Am J Clin Exp Immunol 2014;3(1):1-19
www.ajcei.us /ISSN:2164-7712/AJCEI1310003

Review Article

Chemerin regulation and role in host defense

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Received October 14, 2013; Accepted December 25, 2013; Epub February 27, 2014; Published March 15, 2014

Abstract: Chemerin is a widely distributed multifunctional secreted protein implicated in immune cell migration, adipogenesis, osteoblastogenesis, angiogenesis, myogenesis, and glucose homeostasis. Chemerin message is regulated by nuclear receptor agonists, metabolic signaling proteins and intermediates, and proinflammatory cytokines. Following translation chemerin is secreted as an inactive pro-protein, and its secretion can be regulated depending on cell type. Chemerin bioactivity is largely dependent on carboxyl-terminal proteolytic processing and removal of inhibitory residues. Chemerin is abundant in human epidermis where it is well-placed to provide barrier protection. In host defense, chemerin plays dual roles as a broad spectrum antimicrobial protein and as a leukocyte attractant for macrophages, dendritic cells, and NK cells. Here we review the mechanisms underlying chemerin regulation and its function in host defense.

Keywords: Chemerin, host defense, chemoattractant, adipokine, antimicrobial peptide

Introduction

Chemerin, a multifunctional protein, is known primarily for its chemotactic and adipokine properties. Chemerin was first discovered in the late nineties as retinoid (tazarotene)-responsive gene in skin and as a result was named tazarotene-induced gene 2 (TIG2) [1]. Approximately six years later chemerin was rediscovered through G protein-associated receptor screening assays as a ligand for the orphan seven-pass transmembrane receptor chemokine-like receptor 1 (CMKLR1, also known as ChemR23, DEZ, and recently renamed "chemerin receptor" by the International Union of Basic and Clinical Pharmacology) [2-4]. It is now known that several immune cell subsets, such as plasmacytoid dendritic cells (pDCs), macrophages and NK cells express CMKLR1 and respond to chemerin either through chemotaxis or modulation of their defense function [3-7]. In subsequent years chemerin was 'rediscovered' as an adipocyte signaling molecule important in adipogenesis [8-10]. Chemerin also plays a role in angiogenesis [11], osteoblastogenesis [10], myogenesis [12], and in regulating glucose homeostasis [13, 14]. Moreover, this protein has been implicated in inhibiting bacteria growth [5, 15]. Therefore, the original notion of chemerin mainly as a leukocyte chemoattractant has been substantially challenged and broadened over the years.

Chemerin is predicted to belong to the structural cathelicidin/cystatin family of proteins comprised of antibacterial polypeptide cathelicidins and inhibitors of cysteine proteinases (cystatins). Chemerin is widely expressed and secreted as a precursor protein that can be converted to a variety of isoforms. These isoforms are likely equipped with different functions. The isoforms are generated by proteolytic cleavage of the carboxyl-terminus by a myriad of serine and cysteine proteases as well as carboxypeptidases that can cleave chemerin in a direct or sequential manner [5, 6, 16-18]. Taken together, chemerin emerges as a protein of interest for a number of disciplines, including immunology, dermatology, metabolism and development. Here we review the progress of

Table 1. Regulation of chemerin expression by inflammatory and metabolic factors

Cell type	Nuclear receptors/nuclear receptor ligands	Metabolic factors	Immunomodulatory mediators
Epithelial cells			
Human skin keratinocytes	tazarotene (RARβ/γ)↑, SR 11217/SR 11237 (RXR)—, 1,25D3— [1]		
Fetal human intestinal cells	ATRA (RARβ)↑ [25]		TNFα↑ [25]
Human intestinal enterocyte-like Caco2 cells			TNFα↑ [25]
Human renal tubular epithelial cells	1,25D3—, DEX— [51]		TNFα↓, IFNγ↓, IL1β—, TGFβ— [51]
Fibroblasts			
Human skin fibroblasts	1,25D3↑ [24], — [1]		IFNγ—, IFNα—, TNFα—, CXCL8—, TGFβ—, [24]
Human synoviocytes			TNFα↑, IFNγ↑, IL1β—, IL6—, TGFβ— [64]
Chondrocytes			
T/C-28a2, human immortalized chondrocytes	IL1β↓ [103]	adiponectin↓, leptin↓ [103]	LPS↓ [103]
ATDC-5 mouse chondrogenic cell line	DEX↑ [103]	adiponectin—, leptin— [103]	IL1β↑, LPS— [103]
Osteoclasts-supporting mouse stromal ST2 cell line	1,25D3↑, DEX↑ [26]		
Adipocytes			
Human adipocytes/adipocyte tissue explants	PPARγ (troglitazone) (mRNA)—(protein)↓ [14], gonadal and adrenal steroids— [44]	adiponectin— [14] insulin↑, metformin↓ [44], FFA (SREBP2)↑ [33]	TNFα↑ [14],
Mouse adipogenic cell line 3T3-L1	PPARγ (troglitazone)↑ [29]		TNFα↑ [50], IL1β↑ [58]
Bone marrow ouse mesenchymal stem cell	PPARγ (rosiglitazone)↑ [10]		
Hepatocytes			
Human hepatoma HepG2 cell line	FXR (GW4064)↑ [30]		
Mouse hepatocytes	FXR (GW4064)↑ [30]		TNFα— [50]

↑upregulation, ↓downregulation, —no effect. Abbreviations: 1,25D3, 1,25 dihydroxyvitamin D3; ATRA, all-trans-retinoic acid; DEX, dexamethasone; FFA, free fatty acids; FXR, farnesoid X receptor; GW4064, synthetic ligand for FXR; PPARγ, peroxisome proliferator-activated receptor γ; SR 11217/SR 11237, synthetic ligands of RXR; RARs, retinoic acid receptors; RXRs, retinoid X receptors.

studies involving chemerin, focusing on mechanisms underlying the regulation of chemerin activity and its function in host defense.

Transriptional regulation of chemerin activity

Chemerin is encoded by TIG2 (also known as retinoic acid receptor responder gene 2 (RARRES2)) [1]. Although, liver and adipose tissue are repeatedly reported as the major sites of chemerin production, this protein is expressed in many tissues including adrenal gland, placenta, pancreas, lung and skin [3, 4, 9, 15, 19].

Due to the broad expression of chemerin and its association with inflammatory and metabolic processes, chemerin transcription is likely to be a part of local and systemic programs of gene expression that are crucial in immunity and metabolism. This is supported by the regulation of chemerin production by variety of inflammatory and metabolic mediators that can be broadly classified as; i/ agonists of nuclear receptors (retinoids, vitamin D, glucocorticoids), ii/ factors mainly associated with metabolic processes (e.g. fatty acids, insulin, glucose) and iii/ immunomodulatory mediators (e.g. cytokines of acute or chronic inflammation and LPS) (**Table 1**).

Regulation of chemerin expression by nuclear receptors

Nuclear receptors are ligand-dependent transcription factors that can both activate or repress gene transcription. These receptors were first identified as transcription factors for known lipophilic hormones such as glucocorticoids or the thyroid hormones [20, 21], but are now known to include other members such as: peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor activated by lipids; farnesoid X receptor (FXR) activated by bile acids; RARs (retinoic acid receptors), activated by all-trans-retinoic acid (ATRA) and 9-cis retinoic acid; RXRs (retinoid X receptors), activated by 9-cis retinoic acid; and vitamin D3 receptor (VDR) [20, 22, 23]. Unlike water-soluble protein ligands that initiate signal transduction follow-

ing binding to their cognate receptors at the cell surface, fat-soluble agonists of nuclear receptors can pass through the cell membrane and interact with their cognate receptors. All nuclear receptors contain a highly conserved zinc finger DNA binding domain that binds to similar response element [21].

Tazarotene, a synthetic retinoid acid analog used for treatment of hyperproliferative skin diseases like psoriasis, was the first identified factor to induce chemerin synthesis [1]. In skin, chemerin is distributed across two locations, epidermis and dermis. In healthy skin chemerin is highly expressed by epidermal keratinocytes but negligibly by cells in the dermis. In contrast, in psoriatic skin there are low levels of chemerin in the epidermis with elevated levels in the dermis [1, 15, 24]. Tazarotene, which selectively activates gene expression through retinoic acid receptors β and γ (RARβ/γ), up-regulated TIG2 expression in the epidermis of psoriatic lesions and 3-dimensional normal skin equivalents (skin rafts) [1]. These data clearly demonstrate that TIG2 is a retinoid-responsive gene in skin. Two-dimensional cultures of skin-derived keratinocytes or fibroblast, however did not significantly induce chemerin synthesis in response to tazarotene or ATRA, a high-affinity ligand for RAR-β [1, 24]. Part of the reason for this might be that these types of *in vitro* culture models do not effectively recapitulate the skin microenvironment required for proper regulation of chemerin expression. Nevertheless, primary cultures of other epithelial cells, such as fetal intestinal epithelial cells, were reported to upregulate TIG2 levels following treatment with ATRA [25]. In contrast to the stimulation of TIG2 expression in skin rafts by the RAR ligands, synthetic agonists of RXRs were not effective in inducing TIG2 expression in skin rafts *in vitro* [1], indicating a selectivity of retinoid receptors in promoting TIG2 transcription.

In addition to RAR-ligands, other agonists of the steroid/thyroid/vitamin D3 nuclear receptor family, such as 1,25 dihydroxyvitamin D3 (1,25D3) or synthetic glucocorticoid-derivative dexamethasone (DEX), were also examined as potential regulators of TIG2 expression. For example, 1,25D3, the hormonally active form of vitamin D (also known as calcitriol), as well as DEX markedly induced TIG2 mRNA in the stromal cell line ST2 during osteoclast differentiation [26]. On the other hand, 1,25D3 and DEX did not modify chemerin expression in renal tubular epithelial cells, suggesting cell-specific regulation of TIG2 expression by these factors [26]. Less clear is the role of 1,25D3 in controlling TIG2 expression in skin-derived cells. Whereas exogenous 1,25D3 did not alter TIG2 levels in keratinocyte- and fibroblast-containing skin rafts [1], this sterol significantly induced chemerin expression in primary cultures of fibroblasts isolated from either healthy or psoriatic skin [24].

Chemerin has also been identified as a target for peroxisome proliferator-activated receptor γ (PPARγ). PPARγ, a member of the nuclear hormone receptor superfamily is known to play a critical role in adipogenesis [27, 28]. Bone marrow mesenchymal stem cells (BMSCs) and 3T3-L1 cells that have the potential to differentiate into adipocytes are often used to study adipogenesis. Whereas BMSCs treated with PPARγ agonist rosiglitazone showed elevated chemerin mRNA levels, PPARγ silencing via shRNA resulted in a nearly complete loss of rosiglitazone-induced TIG2 expression in these cells [10]. Troglitazone, another agonist of PPARγ, increased TIG2 mRNA in 3T3-L1 cells during their differentiation [29]. However, BMSCs-derived mature adipocytes, unlike undifferentiated multipotent BMSCs, revealed a dose-dependent reduction in TIG2 mRNA levels upon rosiglitazone treatment [10], suggesting that PPARγ drives TIG2 expression in adipocyte precursor cells but not in terminally differentiated adipocytes.

Another nuclear receptor, farnesoid X receptor (FXR) has been recently implicated in the regulation of TIG2 expression in liver [30]. FXR, a main receptor for bile acids, is expressed at high levels in liver and intestine where it controls lipid and glucose metabolism. Bile acids, natural detergents, are involved in the absorption of dietary fat and fat-soluble vitamins [31]. GW4064, a synthetic FXR ligand induced a dose-dependent increase in TIG2 levels in human hepatoma HepG2 cells as well as primary mouse hepatocytes from FXR sufficient mice but not hepatocytes from FXR deficient mice [30], suggesting communication between FXR and chemerin in liver.

Taken together, these data support a role for nuclear receptors in regulation of chemerin gene expression. However, their impact on TIG2 levels might be a consequence of direct or indirect modulation of chemerin promoter activity.

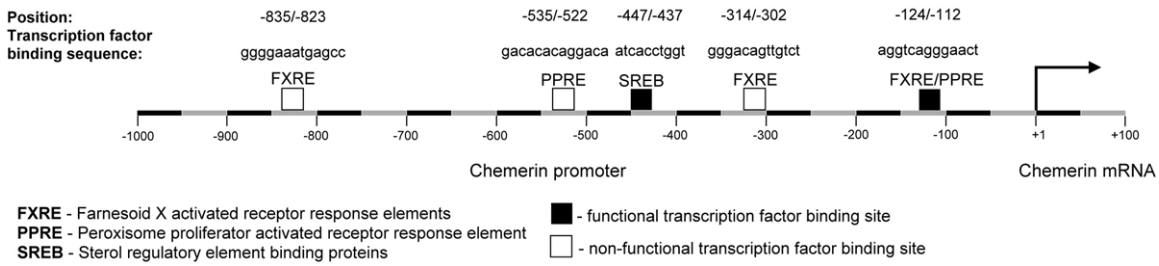

Figure 1. Schematic diagram of the mouse chemerin promoter showing the location and sequences of reported transcription factors binding sites. The genomic sequence of the mouse chemerin promoter was obtained from the NCBI Gene database (National Center for Biotechnology Information website, http://www.ncbi.nlm.nih.gov/gene, Gene ID: 71660).

Chemerin promoter

Computational analysis of the human chemerin promoter sequence identified RARβ binding sites close to the CpG islands [25] that participate in transcriptional and post-transcriptional regulation of gene expression [32]. This could explain why TIG2 expression was affect by tazarotene in skin, or ATRA in intestinal epithelial cells. Indeed, expression of RARβ in fetal intestinal epithelial cells closely followed chemerin expression during their maturation, suggesting the direct involvement of RARβ in controlling TIG2 expression [25].

Although a comprehensive functional analysis of the chemerin promoter has not yet been performed, certain candidate transcription factor binding sites predicted by *in silico* analysis have been validated using engineered cell lines with luciferase reporter constructs, or by electrophoretic mobility shift assays [10, 30, 33]. These studies identified functional response elements for nuclear receptors PPARγ and FXR in the mouse chemerin promoter (**Figure 1**). Members of the PPAR family of nuclear receptors form heterodimers with RXRs, and these heterodimers activate transcription by binding to PPAR response elements (PPREs) in the promoter of target genes [34]. In the chemerin promoter region ranging from -600 bp to +38 bp [10], corresponding to a region between -664 and -27 upstream of the mRNA start site for chemerin the gene (**Figure 1**), two potential PPREs were found, termed proximal and distal with respect to the chemerin start site [10] (**Figure 1**). However, only the promoter region containing the proximal PPRE rendered a fully active chemerin promoter following PPARγ expression/activation in reporter cells [10]. Since mutation of the proximal but not distal PPRE eliminated PPARγ responsiveness, these data suggest that the proximal PPRE is responsible for the regulation of chemerin gene expression by PPARγ [10]. Consistent with these findings, chromatin immunoprecipitation analysis revealed that PPARγ regulates TIG2 expression by localizing to the proximal PPRE element in the chromatin samples isolated from mouse embryonic mesenchymal stem cells, NIH-3T3 and BMSCs [10]. These data support a direct role for PPARγ in controlling TIG2 RNA expression.

Similar to PPARγ, FXR interacts with RXR as a requisite heterodimeric partner and binds to FXR response elements (FXRE) [35]. In line with the regulation of chemerin expression by a synthetic FXR agonist, the chemerin promoter region between -875 and +121 harbors three potential FXR binding sites [30]. One of these FXREs, located within -258/-90 interval (**Figure 1**) was sufficient to confer FXR responsiveness by luciferase reporter assay [30]. Notably, the location and sequence of the functional FXRE overlaps with the previously reported proximal PPRE implicated in PPARγ-activation of chemerin promoter in adipocyte precursors [10]. Furthermore, mutation of the FXRE resulted in loss of activation of the chemerin promoter by the FXR agonist. These findings, coupled with chromatin immunoprecipitation data implicate an association of FXR with this FXRE [30], indicating that the effect of FXR on chemerin expression is a consequence of direct interaction of FXR with the chemerin promoter.

Regulation of chemerin expression by metabolic factors

In addition to PPARγ and FXR ligands, chemerin expression is also affected by other factors

implicated in metabolic regulation such as free fatty acids (FFA). Plasma FFA levels are commonly elevated in obese individuals, mostly due to increased FFA release from hypertrophic adipose tissue [33]. Chronically elevated plasma FFA levels likely contribute to the pathology of hypertension or dyslipidemia [36]. Chemerin synthesis is up-regulated by FFA in 3T3-L1 cells during their differentiation into adipocytes [33]. Elevation in chemerin levels in these cells coincided with activation of sterol regulatory element-binding protein 2 (SREBP2), a transcription factor belonging to the basic-helix-loop-helix leucine zipper class of transcriptional regulators [37]. Following cell cholesterol shortage, SREBP2 is activated by intramembrane proteolysis. SREPB2 processing frees it from the endoplasmic reticulum to move to the nucleus where it binds to sterol regulatory elements in promoter regions of genes required for cholesterol biosynthesis [38]. Excess loading with FFA *in vitro* stimulated SREBP2 activation in 3T3-L1 cells, indicating cholesterol shortage and suggesting that chemerin expression may be affected by cholesterol levels. Indeed, forced cholesterol depletion by pharmacological inhibition of cholesterol synthesis upregulated chemerin expression and SREBP2 activation [33]. Furthermore, siRNA knockdown of SREBP2 reduced basal and FFA-induced chemerin levels, demonstrating that SREBP2 directly regulates chemerin synthesis. An analysis of the chemerin promoter identified a consensus recognition element for basic-helix-loop-helix proteins [39] as a potential SREBP2 binding site, SREB [33] (**Figure 1**). The binding of nuclear proteins from 3T3-L1 adipocytes to this site was confirmed by electrophoretic mobility shift assay [33]. In summary, these data suggest the following chain of events: exogenous FFA treatment induces 3T3-L1 adipocyte hypertrophy which likely leads to intracellular cholesterol shortage; this event triggers the nuclear translocation of SREBP2, which then binds to SREB in the chemerin promoter to induce its expression.

Although the cellular and molecular signaling pathways have yet to be defined, there is also evidence of communication among insulin, glucose and chemerin. In obese diabetic patients circulating chemerin levels were elevated compared with controls [8, 40-43]. Likewise, in patients with endocrine disorders associated with insulin resistance such as polycystic ovary syndrome (PCOS), plasma chemerin levels were elevated [44, 45]. Plasma chemerin levels were significantly increased following insulin infusion in healthy individuals [44]. In human adipose tissue explants, insulin significantly upregulated chemerin production [44]. On the other hand, metformin, an antidiabetic drug that suppresses hepatic glucose production and enhances peripheral glucose uptake by inducing glucose transporter 4 (GLUT4) translocation [46], suppressed chemerin synthesis and secretion in adipocyte tissue explants as well as reduced circulating chemerin levels in PCOS patients [44]. Overall these data suggest that insulin and/or glucose can impact chemerin expression, and that hyperinsulinemia may drive overexpression of chemerin in adipose tissue, leading to elevations in systemic chemerin protein via plasma circulation. Interestingly, data obtained from recently generated chemerin knockout (KO) and transgenic mice suggest a feedback signaling mechanism between plasma chemerin levels and insulin synthesis, as chemerin regulates insulin secretion in the pancreas [47].

Regulation of chemerin expression by cytokines

In addition to its well-defined pro-inflammatory role in acute inflammation, TNFα also contributes to the chronic low-grade inflammation that occurs in obesity [48]. Visceral white adipose tissue (vWAT) plays a key metabolic and endocrine role in overweight individuals and is a target for TNFα. TNFα enhances the sensitivity of vWAT to insulin, for example [42, 48, 49]. *In vitro*, TNFα induced TIG2 mRNA and chemerin protein expression in 3T3-L1 adipocytes, suggesting that TNFα may promote chemerin production in adipocytes *in vivo* [50]. Indeed, TNFα treatment significantly increased plasma chemerin levels in wild type but not TNF receptor (TNFRS1a and 1b) deficient mice. Interestingly, this increase in circulating chemerin protein was not accompanied by an upregulation of TIG2 mRNA in vWAT *in vivo*. Similarly, BMSC-derived primary adipocytes treated with TNFα failed to upregulate TIG2 transcription despite an increase in immunodetectable and bioactive chemerin protein in the conditioned media [50]. These data suggest a complex regulation of chemerin by TNFα in adipocyte tissue

and indicates that tissues other than vWAT are likely responsive to TNFα-induced chemerin expression *in vivo*.

The ambiguity of TNFα in controlling chemerin levels was also reported for renal proximal tubular epithelial cells, where TNFα suppressed TIG2 transcription and secretion of immunodetectable chemerin protein, but increased the level of bioactive chemerin as demonstrated by chemerin-dependent chemotaxis of plasmacytoid dendritic cells (pDCs) [51]. Given that chemerin bioactivity is controlled by proteolysis (see below), these findings suggest that TNFα concomitantly orchestrates proteolytic events required for chemerin conversion to a chemotactically-active form.

The source of chemerin in plasma is not clear at present. In addition to individuals with obesity or type 2 diabetes (T2D), elevated levels of chemerin were reported in several disorders associated with chronic inflammation such as Crohn's disease or chronic hepatitis C [52, 53]. In addition, systemic chemerin levels increased with the severity of liver fibrosis [45, 54]. Due to the strong expression of chemerin in liver and adipose tissue, we and others hypothesize that these organs have the biggest impact on blood chemerin levels. The portal vein delivers vWAT-derived metabolites like FFA as well as adipokines directly to the liver [55, 56], and thus may contribute to inducing hepatic chemerin expression. The analysis of portal, hepatic and systemic venous blood chemerin levels in humans with liver cirrhosis revealed similar chemerin concentrations in portal and systemic circulation, suggesting that vWAT is not a major site of chemerin production. On the other hand, higher chemerin levels in hepatic venous compared to portal venous likely indicate that chemerin is produced and secreted by the liver [57]. However, TNFα had no influence on chemerin synthesis by mouse primary hepatocytes or the liver *in vivo* [50], suggesting that liver is not a substantial source of pro-inflammatory cytokine-induced chemerin production. The relative contribution of liver and WAT to circulating chemerin levels may vary depending on stimulus and/or disease state: whereas elevated chemerin levels found in the blood of obese individuals may to a large extent originate from fat tissue, in liver cirrhosis, the liver may be primarily responsible for elevated plasma chemerin levels.

IL1β and IFNγ are additional proinflammatory cytokines that can modulate chemerin synthesis [58]. IL1β is one of a growing list of newly appreciated adipocyte-derived inflammatory mediators [59]. IL1β can also influence the metabolic function of adipocytes and may, for example, induce insulin resistance [60, 61]. Blocking IL1β improved glycemic control in patients with T2D [62] and delayed the development of T2D in experimental animals [63]. *In vitro*, IL1β increased chemerin protein concentrations approximately 1.3 fold in conditioned media from 3T3-L1 adipocytes and immortalized brown adipocyte tissue (BAT)-derived cells. TIG2 mRNA expression was up-regulated in these cells in a time- and dose-dependent manner as well [58]. IFNγ induced robust chemerin expression by fibroblast-like synoviocytes [64]. Elevated serum levels of IFNγ have been detected in psoriasis [65] or rheumatoid arthritis [66], diseases with postulated chemerin involvement [7, 24, 67, 68]. The cellular and molecular signaling pathways involved in IL1β- or IFNγ-mediated regulation of chemerin expression/secretion have yet to be defined, and it is unclear if this regulation occurs *in vivo*.

Moreover, although the reports referenced above largely focused on the effects of individual mediators on chemerin synthesis, it is likely that chemerin expression is controlled in concert by variety of factors operating in specific anatomic locations dependent on the particular ongoing pathophysiological process. A comprehensive list of mediators that regulate chemerin synthesis is shown in **Table 1**.

Posttranslational regulation of chemerin activity

Chemerin secretion

Chemerin secretion may be constitutive or regulated, and for most cell types the mechanism underlying this process is not well-characterized. As previously mentioned, modulation of TIG2 mRNA is not strictly accompanied by parallel changes in chemerin protein in conditioned media, suggesting that chemerin secretion might be subject to additional control. For example, the strong reduction in chemerin protein levels present in conditioned media of human adipocytes treated with the PPARγ agonist troglitazone was not reflected in TIG2 mRNA levels, which remained unchanged [54].

A

1 *MRRLLIPLALWLGAVGVGV*AELTEAQRRGLQVALEEFHKHPPVQWAFQETSVESAVDTPF

61 PAGIF**VRLEFKLQQTSCRKRDWKKP**ECKVRPNGRKRKCLACIKLGSEDKVLGRLVHCPIE

121 TQVLREAEEHQETQCLRVQRAGEDPHSF**YFPGQFAFS**KALPRS

149 154 157 163

B

CHEMERIN VARIANTS	NATIVE FORMS	ISOFORMS GENERATED BY SPECIFIC PROTEASES	REF
chem163S	plasma		[18]
chem158K	cerebrospinal fluid, synovial fluid, plasma	plasmin, tryptase	[18, 71]
chem157S	ascites	neutrophil elastase, cathepsin L and K, plasmin+carboxtpeptidase N and B, staphopain B	[3, 5, 6, 16-18]
chem156F	CHO conditioned media	kallikrein 7	[101, 105]
chem155A	serum, CHO conditioned media	tryptase, neutrophil elastase, proteinase 3	[18, 76, 105]
chem154F	hemofiltrate	chymase	[76, 105]

Figure 2. Functional regions in chemerin sequence. A: In the human chemerin primary amino acid sequence (NCBI Reference Sequence: NP_002880.1) the following regions are shown: functionally-evaluated chemerin peptides displaying antibacterial activity (green) or chemotactic activity (blue), inhibitory C-terminal region (red), signal peptide (italics), *in silico* predicted C-terminal basolateral sorting signal (underlined blue). B: Proteolytic generation of the indicated chemerin isoforms. Chemerin chemoattractant variants are shown in blue.

Likewise, the increase in chemerin protein levels detected in conditioned media in mouse primary adipocytes treated with TNFα was not reflected in TIG2 mRNA levels, which also remained unchanged [50]. While these examples suggest that adipocytes employ a mechanism to regulate the secretion of chemerin, it is possible that reduced chemerin immunoreactivity in cell conditioned media might result from extensive proteolytic processing of this protein (described in the next paragraph), or from sequestration of secreted chemerin by atypical chemerin receptor CCRL2, which concentrates chemerin on the cell surface and effectively removing it from the fluid phase being analyzed. Nevertheless, it is clear that in the case of anuclear platelets, chemerin is regulated at the level of secretion: chemerin protein is stored in preformed granules that are rapidly released upon platelet activation by thrombin, thrombin receptor-activating peptide, and collagen [16].

The molecular mechanism underlying chemerin secretion are not well-characterized, although it is likely that the protein is sorted via conventional cellular secretory pathways given its conserved consensus amino-terminal signal sequence. Human chemerin is synthesized as a 163-amino acid precursor, (pro-chemerin163S,

with number and capital letter referring to the terminal amino acid position and single amino acid code) (**Figure 2**). After the cleavage of the 20 aa-amino-terminal signal peptide (it is unknown if this occurs co- or post-translationally during translocation to the endoplasmic reticulum), chemerin is secreted as chemerin163S (chem163S). Some tissues, such as intestinal epithelium, might release chemerin in a polarized manner [25]. In enterocyte-like polarized Caco-2 cell monolayers, chemerin concentration varied between the basolateral side (representing the lamina propria) and the apical compartment that represents the intestinal lumen. The prevalence of chemerin in conditioned media collected from the basolateral side suggested a basolateral sorting signal. Whereas *in silico* analysis did not reveal any known apical sorting signal in chemerin sequence, a potential basolateral sorting signal was found in the carboxyl-terminal domain (**Figure 2**). However, this motif, containing a YFPG tetrapeptide with a β-turn conformation, is primarily associated with basolateral localization of transmembrane proteins rather than extracellular secreted proteins like chemerin [25]. Moreover, in contrast to fetal intestinal epithelium, chemerin was found on luminal side of mouse airway epithelium by immunohistochemistry [69], suggesting either that basolateral secretion is not a common feature of polarized epithelial cells or that chemerin localization in epithelial cells is regulated and dependent on cell type or origin.

Proteolytic processing

Although chemerin is subject to transcriptional regulation, posttranslational carboxyl-terminal processing plays a crucial role in determining chemerin bioactivity. Due to the well-described role of chemerin in supporting cell migration, chemerin bioactivity is usually determined based on its chemotactic activity and ability to trigger intracellular calcium mobilization. Chem-163S (**Figure 2**), abundantly present in circulation, is functionally inert. This form of chemerin contains a cystatin-like fold-containing domain and a labile C-terminus that can be cleaved by variety of proteases operating under physiological or pathological conditions (**Figure 2**). Proteolytic cleavage at one of several sites in the carboxyl-terminus of chem163S releases a terminal inhibitory peptide and results in chemoattractant activation [17, 18]. Truncated chem163S lacking 6 (chem157S), 8 (chem155A) or 9 (chem154F) amino acids in the C-terminus have been isolated from several human biological specimens, including ascites, serum and hemofiltrate, respectively [70]. In addition, chemerin devoid of 5 amino acids from C-terminus (chem158K) was reported as a dominant form in synovial fluids from arthritis patients and in cerebrospinal fluid from patients with glioblastoma [71]. Among all chemerin variants known, chem157S appears to be the most effective isoform in triggering chemotaxis of several types of immune cells [72], although chem156F, not yet reported in human biological fluids, is also biologically active [73]. Using chemerin isoform-specific ELISAs that detect chemS163, chem158K and chem157S, the majority of chemerin in inflammatory milieus is proteolytically processed [71]. Whereas normal human plasma contains less than 20% of chem158K and chem157S cleaved isoforms combined, in inflammatory cerebrospinal fluid and arthritis synovial fluid samples, the processed chemerin fraction is nearly 50% and 75%, respectively [71]. These findings indicate that chemerin undergoes extensive proteolytic processing *in vivo*, particularly during inflammation.

Whereas various cells are equipped with proteolytic mechanisms needed to process chemerin to its active isoforms [18, 74], suggesting local regulation of chemerin bioactivity, chemerin also serves as a substrate for systemic proteases, such as enzymes present in plasma.

Proteolytic processing of chemerin can be analyzed *in vitro* by incubation of recombinant chemerin or chemerin purified from natural sources with specific proteases. Using this approach, serine and cysteine proteases, characterized by the usage of serine or cysteine in their catalytic centers, respectively [75], were implicated in the generation of functional chemerin [5]. These enzymes include; serine proteases of the coagulation, fibrinolytic and inflammatory cascades such as neutrophil elastase and cathepsin G, mast cell tryptase and plasma-derived factor XIIa, VIIa and plasmin [17, 18], as well as host cysteine protease such as cathepsins K and L [5]. Plasma carboxypeptidases N and B, which remove the basic amino acids R or K from the C-terminus of proteins/peptides, were also reported to act in concert with plasmin in generation of the most

active chemS157 isoform form plasmin-produced chemK158 intermediate [16]. This example illustrates that chemerin can be proteolytically activated in direct or sequential fashion.

In addition to host proteases that regulate chemerin bioactivity under homeostatic or pathogenic conditions, chemerin activity is also controlled by proteases secreted by human pathogens. Staphopain B, a cysteine protease derived from *Staphylococcus aureus*, is a potent enzymatic chemerin activator that generates the chemS157 variant [6].

As much as proteases are needed to produce active chemerin isoforms, they may also inactivate or degrade the attractant and thus limit the extent of chemerin activity. Tissue-recruited neutrophils secrete chemerin-activating elastase and cathepsin G, but also release proteinase 3 that cleaves chem163S to chemotactically inactive chem155A [76]. Of note, this chemerin isoform differs from the most active chem157S by two C-terminal amino acids. Interestingly, the net result of chemerin proteolysis may depend not only on the interaction of proteases with unprocessed chem163S, but also with other chemerin variants, since chemerin processing intermediates are differentially targeted by proteases. For example, mast cell chymase does not use chem163S as a substrate, but abolishes the activity of functional variants chem157S and chem156F by conversion to the chem154F isoform [76].

The subtle amino acid sequence differences between biologically active and inactive chemerin variants raise the question of whether they compete for the same receptor binding site and can therefore influence each other's activity. This was partially addressed by using chem157S as a cell stimulant in the presence of the chem155A variant that itself shows poor activity by calcium flux assay [72]. Chem155A inhibited 50% of chem157S activity at a molar ratio of 100:1, demonstrating a weak antagonistic effect [72].

The chemerin analog nonapeptide ^{149}YFPGQFAFS^{157}, corresponding to the C terminus of chem157S (**Figure 2**), has been reported to retain most of the activity of the chem157S [73], although in our hands this peptide was approximately 100-fold less-potent than chem157S. Bioactive chemerin protein isoforms are more potent than their C-terminal peptide counterparts, suggesting that the N-terminal portion of the intact protein contributes to chemerin chemotactic activity. Given the isolation of various carboxyl-terminal chemerin variants from endogenous biofluids, most of the effort in defining the posttranslational regulation of chemerin has focused on C-terminal truncations. It is possible, however, that amino-terminal processing may also have a role in defining the bioactivity of chemerin. For example, pharmacological inhibition of aminopeptidases, enzymes that catalyze the cleavage of amino acids from the N-terminus of protein/peptide substrates, significantly increased the concentration of active chemerin in conditioned media from TNFα-treated 3T3-L1 adipocytes [74]. These data suggest that aminopeptidases cleave and inactivate chemerin via amino-terminal truncation. Since aminopeptidases are typically ubiquitous, their significance in regulating chemerin bioactivity requires further study.

Localization-dependent (receptor-based) regulation of chemerin availability

There are three known heptahelical receptors for chemerin: CMKLR1, GPR1, and CC-motif chemokine receptor-like 2 (CCRL2). The three chemerin receptors bind to chemerin with similar low nanomolar affinities [77, 78], but with substantially different functional outcomes. Chemerin binding to CMKLR1 triggers cell migration, intracellular calcium mobilization, and β-arrestin2 association and receptor internalization, all features common to classical G-protein coupled receptors (GPCRs), including most leukocyte-expressed chemoattractant receptors [3, 4, 70, 77, 78]. Chemerin binding to GPR1 triggers β-arrestin2 association and receptor internalization; whether or not chemerin induces GPR1-dependent intracellular calcium signaling is controversial [3, 77]. Furthermore, we found no evidence of chemerin-mediated GPR1+ cell migration (BAZ, unpublished). Thus GPR1 may function as a chemerin-binding 'interceptor', a class of attractant receptors including D6, DARC, CCX-CKR, and CXCR7 that binds, internalizes, and degrades chemoattractants, thereby regulating local attractant levels [79, 80]. Publically available microarray data from the GEO (Gene Expression Omnibus) database and EST (expressed sequence tag) data

from the UniGene database indicate that GPR1 RNA is expressed by placenta, reproductive tissues, skin, adipose tissue, skeletal muscle, and brain. However, it is not known if GPR1 protein is expressed by primary cells *in vivo* and therefore the physiological relevance of GPR1: chemerin interactions remain unclear. The third chemerin receptor, CCRL2, also functions to regulate chemerin concentrations, although the mechanism is thus far unique among heptahelical attractant receptors.

CCRL2 is an atypical chemerin receptor expressed by mast cells, activated macrophages and dendritic cells, and vascular endothelial cells [78, 81, 82]. Both human and mouse CCRL2 protein lack the consensus "DRYLAIV" motif in the second intracellular loop implicated in coupling to heterotrimeric G proteins (huCCRL2, "QRYLVFL"; mCCRL2, "QRYRVSF"), leading to the initial hypothesis that CCRL2 does not function as a classical chemoattractant receptor, Indeed, in contrast to CMKLR1, chemerin does not trigger CCRL2-mediated chemotaxis or intracellular calcium mobilization [78]. Although we found no sign of 'interceptor' activity (e.g. CCRL2-dependent chemerin internalization), CCRL2+ cells could sequester chemerin from solution [78]. Furthermore, chemerin binds to CCRL2 in an orientation that allows for the critical cell-signaling carboxyl-terminal domain of chemerin to remain accessible, as opposed to buried in the receptor binding pocket as is the case when bound to CMKLR1 [78, 81]. Based on these observations, we hypothesized that CCRL2 serves to regulate local concentrations of bioactive chemerin and present chemerin to adjacent CMKLR1$^+$ cells.

Given the expression of CCRL2 on vascular endothelial cells, we hypothesized that circulating chemerin levels may be elevated in CCRL2-deficient mice due to reduced intravascular sequestration. Indeed, plasma levels of total chemerin were slightly but significantly elevated in CCRL2-deficient mice. In mice dosed with endotoxin to induce systemic inflammation and vascular CCRL2 expression, this difference was substantially amplified as total chemerin plasma levels were 2-3-fold higher in CCRL2 KO mice versus WT mice, and 2-fold higher than untreated CCRL2 KO controls. Furthermore, pro-chemerin levels in CCRL2 KO plasma were significantly elevated compared with WT mice, perhaps indicating that CCRL2 is important in fixing pro-chemerin on the vascular EC surface to enable efficient proteolysis. Other cell surface receptors anchor their soluble ligand on the surface of ECs to enable enhanced proteolytic activation. Vascular endothelial cell-expressed endothelial protein C receptor, for example, binds and concentrates protein C, enabling a 20-fold acceleration in its activation by thrombin [83, 84]. Thus we conclude that CCRL2 regulates circulating chemerin levels and its proteolytic processing *in vivo* during systemic inflammation.

Chemerin in host defense

Host defense against pathogens is organized into layered barriers of increasing specificity. Skin is a first line mechanical barrier against infection, and keratinocytes within the skin secrete antimicrobial peptides (AMPs) such as chemerin that serve as an epithelial chemical shield against pathogens. AMPs target a wide array of microorganisms as divergent as fungus and bacteria (i.e. different kingdoms of origin) and can directly kill or inhibit cell growth. Certain AMPs like chemerin serve a second role in host defense as leukocyte attractants. Chemerin attracts cells of the innate immune system that can directly identify and eliminate pathogens, as well as produce cytokines important in priming the highly specific adaptive immune system.

Chemerin as regulator of immune cell trafficking and function

CMKLR1 is expressed by macrophages, DC subsets, and NK cells [4, 85-87], and in most cases these cells respond to chemerin with integrin activation, calcium signaling and chemotaxis [86, 88]. CMKLR1$^+$ leukocytes are multifunctional innate immune effector cells that can initiate pro-inflammatory or immune suppressive responses. The rapid proteolytic activation of chemerin at sites of mechanical barrier breech (e.g. bleeding, damage to cells) may serve to attract these early responder "immunointerpreters" to evaluate local conditions (e.g. sterile bruise vs. microbe invasion) and initiate an appropriate immune response. Thus, depending on the stimuli encountered by recruited CMKLR1$^+$ cells, which is largely dependent on the disease being investigated,

chemerin may exacerbate or ameliorate the local inflammatory response. The role of chemerin and CMKLR1 in a handful of inflammatory disease models has been determined empirically, typically using conventional CMKLR1-deficient mice or in some cases by adding exogenous chemerin to a particular anatomic location to influence cell recruitment. Complicating the interpretation of experimental outcomes in inflammatory disease models is the contemporaneous function of chemerin and CMKLR1 by non-immune cells (e.g. adipocytes). Experimentally-induced alterations in chemerin or CMKLR1 may impact metabolic processes, which may subsequently influence inflammatory disease models in unpredictable ways. Thus the development of conditional CMKLR1 or chemerin KO mice will be an important tool to probe attractant:receptor effects in isolation.

In experimental autoimmune encephalomyelitis (EAE), CMKLR1-deficient mice developed less severe clinical and histological EAE associated with a reduction in macrophage infiltration of the CNS [89]. In a model of cigarette smoke-induced chronic obstructive pulmonary disease (COPD), CMKLR1 KO mice developed less severe pulmonary inflammation associated with a reduction in PMN, DCs and CD4+ T cells infiltration into the airways [69]. In these models, CMKLR1 played a pathogenic role, implying that a pharmaceutical CMKLR1 antagonist may be beneficial in autoimmune demyelinating disease and COPD. In contrast, in a model of acute lung injury induced by LPS injection into the airways, CMKLR1 KO mice developed more severe pulmonary inflammation associated with increased infiltration of PMN into the airways [19]. Co-treatment with exogenous chemerin reversed this outcome. In an infectious model of viral pneumonia using pneumonia virus of mice (PVM, a model of human respiratory syncytial virus), CMKLR1 KO mice developed more severe pulmonary inflammation associated with an increase in PMN infiltration into the lung and airways [90]. Myeloid DC and macrophage infiltration into the lung were increased in the infected CMKLR1 KO mice, while pDC infiltration was reduced. The overall viral burden was elevated and viral clearance was delayed in PVM-infected CMKLR1 KO mice. Lung dysfunction and mortality was enhanced in the CMKLR1 KO mice as well. In a transplantable melanoma model, tumor-expressed chemerin inhibited *in vivo* tumor growth, which was associated with increased tumor-infiltrating NK cells and abrogated by NK cell depletion or CMKLR1-deficiency [91]. In these models, CMKLR1 played a protective role, potentially implying that chemerin itself or a pharmaceutical CMKLR1 agonist might be beneficial in improving acute lung injury, viral pneumonia, or in suppressing cancer.

In humans, the immunohistochemical detection of chemerin, CMKLR1, and leukocytes known to express CMKLR1 in diseased tissues imply a role for this attractant:receptor pair in immune cell recruitment to inflammatory sites. $CMKLR1^+$ pDCs were detected in lesional skin from patients with systemic lupus erythematosus, and the vascular endothelium in the sections stained positive for chemerin [87]. In patients with severe lupus nephritis, $CMKLR1^+$ pDCs were detected in the kidney parenchyma, and chemerin was detected in proximal tubular cells and lymphatic endothelium [51]. $CMKLR1^+$ pDCs were detected in the dermis in early psoriatic skin lesions and in prepsoriatic skin adjacent to active lesions, while chemerin was detected in dermal vascular endothelium, fibroblasts, and mast cells [24]. $CMKLR1^+$ pDCs and NK cells were detected in lesional buccal mucosa biopsies from patients with oral lichen planus, and chemerin was detected in the vascular endothelium as well as by scattered cells in the epithelium [85]. $CMKLR1^+$ DCs were detected in the leptomeninges and perivascular cuffs of chronic and active multiple sclerosis (MS) lesions, and the vascular endothelium in the sections stained positive for chemerin [92]. The accumulation of $CMKLR1^+$ effector cells in inflamed human skin, kidney, and CNS is largely associated with local blood vessel immunoreactivity for chemerin and thus consistent with their role in leukocyte recruitment.

Chemerin-dependent $CMKLR1^+$ macrophage recruitment to adipose tissue may contribute to the chronic, low-grade systemic inflammation associated with obesity and its sequelae. Adipose tissue is one of the major sites of chemerin expression, and the secretion of chemerin from adipose tissue increases with adipocyte differentiation and obesity (reviewed in [93]). There is a significant increase in macrophage infiltration in adipose tissue in obese

patients [94]. In chemerin-deficient mice, there was a significant reduction in macrophage accumulation in epididymal adipose tissue compared to WT (reduction in percent Mac3-positive cells) [47]. However, this effect was not recapitulated in CMKLR1-deficient mice, as there was no difference in percent macrophages in white adipose tissue versus WT [95]. It is possible that other chemerin receptors can compensate for the loss of CMKLR1 in coordinating macrophage infiltration into adipose tissue.

In addition to CMKLR1, there are a small number of *in vivo* studies that implicate chemerin receptor CCRL2 in leukocyte trafficking and the pathophysiology of inflammatory disease. Using CCRL2 KO mice, we found that the receptor is required for maximal ear swelling and leukocyte infiltration in passive cutaneous anaphylaxis (PCA), a mast cell-dependent *in vivo* allergy model [78]. Specifically, using mast cell-deficient animals engrafted with either WT or CCRL2 KO bone marrow-cultured mast cells, we showed that mast cell-expressed CCRL2 is required for optimal induction of PCA in mice sensitized with a low dose of antigen-specific IgE (high levels of sensitizing antigen abrogated this difference). In a model of ovalbumin (ova)-induced airway inflammation, CCRL2 KO mice were protected against pulmonary inflammation, which was associated with a defect in the trafficking of antigen-laden lung DC to mediastinal lymph nodes [35]. In the PCA model and the ova-induced airway inflammation models, it is not known whether the protection in CCRL2 KO mice is related to the role of CCRL2 as an atypical chemerin receptor.

As mentioned above, chemerin co-localized with vascular endothelial cells in MS, lupus, psoriasis, and in the endothelial venules of secondary lymphoid tissues [87]. *In vitro*, we showed that chemerin binding to CCRL2 on endothelial cells triggered robust CMKLR1$^+$ lymphoid cell adhesion dependent on $\alpha_4\beta_1$ integrin/VCAM-1 [81]. Furthermore, CMKLR1$^+$ NK cell recruitment to the airways was significantly impaired in CCRL2 KO mice following acute LPS-induced pulmonary inflammation *in vivo* [81]. CCRL2 expression by vascular endothelial cells thus provides a specific mechanism for the local enrichment of chemerin at inflammatory sites, regulating the recruitment of CMKLR1$^+$ cells.

Defensive function of chemerin in body barriers

Chemerin protein is abundant in a variety of normal epithelial cell types, including human and mouse epidermis [15, 19, 24], human fetal and mouse adult enterocytes [19, 25], and mouse epithelium lining the airways [19, 69]. By immunohistochemistry, the bronchial epithelium is the predominant source of chemerin in the normal mouse lung, likely responsible for the secretion of chemerin into the airways following cigarette smoke exposure [69]. Thus the strategic positioning of chemerin at body barriers alone suggests a role in antimicrobial defense.

Chemerin is predicted to share similar tertiary structure with antibacterial cathelicidins [3, 5]. Although a crystal structure is not yet available yet, consensus predictions [96] made on the basis of results returned by many popular fold recognition servers indicate a structural similarity between human chemerin (GI: 1971-00983) and folds characterizing the cystatin-like superfamily (Pfam Clan: CL0121). The two highest scoring structural templates were cystatin C, a member of the cystatins family (PDB entry 1CEW; Pfam ID: PF00031) and protegrin-3 precursor, a member of cathelicidins family (PDB entry 1KWI; Pfam ID: PF00666). Interestingly, the 3D structure of both template proteins are very similar to each other (**Figure 3A**) which is reflected in a relatively low value of RMSD (root mean square deviation), that after structural superposition (FAST algorithm, [97]) is about 0.181 nm. The alternative sequence alignments returned by fold recognition servers and collected by Genesilico.pl [98] indicate relatively low sequence identity (18-22%) between chemerin and both cystatin C and protegrin-3 precursor. Therefore, the produced homology models are labeled as plausible according to LiveBench evaluation [99] which indicates that the chemerin fold is most likely to be correctly predicted, but the conformation of loops and the precise location of boundaries of secondary structure elements are necessarily subject to adjustments once more detailed information becomes available.

Despite the predicted structural resemblance to cystatins, we found that chemerin does not act functionally as a cystatin, since it does not inhibit cysteine proteases, but instead serves

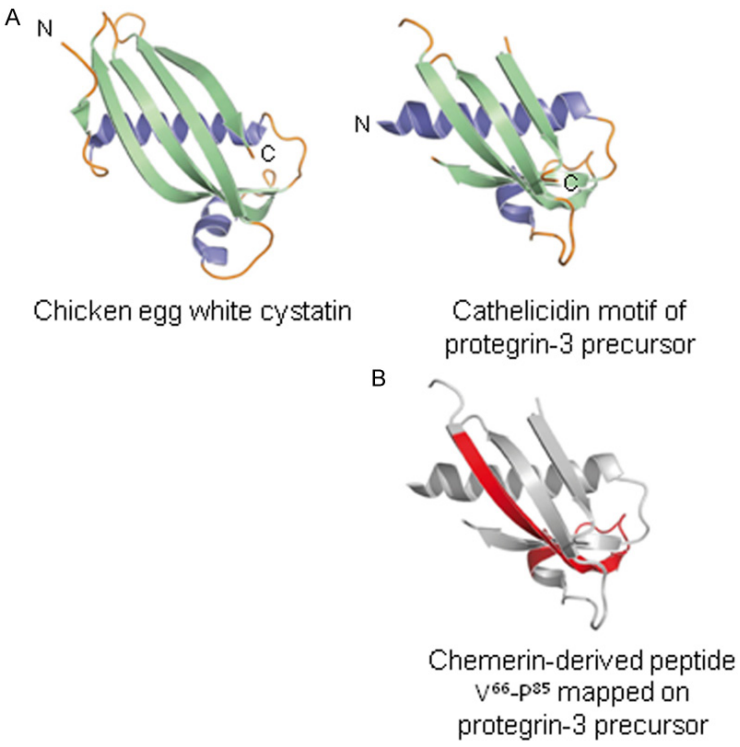

Figure 3. Location of anti-bacterial activity in the predicated chemerin structure. A: The 3D structure of chicken egg white cystatin (PDB entry: 1CEW) and porcine protegrin-3 precursor (PDB entry: 1KWI). Helices, β-strands, and loops are shown, respectively, in blue, green, and orange. N- and C-termini are marked with letters. B: V^{66}-P^{85} peptide (red) mapped on the secondary structure elements of porcine protegrin-3 precursor according to the alignment with human chemerin sequence constructed with hhsearch program [104]. The chemerin sequence VRLEFKLQQTSCRKRDWKKP (positions 66-85) aligns with the KPVSFTVKETVCPRPTRQPP sequence of protegrin-3 (75-94), which adopts a twisted β-strand that terminates at P88 and a four residue helical fragment starting with P92. The 3D structure of several discontinuous fragments of protegrin-3 remain unresolved.

as substrate for these enzymes [5]. However, in line with the predicted similarity to antibacterial cathelicidin, human recombinant chemerin as well as endogenous chemerin in exudates from organ cultures of primary human skin keratinocytes significantly inhibited bacteria growth [5, 15]. As is the case for chemoattractant activity, the inhibitory C-terminal peptide present in the chemerin holoprotein chem163S must be removed for full antibacterial effects. Using a panel of overlapping chemerin-derived synthetic peptides, we also demonstrated that chemerin antimicrobial activity can be largely narrowed down to an internal 20-amino acid long peptide V^{66}-P^{85}, which is comparable in potency to other AMPs [15]. Since this region is localized in the middle of the chemerin sequence (**Figures 2** and **3**), all chemerin isoforms truncated at the C-terminal end will likely be equipped with antimicrobial activity, despite their differing potentials to signal through CMKLR1. Indeed, chemotactically active chemS157 and chemotactically inert chemR125 have similar antimicrobial activity against *E. coli* [5]. Although it remains to be determined which chemerin isoform(s) are present in the epidermis, a pathogen-challenged epithelium will likely deliver specific protease(s) required to activate the antibacterial activity of chemerin [100]. Kallikrein 7, a serine protease constitutively expressed in skin and recently reported to convert chem163S to chem156F, is a likely protease candidate to generate active chemerin in this tissue [101]. Alternatively, epithelium-colonizing pathogens might themselves provide another source of proteinases capable of generating chemerin variants equipped with bactericidal potential. For example, chem157S generated by *S. aureus* would be expected to act as both chemoattractant and AMP. Although the growth of Gram-positive *S. aureus* is inhibited by chemerin peptide V^{66}-P^{85}, other microorganisms such as Gram-negative *E. coli* or fungus *Candida* are more sensitive to this peptide [15]. Therefore, *S. aureus,* via secretion of specific proteolytic enzymes such as staphopain B, may activate chemerin to create a relatively hospitable niche for itself and prevent displacement by other skin-colonizing microorganisms that are more sensitive to chemerin-mediated killing.

Keratinocytes are the main source of chemerin in healthy skin by immunostaining, while in chronically inflamed skin (e.g. psoriasis lesion) chemerin co-localizes with dermal infiltrating pDCs [20]. Psoriatic skin-derived chemerin stimulates CMKLR+ cell migration, indicating that chemerin is activated locally in the skin likely through neutrophil-derived proteases [7,

100]. Given the presence of chemerin-mediated attractant activity and chemerin-responsive pDCs in psoriatic dermis [7], this protein may contribute to the recruitment of pDCs to inflamed skin. Thus, the concerted action of chemerin and skin-associated proteases enables a dual role for chemerin in skin immunity as an AMP and chemoattractant: chemerin may directly contain microbes or act together with recruited immune cells to better control microbial infections.

Skin keratinocytes are not the only epithelial cell engaged in immune cell cross talk via chemerin. For example, chemerin is secreted by human fetal enterocytes [25]. Recruitment of macrophages to the intestinal mucosa is believed to play a role in clearance of luminal bacteria that breach the epithelium and gain access to the lamina propria. Given their phagocytic and bactericidal potential, intestinal macrophages might be particularly important in the intestine of premature infants who are predisposed to bacterial translocation due to a relatively permeable gut epithelium. Macrophage chemotaxis to conditioned media from fetal epithelial cells was chemerin-dependent, suggesting that chemerin may serve as a macrophage chemoattractant in fetal gut [25]. This chemotactic function in the developing intestine is consistent with the basolateral secretion of chemerin by epithelial cells to the subepithelial compartments [25].

Interestingly, having dual antimicrobial and chemotactic activities is not unprecedented for a chemoattractant, as many chemokines (such as CCL20, CXCL10 or CXCL14) have similar activities [102]. Due to constant microbial challenge the skin and gastrointestinal epithelium might be particularly suited to reveal the complexity of these multifunctional molecules in host defense.

Acknowledgements

This work was supported in part by the grant from the Foundation for Polish Science TEAM/2010-5/1, co-financed by the European Union within European Regional Development Fund and Polish National Science Center grant 0724/B/P01/2011/40 (to JC); and by DoD grant W81XWH-11-1-0512 and NIH grant R01 AI-079320 (to BAZ).

Disclosure of conflict of interest

None.

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