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New aspects of the C5a receptor

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ABSTRACT

The process of apoptotic cell death for maintenance of cell homeostasis is now believed to be flexible. To examine the mechanism for this flexibility, the process of programmed cell death is sometimes divided into three phases: initiation, effector and execution. We have demonstrated that apoptotic cells commonly express a *de novo* synthesized C5a receptor (C5aR), which belongs to the G protein-coupled receptor (GPCR) family. A natural agnostic ligand of the C5aR, C5a, is produced from plasma C5 by C5 convertase in the early phase of acute inflammation. Although it is not realistic, we found that C5a can adjust apoptotic cell lifespan long. We recently have read interesting reports that apoptotic cells can release natural agnostic ligands at the initiation phase and corresponding GPCRs are already expressed on cell surfaces of apoptotic cells. Conversely, we found that apoptotic cells commonly release an alternative antagonistic/agnostic ligand of the *de novo* synthesized C5aR, ribosomal protein S19 (RP S19) polymer. The RP S19 polymer can adjust apoptotic cell lifespan short. Importantly, the C5a-dependent regulation is limited by the C5aR sensitization, but the RP S19 polymer-dependent regulation is unlimited by the C5aR desensitization. Therefore, we suggested that apoptotic cells commonly release agnostic ligands in the initiation phase that should lengthen intermittently a period of the initiation phase. Next, apoptotic cells commonly release antagonistic/agnostic ligands in the effector phase that should continue shortening a period of the effector phase. In addition, we know that an inherited erythroblastopenia is associated with mutations in the *RP S*19 gene. However, the roles of RP S19 in the formation of erythroblast-macrophage islands are not clearly understood. We recently have found that a different arm that the RP S19 polymer has connects the *de novo* synthesized C5aR on erythroblasts and the generally expressed C5aR on macrophages. Therefore, we suggested that apoptotic cells commonly release antagonistic/agnostic ligands in the execution phase that should continue connecting apoptotic cells and macrophages in the execution phase for shortening a period of the execution phase. In this review, we introduce new aspects of the C5aR in apoptotic cells and discuss the effects of the long lifespan of apoptotic cell-like neutrophils on the development of periodontitis.

KEYWORDS

Adhesion; Apoptosis; C5a Receptor; Differentiation; G Protein-Coupled Receptor; Neutrophils; Periodontitis; Ribosomal Protein S19

1. INTRODUCTION

To maintain homeostasis, the fate of every cell is determined by programmed cell death [1]. Although macrophages mainly clear apoptotic cells before apoptotic cells can become secondarily necrotic, the progression of apoptosis remains unclear. We do not pathologically observe neutrophil migration into apoptotic cells. For the purpose of understanding the apoptotic process, programmed cell death can be divided into three phases: initiation, effector, and execution [2].

Surprisingly, apoptotic cells release chemoattractants such as fractalkine and sphingosine-1 phosphate and the nucleotides ATP and uridine triphosphate during the initiation phase for apoptotic cell clearance by macrophages at the execution phase [3-5]. These chemoattractants are called find-me signals. We suggested another role of find-me signals in the initiation phase. Their receptors are commonly coupled with G$\alpha i\beta\gamma$ protein (GiPCRs) and/or G$\alpha q\beta\gamma$ protein (GqPCRs), which are classical but not pure chemoattractant receptors [6]. Classical chemoattractant receptors commonly mediate chemotaxis

and secretion of at least macrophages and neutrophils, signaling through downstream extracellular signal-regulated kinase 1/2 (ERK1/2), and leading to an enhancement in cytoplasmic calcium mobilization. In contrast, pure chemoattractant receptors specifically mediate the chemotaxis of macrophages, signaling through an unknown downstream signal without an enhancement in cytoplasmic calcium mobilization. These effects suggest the presence of neutrophil-specific chemotaxis inhibitory molecules during the initiation phase.

To examine the roles of macrophages in the execution phase of apoptosis, we often consult the roles of macrophages in acute inflammation. The process of acute inflammation is sometimes divided into two phases, inflammation and resolution [7]. Lactoferrin, which is stored in primary granules inside neutrophils, commonly functions as an anti-microbial molecule during the inflammation phase by binding to lipopolysaccharides in the membrane of gram-negative bacteria [8]. Interestingly, the infiltrating neutrophils, which are classified as apoptotic cells, appear to synthesize lactoferrin *de novo* during the resolution phase, and the released lactoferrin functions in an autocrine manner as a neutrophil-specific chemotaxis inhibitory molecule [9]. Thus, lactoferrin appears to be one of the neutrophil-specific chemotaxis inhibitory molecules in both the inflammation and resolution phases of acute inflammation. For apoptotic cells, however, further examination will be required regarding monocyte-specific chemoattractants or neutrophil-specific chemotaxis inhibitory molecules during the initiation phase of apoptosis.

Lactoferrin released from neutrophils is also an anti-apoptotic signaling molecule functioning in an autocrine manner [10]. Thus, until now, it was thought that while GPCR agonists were associated with pro-chemotaxis and anti-apoptosis, GPCR antagonists were associated with anti-chemotaxis and pro-apoptosis. However, lactoferrin is both anti-chemotactic and anti-apoptotic. A fourth molecule demonstrating pro-chemotaxis and pro-apoptosis has yet to be discovered.

It is reasonable to believe that a long lifespan is an advantage for neutrophils in destroying invasive agents or particles during the inflammatory phase of acute inflammation. In addition, GPCR ligands reportedly can regulate and lengthen the lifespan of apoptotic cells [11]. GPCRs on macrophages for find-me signals are already expressed on apoptotic cells during the initiation phase. Therefore, we suggest that find-me signals may work as accumulation (un-spread) factors and/or regulators to lengthen the lifespan of apoptotic cells and neutrophils. Apoptotic cells and neutrophils need time to present apoptotic cues for macrophages on the cell surface.

We have demonstrated that apoptotic cell lifespan is partially regulated by a balance between survival signals emanating from GiPCRs and death signals coming from death receptors [12]. There are interesting reports that constitutively active GPCRs spontaneously change their conformations from inactive forms to active forms when stimulated by factors in their microenvironment [13]. The survival signals derived from constitutively active GPCRs without ligands would be weaker than those derived from GPCRs with ligands. However, a decrement in the survival signal from constitutively active GPCRs is not sufficient to shorten the lifespan of apoptotic cells including neutrophils. Therefore, we suggest that apoptotic cells decrease their own lifespan in an autocrine manner by *de novo* synthesis of GiPCRs and/or GiPCR antagonists (**Figure 1**). Apoptotic cells must be cleared by macrophages before presenting inflammatory cues to the cell surface.

Before becoming secondarily necrotic, apoptotic cells timely express adhesion molecules for bridging with macrophages to enhance the clearance efficiency of these macrophages. Apoptotic cells expose phosphatidylserine, or macrophages release milk fat globule-EGF factor 8 protein and expose alpha(v)beta(3) integrin [14]. Milk fat globule-EGF factor 8 can bind to apoptotic cells and macrophages via phosphatidylserine and alpha(v)beta(3) integrin, respectively. The up-regulated intercellular adhesion molecule 3 on apoptotic neutrophils was recently found to directly bind with alpha(L)beta(2) integrin on macrophages [15].

We collected synovial tissue from patients with rheu-

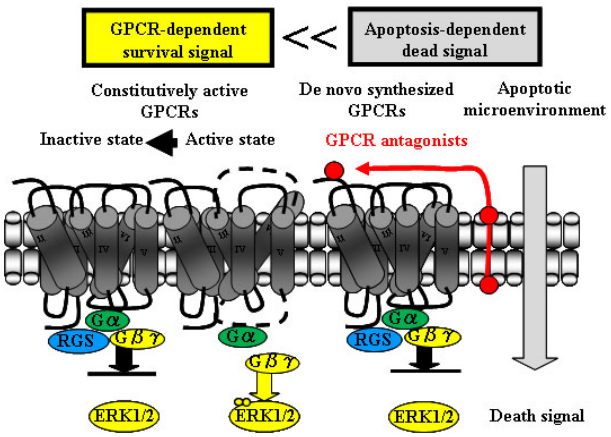

Figure 1. Pro-apoptosis during the late phase of apoptosis. When the microenvironment becomes apoptotic, cells express GPCRs and generate GPCR antagonistic ligands. These antagonists are released from the apoptotic cells and bind to their GPCRs in an autocrine manner. The GPCR-mediated signal transduction pathway induces RGS production. RGS immediately stops G protein-dependent ERK1/2 phosphorylation by an association of Gα and G$\beta\gamma$ subunits with not only the *de novo* synthesized GPCRs but also the constitutively active GPCRs. The intensity of the resulting apoptosis-dependent death signal is stronger than that of the GPCR-dependent survival signal.

matoid arthritis and found a predominant pathological monocyte accumulation [16]. To elucidate the mechanism for this accumulation, we have studied monocyte-specific chemoattractants for approximately 20 years. We previously purified the RP S19 polymer in rheumatoid arthritis-synovial tissue extracts [17]. Recently, we found that apoptotic cells commonly express the C5aR and generate the alternative C5aR ligand RP S19 polymer during the effector phase of apoptosis [18]. In the present review, we introduce new aspects of the C5aR in apoptotic cells and neutrophils and discuss the effects of the long lifespan of neutrophils on the development of periodontitis, similar to rheumatoid arthritis.

2. THE C5AR

The promoter region of the mouse C5aR gene possesses a CCAAT sequence that can be activated by the binding of nuclear factor Y (NF-Y) [19]. NF-Y recruits RNA polymerase II and induces *transcription of CCAAT box-containing genes*. We recently found the CCAAT-box in the promoter region of the human C5aR gene and the C5aR protein expressed in human promyelocytic leukemia HL-60 cells, human pancreatic cancer AsPC-1 cells, and mouse fibroblastic NIH3T3 cells at 12 hr after inducing apoptosis by using heat-shock, $MnCl_2$, and thapsigargin, respectively [12]. Therefore, we demonstrated that the *de novo* synthesis of C5aR is a common event in apoptotic cells, even though cells do not constantly express the C5aR gene.

The C5aR gene does not have splice variant mRNAs. This means that C5aR expresses the same structure on all cell types. We know that C5a is generally cleaved from C5, which is a plasma protein released from hepatocytes, by C5 convertase (C4b2a3b and C3b3bBb). C5a functions as a C5aR agonist in the inflammatory phase of acute inflammation. We observed an anti-inflammatory phenomenon in C5 deficient mice [20]. Therefore, we believe that the C5aR transmits a pro-inflammatory signal. In contrast, we observed a pro-inflammatory phenomenon in C5aR deficient mice [21]. These data suggest that the C5aR uses an alternative ligand to transmit anti-inflammatory signals.

2.1. The Classical C5aR Ligand

Based on the NMR structure of C5a and experiments mutating the C5aR [22], (the crystal structure of C5aR has not been produced yet), we suggest that the N-terminal positive charge cluster of C5a—His_{15}, Asp_{24}, Cys_{27}, Arg_{40}, Arg_{46}, and Ser_{66}—near the three disulfide bonds between Cys_{21}-Cys_{47}, Cys_{22}-Cys_{54}, and Cys_{34}-Cys_{55} first binds to the N-terminal negative charge cluster of C5aR—Asp_{15}-Asp-Lys-Asp_{18}. Then, the C-terminus of C5a, Leu_{72}-Gly-Arg_{74}, binds to Arg_{175}, Glu_{199}, Arg_{206}, or Asp_{282} for induction of the conformational change [23, 24]. Cook's and Hagemann's groups discuss the possibility of an interaction of a homo-polymer formation not only with the C5aR but also with the C5aR ligand [25]. The idea may lead to a correct answer about various signal transduction pathways mediated by the C5aR.

In addition, we think that the distance between the first and second binding moieties is important for C5a binding to the C5aR in the purposed two-step binding theory. We re-modeled the solution structure of C5a (MMDB ID: 73492, PDB ID: 1CFA) utilizing the Molecular Operating Environment software system [26]. The resulting 22.6 Å distance from His_{15} to Gly_{73} may be the key to discovering the correct binding structure of C5aR.

2.2. The Classical Signal Transduction Pathway for the C5aR

When the GDP form of the Gαi2 subunit is exchanged with the GTP form via the guanidine exchange factor activity of C5aR, the G$\beta\gamma$ subunits commonly interact with phospholipase Cβ2 (PLCβ2) at the EF hand domain (Ile_{141}-Lys_{176}) in a calcium-dependent manner for converting phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphates (IP3) and diacylglycerol [27]. However, the source for the calcium interacting with PLCβ2 is still unclear. For the P2Y (GqPCR) ligand ATP, which is released from apoptotic cells at the initiation phase, a source of calcium is the extracellular calcium entering the cell through the ligand-gated ion channels P2Xs [3]. After the binding of IP3 to the IP3 receptor expressed on the endoplasmic reticulum, intracellular calcium is released, and the calcium-released activation of the calcium channel expressed on the plasma membrane is then opened for activation of protein kinase C [28]. Both the phosphatidylinositol 3-kinase signal and the ERK1/2 signal are transmitted through the small GTP binding protein Ras and the MAPK kinase Raf. Meanwhile, the cytoplasmic phospholipase A2 (cPLA2) activated by extracellular calcium through calcium-released activation of the calcium channel hydrolyses arachidonic acid for an extension of the cytoplasmic calcium oscillation induced by the extracellular calcium (**Figure 2** left).

3. AN ALTERNATIVE C5AR LIGAND

RP S19 is cross-linked between Lys_{122} and Gln_{137} by type II tissue transglutaminase (TGII) in apoptotic cells at the effector phase of apoptosis and by activated coagulation factor XIII on platelets at the resolution phase of acute inflammation [29]. The RP S19 polymer-induced chemotaxis is inhibited by a C5aR antagonistic peptide [18, 30,31]. Based on experiments mutating RP S19, we found that an N-terminal positive charge cluster, Lys_{38}-Lys_{43}, is the first binding moiety and Leu_{131}-Asp-Arg_{133} is

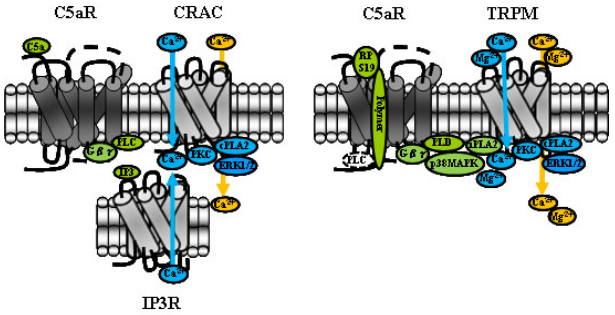

Figure 2. The C5aR-mediated cytoplasmic calcium oscillation. Left: The C5a-induced C5aR-mediated cytoplasmic calcium oscillation is extended by both the intracellular calcium through the inositol 1,4,5-triphosphate receptor (IP3R) and the extracellular calcium through calcium-released activation of the calcium channel (CRAC). Right: The RP S19 polymer-induced C5aR-mediated cytoplasmic calcium oscillation is extended by the extracellular calcium through calcium and/or magnesium channels (TRPM).

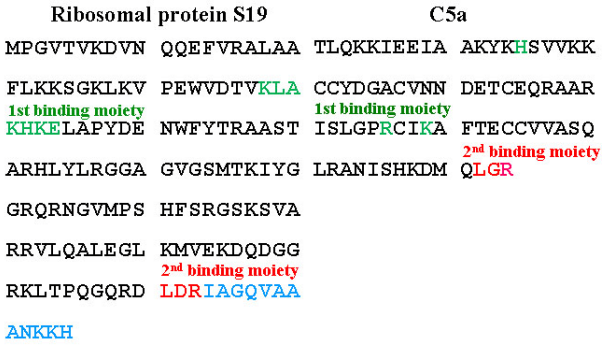

Figure 3. Distinct amino acid sequences for RP S19 and C5a. After the Lys_{38}-Lys_{43} moiety of RP S19 binds to the C5aR, the Leu_{131}-Asp-Arg moiety enters the active pocket of the C5aR. The Ile_{134}-His_{145} switches the RP S19 polymer-dependent function from agonistic to antagonistic.

the second binding moiety. Although the RP S19 monomer does not cross-link with the anti-C5a antibody and never binds to the C5aR, the RP S19 polymer reacts with the anti-C5a antibody in western blot analysis and competitively inhibits the binding of ^{125}I-labeled C5a to C5aR [30]. We constructed a tertiary structural model of RP S19 base on the tertiary structural data of RP S19 from *Archaebacterium* by utilizing the Molecular Operating Environment software system and by studying the crystal structure of RPS19 obtained from *Pyrococcus abyssi* [26,32]. We determined that the distance between the first and second binding moieties of the RP S19 molecule is 35.71 Å. However, the distance from His_{52} on one RP S19 molecule to Asp_{132} on another RP S19 molecule in the RP S19 polymer is adjusted to be 22.6 Å. These results demonstrate that the RP S19 polymer is the alternative C5aR ligand.

Important questions remain regarding when and where the RP S19 monomer is cross-linked by TGII to predominantly attract macrophages. Human promyelocytic leukemia HL-60 cells and human pancreatic cancer AsPC-1 cells increase TGII activity at 12 hr after the induction of apoptosis by heat-shock [33,34]. In the same experimental setting, the monocyte chemotactic activity in each culture supernatant is increased at 24 hrs. Moreover, we recently demonstrated the production of the RP S19 polymer in apoptotic cells and a release of the RP S19 polymer in culture supernatants by western blot analysis with the anti-C5a antibody [12,35].

The amino acid sequence for C5a is distinct from that of RP S19 in that RP S19 has 12 additional amino acid residues on the C-terminus (**Figure 3**). We prepared recombinant C5a, RP S19 polymer and C-terminus deficient RP S19 polymer [36] and found that although the RP S19 polymer attracts macrophages but not neutrophils, the C-terminus deficient RP S19 polymer attracts both macrophages and neutrophils with a chemotactic potency similar to C5a. These results were validated by using guinea pig skin because human C5a and RP S19 polymer can bind to the guinea pig C5aR [37]. In addition, we confirmed these results using the C5a peptide, Tyr-Ser-Phe-Lys-Asp-Met-Gln-Leu-Gly-Arg, the RP S19 peptide, Ac-Gly-Gln-Arg-Asp-Leu-Asp-Arg-Ile-Ala-Gly-Gln-Val-Ala-Ala-Ala-Asn-Lys-Lys, and the C-terminus-deficient RP S19 peptide, Ac-Gly-Gln-Arg-Asp-Leu-Asp-Arg-Ile-Ala-Gly-Gln-Val-Ala-Ala-Ala-Asn. Interestingly, the results obtained by using these peptides demonstrate that although the RP S19 monomer protein does not bind to the C5aR, the RP S19 peptide attracts macrophages but not neutrophils. Thus, we strongly suggest that the distance between the first and second binding moieties of RP S19 to the C5aR plays a major role in the two-step binding theory. We also prepared recombinant C5a and C5a/RP S19, which is the 12 amino acids on the C-terminus of RP S19 connected to the C-terminus of the Gly73Asp-mutant C5a [38]. Although C5a attracts both macrophages and neutrophils, C5a/RP S19 attracts only macrophages. These results were validated by using guinea pig skin. Further study will be needed to elucidate the roles of the RP S19 polymer in the effector phase of apoptosis.

3.1. An Alternative Signal Transduction Pathway Mediated by the Monocyte C5aR

The RP S19 polymer binds to the monocyte C5aR and shifts a G$\beta\gamma$ subunit-dependent downstream signal from ERK1/2 pathway to p38 mitogen-activated protein kinase (p38MAPK) pathway independent of intracellular (endoplasmic reticulum) calcium release [39]. The p38MAPK signal is artificially produced by C5a stimulation following simultaneous treatment with phosphatidylinositol 3-kinase and PLC inhibitors (LY294002 and

U73122) at concentrations equal to half of their respective IC_{50} values. We also determined that the RP S19 C-terminus interacts with the Gla domain of prothrombin at low calcium concentrations [40]. These data indicate that the RP S19 C-terminal $Lys_{143}Lys_{144}$ moiety acts as a competitor against calcium for the activation of PLC $\beta 2$ in macrophages. There is an interesting report that phospholipase D (PLD) downstream of the interaction of the $G\beta\gamma$ subunits with ADP-ribosylation factor exchange factors not only catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid and choline but also drives the p38MAPK pathway [41,42]. We found that the RP S19 polymer-induced p38MAPK phosphorylation upstream of both alternative extracellular calcium oscillation and cell migration was inhibited by the PLD inhibitor 5-fluoro-2-indolyl des-chlorohalopemide (unpublished data), indicating a participation of PLD in the $G\beta\gamma$ subunit-dependent p38MAPK pathway mediated by the monocyte C5aR (**Figure 2** right). Importantly, C5aR internalization is not required in the PLD-dependent pathway. Further studies will be required to determine the mechanism for the monocyte C5aR ligand-specific selection of second messengers.

It has been reported that a reciprocal activation between calcium-independent PLA2 (iPLA2) and cPLA2 at the front side of cells affects the maximum potency of cell migration [27]. We confirmed that a non-specific PLA2 inhibitor is efficient for creating cytoplasmic calcium oscillation by extracellular calcium through extracellular calcium and/or magnesium channels including the transient receptor potential melastatin 7 channels in human mast HMC-1 cells, human peripheral blood macrophages and HL-60-derived macrophage-like cells [18]. The ready state of iPLA2 bound by calmodulin at hydrophobic anchor residues (Val_{691}-Phe_{709}) is altered to the active state via the replacement of calcium from the EF hand domains [43]. The ready state of the transient receptor potential melastatin 7 channel is also maintained at least in part by the binding of calmodulin to the hydrophobic anchor residues of Met_1-Arg_{14} [44,45]. Again, these data indicate the presence of a specific ligand-gated ion channel for the C5aR ligands.

3.2. An Alternative Signal Transduction Pathway Mediated by the Neutrophil C5aR

The C-terminus of RP S19 binds in an autocrine manner to a cell type-specific molecule in addition to the C5aR generally expressed on neutrophils and the C5aR synthesized *de novo* on apoptotic cells [12]. The complex of the RP S19 C-terminus with the cell type-specific molecule blocks the interaction between the $G\alpha i\beta\gamma$ protein and any second messenger. However, C5a can partially delay the apoptotic process in neutrophils via an activation of the ERK1/2 pathway [46]. In contrast, we showed that the RP S19 polymer promotes the apoptotic process in apoptotic cells [12,35]. The RP S19 polymer induces the *de novo* synthesis of the regulator of G protein signaling 3 (RGS3) during the effector phase of apoptosis. The apoptotic process is extended by neutralizing the RP S19 polymer with an anti-RP S19 antibody or by blocking the apoptotic C5aRs with C5aR antagonistic peptides. The ERK1/2 signal in HL-60 cells is strengthened in an RGS3 knockdown and weakened by RGS3 overexpression. Moreover, the regulation of RGS3 expression is efficient in HL-60 cells and inefficient in human monocytic cancer THP-1 cells. The HL-60 cells or THP-1 cells can differentiate into neutrophil-like cells or monocyte-like cells by treatment with dimethyl sulfoxide or phorbol 12-myristate 13-acetate, respectively. Therefore, we suggest that the RP S19 polymer-dependent pro-apoptotic signal is specifically increased in apoptotic cells including neutrophils. The interaction of the apoptotic cell type-specific molecule with the RP S19 polymer likely induces the generation of RGS3 (**Figure 4**). We hypothesize that the RP S19 polymer plays a role in forming apoptotic cell-macrophage islands unlimited by C5aR internalization that initiate efficient processing of apoptotic cell clearance without any inflammatory cues as part of the cell maintenance mechanism.

3.3. An Alternative Signal Transduction Pathway Mediated by the Erythroblast C5aR

In a recently reported case of Diamond black-fan anemia, an inherited erythroblastopenia was associated with mutations in the *RP S19* gene [47]. Matsson *et al.* suggested a role for the constitutive pro-apoptotic signal through a

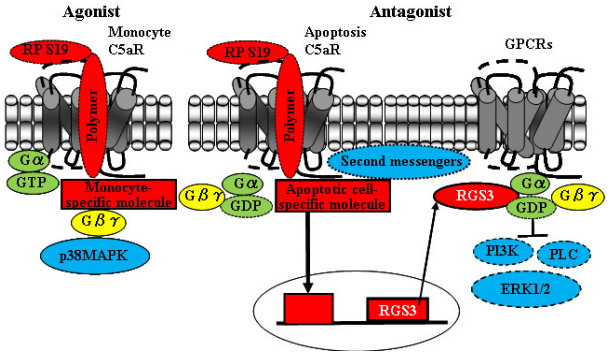

Figure 4. The RP S19 C-terminus-dependent duel agonistic and antagonistic mechanisms. Agonist; an RP S19 polymer is bound to a monocyte C5aR, and the Gi protein interaction with second messengers is partially blocked by the RP S19 C-terminus, resulting in signal transduction mediated through the p38 MAPK pathway. Antagonist; an RP S19 polymer is bound to a neutrophil C5aR, and the Gi protein interaction with second messengers is completely blocked by the RP S19 C-terminus-apoptotic cell-specific molecule complex. The apoptotic cell-specific molecule promotes RGS3 expression.

defect in the ribosome formation mediated by the mutant RP S19. However, the roles of the erythroblast stage-specific activation of pro-apoptotic signals and the formation of erythroblast-macrophage islands under normal conditions are not clearly understood. Recently, we found that human erythroleukemia K562 cells synthesize C5aRs *de novo* and release RP S19 polymers during hemin-induced erythropoiesis [48]. The K562 cell differentiation is stopped before the formation of erythroblast-like cell-macrophage islands by either neutralization of the RP S19 polymer with an anti-RP S19 antibody or blockade of the C5aR with C5aR antagonistic peptides. These data indicate that the RP S19 polymer plays a role in forming erythroblast-macrophage islands via a reciprocal expression of C5aRs unlimited by C5aR internalization that ensures efficient differentiation and apoptotic cell clearance without any inflammatory cue as part of the cell maintenance mechanism. To validate our new idea, we are now developing RP S19 polymer-dependent functionally deficient mice.

4. CHEMOTACTIC GIPCRS IN APOPTOSIS

We previously examined the CCAAT sequence at −75 bp in the promoter region of the C5aR gene (NM_001736.3) that is bound by the apoptosis-induced activated transcription factor NF-Y. We demonstrated that the apoptotic cell lifespan is shortened by the RP S19 polymer-dependent generation of RGS3, which decreases ERK1/2 phosphorylation via *de novo* synthesized GiPCRs through re-association of the Gαi and G$\beta\gamma$ subunits of the G$\alpha i\beta\gamma$ protein (**Figure 4**). To confirm our hypothesis, we searched for CCAAT sequences in the *fMet-Leu-Phe* (*fMLP*) *receptor* (*FPR*) gene and the *leukotriene B*4 (*LTB*4) *receptor* (*BLT*) gene.

In addition to FPR1, there are two other receptor subtypes, FPR2 and FPR3 [49]. The function of FPR2 on the plasma membrane is similar to the prototypical receptor FPR1. Trp-Lys-Tyr-Met-Val-Met is a selective agonist of FPR2, which is an acetylated amino-terminal peptide derived from the cleavage of the human heme-binding protein [50]. The coupling of the FPR2 with G$\alpha i\beta\gamma$ protein is also similar to that of the FPR1. However, the downstream signal pathways for the FPR2 remain a mystery, indicating the need to find or develop additional FPR2 ligands. An interesting study reported that the N-terminus of annexin 1 (Ala_2-Lys_{26}) functions as an FPR antagonist in the effector phase of apoptosis in neutrophils [51]. The FPR3 binds the same FPR2 ligand; however, the FPR3 is coupled with the G$\alpha 16\beta\gamma$ protein and localized on small intracellular vesicles. We found that CCAAT sequences are located at −548 bp, −859 bp, and −524 bp in the promoter region of the *FPR*1 gene (NM_002029.3), *FPR2* gene (NM_001462.3), and *FPR*3 gene (NM_002030.3), respectively. The distance between a binding sequence of a transcription factor and a start codon is inversely related to the intensity of the gene expression. Therefore, further study is needed examining the relationship between FPR and pro-apoptosis.

The BLT receptor is divided into two subtypes, BLT1 and BLT2 [52]. Their homology is 45.2%. The affinity of LTB4 is 20 times lower than that of LTB1 for the BLT2 receptor subtype. In contrast to the BLT1 receptor, the BLT2 receptor appears to activate at least three Gα subunits, Gαi, Gαq-like, and Gαz. The loose selection mechanism of the BLT2 receptor for the G$\alpha\beta\gamma$ proteins makes it difficult to recognize the roles of this receptor subtype in inflammation and apoptosis. We found that the CCAAT sequence is located at −143 bp or -54 bp in the promoter regions of the *BLT1* gene (NM_181657.3) or *BLT*2 gene (NM_019839.4), respectively. We also determined that the pro-apoptotic potency of HL-60 cells induced by $MnCl_2$ is partially suppressed by the simultaneous presence of LTB4 (unpublished data). We suggest that apoptotic cells express BLT receptors and release an unknown BLT antagonist in an autocrine manner for promoting pro-apoptosis, as previously shown for the C5aR.

5. THE C5AR IN PERIODONTITIS

Distinct pathological findings such as continuously acute inflammation in articular cavities and chronic inflammation in synovium are observed in patients with rheumatoid arthritis [53]. Similarly, continuously acute inflammation in periodontal pockets and chronic inflammation in junctional epithelium and periodontal membranes are observed in patients with periodontitis. The gram-negative bacteroides such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* have long been studied as a potential cause of periodontitis [54,55]. Bacteroides are commonly found not only in periodontal pockets but also in the junctional epithelium where they directly digest fibroblasts of the junctional epithelium and the periodontal membranes by an enzymatic function of collagenases. Importantly, lipopolysaccharide, which is a membrane component of the gram-negative bacteroides, induces the production of several types of cytokines in macrophages, such as tumor necrosis factor α, interferon α/β, interleukin-6 and interleukin-17, that shift acute inflammation to chronic inflammation. We have discussed in depth the macrophage-modified mechanisms for development of chronic inflammation. In contrast to macrophages, excessive activation of the infiltrating neutrophils is found in periodontal pockets [56]. We have not fully discussed the neutrophil-modified mechanisms for the change from acute to chronic inflammation.

Therefore, in this section of the review we examine

published reports to study the potential effects of bacteroides on the neutrophil C5aR. *Porphyromonas gingivalis* generates an arginine-specific cysteine proteinase and a lysine-specific cysteine proteinase. The arginine-specific cysteine proteinase cleaves an N-terminal C5a fragment from plasma C5 [57]. Wingrove *et al.* suggested that there is a C5a-dependent predominant infiltration of neutrophils into periodontal pockets. The lysine-specific cysteine proteinase cleaves an N-terminal fragment of the C5aR, Pro_9-Thr_{29} [58]. The cleaved fragment contains an Asp_{15}-Asp-Lys-Asp_{18} moiety, which is the first binding site of C5a on the C5aR. We think that the RP S19 polymer but not C5a can function in the periodontal pocket. However, the binding affinity of the neutrophil C5aR in the circulation is lower than that in the periodontal pocket, resulting in a weak apoptotic signal induced by the RP S19 polymer and offering one reason why the neutrophils infiltrating the periodontal pocket gain excessive activity.

In addition, *Tannerella forsythia* generates a metalloproteinases karilysin and prevents classical and lectin two complement systems by the efficient degradation of mannose-binding lectin, ficolin-2, ficolin-3 and C4 [59]. Interestingly, karilysin cleaves C5a from plasma C5. Jusko *et al.* focus on the C5a-induced migration of neutrophils. In contrast, we suggest a cleavage of the neutrophil C5aRs in the gingival pocket by *Tannerella forsythia*, as previously shown for *Porphyromonas gingivalis* [58].

Aggregatibacter actinomycetemcomitans generates two exotoxins, a cytolethal distending toxin and a leukotoxin [60]. The cytolethal distending toxin directly promotes cell cycle arrest at both the G1 and G2/M phases in gingival fibroblasts and periodontal ligament cells [61]. Leukotoxin, a large pore-forming protein that belongs to the Repeat in Toxin family, induces apoptosis via the lymphocyte function associated receptor 1 (LFA-1) of neutrophils. The LFA-1 functions as an adhesion molecule, binding to intercellular adhesion molecule 1 on antigen-presenting cells for promoting proliferation/differentiation of T cells. We are interested in the different signal transduction pathways mediated by the same LFA-1 but in a cell type-specific manner, as previously shown for the C5aR [39,48]. It is very complicated, because the oral manifestation appears to change with the type and quantity of bacteria in a circadian rhythm.

We developed an experimental rat model of gingivitis (ODUS/Odu) derived from the Wistar-Kyoto strain [62]. Five bacteroides, *Haemophilus actinomycetemcomitans Y*-4, *Bacteroides gingivalis* 381, *Bact. intermedius ATCC* 25261, *Capnocytophaga sp. M*-12 and *Eikenella corrodens ODU*, have been detected in extracts of rat dental plaque [63]. We are very interesting in the enzymatic effects of the above listed bacteroides on C5a and the neutrophil C5aR.

6. CONCLUSIONS

In this review, we demonstrate that apoptotic cells commonly release agnostic ligands in the initiation phase that should lengthen intermittently a period of the initiation phase via the generally expressed GPCRs in an autocrine manner. Next, apoptotic cells commonly release antagonistic/agnostic ligands in the effector phase that should continue shortening a period of the effector phase via the *de novo* synthesized GPCRs in an autocrine manner. The antagonistic/agnostic ligands in the execution phase should continue connecting apoptotic cells and macrophages in the execution phase via the GPCRs for shortening a period of the execution phase.

We discuss the enzymatic effects of the bacteroides in periodontitis at least on C5aR, FPR and BLT of neutrophils. The process of apoptotic cell death for maintenance of cell homeostasis should be wrong. We believe that the neutrophil C5aR is one of the therapeutic targets for periodontitis.

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