Potential applications of components of aged garlic extract in mitigating pro-inflammatory gene expression linked to human diseases (Review)

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Abstract. In the present review, simple approaches for the screening and characterization of natural compound agents that alter pro-inflammatory gene expression are described, with a particular focus on aged garlic extract (AGE), which has been the subject of several investigations that have supported its potential application as an anti-inflammatory agent. Additionally, evidence regarding the possible effects and mechanisms of action of two major AGE components, S-allyl cysteine (SAC) and S-1-propenyl-l-cysteine (S1PC), is reviewed. The proposed molecular targets of SAC and S1PC are IKKβ kinase, the Kelch-like ECH-associated protein 1-nuclear factor erythroid 2-related factor 2 complex, peroxisome proliferator-activated receptor-γ, histone deacetylase and toll-like receptor 4 (TLR4). Targeting these molecules causes a marked reduction in NF-κB activity accompanied by a notable decrease in the transcription of NF-κB-regulated genes. Another main objective of the present review was to discuss the possibility that AGE and its bioactive components could be employed in the treatment of several human pathologies that are characterized by a hyperinflammatory state resulting from dysregulation of the TLR4 and NF-κB pathways. SAC is of interest in the treatment of lung pathologies, neurological diseases, osteoarthritis, muscular atrophy, cardiovascular diseases, diabetes and cancer. Additionally, the anti-oxidative activities of AGE, SAC and S1PC are compatible with their employment in the treatment of diseases characterized by oxidative stress, such as sickle cell disease and β-thalassemia.

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Abbreviations: AGE, aged garlic extract; SAC, S-allyl cysteine; S1PC, S-1-propenyl-l-cysteine; COVID-19, Coronavirus Disease-2019; SARS-CoV-2, severe acute respiratory syndrome corona virus 2; COPD, chronic obstructive pulmonary disease; TLR, toll-like receptor; PAO, *Pseudomonas aeruginosa*; ROS, reactive oxygen species; HDAC, histone deacetylase

Key words: AGE, inflammation, IL-8, TLR4, NF-κB

Contents

1. Introduction

The present review is centered on the characterization and biomedical applications of natural compounds, with a particular focus on aged garlic extract (AGE). Commercially available AGE is an odorless preparation obtained by immersing fresh garlic slices in an aqueous ethanol solution for several months at room temperature (1-5). A number of studies have supported the possible employment of AGE as an anti-inflammatory agent (6-10). In this context, the availability of simple experimental approaches for the screening and characterization of molecules that interfere with pro-inflammatory gene expression is crucial for advancing the knowledge on anti-inflammatory agents present within

AGE. An introductory example of experimental cellular model systems of interest in the field of respiratory diseases is shown in Fig. 1. These experimental systems may be useful in generating datasets that support the possible employment of validated agents for the treatment of human pathologies characterized by a hyperinflammatory state. The study of pro-inflammatory genes is of notable importance since the expression of these genes is critical in the development of a number of diseases, such as COVID-19, cystic fibrosis and chronic obstructive pulmonary disease (COPD). Table I reports examples of relevant pro-inflammatory genes that have been targets for anti-inflammation therapy in pre-clinical or clinical studies (11-23). For instance, interleukin (IL)-8 antagonists and monoclonal antibodies against IL-8 have been proposed for the therapy of cystic fibrosis (11,12), COPD (13), severe asthma (14) and osteoarthritis (15). Additionally, anti-IL-6 therapy has been proposed for COVID-19, long-COVID and post-acute sequelae of SARS-CoV-2 infection (16-18). Furthermore, Toll-like receptor 4 (TLR4) inhibitors may be considered for treating COVID-19 (19-21), osteoarthritis (22) and other inflammation-related diseases, such as cystic fibrosis (23).

The present review will discuss experimental model systems and their application in characterizing the activity of AGE and selected components. Then, recent evidence regarding the possible effects and mechanism of action of two major a sulfur-containing AGE components, S-allyl cysteine (SAC) (24,25) and S-1-propenyl-l-cysteine (S1PC) (26), will be reviewed. In this respect, SAC and S1PC showed stable properties under cell culture conditions, and their acute/subacute toxicity was minor in mice and rats (24,26). In particular, the pharmacokinetics of SAC was investigated on human volunteers, indicating that SAC was rapidly absorbed from the gastrointestinal tract (24).

Finally, the possible application of AGE, SAC and S1PC in the development of protocols for possible therapeutic interventions will be discussed.

2. Experimental model systems to identify and characterize inhibitors of pro-inflammatory genes

The cell models used for testing inhibitors of pro-inflammatory genes are typically mammalian cells infected with a microorganism, treated with a viral protein or treated with other agents stimulating pro-inflammatory genes. Table II provides a representative list of experimental models that have been used to characterize the effects of different classes of inhibitors of pro-inflammatory genes. One of the most used and well-characterized cell models is bronchial epithelial IB3-1 cells infected with *Pseudomonas aeruginosa*. Detailed descriptions of this experimental model system can be found in the publications by DiMango *et al* (27), who reported the origin of the IB3-1 cell line (derived from a cystic fibrosis patient with a DF508/W1282X genotype) and the protocols for infection with *P. aeruginosa*. The non-mucoid laboratory strain of *P. aeruginosa*, PAO1, is typically employed for infection. In the publication by DiMango *et al* (27), information can be found on PAO1 culturing and on the experimental conditions to prepare aliquots to stimulate IB3-1 cells. One of the most relevant effects of the *P. aeruginosa* infection of IB3-1 cells is the induction of a pro-inflammatory phenotype marked by the activation of several pro-inflammatory genes, the most relevant being the IL-8 genes. The role of transcription factors in the *P. aeruginosa* infection of IB3-1 cells was first reported by DiMango *et al* (27), who described NF-κB activation in this model. The cooperative action of the transcription factors NF-κB, NF-IL6, activator protein 1, CHOP and cAMP responsive element binding protein, in the *P. aeruginosa*-dependent induction of IL-8 gene transcription in IB3-1 cells (27) was discussed in depth by Bezzerri *et al* (28). Notably, using this experimental model system, Finotti *et al* (29) demonstrated that decoy oligonucleotide molecules targeting NF-κB were able to inhibit the transcription of the IL-8 gene. Furthermore, using a chromatin immunoprecipitation assay, these authors found that the NF-κB decoy was able to strongly inhibit NF-κB recruitment to the IL-8 gene promoter (29). A microarray-based study that demonstrated the involvement of microRNAs in the *P. aeruginosa* infection of IB3-1 cells was reported by Fabbri *et al* (30), providing new information on possible employment of microRNA targeting for the development of therapeutic protocols for cystic fibrosis. Several studies have been performed using this system to demonstrate the potential anti-inflammatory properties of medicinal plant extracts or low-molecular weight drugs (31-33). For instance, Lampronti *et al* (31) reported the notable effects of *Nigella arvensis* extracts, which inhibit IL-8 expression in *P. aeruginosa*-infected IB3-1 cells. Furthermore, it was demonstrated that β-sitosterol is the compound responsible for this activity, while stigmasterol and campesterol are inactive in this context. These results support the conclusion that *P. aeruginosa*-infected IB3-1 cells serve as a suitable experimental model system to identify inhibitors of pro-inflammatory gene expression (30-33).

Similar experimental model systems have been proposed that are based on the *P. aeruginosa* infection of other cell lines, such as NuLi (30,31), CuFi (30,31), A549 (34). Hawdon *et al* (34), Cerqueira *et al* (35) and Aval *et al* (36) demonstrated the potential anti-inflammatory effects of resveratrol and picetannol, using *P. aeruginosa*-infected A549 alveolar epithelial cells. In addition to *P. aeruginosa* infection, IB3-1 cells can be stimulated to upregulate pro-inflammatory genes by other stimuli, such as lipopolysaccharide (37), IL-1β (38), TNF-α (39-41) and the SARS-CoV-2 spike protein (42,43). Notably, the effects of the SARS-CoV-2 spike protein on a variety of cellular systems were reproduced by treatment with the spike-mRNA-based vaccine, BNT162b2 (44-46). Another experimental model system of particular interest in the context of the present review is the T-lymphoid Jurkat cell line, very useful to determine the effects of inhibitory agents on TLR4-dependent NFκB activation (47). Geng *et al* (48) used an electrophoretic mobility shift assay (EMSA) performed on nuclear extracts prepared from TNF-α stimulated Jurkat cells. This study demonstrated a powerful inhibitory effect of SAC on NF-κB activation, reinforcing the information on anti-inflammatory properties of this AGE component found using other experimental model systems (45).

In conclusion, several experimental model systems have been developed and validated for identifying agents that exhibit potent inhibitory activity on pro-inflammatory gene

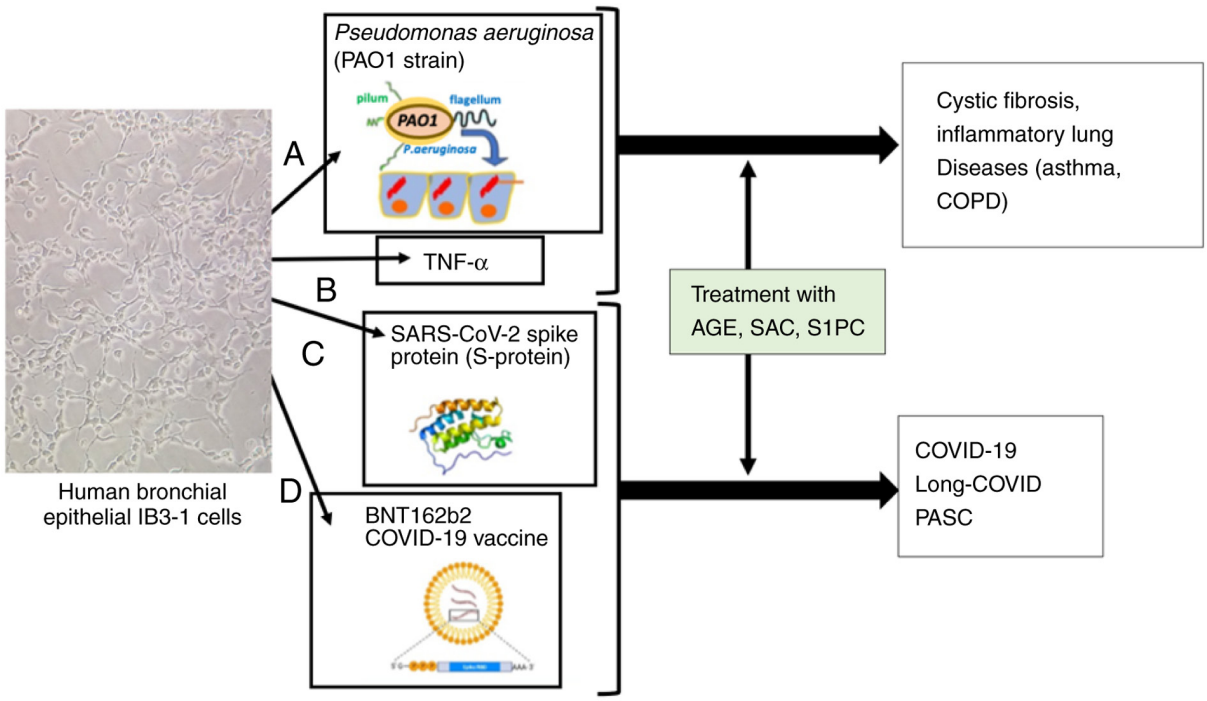

Figure 1. Examples of experimental approaches for the screening and characterization of anti-inflammatory agents to be proposed for the experimental therapy of respiratory diseases, such as cystic fibrosis, asthma, COPD, COVID-19, PASC and long-COVID. The experimental model systems described are based on IB3-1 human bronchial epithelial cells (A) infected with PAO, or exposed to (B) TNF-α, (C) SARS-CoV-2 spike protein and (D) the COVID-19 BNT162b2 vaccine. The image of IB3-1 cells was modified from Gasparello *et al* (45) (copyright can be found at https://doi.org/10.3390/molecules29245938). AGE, aged garlic extract; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-2019; PAO, *Pseudomonas aeruginosa*; PASC, post-acute sequelae of SARS-CoV-2 infection; S1PC, S-1-propenyl-l-cysteine; SAC, S-allyl cysteine; SARS-CoV-2, severe acute respiratory syndrome corona virus 2.

expression. Selected potential anti-inflammatory agents may also be tested *in vivo* using validated animal models of chronic *P. aeruginosa* lung infection that mimic cystic fibrosis, as proposed by Hoffmann *et al* (49). In the near future, we expect that the effects of AGE and its constituents will be analyzed using *P. aeruginosa*-infected primary bronchial epithelial cells. The results obtained should be considered with great attention, taking into account the anti-microbial effects of garlic-derived compounds as potential confounding factors (50,51) requiring adequate further control studies.

3. AGE: Studies supporting anti-inflammatory activity

Among the plant extracts hypothesized to retain anti-inflammatory activities, AGE is of great interest (52,53). The preparation and characterization of AGE has been described in a number of publications such as those by Ohkubo *et al* (1) and Kurita *et al* (54). Briefly, AGE can be prepared by immersing fresh garlic in 15% aqueous ethanol solution for several months at room temperature (1). AGE is a commercial odorless preparation with antioxidant properties for scavenging reactive oxygen species (ROS) (54) and retains immunomodulatory and anticancer properties (54). It should be noted that interest in the preparation of AGE and AGE-related products is evidenced by the high number of patents that have been deposited over the years (a partial list of patents and patent applications is shown in Table III). This information should be considered with great attention for the development of biomedical approaches, despite the fact that the patenting of preparations for 'aged garlic' is outside the major objectives of the present short review. A specialized further review would be useful to describe this interesting activity, which precedes the industrial exploitation of AGE and AGE-related compounds, considering that few reports are available on the patents on garlic and garlic-related products (55). In this context, two notable components of AGE are SAC (25) and S1PC (26). SAC and S1PC showed stable properties under cell culture conditions, and their acute/subacute toxicity was minor in mice and rats (24,26). In particular, the pharmacokinetics of SAC was investigated on human volunteers, indicating that SAC was rapidly absorbed from the gastrointestinal tract (24). Other AGE components have been described by Kodera *et al* (56), including S-(2-propyl) 2-propen-1-sulfinothioate (allicin), 3-(allyltrisulfanyl)-2-aminopropanoic acid, S-allylmercaptocysteine, allixin and tetrahydro-β-carboline derivatives.

4. SAC and S1PC: Proposed mechanisms of action

With respect to the anti-inflammatory activity of AGE, several studies are available on the effects of AGE components on the TLR4/NF-κB pathways, which play a central role in inflammatory processes (57-63). Regarding NF-κB, without external stimuli, an inactive trimer is formed in the cytoplasm that is composed of the inhibitory protein, IκB, and the p50/p65 NF-κB dimer. In these conditions, NF-κB is not translocated to the nucleus and the NF-κB regulated genes are mostly inactive, unless activated by other transcription factors (64,65). However, when external stimuli act on the corresponding receptors (for instance, when TLR4 is activated by a number of stimuli, some

Table I. Examples of pro-inflammatory genes involved in human diseases characterized by hyper-inflammation.

First author/s, year	Clinically relevant pro-inflammatory gene target	Disease	Pre-clinical and clinical studies: Key results	(Refs.)
McElvaney and McElvaney, 2018; Escotte *et al*, 2003	IL-8	Cystic fibrosis	IL-6 and IL-8 production is reduced by fluticasone in cystic fibrosis bronchial epithelial cells (12)	(11,12)
Rennard *et al*, 2015		COPD	The CXCR2 antagonist MK-7123 is proposed for the treatment of COPD (NCT01006616)	(13)
Planagumà *et al*, 2015		Severe asthma	A promising anti-inflammatory treatment targeting CXC receptors 1 and 2 is described for the reduction of neutrophil migration and activation in respiratory diseases	(14)
Yang *et al*, 2024		Osteoarthritis	Osteoarthritis progression is attenuated by injecting a IL-8 neutralizing monoclonal antibody	(15)
Rosas *et al*, 2021; Pinzon *et al*, 2021	IL-6	COVID-19	IL-6 inhibitors are effective therapeutic agents for COVID-19	(16,17)
Simonetti *et al*, 2024		Long-COVID	IL-6 receptor inhibitors tocilizumab and sarilumab are proposed for long-COVID	(18)
Holms, 2022		PASC	Ezrin peptides are potent inhibitors of IL-1, IL-6, IL-8 and TNF-α expression, and can be used for potential therapeutic protocols for long-COVID/PASC	(19)
Dinarello *et al*, 2012	IL-1β	RA	The IL-1 receptor antagonist anakinra, the soluble decoy receptor rilonacept and the neutralizing monoclonal anti-IL-1β antibody canakinumab can be employed in RA	(20)
Asaba *et al*, 2024	TLR4	COVID-19	TLR4 inhibitors decrease inflammation and severity in COVID-19 infections	(21)
Bartels *et al*, 2024		Osteoarthritis	Inhibition of TLR4 signaling strongly inhibits joint inflammation in osteoarthritis	(22)
Greene *et al*, 2008		Cystic fibrosis	TLR4 is a therapeutic target in cystic fibrosis	(23)

COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-2019; CXCR2, C-X-C motif chemokine receptor 2; PASC, post-acute sequelae of severe acute respiratory syndrome corona virus 2 infection; RA, rheumatoid arthritis; TLR4, toll-like receptor 4.

of which are listed in Table II), phosphorylation of IκB occurs, leading to dissociation of IκB from the trimer and its proteasome-mediated degradation, resulting in NF-κB activation. In these conditions, the p50/p65 NF-κB protein translocates to the nucleus and specifically interacts with NF-κB binding sites present in the promoters of NF-κB-regulated genes, such as the IL-6, IL-1β and IL-8 genes (66), all of which are involved in inflammatory processes that characterize several human diseases (as previously discussed in Table I). There is consensus that SAC interferes with NF-κB activation, thereby causing inhibition of the NF-κB-regulated genes (67,68). Table IV summarizes the evidence supporting the role of SAC in modulating the NF-κB pathway. Fig. 2 summarizes the biological effects of SAC (and of the analogue, S1PC) on different steps of the NF-κB pathway.

A notable study on the possible molecular targets of SAC has been reported by Park *et al* (57) and reviewed by Colín-González *et al* (58). In the study by Park *et al* (57), it was demonstrated that SAC is able to decrease IKKβ activity and the phosphorylation of IκBα, resulting in the attenuation of NF-κB. In another study, Shao *et al* (59) performed molecular docking analysis to determine that SAC may interact with the Kelch-like ECH-associated protein 1-nuclear factor erythroid 2-related factor 2 complex, PPARγ, histone deacetylase (HDAC)1 and TLR4. The possible interactions with TLR4 is of particular interest since this receptor is an upstream regulator of NF-κB signaling (60-63).

In our laboratory, the interaction between SAC (and the analogue S1PC) and the intracellular domain of TLR4 has been simulated using the well-known docking software, AutoDock Vina (45,46). Furthermore, to confirm the reliability of the identified molecular interactions and to predict the possible impact on TLR4 function, the computed docking models underwent molecular dynamics simulation. These studies are reported in Gasparello *et al* (45) and Papi *et al* (46) and suggest that the complexes remained stable as the hydrogen bonds formed between SAC (and S1PC) and TLR4 were retained during the entire molecular dynamics simulation, confirming a plausible interaction between the small molecules and TLR4. In addition, the results obtained suggest that binding with SAC and

Table II. Examples of *in vitro* experimental model systems to identify inhibitors of pro-inflammatory genes.

First author/s, year	Experimental model system	Characterized molecule	(Refs.)
Di Mango *et al*, 1998; Fabbri *et al*, 2014; Lampronti *et al*, 2017	Cystic fibrosis IB3-1 epithelial cells infected with *Pseudomonas aeruginosa*	Dexamethasone (27); miR-93-5p (30); β-sitosterol (31)	(27,30,31)
Hawdon *et al*, 2010; Cerqueira *et al*, 2013; Aval *et al*, 2013	A549 alveolar epithelial cells infected with *Pseudomonas aeruginosa*	Resveratrol (35); picetannol (36)	(34-36)
Dechecchi *et al*, 2008; Gambari *et al*, 2012; Borgatti *et al*, 2011	Cystic fibrosis epithelial cells treated with TNF-α	Miglustat (39); corilagin (40); furocoumarin derivatives (41)	(39-41)
Geng *et al*, 1997	T-lymphoid Jurkat cells treated with TNF-α	S-allyl cysteine (47)	(48)
Gasparello *et al*, 2021; Gasparello *et al*, 2021b	Bronchial epithelial cells treated with SARS-CoV-2 spike protein	Sulforaphane (42); miR-93-5p (43)	(42,43)
Cosenza *et al*, 2023; Gasparello *et al*, 2024; Papi *et al*, 2025	Human cell lines exposed to anti-SARS-CoV-2 BNT162b2 vaccine	Aged garlic extract (45); S-allyl cysteine (45); S-1-propenyl-l-cysteine (46)	(44-46)

miR, microRNA; SARS-CoV-2, severe acute respiratory syndrome corona virus 2.

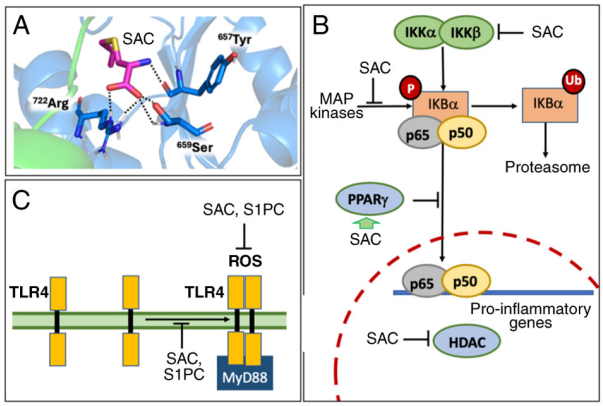

Figure 2. Proposed effects of SAC on the TLR4/NF-κB pathway. (A) Docking experiments showing the interaction between SAC and the Toll/interleukin-1 receptor domain of TLR4 (45). Inhibitory effects of SAC (B) on MAP kinases, IKKβ, PPARγ and HDAC (62-64) and (C) on ROS and TLR4 dimerization are shown. HDAC, histone deacetylase; MyD88, myeloid differentiation primary response 88; P, phosphorylated; PPARγ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; S1PC, S-1-propenyl-l-cysteine; SAC, S-allyl cysteine; TLR4, toll-like receptor 4; Ub, ubiquitinated.

S1PC reduces the intermolecular interaction between the TLR4 domains of the TLR4 dimer, supporting additional effects on NF-κB, as outlined in Fig. 2. The predicted interaction between SAC and the TLR4 complex is depicted in Fig. 2A. The binding mode predicted for S1PC with TLR4 was only slightly different from SAC/TLR4, and SAC and S1PC shared interactions with both ^{657}Tyr and ^{722}Arg (45,46). For both small molecules, the estimated interaction energy with TLR4 was approximately -65 Kcal/mol (sum of short-range Lennard-Jones and short-range Coulomb contributions over the molecular dynamics simulation), suggesting the two compounds may have highly comparable and favorable interactions with the protein target. This finding suggests that one effect of SAC and S1PC on target cells might involve inhibition of TLR4, causing a marked reduction in NF-κB signaling and a notable inhibition of the transcription of NF-κB-regulated genes (Fig. 2B and C).

SAC reduces ROS (48), exerting clear and expected anti-inflammatory effects, as an interactive role of TLR4 and ROS has been described (69) and inhibition of ROS generation attenuates the TLR4-mediated proinflammatory phenotype, as reported by Pi *et al* (70). In addition to these direct and indirect effects on TLR4 (summarized in Fig. 2C), SAC might inhibit the NF-κB activity via several mechanisms of action (summarized in Fig. 2B), including via a strong inhibitory effect on IKKβ kinase, which blocks IκBα phosphorylation (64,65). This has a notable consequence for NF-κB signaling. It is well established that the activation of NF-κB requires phosphorylation of the inhibitory subunit, IκBα, the ubiquitination of IκBα (71,72) and finally the proteasome-dependent degradation of IκBα (72,73). In these conditions the p65/p50 NF-κB complex translocates to the nucleus and activates NF-κB-regulated genes. In addition to an inhibitory effect on IKKβ kinase, SAC has been demonstrated to cause a MAP kinase-mediated inhibition of IκBα phosphorylation (64), with a marked inhibition of the formation of poly-ubiquitin IκBα, of IκBα degradation and of NF-κB nuclear translocation. Finally, SAC has been reported to enhance the expression of PPARγ (74) a recognized NF-κB inhibitor (75), and to modulate HDAC activity (76).

5. TLR4, NF-κB and the expression of pro-inflammatory genes in human pathologies: Possible therapeutic applications of AGE and AGE constituents

The interplay among TLR4, NF-κB and human pathologies has been reported by a number of investigations and reviewed by several articles (77-82). For instance, Aboudounya and Heads (77) reported that SARS-CoV-2 binds and activates TLR4 to increase angiotensin converting enzyme 2 expression, facilitating entry of the virus into cells and resulting in hyper-inflammation. In a recent review, Asaba *et al* (21) described

Table III. Examples of in patents and patent applications to prepare 'aged garlic' and garlic products.

Patent number	Title and assignee	Publication date	Comments/description
US2554088A	Extraction of garlic; assignee: Winthrop Stearns, Inc.	May 22, 1951	This invention relates to an antibiotic substance and to a process for obtaining it from garlic cloves.
CN-101863807-B	Preparation method of garlic extract; assignee: Sichuan Xiangzhen Enterprise Co., Ltd.	June 10, 2010	A method for preparation of a garlic extract solution is described based on the following steps: Peeling, washing and slicing garlic, adding water for soaking, and filtering with a 50-100-mesh sieve.
US8187654B2	Process for preparing aged garlic; assignee: Blackgarlic, Inc.	May 29, 2012	The invention concerns a method of producing aged garlic with increased antioxidation capability compared with that of raw garlic.
US20110293803	Method for producing an aged black garlic concentrate; assignee: Saenamhae Nonghyup, KR	January 12, 2011	Aged black garlic concentrate is produced according to the following steps: Pretreating for cleaning; sealing the garlic in a polyethylene bag and storing in a tray; putting the tray into an aging device; and steam-treating by applying steam and heat for 30-60 min maintaining the aging device at a temperature of 80-90˚C.
JPS60262565A	Preparation of garlic juice; assignee: Meiji Seika Kaisha, Ltd.	December 25, 1985	A liquid containing crushed bulbs of garlic is combined with vitamin B1, saccharides and centrifuged, then the supernatant is combined with soybean powder to prepare odorless garlic juice without generation of irritating odor of garlic while they are processed.
CN110623255A	Method for producing aged garlic; assignee: Pizhou Mid Autumn Food Co., Ltd.	December 31, 2019	A method for producing aged garlic in which oxidation resistance is remarkably higher than that of raw materials such as increased garlic, which is used for producing garlic in which the necessary effectiveness of aged garlic as a raw material is not lowered, S-arylcysteine is not present at all in the produced garlic, and the polyphenol content is increased.
JP5968729B2	Production of black garlic extract and its use; assignee: MOMOYA KK Momoya Co Ltd.	August 10, 2016	A technique for producing black garlic extract, that maintains, increases or enriches highly bioactive substances (such as S-allyl cysteine and cycloallyin) by heat-treating garlic raw material for a short time.
EP1752051A1	Method of producing aged garlic; assignee: COST PLUS KOREA Co Ltd	February 14, 2007	The invention concerns a method of producing aged garlic in which its antioxidation capability is increased compared with that of raw garlic, which is used as a raw material.
WO2004077963A1	Processed plant product and method of producing the same; assignee: Wakunaga Pharmaceutical Co., Ltd.	September 16, 2004	The plant product is obtained by maintaining allium in a low-oxygen or oxygen-free condition at a temperature ranging between 1˚C and 40˚C in the absence of any medium for 1 week or longer.
JP4380951B2	Garlic fermentation composition; assignee: Wakunaga Pharmaceutical Co., Ltd.	December 9, 2009	An antioxidant composition obtained by fermenting enzyme-deactivated garlic with koji mold, or a composition obtained by adding beans and/or cereals to enzyme-deactivated garlic and fermenting with koji mold.
US6146638A	Fermented garlic composition; assignee: Wakunaga Pharmaceutical Co., Ltd.	November 14, 2000	A composition is described that is prepared by fermenting enzymatically-deactivated garlic with aspergillus and/or monascus. The composition is free from any unpleasant odor and useful as a prophylactic or therapeutic agent for several diseases, such as diabetes, hepatic diseases, cancer, immunopathy and hyperlipemia.

https://patents.google.com/(accessed November 8, 2024). http://www.freepatentsonline.com/(accessed November 9, 2024).

Table IV. Effects of SAC on different molecular targets involved in the NF-κB pathway.

First author/s, year	Molecular target	Employed experimental model system	Methodological approach	Comments	(Refs.)
Park *et al*, 2014; Colín-Gonzáles *et al*, 2015; Shao *et al*, 2020	IKKb kinase	Gastric ulcers induced in mice by intragastric administration of indomethacin (57)	IKKb kinase assay (57); molecular docking (59)	SAC is able to decrease IKKb activity (57) and was found to decrease phosphorylation of IkBa, leading to attenuation of NF-κB.	(57-59)
Shao *et al*, 2020	Keap1-Nrf2 complex PPARγ HDAC	*In vitro*: TBHP-treated murine chondrocytes; *in vivo*: Destabilization of the medial meniscus mice (59)	Molecular docking (59)	SAC is an activator of PPARγ (a strong inhibitor of NF-κB signaling).	(59)
Shao *et al*, 2020; Gasparello *et al*, 2024	TRL4	BNT162b2 stimulated IB3-1 cells (45)	Molecular docking (59); molecular docking and dynamics (45)	The possible interactions with TLR4 are relevant, considering the TLR4 role in the regulation of NF-κB signaling.	(59,45)

HDAC, histone deacetylase; Keap1, Kelch-like ECH-associated protein 1; PPARγ, peroxisome proliferator-activated receptor-γ; SAC, S-allyl cysteine; TBHP, tert-butyl hydroperoxide; TLR4, toll-like receptor 4.

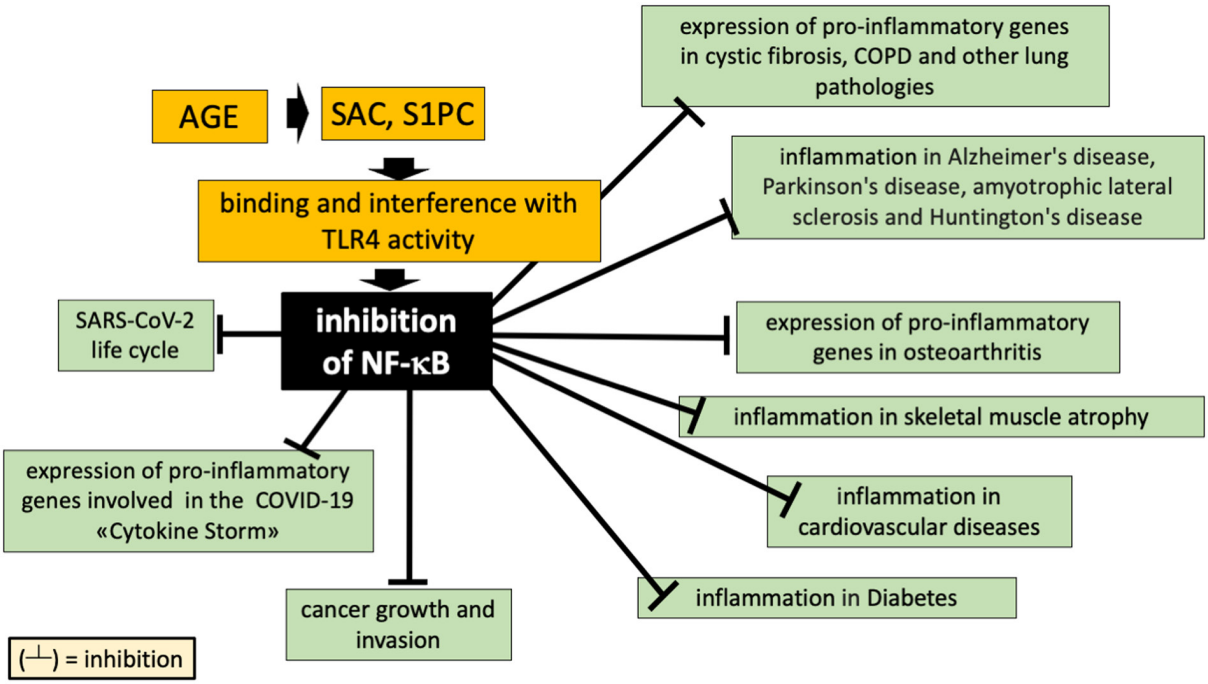

Figure 3. Inhibitory effects of AGE, SAC and S1PC on the NF-κB-associated expression of pro-inflammatory genes in human diseases. AGE, aged garlic extract; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-2019; S1PC, S-1-propenyl-l-cysteine; SAC, S-allyl cysteine; SARS-CoV-2, severe acute respiratory syndrome corona virus 2; TLR4, toll-like receptor 4.

the interplay between TLR4 and SARS-CoV-2, focusing on inflammation and the severity SARS-CoV-2 infections, suggesting TLR4 as a possible target for anti-SARS-CoV2 agents. Sahanic *et al* (78) demonstrated that SARS-CoV-2 activates the TLR4/MyD88 pathway in human macrophages causing a strong pro-inflammatory response in severe

COVID-19. In a notable study, Alamin *et al* (79) identified common molecular signatures characterizing the molecular effects of SARS-CoV-2 infections and different lung diseases. This study was based on the analysis of RNA-sequencing and microarray gene expression datasets (from the Gene Expression Omnibus platform of the National Center for Biotechnology Information) and considered the following lung diseases: COPD, idiopathic pulmonary fibrosis, ILD (interstitial lung disease), asthma, tuberculosis, cystic fibrosis, pneumonia, emphysema and bronchitis. The full information about these datasets and the description of the complete workflow of the study can be found in Alamin *et al* (79). Notably, TLR4 was identified in most of these diseases, among the top-ranked differentially expressed genes.

Since AGE (and its constituents, SAC and S1PC) might interfere with the TLR4 and NF-κB pathways (as outlined in Fig. 2), several pathologies caused by dysregulation of the TLR4/NF-κB axis might be affected by treatments with these natural products. Among these target pathologies, the most impactful for the worldwide health systems are COVID-19, lung infectious diseases, cystic fibrosis and COPD (https://www.healthdata.org/research-analysis/gbd; accessed February 20, 2025). Notably, and in agreement with the approach by Alamin *et al* (79), Hasan *et al* (80) reported using a differential gene expression pattern analysis, common pathophysiological processes highly similar in COVID-19 and cystic fibrosis, including the Toll-like receptor signaling pathway. Fig. 3 summarizes the pathologies with a dysregulated TLR4/NF-κB axis and/or oxidative stress, which leads to the activation of a hyperinflammatory state (81-94). In all these pathologies, novel anti-inflammatory molecules, such as AGE, SAC and S1PC, hold promise (81-94).

6. Conclusions and future perspectives

The main purpose of the present review was to discuss the possibility that AGE and its components, SAC and S1PC, might be employed in the treatment of several human pathologies that are characterized by a hyperinflammatory state. A partial list of published examples is reported in Fig. 3 (81-94). In most of these pathologies, dysregulation of the TLR4 and NF-κB pathways have been observed. In this respect, a large consensus has been reached sustaining the concept that AGE, and the most important constituents SAC and S1PC, inhibit TLR4 and NF-κB. SAC has been shown to be of interest for the treatment of lung pathologies (81,82), neurological diseases (83,84), osteoarthritis (85), muscular atrophy (86,87), cardiovascular diseases (88,89), diabetes (90,91) and cancer (92-95). In addition, in the possible use of AGE in the therapy of human pathologies, the antioxidative effects of AGE should be noted, whereby the oxidative stress of cells is altered in different pathologies. This is important for evaluating AGE and its constituents as treatments for hematological diseases characterized by oxidative stress of the erythroid cells. For instance, AGE and AGE components have been proposed for the treatment of sickle-cell diseases (95-97). In this respect, a Trade Mark for S-1-propenyl-l-cysteine (S1PC™) has been recently obtained by Wakunaga Holdings Co., Ltd., Japan (registered on July 9, 2024; https://branddb.wipo.int/; accessed on March 20, 2025).

To maximize the impact of the studies described in the present review, further research and development are necessary. Clinical trials are expected to verify possible applications on human pathologies in the real world. Taking advantage of the increase in genomic information, one notable issue is related to the design of personalized therapeutic intervention. In this context, pharmacogenomic studies should be considered and undertaken within clinical trials, to predict the possible response (or no response) to the proposed AGE-based treatments. In this respect, combined therapies using AGE (or its constituents) alongside established therapeutic drugs or RNA- and DNA-based drugs is expected to be an attractive area of investigation. For instance, Reyes-Soto *et al* (94) found that SAC exhibits cytotoxicity in glioblastoma cells and improves the effect of temozolomide through the regulation of oxidative responses. Regarding the effects on TLR4, NF-κB and pro-inflammatory gene expression, we anticipate development based on the co-treatment with other currently available molecular inhibitors, including RNA-based therapies using microRNAs, used to specifically downregulate TLR4 and NF-κB, as previously suggested (98,99).

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

EA, GM, RG and AF conceived the study. GM and AF curated data. EA, GM, RG and AF performed formal analysis. RG, AF, EA and GM acquired funding. RG wrote the original draft. EA, GM, RG and AF reviewed and edited the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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