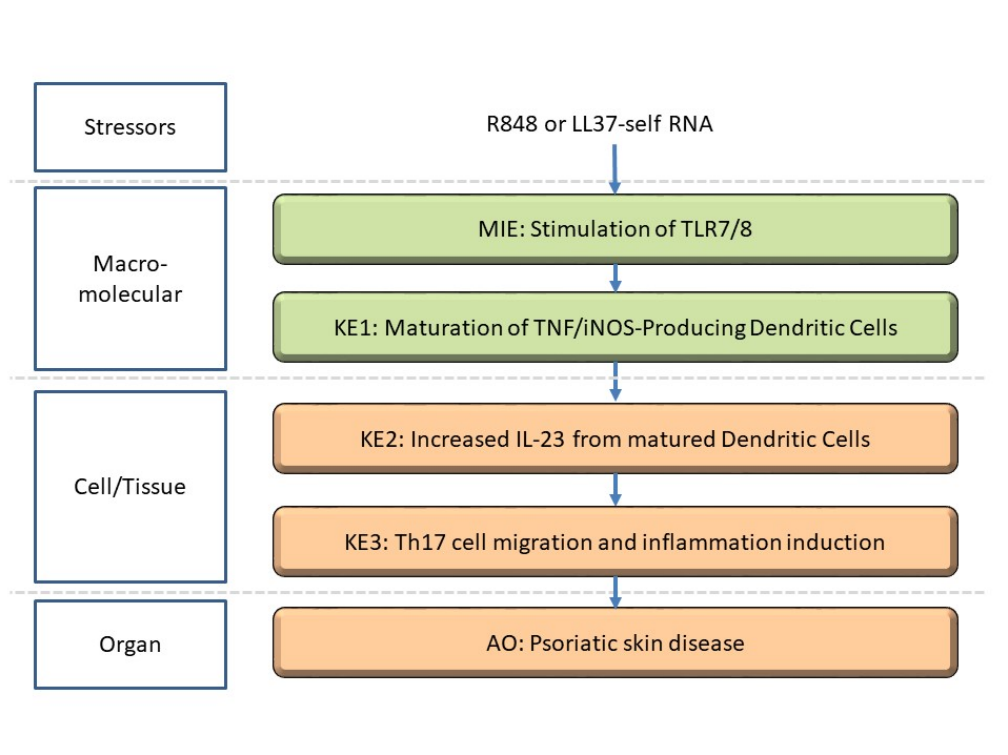
AOP ID and Title:

AOP 313: Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease
Short Title: Skin disease by stimulation of TLR7/8

Graphical Representation



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Abstract

Toll-like receptor (TLR) 7 and TLR8 are pattern recognition receptors that are known to activate antiviral reaction of immune system, hyperactivation of which can lead to psoriatic skin disease when hyperactivation of them occurred. The relationship between TLR7/8 and immune functions is well understood, and antiviral compound that work by stimulating TLR7/8 have been developed. TLR7/8 agonists such as imidazoquinolin compounds stimulate these TLRs through the formation of homodimer. This signal activates the IL-23/IL-17 axis, which leads to psoriasis and other related skin diseases.

Activation of the IL-23 / IL-17 axis and causes abnormal proliferation and inflammation of the epidermis, which is a pathological condition of psoriasis. This AOP shows an association between TLR7 / 8 stimulation and psoriatic skin disease.

TLR7-mediated signaling in plasmacytoid dendric cells (pDC) is mediated in a MyD88-dependent fashion, which initiates an IRF7, IRAK1, TRAF6, TRAF3, and IKKα-mediated response, secreting vast amounts of IFN type 1. Similarly, upon engagement of ligands in endosomes, TLR8 initiate the MyD88-dependent pathway culminating in synthesis and release of proinflammatory mediators, such as TNF-α via NF-κB activation. IFN-α and TNF-α cooperatively mature myeloid dendritic cells. TLR7/8 agonist stimulates a specific population of inflammatory dermal dendritic cells referred as TNF and inducible nitric oxide synthase–expressing DCs (Tip-DCs) to produce IL-23 after maturation by enhanced transcriptional activity.

IL-23R is mainly expressed in Th17 cells. In chronic psoriasis, the cytokines IL-12 and IL-23 produced by resident DC are the main causes. Not only does the expression of IL-23 increases in the skin tissue of the lesion, Th17 cells also increase.

Mature Th17 cells are activated by IL-23 stimulation. Signaling through IL-23 produces cytokines IL-17 and IL-22 that mediate the psoriasis response and promote neutrophil migration into the epidermis, epidermal cell proliferation, and similar responses, which lead to the development of a psoriasis rash. In mice, psoriasis-like hyperplasia is induced by the application of IL-23 but does not occur in IL-17A and IL-22 KO mice, so IL-17A and IL-22 play an important role downstream of IL-23.

IL-17 receptor form heterodimers, and IL-17RA / IL-17RC appears in a variety of cells, including fibroblasts and epidermal cells. IL-17RE / IL-17RA expressed in epidermal cells and IL-17C binding are also important in the pathology of psoriasis. Immunohistochemically, IL-17A is expressed only in cells of the dermal papilla layer, while IL-17C is widely expressed in cells such as hyperproliferative overexpressed keratinocytes, leukocytes, and vascular endothelial cells. IL-17C produces keratinocytes by bacterial stimulation and further stimulates keratinocytes to induce the production of various cytokines and chemokines. Keratinocytes are known to be self-activated by IL-17C.

IL-17 and IL-22 secreted from Th17 act on keratinocytes, causing abnormalities in keratinocytes through the secretion of inflammatory cytokines, chemokines, growth factors, and antimicrobial peptides, and thereby exacerbating the skin symptoms of psoriasis.

The creation of this AOP began with an examination of important event relationships brought about by TLR7 / 8 activity due to environmental or genetic factors and resulting in abnormal differentiation of keratinocytes, which leads to thickening of the epidermis and its resultant autoimmune skin disease, psoriasis

Background

Psoriasis is an chronic autoimmune disease characterized by chronic epithelial inflammatory disease induced by environmental factors such as infection, stress, smoking or alcohol consumption as well as by genetic factors. The onset of psoriasis has been reported to be triggered by drugs and chemical substances use, including beta-blockers, chloroquine, lithium, ACE inhibitors, indomethacin, terbinafine, and interferon alpha. Diagnosis is based on the type and distribution of the lesions.

Psoriasis occurs when abnormal differentiation (keratosis) of keratinocytes leads to thickening of the epidermis. Patients often exhibit an erythema with a clear border and epidermal hyperplasia, stratum corneum hyperplasia, heterocytosis in the stratum corneum, mixed skin moist cells of neutrophilic granulocytes and T cells in the epidermis. Dendritic cells (DC) and macrophages are associated with silver-white plaque. Neutrophilic effusion (Munro microabscesses) are observed in the epidermis, and CD8+ T cells (Tc17) increase the expression of angiogenesis related genes.

The main therapeutic agents are mild topical treatments such as emollients, salicylic acid, coal tar preparations, anthralin, corticosteroids, vitamin D3 derivatives, retinoids, calcineurin inhibitors or tazarotene. UV therapy is also used for moderate or severe psoriasis. Widespread psoriasis is treated with systemic therapies such as immunomodulators methotrexate, cyclosporin, retinoids and other immunosuppressants used alone or in combination.

Although there are stressors that are well known to induce psoriasis-like skin inflammation in mice, this AOP is based primarily on an understanding of stimulation caused by imiquimod, resiquimod or LL37-selfRNA complexes, for which a significant body of scientific literature has been published.

As a test model for psoriasis, an Autoimmune skin disease, mouse tests that induce skin inflammation like psoriasis are frequently conducted using the imidazoquinoline derivative imiquimod. This AOP is primarily based on an understanding of stimuli caused by imiquimod, resiquimod, or LL37-selfRNA complexes.

Imiquimod is derived from imidazoquinoline and is often used to create mouse models. It is our hope that this AOP will contribute to greater knowledge about the development of psoriatic skin diseases that start from stimulation of TLR as well as the development of new treatment targets for psoriasis.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	1706	Stimulation, TLR7/8	Stimulation of TLR7/8
2	KE	1822	Maturation of TNF/iNOS-Producing Dendritic Cells	Maturation, TNF/iNOS-Producing Dendritic Cells
3	KE	1707	Increase, IL-23 from matured dendritic cells	Increase of IL-23

Sequence	Type	Event ID	Title	Short name
4	KE	1708	Th17 cell migration and inflammation induction	Th17 cell migration and inflammation induction
5	AO	1709	Psoriatic skin disease	Skin disease

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Stimulation, TLR7/8	adjacent	Increase, IL-23 from matured dendritic cells	High	High
Increase, IL-23 from matured dendritic cells	adjacent	Th17 cell migration and inflammation induction	High	High
Th17 cell migration and inflammation induction	adjacent	Psoriatic skin disease	High	High

Stressors

Name	Evidence
Imiquimod	High
Resiquimod	High

Overall Assessment of the AOP

TLR7/8 is stimulated when imidazoquinolin compounds or stimilar agonists from homodimers TLR7-mediated signaling in plasmacytoid dendritic cells (pDC) is mediated in a MyD88-dependent fashion, which initiates an IRF7, IRAK1, TRAF6, TRAF3, and IKKα-mediated response,thereby secreting large amounts of IFN-α. Similarly, the engagement of ligands in endosomescauses TLR8 initiate the MyD88-dependent pathway, culminating in synthesis and release of TNF-a and other proinflammatory mediators, via NF-κB activation.

IFN-α and TNF-α cooperatively mature myeloid dendritic cells. TLR7/8 agonist stimulates a specific population of inflammatory dermal dendritic cells referred as Tip-DCs to produce IL-23 after maturation by enhanced transcriptional activity.

Naive T cells differentiate into Naive Th17 by both IL-6 and TGF-β cells that express the transcription factors ROR-γt, ROR-α, and STAT3. These naive Th17 cells are self-activated by IL-21 in an autocrine manner and mature into Th17 cells which express IL-23 receptor on cell surface. Mature Th17 cells are activated by IL-23 stimulation. IL-23-mediated signal transduction produces cytokines IL-17.

IL-17 mediates the psoriasis response, promoting such activities as neutrophil migration to the epidermis,and proliferation of epidermal cells, which leads to the outbreak of psoriasis rash. Thus, psoriatic skin is induced mainly by overproduction of IL-17, which leads to a variety of adverse effects. We have identified a number of key events (KEs) along this pathway and created an AOP for stimulation of TLR7/8 that leads to psoriatic skin disease based on these key event relationships(KERs).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	Moderate	NCBI

Sex Applicability

Sex	Evidence
Mixed	High

The proposed AOP for psoriasis-like skin thickening resulting from abnormal differentiation of keratinocytes, starting with Toll-like receptor (TLR) 7/8 activity, is independent of life stage, gender, or age (Lowes et al. 2007). The pathogenesis of psoriasis, an

autoimmune disease, is genetically predisposed (3), but the autoantigen that causes psoriasis has not been identified (Zaba et al. 2008). Other causes of psoriasis are caused by external and internal triggers such as mild trauma, sunburn, infection, systemic drugs, and stress (Hansel et al. 2011). Stimulation of TLR7 / 8 releases INF-α and TNF-α in large amounts to produce IL-23, and Th17 cells mature by the stimulation to produce IL-17 and IL-22. In psoriasis skin formation, cytokines such as TNF-α, IL-23, and IL-17 work continuously. Since TNF-α inhibitors significantly suppressed IL-17A and IL-23p19 expression in psoriatic eruptions (Leonardi et al. 2012), by suppressing self-activation of Tip-DC by TNF-α, It can be seen that IL-23 and IL-17A production was suppressed. Anti-IL-17 and anti-IL-17RA antibodies suppress IL-17A and IL-17C, which are highly expressed in psoriatic eruptions. In particular, anti-IL-17RA antibody has been shown to normalize the expression of keratinocyte-related genes and IL-17C production two weeks after administration, followed by normalization of IL-17A production from leukocytes.

In mice, subcutaneous administration of IL-23 induced psoriatic eruption and IL-17A expression (K. A. et al. 2013), and IL-17C transgenic mice overexpressing IL-17C in keratinocytes showed psoriatic eruption. As shown in (8), the reaction of psoriasis-like eruption occurs in mice due to the chain of stimulation to T cells and epidermal cells starting from TLR.

Essentiality of the Key Events

Stressor, MIE and later events:MyD88 knock out(KO) mice

TLR7 (TLR7 / 8 in human) recognizes the imidazoquinoline derivative, binds to the adapter molecule MyD88, activates IRAKs (IL-1 receptor associated kinases), interacts with TRAF6 (TNF receptor associated factor 6) and IKK (Activates the IκB kinase complex). It phosphorylates IκB, induces its degradation, and transfers the transcription factor NF-κB to the nucleus. This pathway is called MyD88-dependent pathway and is essential for the production of inflammatory cytokines such as TNF-α (Akira S, Takeda K .: Nat Rev Immunol. Jul; 4: 499-511, 2004). When pDC is stimulated with a TLR7 / 8 ligand, the transcription factor IRF7 constitutively expressing pDC and MyD88 associate directly. IRF7 activity does not occur when pDCs of MyD88 KO mice are stimulated with TLR7 / 8 ligand. IRF7 is also activated by binding to TRAF6, leading to IFN-α production, which requires the Myd88 / TRAF6 / IRF7 complex. (Satoshi U, Shizuo A: Virus 54; 2: 145-152,2004)

Imiquimod 5% cream was applied to the left flank of female SKH-1 hairless mice (25 g body weight). The IFN-α and TNF-α concentrations in the skin after 1 and 2 hours of application increased these concentrations compared to the untreated skin.

In C57BL / 6 mice (8-12 weeks old) sensitized with 0.5% dinitrofluorobenzene (DNFB) as an antigen, imiquimod 5% cream was applied to the auricle once a day for 3 days. The application of imiquimod 5% cream promoted edema of the ears of mice (promoted DTH) compared to the base cream group. Imiquimod activates antigen-specific T cells by topical application to the skin. (Beserna Cream Interview Form Mochida Pharmaceutical Co., Ltd.)

KE-1 and later event:IL-17, IL-22 KO mice

In mice, psoriasis-like hyperplasia is induced by the application of IL-23, but this effect does not occur in IL-17A and IL-22 KO mice. IL-17A deficient mice show little epidermal hyperplasia after intradermal administration of IL-23. WT mice treated with anti-IL-17A Ab did not show IL-23-induced epidermal hyperplasia. IL-17 KO mice treated with IL-23 do not induce TNF-α mRNA and do not cause epidermal thickening. IL-22 did not increase in IL-17-/-mice after IL-23 administration, and IL-17 clearly increased in IL-22-/-mice. In IL-17-/-, IL-22-/-and WT mice treated with IL-23, immunohistochemically CD3 + T cells, CD11c (dendritic cells), F4 / 80 (macrophages), Gr-1 (Neutrophils) were analyzed. There was no difference in F4 / 80 and Gr-1 + cells in IL-17A-/-compared to WT mice, and CD3 + T cells decreased, but there was no obvious difference in IL-22-/-mice .

These data suggest that cytokines alone are not sufficient to mediate IL-23-induced epidermal changes, and that IL-17 and IL-22 are downstream mediators of mouse skin IL-23-induced changes. Therefore, Th17 cytokines are required for the generation of IL-23-mediated skin lesions.

KE-2 and later events: Mouse psoriasis-like dermatitis model

When TPA (12-O-tetradecanoy1phorbol-13-acetate) on the dorsal skin of K14 / mIL-1F6 gene-modified mice overexpress mouse IL-1F6 (IL-36a) selectively under the keratin 14 promoter was applied, skin pathological findings specific to psoriasis were observed, such as epidermal hyperplasia, epidermal exfoliation and micro-abscess formation, and wet inflammatory cells in the dermis. Quantitative RT-PCR measures mRNA expression levels of inflammatory chemokines and cytokines in skin tissues, and includes inflammatory chemokines: CCL3, CCL4, CXCL10, CXCL1, and cytokines: IL-23, IL-12, IL-1β, etc. These expressions were elevated. (Kyowa Hakko Kirin Co., Ltd.)

References

Appendix 1

List of MIEs in this AOP

Event: 1706: Stimulation, TLR7/8

Short Name: Stimulation of TLR7/8

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:313 - Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

TLR7 and TLR8 are conserved among humans and mice (Gupta et al. 2016). In addition, these molecules are also conserved among humans and rhesus monkeys.

Alignment of amino acid residues between human toll-like receptor 7 (NP_057646.1) and murine toll-like receptor 7 (NP_573474.1, NP_001277684.1, NP_001277685.1, NP_001277686.1, NP_001277687.1, XP_006528776.1, XP_011246087.1 and XP_011246088.1) was 80.74-80.76% identification. Murine TLR7 proteins have 1050 or 1053 amino acids. Human and rhesus toll-like receptor 7 (NP_001123898.1) was 98.00% identification. Rhesus TLR7 protein has 1049 amino acid residues.

In addition, alignment of amino acid residues between human toll-like receptor 8 (NP_619542.1) and murine toll-like receptor 8 (NP_001300689.1) was 70.97% identification. Likewise, human and rhesus toll-like receptor 8 (NP_001123899.1) was 96.73% identification. Murine and rhesus TLR8 referred here have 1029 and 1039 amino acids residues, respectively.

Studies of DC subsets isolated from humans and mice have revealed that TLRs have distinct expression patterns. TLR7 is expressed in freshly isolated human pDCs, whereas TLR8 is expressed in $CD11c^+$ human myeloid DCs (mDCs). In some studies, TLR7 expression was detected on both pDCs and mDCs, whereas other reports showed that TLR7 was exclusively expressed in pDCs (Iwasaki and Medzhitov. 2004).

In mice, $CD4^+$ dendritic cells (DC), CD4/CD8 double negative DC and pDC express TLR7. All splenic DC subsets express TLR8. Moreover, mouse $CD8\alpha^+$ DCs lack TLR7 expression and fail to respond to TLR7 agonists. (Iwasaki and Medzhitov. 2004).

Key Event Description

Toll-like receptors (TLRs) are members of interleukin-1 (IL-1) receptor/TLR superfamily, as they share the intracellularToll-IL-1 receptor (TIR) domain with the IL-1 receptor.

Toll-like receptor (TLR) 7 and TLR8 is known to mediate the recognition of guanosine- and uridine-rich single-stranded RNA (ssRNA) derived from ssRNA viruses and synthetic antiviral imidazoquinoline components specifically that lead to activation of sequential signalling pathway (Akira et al. 2006, Blasius and Beutler. 2010). They also mediate the recognition of self RNA that is released from dead or dying cellsand activation of Myeloid differentiation primary response 88 (MyD88)-dependent signals can occur that leads to inflammation process as well as ssRNA derived from viruses.

TLR7 are exclusively expressed in plasmacytoid DCs (pDCs), which have the capacity to secrete vast amounts of type I interferon (IFN) in rapid response to viral infection (Gilliet et al. 2008, Reizis et al. 2011). TLR8 is express in various tissues, with its highest expression in monocytes. Myeloid DCs (mDCs) also express TLR8 in human (Iwasaki and Medzhitov. 2004). Thus, TLR8 ligands can directly activate mDCs via TLR8. TLR8 signalling activates mDCs to secrete TNF-α and IL-6 (Ganguly et al. 2009). TLR7 and TLR8 are localize in the endoplasmic reticulum of expressing cells (Lai et al. 2017).

Human TLR7 (hTLR7) and human TLR8 (hTLR8) contain 1049 and 1041 amino acid residues, respectively with molecular weight of 120.9 kDa and 119.8 kDa, respectively (Chuang and Ulvitch. 2000). The full-length hTLR7 protein includes a signal peptide of 26 amino acids (1–26 aa). The mature hTLR7 protein ectodomain, trans-membrane, and TIR domain are composite structure of 27–839, 840–860, and 889–1,036 amino acids, respectively (Gupta et al. 2016).

hTLR7 and hTLR8 form a subfamily of proteins that each contain an extracellular domain of >800 residues and share functional and structural features. hTLR7 and hTLR8 contains 27 and 26 leucine-rich repeats (LRRs), which is the largest number of LRRs among TLRs whose structures have been reported (Tanji et al. 2013).

As mentioned above, TLR7 and TLR8 are localize in the endoplasmic reticulum of expressing cells. They are deliver to the endosomes by interacting UNC93B1, which is a 12 membrane-spanning protein (Kawai and Akira. 2011, Itoh et al. 2011). After the trafficking, they initiate cellular responses upon their activation by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Lai et al. 2017).

Structural characterization was conducte with recombinant TLR7 from monkey (*Macaca mulatta*; 96.8% sequence identity with human TLR7) expressed in *Drosophila* S2 cells (Zhang et al. 2016). Rhesus TLR7 exists as a monomer in the absence of ligands. This TLR7 is activate by dimerization triggered by guanosine and uridine-containing ssRNA, which are degradation products of ssRNA, synergistically. Specifically, this TLR7 molecule has two ligand-binding sites. The first site conserved in TLR7 and TLR8 is use for small ligand-binding essential for its activation. The second site spatially distinct from that of TLR8 is use for ssRNA-binding that enhances the affinity of the first-site ligands. The first site preferentially recognize guanosine and the second site specifically bound to non-terminal uridine in ssRNA which have more than 3 bases. Rhesus TLR7 is also activate by dimerization induced by resiquimod alone, which is bound to only the first site (Zhang et al. 2016).

In contrast, hTLR8 exists as preformed dimer before ligand recognition. hTLR8 molecule has two ligand-binding sites as well as TLR7. The first site preferentially recognize uridine and second site recognize short-oligonucleotide. hTLR8 transforms into activated form upon binding of these two degradation products of ssRNA. hTLR8 is also activated by transformation induced by resiquimod alone, which is bound to only the first site (Tanji et al. 2015).

The key residues involved in TLR7 dimerization are LYS410, ASN503, SER504, GLY526, ASN527, SER530, THR532, ARG553, and TYR579 (Gupta et al. 2016).

Cellular signalling initiated by TLR7 activation with ssRNA in pDC is mediated in a Myeloid differentiation primary response 88 **(**MyD88)-dependent fashion, and activates NF-κB and IRF7, which results in induction of inflammatory cytokines and type I interferon, respectively (Kawai and Akira. 2011).

MyD88-dependent IRF7 activation in pDCs is mediate by activation of IRAK1, TRAF6, TRAF3, and IKKα and is facilitate by IFN-inducible Viperin expressed in the lipid body (Kawai and Akira. 2011).

IRF7, which is constitutively expresse by pDCs, binds MyD88 and forms a multiprotein signalling complex with IRAK4, TRAF6, TRAF3, IRAK1 and IKKα (Kawai and Akira. 2008). In this complex, IRF7 becomes phosphorylate by IRAK1 and/or IKKα, dissociates from the complex and translocates into the nucleus to induce transcription of type I IFN by binding to its promoter proximal region (Kawai and Akira. 2008, Génin et al. 2009).

Signalling initiated by TLR8 engagement with ssRNAs in endosomes is also mediated the MyD88-dependent pathway culminating in synthesis and release of proinflammatory mediators, such as TNF-α via NF-κB activation (Tanji et al. 2015).

How it is Measured or Detected

In general, quantification of TLR7/8 activation can be done by:

- Reporter gene assay
- ELISA

Measurement of transcriptional activation of human TLR and NF-κB-luciferase co-transfected cells

HEK293 cells were transiently co-transfected with human TLR7 and a NF-κB-luciferase reporter. The cells were incubated for 48 hours following transfection and then stimulated with various concentrations of resiquimod or imiquimod. Luciferase activity was measured 48h post-stimulation and the results are reported as fold-increase in luciferase production relative to medium control (Gibson et al. 2002). Likewise, resiquimod (0.001-10 µg/mL) induced NF-κB activation in HEK293 cells transfected with human TLR7 or human TLR8 and a NF-κB-luciferase reporter is detected in the same manner (Jurk et al. 2002).

Measuring of cytokine levels in supernatants

IFN-α in cell-free supernatants collected after stimulation of human PBMC and/or pDC-enriched cells by imidazoquinoline derivatives is detected by ELISA (Gibson et al. 2002).

TNF-α and IL-6 in cell-free supernatants collected after stimulation of mDCs by RNA-LL37 are measured by ELISA (Ganguly et al. 2009).

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List of Key Events in the AOP

Event: 1822: Maturation of TNF/iNOS-Producing Dendritic Cells

Short Name: Maturation, TNF/iNOS-Producing Dendritic Cells

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:313 - Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Domain of Applicability

Tip-DCs are also observed in mice. Murine Tip-DCs are defined as splenic $CD11c^+$, $CD11b^+$, $MHC\text{-}II^+$, $CD40^+$, and $CD86^+$ cells producing iNOS and TNF. CD11b expression is observed in murine Tip-DC, however it is lacking on human cells (Lowes et al. 2005).

Key Event Description

Monocytes are formed in the bone marrow and continuously enter the blood circulation, where they constitute 10% of the total

leukocyte population in humans (Sprangers et al. 2016). They are recruited to inflammatory sites and differentiate into immature dendritic cells in situ (Tang-Huau and Segura. 2019). These immature dendritic cells, known as monocyte-derived dendritic cells (mo-DC) are distinguished from conventional or classical DCs which arise from a common DC precursor (Guilliams et al. 2014). They possess typical DC functions of antigen-presenting cells, including the ability to efficiently stimulate naive T cells and the capacity to express CCR7, and potentially enabling their migration to lymph nodes (Tang-Huau and Segura. 2019).

Mo-DC are HLA-DR^{+}CD11c^{+}CD14^{int}CD206^{+}CD1c^{+} cells. By contrast, they lack the macrophage markers CD16 and CD163. They also display a typical DC morphology: they are small size, possess dendrites and lack large cytoplasmic vacuoles (Tang-Huau and Segura. 2019). Human mo-DC are present in lungs, intestine and peritoneum in the steady-state. Peritoneal mo-DC secrete IL-6, TNF-α, IL-1β and IL-12p70 upon ex vivo re-stimulation. Mo-DC from bronchoalveolar lavage also secrete TNF-α upon re-stimulation (Tang-Huau and Segura. 2019).

Pathogen-derived components, such as Toll-like receptor ligands as well as inflammatory mediators induce maturation of mo-DC. These stimulants include LPS, ssRNA, IFN-α, TNF-α, IFN-γ or CD40L (León et al. 2005, Farkas and Kemény. 2011). TNF-α and inducible nitric oxide synthase (iNOS)-producing DCs (Tip-DCs) are abundant in inflamed tissue such as skin in patients of chronic inflammatory skin disease, and not present in the steady-state or normal skin tissue. These cells are derived from monocyte infiltrated during inflammation and contribute to innate immune response to pathogens including bacteria and parasites (Guilliams et al. 2014).

From the above, monocyte is considered to infiltrate into inflammatory site and differentiate to mo-DC and Tip-DC, sequentially in chronically inflamed tissue. This maturation process is induced and/or promoted by IFN-α, TNF-α and GM-CSF (Farkas and Kemény. 2011).

Tip-DCs express HLA-DR, CD40, CD86, as well as maturation markers DC-Lamp and CD83 but lack the CD207/Langerin and CD14 markers of Langerhans cells and monocytes. In addition, Tip-DCs found in psoriasis produce the inflammatory mediators IL-8, IL-1, STAT1, CCL 20, IL-20, IL-23p19, and IL-12/IL-23p40, which mediate Th1 and Th17 responses (Wilsmann-Theis et al. 2013).

How it is Measured or Detected

Detection of Tip-DC is considered to be done by:

- Flowcytometry
- RT-qPCR

Analysis of maturation marker expression on cell surface

Maturation markers such as CD80, CD86, CD40 and CD83 can be analyzed by flowcytometry (Wilsmann-Theis et al. 2013).

Quantification of mRNA expression of TNF-α and iNOS

Expression of TNF-α, iNOS, IL-12p35 and IL-23p19 mRNA in in vitro generated Tip-DC are quantified by RT-qPCR. (Wilsmann-Theis et al. 2013).

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Event: 1707: Increase, IL-23 from matured dendritic cells

Short Name: Increase of IL-23

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:313 - Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
dendritic cell

Organ term

Organ term
immune system

Domain of Applicability

Tip-DCs are also observed in mice. Murine Tip-DCs are defined as splenic CD11c$^+$, CD11b$^+$, MHC-II$^+$, CD40$^+$, and CD86$^+$ cells producing iNOS and TNF. CD11b expression is observed in murine Tip-DC, however it is lacking on human cells (Lowes et al. 2005).

Key Event Description

Increased IL-23 synthesis from TNF-α and inducible nitric oxide synthase (iNOS)-producing DCs (Tip-DCs) is a result of maturation from monocyte-derived dendritic cells (mo-DC) to Tip-DC which is HLA-DR$^+$CD40$^+$CD86$^+$DC-Lamp$^+$ and CD83$^+$ (Farkas and Kemény. 2011, Wilsmann-Theis et al. 2013).

Tip-DCs are abundant in inflamed tissue such as skin in patients of chronic inflammatory skin disease, and not present in the steady-state or normal skin tissue. These cells are derived from monocyte infiltrated during inflammation and contribute to innate immune response to pathogens including bacteria and parasites (Guilliams et al. 2014).

Increased production of cytokines including IL-12/IL-23p40 in Tip-DC stimulated with R848, an agonist of Toll-like receptor (TLR) 7/8 was reported (Hänsel et al. 2011). In addition, maturation-dependent cytokine production including IL-23 from 6 hours in-vitro matured Tip-DC were observed when stimulated with CD40L and TLR ligands, such as LPS, PGN, Pam3Cys and R848 (Hänsel et al. 2011).

IL-23 is a heterodimer, sharing a p40 subunit with IL-12 but having a distinct p19 subunit. IL-23 binds to IL-12Rβ1 but not IL-12Rβ2. The receptor for this cytokine is heterodimeric and uses a novel second subunit, IL-23R, which is a member of the hematopoietin receptor family (Lee et al. 2004).

How it is Measured or Detected

Measurement of IL23 protein

IL-23 in cell-free supernatants collected after R848 stimulation to moDCs is detected by ELISA (Schwarz et al. 2013).

Measurement of IL23 RNA levels

Expression of IL-23 mRNA in R848-stimulated moDCs is measured by qRT-PCR (Schwarz et al. 2013).

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Event: 1708: Th17 cell migration and inflammation induction

Short Name: Th17 cell migration and inflammation induction

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:313 - Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

dendritic cell

Organ term

Organ term

immune system

Domain of Applicability

Ras homolog gene family H (RhoH) is a membrane-bound adapter protein involved in proximal T cell receptor signaling, and spontaneously develops chronic dermatitis that closely resembles human psoriasis in RhoH gene-deficient mice. Ubiquitin protein ligase E3 component N recognition 5 (Ubr5) and nuclear receptor subfamily 2 group F member 6 (Nr2f6) expression levels are decreased at the lesion site, and protein levels and DNA binding activity of retinoic acid-related orphan receptors are increased is doing. As a result, T cells differentiated into Th17 cells due to increased production of IL-17 and IL-22. These results indicate that RhoH suppresses the differentiation of naive T cells into effector Th17 cells. RhoH is a gene expressed in blood cells, and when RhoH expression decreases in T cells, Th17 cells increase, IL-22 is produced in large quantities, and the epidermis thickens, leading to the formation of psoriasis pathology. Humans with low RhoH expression may become more severe if they suffer from psoriasis. Journal of Allergy and Clinical Immunology

The effect of the unique gut flora in psoriasis on the development and reactivity of inflammatory cells on the IL-23 / Th17 axis was analyzed in imiquimod-induced psoriasis model mice. Th17, γδ TCR-bearing lymphocytes in the spleen were measured from sterile (GF) mice, broad-spectrum antibiotic mixture-administered (ATB) mice, and conventional (CV) mice. GF mice and ATB-treated mice had fewer Th17 cells and γδTCR + cells than CV mice. This is thought to be due to the symbiotic bacteria that lack microbiota or changes due to ATB treatment reduce pro-inflammatory T cell response and regulate T cell development. In other words, it is proof that the interaction between the microorganisms of Clostridiales and Elysiperotricales and the host affects the reactivity of Th17 cells and is involved in the etiology of imiquimod-induced skin inflammation. The positive effect of antibiotic regulation of the gut flora on skin severity suggests the involvement of the gut and skin axes and is part of the management of psoriasis patients. (Zizana Z et al. 2016) * Wide-area antibiotic mixture (ATB): A mixture of metronidazole, colistin, streptomycin, and vancomycin.

Key Event Description

Psoriasis is known to play a major role in the etiology of T cell dysfunction, especially in over activation of the Th17 pathway, which Th17 cells were associated with Th1 and Th2 (Lisa C. et al. 2007) Th17 cell was identified as a cell population that produces different IL17. Abnormal activation of Toll-like receptors (TLR7, 8 and 9) is also involved in the initiation and maintenance of psoriasis. IMO-3100 (an antagonist of TLR7 and 9) and IMO-8400 (an antagonist of TLR7, 8 and 9) has been shown to reduce psoriasis-like skin lesions induced by intradermal administration of IL-23 on the back of mice (Mayte S-F et al. 2013). Immune cell infiltration in psoriasis lesions is composed of CD3 + Th1cell, Th17 cells and CD11c + dendritic cells (DC) (Chamian F et al 2005).

Cytokines such as TNF-α, IFN-γ, IL-17, IL-22, IL-23, IL-12, and IL-1β produced from these cells cause an inflammatory cascade. In particular, the IL-23 / Th17 axis plays an important role, and IL-23h is produce in DC, promotes the differentiation of naive CD4 + T cell progenitor cells into the Th17 phenotype, and stimulates the survival and expansion of the Th17 population (Harrington LE et al. 2005) (Veldhoen M et al. 2006). IL-17 produced from Th17 cells regulates the expression of defensin, S100 family protein and LL-37. These are innate immune responses in the skin and show higher expression of IL-23 in keratinocytes and dermal tissues of psoriatic lesions than in non-lesions (Liang SC et al. 2006).

Overproduction of Th1 and TH17 cytokines is a major cause of psoriasis, and glucocorticoid (GC) regulates epidermal differentiation and acts as a potent anti-inflammatory compound to suppress the pathology of psoriasis. Synthetic glucocorticoids are uses to suppress inflammatory disease including psoriasis, and induce the glucocorticoid-induced leucine zipper (GILZ), a protein that inhibits major immune cell signaling pathways. CILZ is deficient in lesioned skin of psoriasis patients and shows a negative correlation with the expression of pro-inflammatory cytokines IL-1, IL-23, IL-22, and STAT3. *Lisa et al.* was identified a T cell-specific role of CILZ that limits Th17 cell formation in vitro in response to the Th17-promoting cytokines IL-1β and IL-23 (Lisa M et al.2019). CILZ has the clinical significance of psoriasis as well as the non-redundant function of controlling pathogenic Th17 responses (Lisa M et al.2019).

One of the causes of psoriasis is an increase in pathogenic Th17 cells in people with a genetic predisposition stimulated by the production of Th17 polarized cytokines by bone marrow cells. The antibacterial peptide LL37, which forms a complex with nucleic acids released from cells, is an autoantigen that promotes the activation of cutaneous plasmacytoid dendritic cells and myeloid DCs, and Th17 cells are effector cytokines such as IL-17A. It activates keratinocytes directly through release. Activated keratinocytes proliferate abnormally and release inflammatory mediators and chemokines to amplify the inflammatory response (Boehncke WH et al.2015).

How it is Measured or Detected

IL17 + cell count measurement

Flow cytometric analysis of psoriasis skin biopsy showed increased IL-17 + and IL-22 + CD4 + T cells,

Measurement of IL17 protein levels (in skin and serum)

Increased frequency of IL-17 +, CCR6 +, and CCR4 + T cells. IL-22-producing cells (Th-22 cells) that do not produce IL-17 or IFNγ also increased (Benham et al. 2013).

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List of Adverse Outcomes in this AOP

Event: 1709: Psoriatic skin disease

Short Name: Skin disease

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:313 - Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	AdverseOutcome

Biological Context

Level of Biological Organization

Individual

Domain of Applicability

Mouse psoriasis-like dermatitis model: K14 / mIL-1F6 gene-modified mice overexpress mouse IL-1F6 (IL-36a) selectively under the keratin 14 promoter, and TPA: 12-O- tetradecanoy1phorbol-13-acetate(TPA) was applied, skin pathological featuresfindings specific to psoriasis-such as epidermal hyperplasia, epidemal exfoliation and micro-abscess formation, and wet inflammatory cells in the dermis-were observed. Quantitative RT-PCR. Measures mRNA expression levels of Inflammatory chemokines and cytokines in skin tissues, and includes inflammatory chemokines: CCL3, CCL4, CXCL10, CXCL1 and cytokines: IL-23, IL-12, IL-1β etc. Expression was observed. (Kyowa Hakko Kirin Co., Ltd.)

Epidermal keratinocyte expression genes that were elevated in psoriatic lesions of patients with psoriasis with stage-type skin eruption: mRNA expression level of keratin6a and 16, s100A7A, S100A12, DEFB4, IL-1F6, CCL20, IL-17C, etc. was rapidly reduced by 700 single intravenous dose of brodalumab and decreased to non-lesional skin level two weeks after administration.On the other hand, leukocyte expression genes with increased expression in psoriatic lesion skin: IL-17A, IL-17F, IL-23F, IL-12B, IL-22, IFN-γ and other mRNA expression levels decreased with brodalumab administration However, at 2 weeks after administration, the level did not decrease to the level of the non-lesional skin. Since the expression of pathophysiology-related genes is reduced prior to the decrease in the expression of leukocyte expression genes and the decrease in the PASI score, brodalumab expresses pathophysiology-related genes by blocking IL-17 signaling in the epidermal keratinocytes of psoriatic lesions It is possible to improve the skin eruption promptly. (Kyowa Hakko Kirin Co., Ltd. In-house materials)

The monoclonal antibody-mediated IL-17A (secukinumab / ixekizumab) and the receptor subunit IL-17RA (brodalumab) are effective approaches in the treatment of psoriasis. Blocking IL-17RA results in inhibition of the IL-17 family, including IL-17A, IL-17F, IL-17C, and IL-17E. Other drugs are under clinical development that target bimekizumab targeting IL-17A and IL-17F, IL-23 upstream of the IL-17 pathway, or signal transduction substances downstream. (Conrad C et al. 2018)

Key Event Description

Psoriasis is a complex inflammatory disease caused by activation of Th1 and TH17 cells. The epidermis is composed of keratinocytes that differentiate to form a permeable barrier. Abnormal balance between proliferation and differentiation of keratinocytes affects barrier function and causes inflammatory skin lesions. Psoriasis is a complex combination of genetic and environmental risk factors, and dysregulation of Th1 and Th17 lines is due to overproduction of cytokines including IFN-α, TNF-α, IL-23, IL-17, and IL-22. Causes overgrowth and skin immunity. (Harrington LE et al. 2005)

Histopathological features of psoriasis lesions include epidermal thickening, epidermal differentiation, and epidermal protrusions (reticular ridges), with intraepithelial neutrophil moistening (manlo-like abscess) and marked immune moistening consisting of T cells and dendritic cells. See an increase in. (Boehncke WH et al. 2018) Commonly used psoriasis model mice were produced by topical application of the TLR7 agonist imiquimod, which induces the IL-23-Th17 cell axis and histopathology of human psoriasis pathology. Strictly reproduce the target and molecular features. (Wagner EF et al.2010)

Psoriasis vulgaris shows overexpression of the S100 protein family soriacin (sorazine), cobunericin (kebuneridine), and epidermal antibacterial peptide (AMP). AMP is induced by IL-17 and itself functions as a chemotactic factor and cytokine. Mobilize CD4 + T cells and neutrophils that exacerbate inflammation. (Kanagawa Psoriasis Treatment Study Group)

Serum IL-17 levels in psoriasis patients are significantly higher than in healthy individuals, and brodalumab, a neutralizing antibody against the IL-17A receptor, has been shown to be effective in the treatment of psoriasis (Gilliet et al. 2004). In addition, antibody preparations against IL-17 ixekizumab (John K. et al. 2002) and Szeimies et al. 2004 were used to treat psoriasis, and with positive results, Th17-mediated pathways are important for the etiology of psoriasis. It is believed to play a role. Immunohistological examination of biopsies of skin areas with psoriatic plaques, including surrounding normal skin, shows an increase in the number of activated dendritic cells, especially CD1a-positive Langerhans cells in the epidermis of psoriatic lesions. In CD83-positive CD1a-negative Langerhans, -negative CD11c-positive dermal dendritic cells increased in the epidermis at the border of psoriatic plaques. In normal skin, there were significantly fewer CD3-positive T lymphocytes than lesions.

CD4 and CD8 T cells infiltrate both the epidermis and dermis and show increased expression of IL17A, IL22, and IFNG in epidermal CD4 and CD8 T cells near keratinocytes, but with lower dermal T cell upregulation. IL-22, produced primarily by lesion epidermal CD4 T cells, is associated with keratinocyte activation and the formation of epidermal thickening, a prominent morphological feature of psoriasis. Lesion epithelial CD8 T cells mainly produce IL-17A and promote the production of inflammatory cytokines and chemokines by keratinocytes. IL-17A is an important mediator of psoriatic inflammation through skin recruitment and activation of leukocytes. (Cheuk et al. 2014)

In patients with psoriasis, inflammatory keratin K6 and K16-positive keratinocytes were found, even in areas of normal-appearing skin that were not affected by psoriasis lesions. In addition, the transcription factor C / EBPβ, which is normally expressed only in the stratum granulosum of the healthy epidermis, was expressed throughout the epidermis, including the epidermis of the lesion. This suggests that early inflammatory changes have already occurred in areas that have not yet shown obvious skin lesions, and that these changes are caused by dendritic cells rather than lymphocytes. (Komine et al. 2007)

How it is Measured or Detected

Biopsy of the skin area and surrounding normal skin of patients with psoriasis vulgaris

The dendritic cell surface marker and lymphocyte surface marker of the section were used as the primary antibody.

In the vicinity of psoriasis lesions, an increased number of activated dendritic cells was observed, with CD1a-positive Langerhans cells in the epidermis and CD83-positive CD1a-negative Langerin-negative CD11c-positive dermal dendritic cells at the epidermal border.

There were significantly fewer CD3-positive T lymphocytes than lesions in normal skin.

Inflammatory keratin K6 and K16 positive keratinocytes were found in the normal part.

The transcription factor C / EBPβ, which is normally expressed only in the granular layer of the normal epidermis, was expressed in the entire epidermis in the same manner as the lesion. (Komine et al. 2007)

Serum amyloid A: SAA measurement ... measured in 35 psoriasis patients and healthy humans

DNA microarray analysis in lesions of psoriasis patients ... SAA levels are about 5 times higher than in normal skin. The average SAA of psoriasis patients was 19.1 ug / ml, and the average SAA after treatment was 6.9 ug / ml.

There is a correlation between SAA and psoriasis severity score (PASI).

Amyloid A deposition was observed in the skin-stained area of the psoriasis lesion skin area (Tanizaki H et al. 2013)

Regulatory Significance of the AO

Psoriasis model mice have been developed as a tool for understanding the etiology of this disease and as a preclinical model. Five representative models are based on K14-amphiregulin, K5-Stat3C, K5-Tie2, K5-TGF-β1, and imiquimod. There were statistically significant similarities between the gene expression patterns associated with epidermal development and keratinization in these

models and the gene expression patterns in human psoriasis. Direct high-level activation of keratinocytes via autoclean growth factor (amphiregulin) has the ability to induce cytokine-related genetic circuits that closely resemble human psoriasis. Through transgenes, inward mutations (CARD14), injury, and exposure to specific T cell-producing cytokines, activated keratinocytes induce and lead to a chronic inflammatory response that is highly consistent with psoriasis. However, there were frequent differences in the expression of immune-related genes between the models. (Cook PW et al. 1997)

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2017: Stimulation of TLR7/8 leads to Increase of IL-23

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Thirteen mammalian TLR members (10 in humans and 13 in mice) have been identified so far, of which TLR1, 2, 4, 5, and 6 are membrane bound and catalytic site for pathogenic structural components, whereas TLR3, 7, 8, and 9 expressed within the

endosomal compartment

are dedicated to nucleic acids. TLRs 1–9 are conserved among humans and mice, yet TLR10 is present only in humans and TLR11 strictly restricted to rodents (Gupta et al. 2016).

Mouse TLR10 is not functional because of a retrovirus insertion, and TLR11, TLR12 and TLR13 have been lost from the human genome (Kawai and Akira. 2010).

In addition, alignment of amino acid residues between human toll-like receptor 7 (AAF60188.1) and murine toll-like receptor 7 (AGX25544.1) was 80.74% identification. Both proteins have 1049 amino acid residues.

Structural characterization was conducted with recombinant TLR7 from monkey (*Macaca mulatta*; 96.8% sequence identify with human TLR7) expressed in *Drosophila* S2 cells (Zhang et al. 2016).

Studies of DC subsets isolated from humans and mice have revealed that TLRs have distinct expression patterns. Freshly isolated human pDCs express TLR7 and TLR9, whereas $CD11c^+$ human myeloid DCs (mDCs) express TLR1, TLR2, TLR3, TLR5, TLR6 and TLR8. In some studies, TLR7 expression was detected on both pDCs and mDCs, whereas others found TLR7 was exclusively expressed in pDCs (Iwasaki and Medzhitov. 2004).

In mice, all splenic DC subsets express TLRs 1, 2, 4, 6, 8 and 9. However, mouse pDCs do not express TLR3. Moreover, mouse $CD8\alpha^+$ DCs lack TLR5 and TLR7 expression and fail to respond to TLR7 agonists. In short, $CD4^+$ DC, CD4CD8DN DC and pDC express TLR7 in mice (Iwasaki and Medzhitov. 2004).

Although unpublished, it has been reported that human slanDCs (Tip-DCs) lack the DNA-binding structureTLR9 but can express the endosomal RNA-binding receptorsTLR8 (slanDCs and$CD1c^+$ DCs) and TLR7 (slanDCs but not $CD1c^+$ DCs; Hänsel et al, unpublished data, June 2010) (Hänsel et al. 2011). There are not any other reports which mentioned TLR7 expression in Tip-DCs, so whether or not TLR7 exists in human Tip-DCs is still unknown.

IFN-α, but not TNF-α and IL-6 production by human pDCs after stimulation with self-RNA-LL37 complex was detected (Ganguly et al. 2009). However, in mice, IFN-α production from splenic pDCs was induced by IMQ treatment. The production of TNF-α and IL-23 was also induced by IMQ treatment. Although 4–8% of $mPDCA\text{-}1^-$ $CD11c^+$ DCs were contaminated in prepared $mPDCA\text{-}1^+$ pDC fraction, it was confirmed that splenic $mPDCA\text{-}1^-$ $CD11c^+$ DCs enriched to approximately 80% purity could not produce TNF-α and IL-23 by IMQ stimulation. In $Tlr7^{-/-}$ splenic pDCs, these cytokines (IFN-α, TNF-α and IL-23) were not induced by IMQ treatment, although stimulation by CpG, a TLR9 ligand, resulted in induction of these cytokines at the same level as was produced by wild-type splenic pDCs. These data indicate that, in mice, IMQ application can induce the production via TLR7 of IFN-α, TNF-α and IL-23 from pDCs existing in the skin in vivo (Ueyama et al. 2014).

When BMDCs were generated by 10-day culture with GM-CSF and IL-4 and characterized their phenotypes, CD11c+ BMDCs showed MHC II^{high}, $CD11b^{high}$, $B220^-$, $CD86^{high}$, $Mac\text{-}3^+$, and had the ability to produce high levels of TNF-α and NO/iNOS in response to LPS stimulation, which represents a similar phenotype to Tip-DCs (Xu et al. 2007, Ueyama et al. 2014).

In these BMDCs which represents a similar phenotype to Tip-DCs, IMQ weakly but significantly induced the production of IL-23. In addition, although IFN-α had no effect alone, co-stimulation with IFN-α and IMQ resulted in marked induction of IL-23 production. However, using BMDCs derived from $Tlr7^{-/-}$ mice, these effects of IMQ and IFN-α was not observed, verifying that it is also mediated via TLR7 (Ueyama et al. 2014).

In mice, purified bone marrow dendritic cells (BMDCs) derived from wild-type mice stimulated with IFN-α showed increase in Tlr7 mRNA expression (Ueyama et al. 2014). In addition, TLR7 expression was also observed in the inflamed skin of IMQ-treated mice (Ueyama et al. 2014). These data suggest that the synergistic effect of IMQ and IFN-α on BMDCs was caused by induction of TLR7 expression by IFN-α (Ueyama et al. 2014).

Taken together, in mice, IFN-α produced by IMQ-primed pDCs may enhance the effects of IMQ to activate Tip-DC, resulting in the release of a large amount of IL-23 in IMQ-induced psoriasis-like skin lesion (Ueyama et al. 2014).

Key Event Relationship Description

Toll-like receptors (TLRs) are members of interleukin-1 (IL-1) receptor/TLR superfamily, as they share the intracellularToll-IL-1 receptor (TIR) domain with the IL-1 receptor.

Toll-like receptor (TLR) 7 and TLR8 is known to mediate the recognition of guanosine- and uridine-rich single-stranded RNA (ssRNA) derived from ssRNA viruses and synthetic antiviral imidazoquinoline components (Akira et al. 2006; Blasius and Beutler. 2010). They also mediate the recognition of self RNA that is released from dead or dying cells.

Human TLR7 (hTLR7) and human TLR8 (hTLR8) contains 1049, 1041 amino acid residues with a calculated molecular weight of 120.9 kDa and 119.8 kDa respectively (Chuang and Ulvitch. 2000).

The full-length hTLR7 protein includes a signal peptide of 26 amino acids (1–26 aa). The mature hTLR7 protein ectodomain, trans-membrane, and TIR domain are composite structure of 27–839, 840–860, and 889–1,036 amino acids, respectively (Gupta et al. 2016).

hTLR7 and hTLR8 form a subfamily of proteins that each contain an extracellular domain of >800 residues and share functional and structural features. TLR8 contains 26 leucine-rich repeats (LRRs), which is the largest number of LRRs among TLRs whose structures have been reported (Tanji et al. 2013).

Monkey TLR7 exists as a monomer in the absence of ligands, and TLR7 dimerization is induced by R848 alone, but not by poly U or guanosine alone, although these two ligands synergistically triggered TLR7 dimerization (Zhang et al. 2016). In contrast, hTLR8 exists as preformed dimer before ligand recognition. TLR8 is activated by R848 alone, or both uridine and ssRNA synergistically (Tanji et al. 2013).

The key residues interacting two TLR7 molecules into dimer confirmation are LYS410, ASN503, SER504, GLY526, ASN527, SER530, THR532, ARG553, and TYR579 (Gupta et al. 2016).

TLR3, TLR7, TLR8, and TLR9 localize to the endoplasmic reticulum and are trafficked to the endosomal compartment where they initiate cellular responses upon their activation by PAMPs and DAMPs (Lai et al. 2017).

TLR7 are exclusively expressed in plasmacytoid DCs (pDCs), which have the capacity to secrete vast amounts of type I IFN rapidly in response to viral infection (Gilliet et al. 2008, Reizis et al. 2011).

TLR8 is expressed in various tissues, with its highest expression in monocytes. Myeloid DCs (mDCs) also express TLR8 in human (Iwasaki and Medzhitov. 2004). Thus, TLR8 ligands can directly activate mDCs via TLR8.

TLR7-mediated signaling in pDC is mediated in a MyD88-dependent fashion, which initiates an IRF7-mediated response, secreting vast amounts of IFN type 1 (Kawai and Akira. 2011).

MyD88-dependent IRF7 activation in pDCs is mediated by activation of IRAK1, TRAF6, TRAF3, and IKKα and is facilitated by IFN-inducible Viperin expressed in the lipid body (Kawai and Akira. 2011).

IRF7, which is constitutively expressed by pDCs, binds MyD88 and forms a multiprotein signaling complex with IRAK4, TRAF6, TRAF3, IRAK1 and IKKα (Kawai and Akira. 2008). In this complex, IRF7 becomes phosphorylated by IRAK1 and/or IKKα, dissociates from the complex and translocates into the nucleus.

The interferons (IFNs) are a primary defense against pathogens because of the strong antiviral activities they induce. Three types of IFNs, types I, II and III, have been classified based on of their genetic, structural, and functional characteristics and their cell-surface receptors (Zhou et al. 2014). IFN-α belongs to the type I IFNs, the largest group which includes IFN-β, IFN-ε, IFN-ω, IFN-κ, IFN-δ, IFN-τ and IFN-ζ.

The IFN-α of type I IFN family in humans is composed of 12 subtypes encoded by 14 nonallelic genes including one pseudogene and two genes that encode the same protein. The various IFN-α subtypes have many common points. For example, all are clustered on chromosome 9 (Diaz et al. 1993). IFN-αs, which are composed of 165 to 166 aa, have 80% amino acid sequence identities (Li et al. 2018).

Upon engagement of ssRNAs in endosomes, TLR8 initiate the MyD88-dependent pathway culminating in synthesis and release of proinflammatory mediators, such as TNF-α via NF-κB activation (Tanji et al. 2015).

A distinct population of human blood DCs that are defined by the selective expression of the 6-sulfo LacNAc residue on the P-selectin glycoprotein ligand 1 membrane molecule was described previously. 6-Sulfo LacNAc DCs (slanDCs) stand out by a marked production of TNF-α, and they were recognized as the major source of IL-12p70 among blood leukocytes when stimulated with CD40 ligand or LPS of gramnegative bacteria (Hänsel et al. 2011).

According to the current concept, these inflammatory DCs are $CD1c^{-}$, $CD11c^{+}$ cells locally expressing TNF-α and iNOS. They were also referred to as TNF and inducible nitric oxide synthase–expressing DCs (Tip-DCs) (Lowes et al. 2005) or inflammatory dermal DCs (Zaba et al. 2009). In contrast, resident dermal DCs express CD1c and CD11c and were shown to lack inflammatory markers. The phenotype of slanDCs ($CD11c^{+}$ and $CD1c^{-}$) and their local production of IL-23p19, TNF-α, and iNOS identify slanDCs as being a population of inflammatory dermal DCs and so-called Tip-DCs in psoriasis (Hänsel et al. 2011).

Stimulation of blood DCs with self-RNA–LL37 complexes induced a robust TNF-α response (Hänsel et al. 2011). TNF-α affects Tip-DCs in an autocrine and/or paracrine manner (Zaba et al. 2007).

DC activation is known to be enhanced by IFN-α in the presence of TNF-α (Luft et al. 1998).

R848 induces IL-23 production from activated human monocyte-derived DCs (moDCs) by enhanced transcriptional activity (Schwarz et al. 2013).

IL-23 is a heterodimer, sharing a p40 subunit with IL-12 but having a distinct p19 subunit. IL-23 binds to IL-12Rβ1 but not IL-12Rβ2. The receptor for this cytokine is heterodimeric and uses a novel second subunit, IL-23R, which is a member of the hematopoietin receptor family (Lee et al. 2004).

Evidence Supporting this KER

Biological Plausibility

The molecular structure and function of TLR7 and TLR8 are evident based on sufficient scientific findings as mentioned above. The known mechanisms for stimulation of TLR7/8 by each ligand are initiated by the formation of homodimer. TLR7-mediated signaling in pDC is mediated in a MyD88-dependent fashion, which initiates an IRF7, IRAK1, TRAF6, TRAF3, and IKKα-mediated response, secreting vast amounts of IFN type 1 (Kawai and Akira. 2011).

Similarly, upon engagement of ligands in endosomes, TLR8 initiate the MyD88-dependent pathway culminating in synthesis and release of proinflammatory mediators, such as TNF-α via NF-κB activation (Tanji et al. 2015).

DC activation is known to be enhanced by IFN-α in the presence of TNF-α (Luft et al. 1998).

R848 induces IL-23 production from activated human monocyte-derived DCs (moDCs) by enhanced transcriptional activity (Schwarz et al. 2013).

TNF and inducible nitric oxide synthase–expressing DCs also known as Tip-DCs or inflammatory dermal DCs differentiates from moDCs by inflammation (Hänsel et al. 2011).

As mentioned above, stimulation of TLR7 in pDCs, and TLR8 in moDCs and Tip-DCs leads to activation of Tip-DCs, which leads to the overproduction of IL-23 from matured Tip-DCs.

Empirical Evidence

Much experimental data is available that supports the stimulation of TLR7 in pDC induced by TLR7 agonist, which subsequently promote secretion of IFN-α in MyD88-dependent fashion. For example, three populations of cells were evaluated for type I IFN production following imidazoquinoline stimulation: human PBMC, pDC-depleted PBMC, and pDC-enriched cells. Human PBMC produce IFN-α following imiquimod (0.3–30 μM) or resiquimod (0.03–30 μM) treatment. Peak levels of IFN-α were reached with imiquimod and resiquimod at 3 μM. PBMC, depleted of pDC, did not produce detectable levels of IFN-α in response to imiquimod or resiquimod treatment.

The imidazoquinoline-treated pDC-enriched cultures produced 2–20 times more IFN-α than similarly treated PBMC as measured over the entire dose range. Peak levels of Resiquimod- and imiquimod-induced IFN-α production were reached with 0.3 μM and 30 μM, respectively (Gibson et al. 2002).

In addition, pDCs were stimulated with LL37 premixed with total human RNA extracted from U937 cells to confirm that LL37 can interact with self-RNA and convert it into a trigger of IFN-α production. U937-derived self-RNA induced dose-dependent IFN-α production when mixed with LL37, but not when given alone or mixed with the scrambled peptide GL37. Similar to self-DNA (Lande et al., 2007), pDCs activated by self-RNA mixed with LL37 produced high levels of IFN-α, but did not produce TNF-α or IL-6 or undergo maturation as assessed by measuring the expression of costimulatory molecules CD80 and CD86 (Ganguly et al. 2009).

Importantly, self-RNA isolated from a variety of cell types and tissue samples from various types of skin pathologies induced similar levels of IFN-α when mixed with LL37, indicating that cellular- or disease-dependent variations in RNA composition do not play a role in the responses to self-RNA. These data demonstrate that LL37 can convert otherwise nonstimulatory self-RNA into a trigger of pDC activation to produce IFN-α, and thus enable the RNA released during cell death to induce innate immune activation (Ganguly et al. 2009).

IFN-α induced in pDCs by self-RNA–LL37 complexes was inhibited in a dose-dependent manner by bafilomycin, which blocks endosomal acidification and TLR signaling. To specifically inhibit TLR7, we used the short oligonucleotide C661, which selectively blocks TLR7 (Barrat et al. 2005), as shown by the inhibition of IFN-α induction by the synthetic TLR7 agonist R837 but not the TLR9 agonist CpG2006. Pretreatment of pDCs with C661 specifically blocked the IFN-α induction by self-RNA–LL37 complexes, indicating that pDC activation by self-RNA–LL37 complexes occurs through TLR7 (Ganguly et al. 2009).

Self-RNA–LL37 complexes but not self-RNA alone activated mDCs to produce the proinflammatory cytokines TNF-α and IL-6, but not IFN-α (Ganguly et al. 2009). Self-RNA–LL37 complexes also activated mDCs to undergo maturation as shown by the up-regulation of CD80 and CD86 expression (Ganguly et al. 2009) . mDC activation by self-RNA–LL37 complexes was entirely dependent on self-RNA, given that these responses were abrogated by decreasing the amount of self-RNA in the complexes (unpublished data). In contrast to self-RNA–LL37 complexes, self-DNA–LL37 complexes were unable to activate mDCs (Ganguly et al. 2009) . In accordance with these findings, stimulation of mDCs with supernatants of apoptotic cells combined with LL37 induced the secretion of proinflammatory cytokines, and this secretion was entirely dependent on self-RNA because activity was abolished by depletion of self-RNA but not self-DNA (Ganguly et al. 2009).

Compared with stimulation with either supernatant of activated pDCs or self-RNA–LL37 alone, the combination of both significantly enhanced the activation of mDCs to secrete IL-6 and TNF-α and enhanced their differentiation into mature CD83+ DCs (Ganguly et al. 2009). This activity was completely blocked by antibodies against IFN-α, IFN-β and IFN-αβR (Ganguly et al. 2009). Thus, self-RNA–LL37 complexes can trigger mDC activation and maturation, and this process is enhanced by the concomitant activation of pDCs to produce IFN-α.

Self-RNA was also internalized by mDCs when complexed with LL37 but not when given alone. The cytokine production such as TNF-α and IL-6 of mDCs induced by self-RNA–LL37 complexes but not by the TLR4 agonist LPS was completely inhibited by bafilomycin in a dose-dependent manner, demonstrating that mDC activation by self-RNA–LL37 complexes involved endosomal TLR activation. Using 293T cells transfected with TLR8 and TLR3 expression vectors along with a NF-κB luciferase reporter plasmid, it was confirmed that self-RNA–LL37 complexes activated TLR8 but not TLR3. In support of this finding, synthetic short ssRNA sequences that activate TLR8 in human mDCs (Diebold et al. 2004, Heil et al. 2004) also activated mDCs when complexed

with LL37 but not when given alone (Ganguly et al. 2009).

Dose-dependent DC maturation was observed with increasing concentrations from 10 IU/ml up to 1000 IU/ml of IFN-α2a or IFN-α8 added to cultures containing GM-CSF, IL-4, and TNF-α. Both of the IFNs had a similar capacity to up-regulate HLA-A, B, C, CD80, and CD86 and to down-regulate CD1a and CD11b expression on the cell population (Luft et al. 1998).

DC cultured in GM-CSF, TNF-α, and IL-4-containing medium until day 14, and type I IFNs were added daily between days 14 and 17. Proportions of positive cells for each markers were analyzed by FACS on day 17 (Luft et al. 1998).

When GM-CSF, TNF-α, and IL-4-containing cultures were washed on day 14 and continued until day 17 in serum-free medium containing GM-CSF and IL-4, without or with TNF-α (20 ng/ml, standard conditions), IFN-a (1000 IU/ml), or both, IFN-α alone did not enhance DC maturation as compared with standard conditions. When both of TNF-α and IFN-α exist, optimal maturation was observed than either TNF-α or IFN-α alone. Thus, the enhancement of DC activation by IFN-α under serum-free conditions required the presence of TNF-α (Luft et al. 1998).

LL37 is highly expressed in the inflamed skin of psoriasis but is undetectable in inflamed skin of atopic dermatitis or in healthy skin (Lande et al. 2007). To determine whether extracellular self-RNA–LL37 complexes form in vivo, Staining skin sections with Ribogreen and DAPI revealed that numerous extracellular Ribogreen^{+}/ DAPI^{-} complexes in the dermal compartment of psoriatic skin lesions, but not in skin of atopic dermatitis or healthy skin (Ganguly et al. 2009). These tissue RNA complexes presented several features of self-RNA–LL37 complexes generated in vitro, including the size and bead-like branched structures resulting from the aggregation of smaller particles (Ganguly et al. 2009).

Skin sections of psoriatic tissues were stained with an anti-LL37 antibody and Ribogreen to determine whether the self-RNA complexes in the tissues contained LL37 and it was found that the majority of these complexes contained LL37 (Ganguly et al. 2009). Importantly, psoriatic skin also contained substantial numbers of particulate self-DNA–LL37 complexes.

Serial sections of lesional psoriatic skin were stained for RNA complexes and DC-LAMP, a lysosomal marker specific for mature mDCs to determine whether the presence of tissue self-RNA complexes is associated with the presence of activated DCs in psoriatic skin. Consistent with previous reports (Lowes et al. 2005), it was found that large clusters of DC-LAMP–positive mature mDCs (Ganguly et al. 2009). We also found tissue self-RNA–LL37 complexes within these clusters, and, occasionally, even inside the DCs as shown by the colocalization with endolysosomal compartments stained with DC-LAMP (Ganguly et al. 2009). The number of tissue self-RNA complexes significantly correlated with the numbers of DC-LAMP–positive mDCs in psoriatic skin (Ganguly et al. 2009). Together, these findings strongly support *in vitro* data that self-RNA complexes can activate mDCs and suggest that this pathway is operational in psoriasis.

When mRNA expression normalized to HARP for IL-23 subunits, such as p19 and p40 were quantified by RT-PCR in monocyte-derived DCs (moDCs) matured without and with etanercept, a dimeric human tumor necrosis factor recepter p75-Fc fusion protein made of 2 extra-cellular domains of the human 75kD TNFR linked by the constant Fc portion of human IgG1 (Haraoui and Bykerk. 2007), significant decrease in expression of IL-23 subunits p19 and p40 by etanercept were observed (Zaba et al. 2007). MoDCs cultured with etanercept decreased CD86 expression threefold and HLA-DR expression fivefold. In addition, moDCs cultured with etanercept were also an average of two to threefold less stimulatory than control DCs in a mixed leukocyte reaction. Gene array on control moDCs compared with those cultured with etanercept revealed that CD163, a macrophage scavenger receptor, was up-regulated 6.5-fold (Zaba et al. 2007).

In psoriatic dermis, mRNA expression normalized to HARP for multiple inflammatory products of Tip-DCs, including iNOS, TNF-α and IL23 p40 subunit, are reduced within 1–2 weeks after beginning etanercept, whereas the number of CD11c^{+} DCs in the tissue is minimally affected during this time, suggesting an initial blockade of cytokine production by these cells rather than cell reduction (Zaba et al. 2007). These facts suggest that TNF-α is an autocrine or paracrine inducer of IL-23 from Tip-DC (Zaba et al. 2007).

R848-treatment to moDCs, which were generated from monocytes isolated from buffy coats of healthy donors, resulted in concentration-dependent expression of IL-23. 2×10^{5} moDCs/ml were plated in DC medium and stimulated with 0-5 µg/ml R848. After 24 h of TLR stimulation, supernatants were harvested and cytokine expression was measured by ELISA. In addition, the combination of NOD1 and NOD2 agonists with R848 stimulated high levels of IL-23 secretion (Schwarz et al. 2013).

qRT-PCR for moDCs stimulated with TLR agonists in the absence or presence of NOD1 and NOD2 ligands at 8 h and 24 h post induction revealed that synergistic cytokine expression observed in NOD1/NOD2- and R848-stimulated cells is largely mediated by enhanced transcriptional activity (Schwarz et al. 2013).

In time kinetic studies, moDCs were stimulated with R848 in the absence or presence of MDP and iE-DAP which are ligands of NOD1/2, for 30 min, 2 h, 8 h or 24 h and mRNA levels of IL-23 as well as the cumulative cytokine release were measured by qRT-PCR and sandwich-ELISA, respectively. At the mRNA level, synergistic effects of both NOD ligands with R848 are already detectable after 8 h of stimulation. In agreement with IL-23 mRNA expression, synergistic effects are detectable by ELISA after 8 h; nevertheless, these effects are even more pronounced after 24 h of stimulation (Schwarz et al. 2013).

These findings show that dose responses and temporality of MIE and KE1 seem to be in sequence.

Uncertainties and Inconsistencies

Although unpublished, it has been reported that human slanDCs (Tip-DCs) lack the DNA-binding structureTLR9 but can express the endosomal RNA-binding receptorsTLR8 (slanDCs andCD1c^{+} DCs) and TLR7 (slanDCs but not CD1c^{+} DCs; Hänsel et al, unpublished data, June 2010) (Hänsel et al. 2011). There are not any other reports which mentioned TLR7 expression in Tip-DCs,

so whether or not TLR7 exists in human Tip-DCs is still unknown.

In addition, freshly isolated human pDCs have been reported to express TLR7 and TLR9, whereas $CD11c^+$ human myeloid DCs (mDCs) express TLR1, TLR2, TLR3, TLR5, TLR6 and TLR8. In some studies, TLR7 expression was detected on both pDCs and mDCs, whereas others found TLR7 was exclusively expressed in pDCs. Therefore, it is still unknown that whether or not TLR7 exists in human mDCs, and how much it does contribute recognition of R848 or LL37-RNA in these cells (Iwasaki and Medzhitov. 2004).

Quantitative Understanding of the Linkage

Response-response relationship

MIE:

Much experimental data is available that supports the stimulation of TLR7 in pDC induced by TLR7 agonist, which subsequently promote secretion of IFN-α in MyD88-dependent fashion. For example, HEK293 cells were transiently co-transfected with human TLR7 and NF-κB-luciferase reporter. After 48 hours, the cells were stimulated with various concentrations of resiquimod or imiquimod. Luciferase activity was measured 48h post-stimulation and the results are reported as fold-increase relative to medium control. As a result, dose-dependent increase in NF-κB-dependent luciferase activity in HEK293 transfected with hTLR7 was observed with increasing concentrations from 0.01 μM up to 10 μM of resiquimod, and 0.1 μM up to 15 μM of imiquimod. Maximal NF-κB activation with resiquimod is achieved with 10-30 μM, which yields an 18-fold increase in luciferase production. Maximal NF-κB activation with imiquimod requires 10-15 μM compound and induces a 5-6-fold increase in luciferase production (Gibson et al. 2002).

In addition, three populations of cells were evaluated for type I IFN production following imidazoquinoline stimulation: human PBMC, pDC-depleted PBMC, and pDC-enriched cells. Human PBMC produce IFN-α following imiquimod (0.3–30 μM) or resiquimod (0.03–30 μM) treatment. Peak levels of IFN-α were reached with imiquimod and resiquimod at 3 μM. PBMC, depleted of pDC, did not produce detectable levels of IFN-α in response to imiquimod or resiquimod treatment.

The imidazoquinoline-treated pDC-enriched cultures produced 2–20 times more IFN-α than similarly treated PBMC as measured over the entire dose range. Peak levels of Resiquimod- and imiquimod-induced IFN-α production were reached with 0.3 μM and 30 μM, respectively (Gibson et al. 2002).

In different experiments, pDCs were stimulated with LL37 premixed with total human RNA extracted from U937 cells to confirm that LL37 can interact with self-RNA and convert it into a trigger of IFN-α production. U937-derived self-RNA induced dose-dependent IFN-α production when mixed with LL37, but not when given alone or mixed with the scrambled peptide GL37 (Ganguly et al. 2009).

R848 (0.001-10 μg/mL) induced NF-κB activation in HEK293 cells transfected with human TLR8 in a dose-dependent manner (Jurk et al. 2002). In addition, the production of TNF-α and IL-6, and the maturation

of mDCs induced by self-RNA–LL37 complexes but not by the TLR4 agonist LPS was completely inhibited by bafilomycin in a dose-dependent manner, demonstrating that mDC activation by self-RNA–LL37 complexes involved endosomal TLR activation (Ganguly et al. 2009).

Dose-dependent DC maturation was observed with increasing concentrations from 10 IU/ml up to 1000 IU/ml of IFN-α2a or IFN-α8 added to cultures containing GM-CSF, IL-4, and TNF-α. Both of the IFNs had a similar capacity to up-regulate HLA-A, B, C, CD80, and CD86 and to down-regulate CD1a and CD11b expression on the cell population (Luft et al. 1998).

DC cultured in GM-CSF, TNF-α, and IL-4-containing medium until day 14, and type I IFNs were added daily between days 14 and 17. Proportions of positive cells for each markers were analyzed by FACS on day 17 (Luft et al. 1998).

When GM-CSF, TNF-α, and IL-4-containing cultures were washed on day 14 and continued until day 17 in serum-free medium containing GM-CSF and IL-4, without or with TNF-α (20 ng/ml, standard conditions), IFN-a (1000 IU/ml), or both, IFN-α alone did not enhance DC maturation as compared with standard conditions. When both of TNF-α and IFN-α exist, optimal maturation was observed than either TNF-α or IFN-α alone. Thus, the enhancement of DC activation by IFN-α under serum-free conditions required the presence of TNF-α (Luft et al. 1998).

In accordance with these findings, compared with stimulation with either supernatant of activated pDCs or self-RNA–LL37 alone, the combination of both significantly enhanced the activation of mDCs to secrete IL-6 and TNF-α and enhanced their differentiation into mature CD83+ DCs (Ganguly et al. 2009). This activity was completely blocked by antibodies against IFN-α, IFN-β and IFN-αβR (Ganguly et al. 2009). Thus, self-RNA–LL37 complexes can trigger mDC activation and maturation, and this process is enhanced by the concomitant activation of pDCs to produce IFN-α.

KE 1

R848-treatment to moDCs, which were generated from monocytes isolated from buffy coats of healthy donors, resulted in concentration-dependent expression of IL-23. 2×10^5 moDCs/ml were plated in DC medium and stimulated with 0-5 μg/ml R848. After 24 h of TLR stimulation, supernatants were harvested and cytokine expression was measured by ELISA. In addition, the

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Time-scale

Human PBMC, pDC-deficient PBMC, and pDC -enriched from human PBMC (pDC-enriched) were cultured with various concentrations of resiquimod or imiquimod. After 24 h in culture, cell-free supernatants were collected and IFN-α was analyzed by ELISA (Gibson et al. 2002).

Suspensions containing RNA-LL37 or supernatants of dying cells were added to pDC and mDC cultures. After overnight culture, supernatants of pDCs and mDCs were collected and IFN-α, TNF-α and IL-6 were measured by ELISA (Ganguly et al. 2009). pDCs and mDCs were also stained with fluorochrome-labeled anti-CD80, anti-CD86, and anti-CD83 antibodies and analyzed by flow cytometry. mDCs were also cultured with supernatants of pDCs stimulated for 24 h with self-DNF-LL37 (Ganguly et al. 2009).

In time kinetic studies, moDCs were stimulated with R848 in the absence or presence of MDP and iE-DAP which are ligands of NOD1/2, for 30 min, 2 h, 8 h or 24 h and mRNA levels of IL-23 as well as the cumulative cytokine release were measured by qRT-PCR and sandwich-ELISA, respectively. At the mRNA level, synergistic effects of both NOD ligands with R848 are already detectable after 8 h of stimulation. In agreement with IL-23 mRNA expression, synergistic effects are detectable by ELISA after 8 h; nevertheless, these effects are even more pronounced after 24 h of stimulation (Schwarz et al. 2013).

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Relationship: 2018: Increase of IL-23 leads to Th17 cell migration and inflammation induction

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	adjacent	High	High

Evidence Supporting Applicability of this Relationship

In mice, application of IL-23 causes psoriatic-like epidermal hyperplasia, but this effect does not occur in IL-17A and IL-22KO mice. Therefore, it is thought that IL-17A and IL-22 play an important role downstream of IL-23 Rizzo HL. Et al. 2011 .

Recombinant mIL-23 (rmIL-23) injected into the ear of WT mice induced IL-17A and IL-22 expression, and showed ear swelling and epidermal hyperplasia. When rmIL-23 was injected into IL-22 KO mice, IL-22 was induced, but ear swelling and epidermal hyperplasia were less than in WT mice. When rmIL-23 was injected into IL-17A KO mice, IL-22 was induced, but ear swelling and epidermal hyperplasia hardly occurred. WT mice after administration of IL-22 or IL-17A inhibitor completely inhibited IL-23-induced epidermal hyperplasia. These results indicate that two cytokines, IL-22 and IL-17A, are downstream mediators of IL-23-induced changes in mouse skin and are required for the generation of IL-23-mediated skin lesions. (Hansel et al. 2011)

Key Event Relationship Description

IL-23 is important for differentiation and proliferation of Th17 cells. As a major source of IL-23, Tip-DC is present in the skin lesions of psoriatic patients and works to activate the Th17 pathway (Hansel et al. 2011).

Signaling through the heterodimeric IL-23 receptor (subunits of p19 and p40) of Th17 cells stimulates the production of proinflammatory keratinocyte cytokines that mediate the psoriatic response and induces the production of IL-17. Th17 cells are increased in the peripheral blood and lesion skin of psoriatic patients, and IL-17 and IL-22 produced from Th17 act on epidermal keratinocytes to cause inflammatory chemokines and hyperproliferation (Michelle A. et al. 2005).

IL-17A, which is highly expressed by Th17 cells, has a direct effect on the regulation of genes expressed by keratinocytes that are

involved in innate immune defense, including defensins,8, 9 S100 family proteins, lipocalin, and LL37/cathelicidin, as well as a range of CXCL chemokines that regulate neutrophil trafficking (Gilliet et al. 2004). IL-22, which is expressed by Th22 and Th17 cells, and related IL-20 family members promote keratinocyte hyperproliferation and abnormal differentiation (Krueger et al. 2012).

Evidence Supporting this KER

Biological Plausibility

IL-17A, which is highly expressed by TH17 cells, has a direct effect on the regulation of genes expressed by keratinocytes that are involved in innate immune defense, thorough expressions of defensins,8, 9, S100 family proteins, lipocalin and LL37/cathelicidin, as well as a range of CXCL chemokines that regulate neutrophil trafficking. IL-22, which is expressed by TH22 and TH17 cells, and related IL-20 family members promote keratinocyte hyperproliferation and abnormal differentiation Gilliet et al.2012 .

In vitro Reconstituted Human Epidermis (RHE) model stimulated for 48 hours with medium containing IL-17, IL-22 and TNFα mix (concentration 3 ng / mL) as psoriasis-specific cytokines. Controls were cultured in normal medium. After fixing RHE and embedding in paraffin, 4 µm sections were stained with hematoxylin-eosin or immunolabeled with anti-filaggrin, anti-S100A7, anti-hBD-2 mAb.

RHE stimulated with cytokine mix showed dramatic expression of these protein. In the RHE with normal medium, antibacterial peptide S100A7 was expressed locally, but BD-2 protein was not detected. This is due to the synergistic effect of IL-17 added to the IL-22 / TNFα combination. Filaggrin, S100A7 and BD-2 mRNA expression by RT-qPCR analysis increased 20-fold (S100A7) or -50-fold (BD-2) compared to controls. This is a downstream event that can be modeled using keratinocytes and cytokines and relies on upstream mechanisms of recruitment and activation of other innate adaptive immune cells. Bernard et al. 2012. .

Quantitative Understanding of the Linkage

Response-response relationship

KE1:

IL-23, which maintains Th17 cells, is released from TNF-a and inducible nitric oxide synthase (iNOS) -producing dendritic cells (TIP-DC). TIP-DC activates IL-17, IL-22, IL-23, and TNF-a mRNA expression in psoriatic skin. Cytokine staining analysis of peripheral blood mononuclear cell (PBMC) in patients with psoriasis showed a three-fold increase in Th17 cells compared to normal PBMC. Thl7 cells produce IL-22 and stimulate keratinocyte proliferation. IL-22 activates STAT3 and induces the production of cytokine (such as IL-8), chemokines and the synthesis of antimicrobial peptides (Zaba et al. 2005).

KE 2

The epidermis of psoriasis patients did not have many T cells, but the analysis was similar to peripheral blood and dermis. The proportion of Th17 cells in the dermis was significantly higher than that in normal skin, and TNF and IFN-g were produced from Th17 cells. Skin and peripheral blood contained a subset of Th17 cells producing IFN-g / TNF.

Keratin 16, IL-17, IFN-g, and IL-22 mRNA expression increased in psoriatic skin, but cyclosporine therapy returned these mRNA to normal levels. The average expression of IL-17 / human acidic ribosomal protein (hARP) in non-lesional skin was 0.4 compared to 10.8 in lesional skin, and cyclosporine administration returned to non-lesional levels. That IL-17 mRNA return to baseline, effective treatment supports that Th17 in psoriasis is a central pathogenic.(Lowes et al.2008)

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Relationship: 2019: Th17 cell migration and inflammation induction leads to Skin disease

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Stimulation of TLR7/8 in dendric cells leading to Psoriatic skin disease	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Initiation of plaque formation in the Aldara psoriasis mouse model is dependent on RORγt +, skin infiltrating γδ T cells, and innate lymphocyte cells (ILC). Vγ4 + γδT cells and innate lymphoid cells (ILC) are the dominant and important sources of IL-17A, IL-17F, and IL-22 in the formation of acute psoriatic lesions, rather than Th cells (Pantelyushin et al. 2012).

Amyloid A: SAA, an inflammatory marker, is high in the serum of patients with psoriasis. When C57B6 mice were given SAA protein subcutaneously on the back, epidermal thickening and inflammatory cell wetting were frequent on days 5-7. Skin inflammation was significantly suppressed when IL-12 / IL-23p40 protein, a target molecule of psoriasis biologics, was administered in advance. By SAA administration, a similar reaction to psoriatic eruption was formed in the immunological reaction, and a psoriatic eruption model mouse was established. (J Dermatolog Trest. 2013; 24 (6): 477-80)

Key Event Relationship Description

Th17 cells produce the cytokines IL-17 and IL-22. IL-17 is inflammatory, promotes the migration of neutrophils to psoriatic lesions, contributes to the formation of Munro's micro-abscess, and through DCL and memory T cells, including Th17 cells and CCR6, via CCL20 Incorporate into the affected area. IL-22 causes abnormal keratinocyte proliferation by down-regulating genes that control terminal differentiation, leading to altered differentiation and keratinization. Both IL-17 and IL-22 induce keratinocyte expression of the antibacterial S100A7 (psoriacin). Nograles et al. 2008

STAT3 is important for Th17 differentiation. Cytokine signaling SOCS3-deficient mice show increased IL-17 expression by increasing STAT3 activity in response to IL-23 binding to IL-17. Associated with increased activity of STAT3 in response to IL-23 capable of binding to IL-17 and IL-17F promoters. STAT3 overexpression promotes Th17 differentiation, whereas STAT3 deficiency inhibits Th17 differentiation. STAT3 signaling from IL-6, IL-21, IL-23 regulates the expression of lineage specific master transcription factors RORγt22, 63, 66 and RORα67. It has been found that patients with psoriasis with mutations in STAT3 cannot generate a Th17 response. Martinez et al. 2008

Evidence Supporting this KER

Biological Plausibility

The biological activity of the combination of cytokines was investigated. The combination of IL-17A and IFN-γ or IL-17A and TNF-α has a synergistic effect on CXCL8 production by keratinocytes. IL-17A and IL-22 exert a synergistic effect in upregulation of β-defensin 2: BD-2 and S100A9 production] IL-1α, IL-17, IL-22, Oncostatin M: OSM, and TNFα binding are associated with increased expression of inflammatory molecules such as soriacin / S100A7 or BD-2, IL-8 in vitro by NHEK Although very potent synergistic, removal of IL-22 from the cytokine mixture reduces CXCL8 and BD-2 expression by 30% and removal of IL-17 reduces it by 70%.

Ex vivo studies on human skin explants showed upregulation of BD-2, S100A7, and CXCL8 expression in response to the same combination of cytokines, and intradermal injection of cytokines into mice resulted in neutrophil infiltration and early epidermis CXCL1, CXCL2, CXCL3, S100A9, and BD-3 expression related to epidermal thickening was increased. (Bernard et aaal. 2012)

Empirical Evidence

Resident memory tissue T cells (TRM cells) confer both resistance and immunity depending on the local microenvironment, and CD8 TRM can be tracked by phenotypic markers CD49a and CD103. Circulating effector T cells infiltrate the site of skin inflammation and turn into long-lived epidermal TRM cells when the skin inflammation is resolved. Epidermal TRM cells are thought to form pathological site-specific disease memory at the site of recurrent psoriasis.(Cheuk et al. 2014)

Single cell suspensions of epidermis and dermis were analyzed by flow cytometry within 30 hours of sampling. In active psoriasis, CD8 T cells increased about 100-fold in the epidermis compared to normal skin, whereas CD4 T cells increased 10-fold in the dermis. In healthy skin, 20-30% of epidermal CD8 T cells co-expressed integrin CD103 and CD49a, which are phenotypic markers

of TRM cells. In active psoriasis, approximately half of epidermal CD8 T cells co-expressed these TRM phenotypic markers, a 100-fold increase compared to healthy skin. Cheuk et al. 2014

Uncertainties and Inconsistencies

Cytokines cannot be specified for genes associated with abnormalities in psoriatic skin. Many genes that are up-regulated in psoriatic lesions can be attributed to IFN-γ, including IL-17 and IL-22. Cytokines synthesized by Th1 / Th17 cells regulate different gene expression pathways in epidermal keratinocytes and other skin resident cells. The psoriatic transcriptome may result from activation of multiple independent pathways. Nograles et al. 2008

After daily topical application of Aldara containing imiquimod (IMQ) to humans, significant skin thickening, redness and scaling were observed 3 days later (doi: 10.1172 / JCI61862DS1). The clinical course of plaque formation on the ear and back skin and histopathology were similar. Aldara treatment resulted in impaired keratinocyte hyperproliferation and epidermal differentiation, as indicated by epidermal thickening and hyperkeratosis. There was a terminal neutrophil accumulation in the stratum corneum reminiscent of a Munro micro-abscess in psoriasis. Extensive leukocyte infiltration was observed in the dermis.(Pantelyushin et al. 2012)

Quantitative Understanding of the Linkage

Response-response relationship

KE2

High levels of Th17 cytokines were observed in psoriatic skin induced by CD4 + T cells. IL-23 p40 subunit or IL-22 significantly prevented the development of skin lesions.

IL-22-induced acantosis and inflammation were reduced in IL-22-deficient mice compared to WT mice. Blocking IL-22 increases IL-1α, IL-1β, IL-6, IL-17, IL-17F, and TNF-α. (K. A. et al. 2013)

AO

Anti-IL-17 antibody administration results in decreased keratinocyte proliferation and differentiation, leukocyte infiltration, and keratinocyte release of inflammatory cytokines. In psoriatic lesioned keratinocytes, changes in mRNA and protein expression of IL-17 regulatory products occurred. Within 2 weeks of antibody administration, the expression of LL37 (cathelicidin), β-defensin 2, S100A7, and S100A8 proteins was markedly decreased in keratinocytes, and the expression reached normal levels after 6 weeks. (Krueger et al. 2012)

Time-scale

Epidermal keratinocyte expression genes that were elevated in psoriatic lesions of patients with psoriasis with stage-type skin eruption: mRNA expression level of keratin6a and 16, s100A7A, S100A12, DEFB4, IL-1F6, CCL20, IL-17C, etc. was rapidly reduced by 700 single intravenous dose of brodalumab and decreased to non-lesional skin level 2 weeks after administration. On the other hand, leukocyte expression genes with increased expression in psoriatic lesion skin: IL-17A, IL-17F, IL-23F, IL-12B, IL-22, IFN-γ and other mRNA expression levels decreased with brodalumab administration. However, at 2 weeks after administration, the level did not decrease to the level of the non-lesional skin. Since the expression of pathophysiology-related genes is reduced prior to the decrease in the expression of leukocyte expression genes is reduced prior to the decrease in the expression of leukocyte expression genes and the decrease in the PASI score, Brodalumab is reduced expression of pathophysiology-related genes by blocking IL-17 signaling in the epidermal keratinocytes of psoriatic lesions It is possible to improve the skin eruption promptly. (Kyowa Hakko Kirin Co., Ltd.)

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