 23).

Although one group was able to show enhanced IL-31RA protein levels in IFN-γ–stimulated monocytes by Western blotting (23), little alterations in IL-31RA surface expression could be observed by flow cytometry (our unpublished observations) (22).

To date, signaling pathways that directly influence the expression of IL-31RA expression have not been determined. Based on the predominant role of IFN-γ in stimulating IL-31R expression in human DCs, we investigated molecular mechanisms underlying this process. It has become apparent that other signal transduction proteins like MAPKs (42), PI3K (43), and NF-κB (44) act in cooperation with or parallel to the canonical JAK-STAT pathway. However, the majority of the pleiotropic effects of IFN-γ, in particular the regulation of gene expression in response to IFN-γ, are mediated by the canonical JAK-STAT pathway (45). Although IFN-γ primarily induces activation of STAT1, several studies reported that STAT3 and STAT5 are also activated by IFN-γ (27, 28, 46). Analysis of STAT proteins in IFN-γ–stimulated DCs showed phosphorylation of STAT1, STAT3, and STAT5. However, supershift assays revealed that only STAT1 was able to bind to one of the putative GAS motifs in the proximal promoter. A trend toward increased binding of STAT1 in IFN-γ–stimulated DCs was further observed in ChIP assays. This indicates the direct association of STAT1 and a specific GAS motif within the IL-31RA locus. Notably, supershift assays employing STAT3-specific Abs indicate that STAT3 may be part of a second nucleoprotein complex that occurs in an IFN-γ–independent way. Notwithstanding this possibility, the pivotal role of STAT1 in IFN-γ–induced IL-31RA expression was substantiated by the results of the STAT1-silencing experiments. Thus, to our knowledge, this study is the first to describe a signaling pathway that is directly involved in the regulation of IL-31RA.

To date, a close relationship between IL-31 expression and skin inflammation has been demonstrated (1–3, 12). In addition, increased IL-31 levels were found in the sera of patients with allergic asthma (3). These findings indicate that IL-31 may contribute to the development of Th2-related diseases. Although Th2-related diseases such as AD are characterized by the initial activation of Th2 cytokines, Th1 cytokines such as IFN-γ and IL-12 are considered to be important players during the later (chronic) phase of the diseases (47, 48). Thus, we assume that IFN-γ-dependent IL-31R expression may play a role in the manifestation of Th2-
related inflammatory disorders such as AD. However, this assumption is in contrast to the observation that parasitic infection of IL-31RA−/− mice were not due to the lack of IL-31R-mediated signaling, but rather to the physical absence of IL-31RA. Because the functional IL-31R is composed of IL-31RA and OSMRB, absence of IL-31RA would increase the relative amount of available OSMRB to form a heterodimeric receptor with gp130, and therefore give rise to increased OSM signaling. Thus, the authors speculated that an increase in OSM signaling, as observed in IL-31RA−deficient mice, may account for the enhanced Th2 inflammation in IL-31RA−deficient mice (13, 14). In line with these findings, our present study provides evidence that IL-31 acts as proinflammatory rather than as an anti-inflammatory cytokine. The observed secretion of proinflammatory cytokines and chemokines is in good accordance with IL-31−induced cytokine-secretion profiles described for bronchial epithelial cells (10), colonic myofibroblasts (36), and PBMCs (22). In CD1c+ DCs, IL-31 concentrations needed to induce the release of specific cytokines and chemokines seem to be relatively high. These findings are in agreement with other reports, showing that similar or even higher concentrations of IL-31 are required to induce the release of inflammatory mediators (10, 22, 36, 50) and may be explained by the relatively low expression of OSMRB on the surface of moDCs and CD1c+ DCs.

DC-derived cytokines and chemokines deliver important signals that promote T cell polarization and determine the type of T effector cell. Although IL-4, which is unique for the induction of Th2 responses, is not released by DCs, a number of additional DC-derived factors are known to contribute to Th2 inflammation, among them the chemokines CCL2/MCP-1, CCL17/TARC, and CCL22/MDC. The observation that IL-31−treated DCs show increased CCL2/MCP-1 expression, but barely detectable amounts of IL-12, is of particular interest because recent data from Del Conro et al. (51) demonstrate that CCL2/MCP-1 inhibits TLR-induced IL-12 production in moDCs and, as a consequence, decreases IFN-γ production in DC−T cell cocultures. These findings indicate that IL-31−treated DCs may promote the development of Th2− rather than Th1−mediated immune responses. In addition, the release of MDC/CCL22 further indicates a role for IL-31−stimulated DCs in Th2−mediated inflammation. Elevated levels of MDC/CCL22 and TARC/CCL17 have been detected in skin lesions from AD patients. Additionally, both chemokines were reported to promote trafficking of Th2 cells (52). However, TARC/CCL17 was not produced by IL-31−stimulated DCs. CCL5/RANTES is a chemokine that attracts T cells to inflammatory sites and plays a predominant role in allergic and inflammatory skin diseases such as AD, ACD, or psoriasis (53–56). Two classical proinflammatory cytokines, TNF-α and CXCL8/IL-8, are released by IL-31−activated DCs, as well. TNF-α was described as one of the AD-associated cytokines that is mainly expressed in late phases of the disease (57), whereas CXCL8/IL-8 is specifically upregulated in psoriatic skin and is responsible for the typical intraepidermal collection of neutrophils (56, 58, 59).

The release of IL-31−induced cytokines and chemokines is initiated by binding of IL-31 to IL-31RA, which forms a heterodimeric receptor complex with OSMRB (1, 5–7, 60). IL-31RA, the signaling chain that first binds IL-31 (60), is expressed in several isoforms (1, 6, 7, 26). The longer isoforms contain three tyrosine residues known to be critical for STAT activation (7). As a consequence, IL-31 treatment of cells expressing the long isoforms results in the induction of STAT-mediated signaling, whereas stimulation of cells expressing the short isoforms fails to activate STAT signaling. Notably, we showed upregulation of short and long isoforms in IFN-γ−treated DCs, suggesting that STATs are only partially involved in the activation of DCs. This is in line with our observation that IL-31 signaling in DCs results in the release of several cytokines and chemokines, which were shown to be regulated by STAT-independent signaling mechanisms (61–67). These findings indicate that, in addition to a STAT-dependent mechanism, other signaling pathways may be involved in the activation of DCs by IL-31.

In conclusion, this study demonstrates that IFN-γ/STAT1 signaling renders DCs responsive to the Th2 cytokine IL-31 by inducing IL-31R expression. Subsequent IL-31 stimulation primes DCs to release mediators that are involved in inflammatory skin diseases. An important role of Th2 as well as Th1 cytokines was shown for the immunopathogenesis of AD, one of the most common inflammatory skin disorders. Whereas the classical Th2 cytokines IL-4 and IL-13 are implicated in the initial phase of AD, the Th1 cytokine IFN-γ is instead associated with disease chronicity (47, 48). The finding that IL-31 is associated with AD (3, 12) and our observation showing the predominant role of IFN-γ in the expression of IL-31RA indicate that IL-31−treated DCs may contribute to the progression of IL-31 in the late phase of the disease. Although distinct effects of IL-31−treated DCs on T cell activation and T cell differentiation remain elusive and need to be addressed in further studies, our findings suggest a positive regulatory feedback loop that might enhance inflammation in the chronic phase of pruritic skin diseases, including AD.

Disclosures

The authors have no financial conflicts of interest.

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