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Review Article

Alpha-2-Macroglobulin, a Hypochlorite-Regulated Chaperone and Immune System Modulator

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Alpha-macroglobulins are ancient proteins that include monomeric, dimeric, and tetrameric family members. In humans, and many other mammals, the predominant alpha-macroglobulin is alpha-2-macroglobulin (α_2M), a tetrameric protein that is constitutively abundant in biological fluids (e.g., blood plasma, cerebral spinal fluid, synovial fluid, ocular fluid, and interstitial fluid). α_2M is best known for its remarkable ability to inhibit a broad spectrum of proteases, but the full gamut of its activities affects diverse biological processes. For example, α_2M can stabilise and facilitate the clearance of the Alzheimer's disease-associated amyloid beta (Aβ) peptide. Additionally, α_2M can influence the signalling of cytokines and growth factors including neurotrophins. The results of several studies support the idea that the functions of α_2M are uniquely regulated by hypochlorite, an oxidant that is generated during inflammation, which induces the native α_2M tetramer to dissociate into dimers. This review will discuss the evidence for hypochlorite-induced regulation of α_2M and the possible implications of this in neuroinflammation and neurodegeneration.

1. Structure and Function

α_2M is a secreted protein that is present at 1.5–2 mg mL^{-1} and 1.0–3.6 μg mL^{-1} in human blood plasma and cerebral spinal fluid, respectively [1, 2]. The cage-like structure of α_2M (720 kDa) is formed by the assembly of four 180 kDa subunits into two disulfide-linked dimers, which noncovalently associate to complete the tetrameric quaternary structure of the protein [3]. A bait region that contains a large number of protease cleavage sites is responsible for the incredibly diverse range of proteases that interact with α_2M [4]. Cleavage of the α_2M bait region, which is in close physical proximity to a reactive thioester bond, results in covalent trapping of proteases within a steric cage [5]. This process involves a substantial conformational change that generates a compact tetrameric form [6] and reveals the binding site for the low-density lipoprotein receptor-related protein-1 (LRP1) [7, 8] (Figure 1(a)). For the purpose of this review, the compact tetrameric protease-bound form of α_2M is referred to as transformed α_2M. Transformed α_2M (covalently bound to up to two protease molecules) is rapidly cleared from the circulation via LRP1-facilitated endocytosis (Figure 1(a)). As such, α_2M can efficiently inhibit a myriad of extracellular processes that are dependent on proteolysis.

Consistent with having an ancient origin in innate immunity, α_2M is a promiscuous protein that noncovalently binds to a diverse range of nonprotease ligands including cytokines [9, 10], growth factors [9–14], apolipoproteins [15], and misfolded proteins [16–20]. Many noncovalent ligands of α_2M including the Alzheimer's disease-associated Aβ peptide [21], neurotrophins [14], and tumour necrosis factor-alpha (TNF-α) preferentially bind to transformed α_2M which is generated following the reaction of native α_2M with a protease or with small nucleophilic compounds that also target the α_2M thioester bond [6]. In these cases, it is proposed that transformed α_2M acts to limit the activities of noncovalently bound ligands by facilitating their disposal via LRP1 [10, 22] (Figure 1(a)). On the other hand, α_2M can control signalling

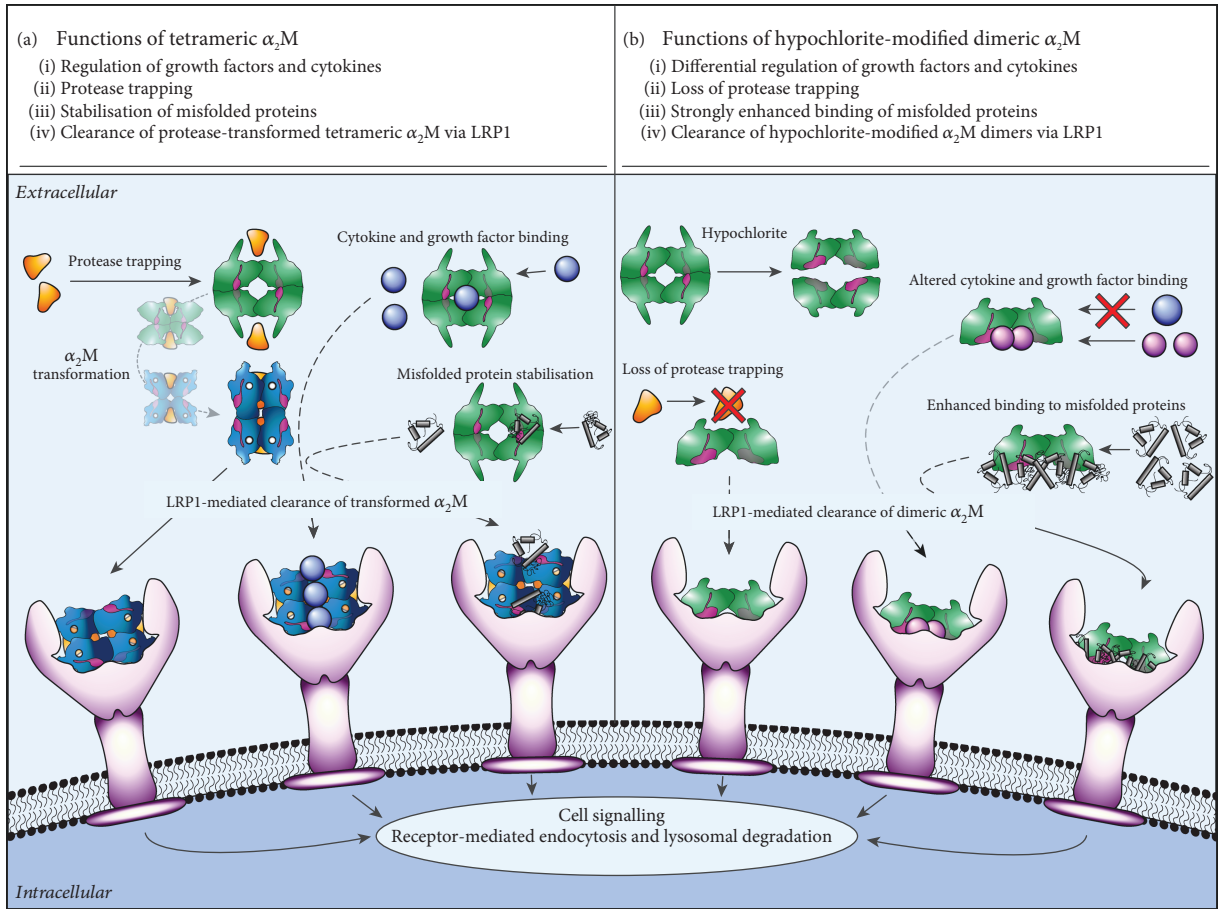

Figure 1: Schematic diagram showing the function consequences of hypochlorite-induced modification of α_2M. (a) Native α_2M, a tetramer (shown in green), is constitutively present in biological fluids and covalently binds to a broad range of proteases. Binding to proteases results in a conformational change that exposes the binding site on α_2M for LRP1, which is responsible for the clearance of the protease-transformed α_2M complex (shown in dark blue). α_2M also binds to a large number of noncovalent ligands including cytokines and misfolded proteins. In many cases, noncovalent binding of ligands occurs preferentially to the protease-transformed conformation (not shown). In the instance that native α_2M binds noncovalently to a nonprotease substrate, protease interaction is required to enable clearance of the complex via LRP1. (b) Reaction with hypochlorite induces the dissociation of the native α_2M tetramer into dimers. This process abolishes the protease-trapping activity of α_2M; however, the binding to some cytokines (i.e., TNF-α, IL-2, and IL-6) and misfolded proteins is enhanced. On the other hand, the binding of α_2M to other noncovalent ligands (i.e., β-NGF, PDGF-BB, TGF-β1, and TGF-β2) is reduced. The dissociation of the native α_2M tetramer into dimers reveals the binding site on α_2M for LRP1. Therefore, α_2M dimers can facilitate the clearance of substrates in a protease-independent manner. N.B.: Inflammatory processes potentially elevate levels of protease-transformed α_2M and hypochlorite-modified α_2M dimers, concomitantly.

pathways via alternative mechanisms. For example, the binding of α_2M to phosphorylated insulin-like growth factor binding protein-1 abrogates its inhibitory effects on insulin-like growth factor-1 (IGF-1); therefore, in some scenarios, α_2M can potentiate growth factor signalling [13]. Another example whereby α_2M is reported to potentiate growth factor signalling involves the pronerve growth factor (pro-NGF), which induces the expression of TNF-α via stimulating the neurotrophin receptor p75 [11]. Although α_2M potentiates pro-NGF signalling *in vitro*, α_2M is reported to inhibit the activity of mature NGF by binding either to NGF or to Trk receptors [12, 23, 24].

The accumulation of misfolded proteins is inherently deleterious to living organisms and underlies the pathology of many human diseases including Alzheimer's disease, Parkinson's disease, and motor neuron disease. α_2M is one of a small number of secreted proteins that are known to possess holdase-type chaperone activity, which is the ability to stabilise misfolded proteins and prevent their aberrant aggregation [16–20, 25]. The chaperone function of α_2M has been demonstrated *in vitro* using a broad range of misfolded clients including denatured globular proteins and aggregation prone, intrinsically disordered substrates (e.g., Aβ peptide and Parkinson's disease-associated alpha-synuclein). Furthermore, it has been shown that α_2M preferentially binds several plasma proteins *in situ* following experimentally-induced shear stress which causes plasma protein aggregation [18, 19]. The likely fate for complexes formed between native α_2M and misfolded proteins is clearance via LRP1 following interaction with a

protease [16, 22, 25–27] (Figure 1(a)). However, protease-transformed α_2M can also inhibit Aβ aggregation via degrading the peptide because trapped proteases remain active following covalent binding to α_2M [18, 19]. The neuroprotective activity of α_2M against the toxicity induced by misfolded proteins has been demonstrated using several *in vitro* models [17, 25, 27, 28] and has also been demonstrated in rats directly injected with toxic Aβ oligomers [29]. Taken together, the results of these studies support the conclusion that the functions of α_2M are broadly important to extracellular proteostasis.

2. α_2M and Neurodegenerative Diseases

Interest in the role of α_2M in Alzheimer's disease spans several decades. In part, this stems from early reports that polymorphisms in α_2M are associated with increased risk of Alzheimer's disease in some populations [30–36]. However, opposing results have also been presented [37, 38], and more recent genome-wide association studies have not found any association [39]. It has recently been reported that serum α_2M is elevated in men with preclinical Alzheimer's disease, which potentially represents a general response to neuronal injury [40]. The significance of elevated levels of α_2M is hard to determine, because aside from influencing Aβ aggregation and clearance, there are many other relevant biological processes that α_2M potentially influences. For example, apolipoprotein E (ApoE) is an endogenous ligand of α_2M in blood plasma, and the binding of α_2M to the ε4 isoform (the strongest known genetic risk factor for Alzheimer's disease) is much less compared to the binding of α_2M to the ε2 and ε3 ApoE isoforms [15]. The functional importance of this interaction has yet to be solved.

There is strong evidence that native α_2M can inhibit the aggregation and toxicity of Aβ peptide (the major constituent of extracellular plaques in Alzheimer's disease). Furthermore, the widely documented ability of α_2M to facilitate the clearance of the Aβ peptide is central to its neuroprotective action [17, 25, 27–29]. α_2M is found colocalised with the Aβ peptide in the brain in Alzheimer's disease [41, 42], which supports the idea that the LRP1-mediated clearance of α_2M-Aβ complexes is impaired or overwhelmed. Similar to α_2M, there are conflicting reports regarding an association between polymorphisms in LRP1 and the risk of Alzheimer's disease (reviewed in [43]). Given that the accumulation of the Aβ peptide in the brain in Alzheimer's disease appears to be the result of a defect in clearance, rather than elevated production of the peptide [44], it is important to understand the contribution of α_2M to the clearance of the Aβ peptide in greater detail.

Roles for α_2M in preventing or promoting neurodegeneration independent of Alzheimer's disease are less clear. Nevertheless, α_2M is reported to bind to a broad range of misfolded proteins including the infectious prion protein that is responsible for transmissible spongiform encephalopathies [45] and α-synuclein, the major constituent of misfolded protein deposits in Parkinson's disease [17]. In the case of the prion protein, it has been reported that binding to α_2M *in vitro* facilitates the conformational change in the prion protein that is responsible for its infectious characteristics [45]. On the other hand, similar to the protective effect of α_2M on Aβ toxicity, the binding of α_2M to α-synuclein is cytoprotective [17]. α_2M also potentially inhibits neurodegeneration by influencing the activity of neurotrophins such as NGF and pro-NGF or by inhibiting the activity of neurotrophin receptors directly [12, 23, 24]. The latter could have relevance in a range of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease in which aberrant neurotrophin signalling is implicated [46]. Moreover, the ability of α_2M to bind to proinflammatory mediators such as TNF-α, IL-6, and IL-1β [47–49] supports the idea that α_2M has generalised importance in controlling inflammatory processes including in the central nervous system.

3. Hypochlorite, a Novel Regulator of α_2M Functions

Hypochlorite (OCl^-) is a powerful oxidant that is produced by the action of the enzyme myeloperoxidase during inflammation. Myeloperoxidase is not detected in the brains of healthy individuals; however, in neuroinflammatory disorders, myeloperoxidase is generated by activated microglia and astrocytes [50–54]. Infiltrating monocytes/macrophages and neutrophils can also contribute to myeloperoxidase production in the brain [50, 55]. Although the reasons for this are unclear, myeloperoxidase-immunoreactivity is also detected in neurons in Alzheimer's disease [50, 51]. Interestingly, in a mouse model of Parkinson's disease, ablation of the myeloperoxidase gene is protective, which supports the conclusion that myeloperoxidase is a major contributor to the oxidative damage generated by pathological neuroinflammatory processes [56].

Hypochlorite production is primarily considered important for defence against invading microbes [57]. The effectiveness of hypochlorite as a microbicidal agent is linked to the potency with which hypochlorite damages proteins, inducing their misfolding [58, 59]. Given that reaction with hypochlorite is not specific to molecules of microbial origin, the generation of hypochlorite is associated with collateral damage to the host organism. As a result of aberrant inflammatory activity, hypochlorite-modified proteins accumulate in a large number of pathologies including Alzheimer's disease [51], atherosclerosis [60], kidney disease [61], rheumatoid arthritis [52] and in experimental animal models of Parkinson's disease [56] and multiple sclerosis [62]. Hypochlorite-induced modification can directly cause proteins to adopt immunostimulatory and cytotoxic properties. For example, hypochlorite-induced modification of apolipoprotein B-100, the major protein component of low-density lipoprotein particles, promotes macrophage foam cell formation and triggers platelet aggregation [63]. Additionally, hypochlorite-modified albumin is known to promote proinflammatory signalling [64], endothelial cell dysfunction [65], and apoptosis [66].

It is well-known that antioxidants are the first line of defence that protects the host from excessive oxidative damage during inflammation. However, evidence has emerged

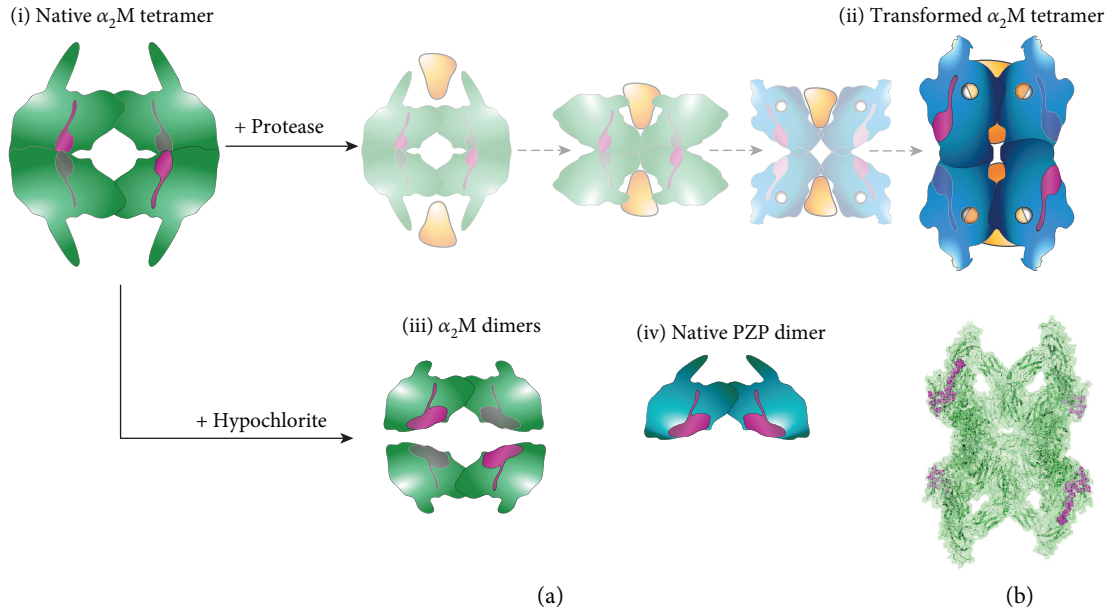

Figure 2: Theoretical model showing the binding sites for monomeric Aβ on native α_2M and PZP. (a) The binding sites for monomeric Aβ (magenta; centred at amino acids 1314–1365 according to [21]) are normally concealed at the noncovalent interface of the (i) native α_2M tetramer. (ii) Binding to proteases (yellow triangles) results in the partial opening of the noncovalent interface between α_2M dimers and exposes the binding sites for monomeric Aβ on each subunit of transformed α_2M. (iii) The binding sites for monomeric Aβ are also exposed by hypochlorite-induced dissociation of the native α_2M tetramer into dimers. (iv) Native PZP (a disulfide-linked dimer) shares 82.7% sequence identity with α_2M in the Aβ binding region (magenta). The dimeric quaternary structure of native PZP results in surface exposure of the binding sites for monomeric Aβ. Although the binding sites for other misfolded proteins are not known, intuitively, they are also located at the normally buried hydrophobic interface of noncovalently associated α_2M dimers. (b) Image of the crystal structure of the transformed α_2M tetramer from PBD 4ACQ [3] with the binding sites for monomeric Aβ shown in magenta, which is comparable to the model shown in (a (ii)). The crystal structures of native α_2M or hypochlorite-modified α_2M dimers have not been solved.

that supports the conclusion that specialised hypochlorite-inducible systems are also important. Around a decade ago, it was demonstrated that the activity of the bacterial chaperone Hsp33 is directly enhanced following reaction with hypochlorite and the chaperone activity of hypochlorite-modified Hsp33 protects bacteria from hypochlorite-induced death [59]. More recently, it has been demonstrated that reaction with hypochlorite induces the dissociation of the native α_2M tetramer into dimers that have dramatically enhanced chaperone activity compared to the native α_2M tetramer [25] (Figure 1(b)). The mechanism responsible for the enhanced chaperone activity of hypochlorite-modified α_2M dimers involves the exposure of the normally buried hydrophobic surfaces that are situated at the interface of noncovalently-associated dimers in the native α_2M tetramer [25] (Figure 2). It has been reported that methionine oxidation is largely responsible for the hypochlorite-induced dissociation of α_2M into dimers [67]; however, aromatic amino acids are also modified by physiologically relevant levels of hypochlorite [25, 68, 69]. The results of biophysical analyses show that physiologically-relevant levels of hypochlorite also alter the secondary structure of α_2M subunits [25, 68]. Precisely how hypochlorite-induced modification of the secondary structure of α_2M influences its functions is not known.

During inflammation, extracellular protease activity and the generation of hypochlorite are both elevated; therefore, it is plausible that protease-transformed α_2M and hypochlorite-induced α_2M dimers are concomitantly generated *in vivo*. Hypochlorite-induced modification of native α_2M exposes its LRP1 binding sites ([25, 70]); therefore, during inflammation, α_2M and its cargoes are potentially cleared via two distinct mechanisms involving LRP1 (Figure 1(a): protease-transformed α_2M and Figure 1(b): hypochlorite-induced α_2M dimers). The dissociation constant for the binding of hypochlorite-modified α_2M to LRP1 is reportedly ~0.7 nM [70] compared to 40 pM—2 nM for the transformed α_2M [71]. Unlike native α_2M, reaction with hypochlorite does not induce transformed α_2M (generated using methylamine) to dissociate into dimers, and the resultant hypochlorite-induced damage reduces the binding of transformed α_2M to LRP1 [70]. Therefore, during inflammation, the generation of hypochlorite potentially enhances the delivery of hypochlorite-modified α_2M dimers that are generated from the native α_2M tetramer to LRP1, while impeding the delivery of transformed α_2M to the same receptor.

Although the chaperone activity of native α_2M is enhanced following hypochlorite-induced modification, similar levels of hypochlorite-induced modification abolish the protease trapping function of α_2M [72, 73]. Collectively, the evidence suggests that reaction with hypochlorite is a rapid switch that regulates the activities of α_2M during inflammation. Supporting this idea, it has been reported that hypochlorite-induced modification of α_2M also regulates its binding to cytokines and growth factors in a manner that increases its binding to TNF-α, IL-2, and IL-6 (involving

preferential binding to hypochlorite-induced α_2M dimers) and decreases its binding to β-NGF, PDGF-BB, TGF-β1, and TGF-β2 *in vitro* [74] (Figure 1(b)). Furthermore, hypochlorite-induced dissociation of α_2M enhances its cytoprotective effect against TNF-α *in vitro* [74]. Interestingly, it has been reported that the complement system, which includes several proteins that are closely related to α_2M, is also activated by reaction with hypochlorite [75, 76]. Therefore, it is tempting to speculate that hypochlorite-induced regulation is a characteristic that is shared by this family of proteins.

Studies of the hypochlorite-induced regulation of α_2M are currently limited to *in vitro* systems; however, using the specific marker for reaction with hypochlorite 3-chlorotyrosine, it has been shown that α_2M is modified by hypochlorite in synovial fluid from inflamed joints [69]. Moreover, considering that hypochlorite levels are predicted to reach the low millimolar range in tissues during inflammation [77], it is plausible that hypochlorite-modified α_2M dimers are generated in biological fluids during inflammation. Of the studies reporting an association between mutation in α_2M and risk of Alzheimer's disease, one study has reported that there is a synergistic effect between polymorphisms in α_2M and myeloperoxidase and an increased risk of Alzheimer's disease [36]. The results of the latter study support the idea that the functions of these two proteins might interrelate in a way that is important to neurodegeneration. It is not currently known if any of the other identified extracellular chaperones (e.g., clusterin and haptoglobin) might also have their activities regulated by hypochlorite-induced modification, but this is an area worthy of future investigation.

4. PZP, a Dimeric α_2M-like Molecule

The major structural modification induced by reaction with hypochlorite that is responsible for functionally controlling α_2M is the dissociation of the native α_2M tetramer into dimers. Strikingly, many mammals are capable of generating large amounts of a dimeric α_2M-like protein known as pregnancy zone protein (PZP). In humans, α_2M and PZP share very high sequence homology in all domains (71% amino acid identity), with the exception of the bait region [4, 78]. As a result, the ability of PZP to inhibit proteases is much more restricted compared to that of α_2M. Few *in vitro* studies have focused on characterising the functions of PZP; however, it has been proposed that PZP contributes to regulating glycodelin-A (a paracrine mediator in early pregnancy) and TGF-β2 (important for embryonic development) [12, 79–81]. Consistent with this idea, PZP is usually lowly abundant in biological fluids but is markedly upregulated in pregnancy [82]. On the other hand, glycodelin-A and TGF-β2 are also ligands for constitutively abundant α_2M ([12, 79–81]); therefore, the precise importance of PZP as a modulator of these signalling pathways remains unclear. Similarly, several neurotrophins are shared ligands of PZP and α_2M, but the precise biological importance of these interactions is not known [12]. Pregnancy-independent expression of PZP is widely reported in diseases such as Alzheimer's disease [83, 84], Parkinson's disease [85], rheumatoid arthritis [86], Behcet's syndrome [87], psoriasis [88, 89], Chagas disease [90], viral infection [91, 92], inflammatory bowel disease [93], and cancers [94, 95]. The latter observations support the idea that the upregulation of PZP could be a general stress response that is related to chronic inflammation. This limits the usefulness of PZP as a diagnostic marker; however, the results of studies of lymphoma and arthritis patients suggest that PZP levels are potentially useful for monitoring disease progression [95, 96].

The ability of native tetrameric α_2M to inhibit Aβ aggregation is restricted to binding to soluble Aβ oligomers formed early during the aggregation pathway [20]. In contrast, transformed α_2M and hypochlorite-modified α_2M dimers bind to monomeric Aβ [21, 25], presumably via the hydrophobic binding site (centred at amino acids 1314–1365) identified by [21] (Figure 2). Intuitively, surface exposure of this site contributes to the efficiency with which hypochlorite-modified α_2M dimers inhibit Aβ amyloid formation compared to native α_2M [25]. Similarly, the results of recent studies show that PZP binds to the monomeric Aβ peptide and prevents the aggregation of the Aβ peptide much more efficiently than native α_2M [97]. Whether or not PZP contributes to the clearance of the Aβ peptide *in vivo* is currently unknown; however, it has been demonstrated that PZP levels are elevated in women with presymptomatic Alzheimer's disease and PZP is found colocalised with microglia around Aβ plaques in the brain in Alzheimer's disease [83, 84]. Combined, these observations suggest that PZP is likely to participate in Aβ homeostasis. Whether or not the role of PZP overlaps with or is discrete from that of α_2M remains to be determined.

5. Concluding Remarks

α_2M is a remarkably multifunctional protein that can influence a broad range of biological processes. Direct injection of α_2M into inflamed joints has been shown to have protective effects in a rodent model of osteoarthritis ([98]); however, the efficacy and safety of this as a human therapy is not yet known. An alternative α_2M-based anti-inflammatory strategy involves the oral administration of proteases, which is proposed to increase levels of transformed α_2M in blood plasma [99, 100]. This strategy is clearly limited by the poor bioavailability of orally administered proteases, but this problem could potentially be overcome by the identification of bioavailable small molecule modifiers of α_2M function.

Growing evidence suggests that hypochlorite-induced dissociation of α_2M into dimers is a rapid switch that enhances the ability of α_2M to facilitate the clearance of disease-associated misfolded proteins and proinflammatory cytokines during inflammation. This is potentially a broadly important process that occurs in response to inflammation, including in neurodegenerative disorders in which neuroinflammation is known to be an early event that precedes other pathological changes (reviewed in [101]). A deeper understanding of the physiological relevance of hypochlorite-induced α_2M dimers has the potential to shed much needed light on the participation of α_2M in controlling inflammatory processes and extracellular protein homeostasis during neuroinflammation.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] M. J. Garton, G. Keir, M. V. Lakshmi, and E. J. Thompson, "Age-related changes in cerebrospinal fluid protein concentrations," *Journal of the Neurological Sciences*, vol. 104, no. 1, pp. 74–80, 1991.

[2] L. Sottrup-Jensen, "Alpha-macroglobulins: structure, shape, and mechanism of proteinase complex formation," *The Journal of Biological Chemistry*, vol. 264, no. 20, pp. 11539–11542, 1989.

[3] A. Marrero, S. Duquerroy, S. Trapani et al., "The Crystal Structure of Human α2-Macroglobulin Reveals a Unique Molecular Cage," *Angewandte Chemie (International Ed. in English)*, vol. 51, no. 14, pp. 3340–3344, 2012.

[4] L. Sottrup-Jensen, O. Sand, L. Kristensen, and G. H. Fey, "The alpha-macroglobulin bait region. Sequence diversity and localization of cleavage sites for proteinases in five mammalian alpha-macroglobulins," *The Journal of Biological Chemistry*, vol. 264, no. 27, pp. 15781–15789, 1989.

[5] A. J. Barrett and P. M. Starkey, "The interaction of alpha 2-macroglobulin with proteinases. Characteristics and specificity of the reaction, and a hypothesis concerning its molecular mechanism," *The Biochemical Journal*, vol. 133, no. 4, pp. 709–724, 1973.

[6] A. J. Barrett, M. A. Brown, and C. A. Sayers, "The electrophoretically 'slow' and 'fast' forms of the alpha 2-macroglobulin molecule," *The Biochemical Journal*, vol. 181, no. 2, pp. 401–418, 1979.

[7] J. D. Ashcom, S. E. Tiller, K. Dickerson, J. L. Cravens, W. S. Argraves, and D. K. Strickland, "The human alpha 2-macroglobulin receptor: identification of a 420-kD cell surface glycoprotein specific for the activated conformation of alpha 2-macroglobulin," *The Journal of Cell Biology*, vol. 110, no. 4, pp. 1041–1048, 1990.

[8] T. Kristensen, S. K. Moestrup, J. Gliemann, L. Bendtsen, O. Sand, and L. Sottrup-Jensen, "Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the alpha 2-macroglobulin receptor," *FEBS Letters*, vol. 276, no. 1-2, pp. 151–155, 1990.

[9] S. L. Gonias, A. Carmichael, J. M. Mettenburg, D. W. Roadcap, W. P. Irvin, and D. J. Webb, "Identical or overlapping sequences in the primary structure of human alpha(2)-macroglobulin are responsible for the binding of nerve growth factor-beta, platelet-derived growth factor-BB, and transforming growth factor-beta," *The Journal of Biological Chemistry*, vol. 275, no. 8, pp. 5826–5831, 2000.

[10] J. LaMarre, G. K. Wollenberg, S. L. Gonias, and M. A. Hayes, "Cytokine binding and clearance properties of proteinase-activated alpha 2-macroglobulins," *Laboratory Investigation*, vol. 65, no. 1, pp. 3–14, 1991.

[11] P. F. Barcelona and H. U. Saragovi, "A pro-nerve growth factor (proNGF) and NGF binding protein, α2-macroglobulin, differentially regulates p75 and TrkA receptors and is relevant to neurodegeneration ex vivo and in vivo," *Molecular and Cellular Biology*, vol. 35, no. 19, pp. 3396–3408, 2015.

[12] E. L. Skornicka, X. Shi, and P. H. Koo, "Comparative binding of biotinylated neurotrophins to alpha(2)-macroglobulin family of proteins: relationship between cytokine-binding and neuro-modulatory activities of the macroglobulins," *Journal of Neuroscience Research*, vol. 67, no. 3, pp. 346–353, 2002.

[13] M. Westwood, J. D. Aplin, I. A. Collinge, A. Gill, A. White, and J. M. Gibson, "Alpha 2-macroglobulin: a new component in the insulin-like growth factor/insulin-like growth factor binding protein-1 axis," *The Journal of Biological Chemistry*, vol. 276, no. 45, pp. 41668–41674, 2001.

[14] B. B. Wolf and S. L. Gonias, "Neurotrophin binding to human alpha 2-macroglobulin under apparent equilibrium conditions," *Biochemistry*, vol. 33, no. 37, pp. 11270–11277, 1994.

[15] L. Krimbou, M. Tremblay, J. Davignon, and J. S. Cohn, "Association of apolipoprotein E with alpha2-macroglobulin in human plasma," *Journal of Lipid Research*, vol. 39, no. 12, pp. 2373–2386, 1998.

[16] K. French, J. J. Yerbury, and M. R. Wilson, "Protease activation of alpha2-macroglobulin modulates a chaperone-like action with broad specificity," *Biochemistry*, vol. 47, no. 4, pp. 1176–1185, 2008.

[17] D. R. Whiten, D. Cox, M. H. Horrocks et al., "Single-Molecule Characterization of the Interactions between Extracellular Chaperones and Toxic α-Synuclein Oligomers," *Cell Reports*, vol. 23, no. 12, pp. 3492–3500, 2018.

[18] A. R. Wyatt, P. Constantinescu, H. Ecroyd et al., "Protease-activated alpha-2-macroglobulin can inhibit amyloid formation via two distinct mechanisms," *FEBS Letters*, vol. 587, no. 5, pp. 398–403, 2013.

[19] A. R. Wyatt, N. W. Zammit, and M. R. Wilson, "Acute phase proteins are major clients for the chaperone action of α2-macroglobulin in human plasma," *Cell Stress & Chaperones*, vol. 18, no. 2, pp. 161–170, 2013.

[20] J. J. Yerbury, J. R. Kumita, S. Meehan, C. M. Dobson, and M. R. Wilson, "Alpha2-macroglobulin and haptoglobin suppress amyloid formation by interacting with prefibrillar protein species," *The Journal of Biological Chemistry*, vol. 284, no. 7, pp. 4246–4254, 2009.

[21] J. M. Mettenburg, D. J. Webb, and S. L. Gonias, "Distinct binding sites in the structure of alpha 2-macroglobulin mediate the interaction with beta-amyloid peptide and growth factors," *The Journal of Biological Chemistry*, vol. 277, no. 15, pp. 13338–13345, 2002.

[22] M. Narita, D. M. Holtzman, A. L. Schwartz, and G. Bu, "α2-Macroglobulin complexes with and mediates the endocytosis of β-amyloid peptide via cell surface low-density lipoprotein receptor-related protein," *Journal of Neurochemistry*, vol. 69, no. 5, pp. 1904–1911, 2002.

[23] P. H. Koo and W. S. Qiu, "Monoamine-activated alpha 2-macroglobulin binds trk receptor and inhibits nerve growth factor-stimulated trk phosphorylation and signal

transduction," *The Journal of Biological Chemistry*, vol. 269, no. 7, pp. 5369–5376, 1994.

[24] D. J. Liebl and P. H. Koo, "Serotonin-activated alpha 2-macroglobulin inhibits neurite outgrowth and survival of embryonic sensory and cerebral cortical neurons," *Journal of Neuroscience Research*, vol. 35, no. 2, pp. 170–182, 1993.

[25] A. R. Wyatt, J. R. Kumita, R. W. Mifsud, C. A. Gooden, M. R. Wilson, and C. M. Dobson, "Hypochlorite-induced structural modifications enhance the chaperone activity of human 2-macroglobulin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 20, pp. E2081–E2090, 2014.

[26] Z. Qiu, D. K. Strickland, B. T. Hyman, and G. W. Rebeck, "Alpha2-macroglobulin enhances the clearance of endogenous soluble beta-amyloid peptide via low-density lipoprotein receptor-related protein in cortical neurons," *Journal of Neurochemistry*, vol. 73, no. 4, pp. 1393–1398, 1999.

[27] J. J. Yerbury and M. R. Wilson, "Extracellular chaperones modulate the effects of Alzheimer's patient cerebrospinal fluid on Abeta(1-42) toxicity and uptake," *Cell Stress & Chaperones*, vol. 15, no. 1, pp. 115–121, 2010.

[28] C. Fabrizi, R. Businaro, G. M. Lauro, and L. Fumagalli, "Role of alpha2-macroglobulin in regulating amyloid beta-protein neurotoxicity: protective or detrimental factor?," *Journal of Neurochemistry*, vol. 78, no. 2, pp. 406–412, 2001.

[29] R. Cascella, S. Conti, F. Tatini et al., "Extracellular chaperones prevent Aβ42-induced toxicity in rat brains," *Biochimica et Biophysica Acta*, vol. 1832, no. 8, pp. 1217–1226, 2013.

[30] V. Alvarez, R. Alvarez, C. H. Lahoz et al., "Association between an alpha(2) macroglobulin DNA polymorphism and late-onset Alzheimer's disease," *Biochemical and Biophysical Research Communications*, vol. 264, no. 1, pp. 48–50, 1999.

[31] D. Blacker, M. A. Wilcox, N. M. Laird et al., "Alpha-2 macroglobulin is genetically associated with Alzheimer disease," *Nature Genetics*, vol. 19, no. 4, pp. 357–360, 1998.

[32] A. Liao, R. M. Nitsch, S. M. Greenberg et al., "Genetic association of an alpha2-macroglobulin (Val1000Ile) polymorphism and Alzheimer's disease," *Human Molecular Genetics*, vol. 7, no. 12, pp. 1953–1956, 1998.

[33] E. Mariani, D. Seripa, T. Ingegni et al., "Interaction of CTSD and A2M polymorphisms in the risk for Alzheimer's disease," *Journal of the Neurological Sciences*, vol. 247, no. 2, pp. 187–191, 2006.

[34] A. J. Saunders, L. Bertram, K. Mullin et al., "Genetic association of Alzheimer's disease with multiple polymorphisms in alpha-2-macroglobulin," *Human Molecular Genetics*, vol. 12, no. 21, pp. 2765–2776, 2003.

[35] X. Xu, Y. Wang, L. Wang et al., "Meta-analyses of 8 polymorphisms associated with the risk of the Alzheimer's disease," *PLoS One*, vol. 8, no. 9, article e73129, 2013.

[36] M. Zappia, I. Manna, P. Serra et al., "Increased risk for Alzheimer disease with the interaction of MPO and A2M polymorphisms," *Archives of Neurology*, vol. 61, no. 3, pp. 341–344, 2004.

[37] L. Chen, L. Baum, H. K. Ng et al., "Apolipoprotein E promoter and α2-macroglobulin polymorphisms are not genetically associated with Chinese late onset Alzheimer's disease," *Neuroscience Letters*, vol. 269, no. 3, pp. 173–177, 1999.

[38] F. Wavrant-DeVrièze, V. Rudrasingham, J. C. Lambert et al., "No association between the alpha-2 macroglobulin I1000V polymorphism and Alzheimer's disease," *Neuroscience Letters*, vol. 262, no. 2, pp. 137–139, 1999.

[39] L. Shen and J. Jia, "An overview of genome-wide association studies in Alzheimer's disease," *Neuroscience Bulletin*, vol. 32, no. 2, pp. 183–190, 2016.

[40] V. R. Varma, Predictors of Cognitive Decline Among Normal Individuals (BIOCARD) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) studies, S. Varma et al., "Alpha-2 macroglobulin in Alzheimer's disease: a marker of neuronal injury through the RCAN1 pathway," *Molecular Psychiatry*, vol. 22, no. 1, pp. 13–23, 2017.

[41] D. R. Thal, R. Schober, and G. Birkenmeier, "The subunits of alpha2-macroglobulin receptor/low density lipoprotein receptor-related protein, native and transformed alpha2-macroglobulin and interleukin 6 in Alzheimer's disease," *Brain Research*, vol. 777, no. 1-2, pp. 223–227, 1997.

[42] D. Van Gool, B. de Strooper, F. Van Leuven, E. Triau, and R. Dom, "α2-macroglobulin expression in neuritic-type plaques in patients with Alzheimer's disease," *Neurobiology of Aging*, vol. 14, no. 3, pp. 233–237, 1993.

[43] M. Shinohara, M. Tachibana, T. Kanekiyo, and G. Bu, "Role of LRP1 in the pathogenesis of Alzheimer's disease: evidence from clinical and preclinical studies," *Journal of Lipid Research*, vol. 58, no. 7, pp. 1267–1281, 2017.

[44] R. Deane, R. Bell, A. Sagare, and B. Zlokovic, "Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease," *CNS & Neurological Disorders Drug Targets*, vol. 8, no. 1, pp. 16–30, 2009.

[45] V. Adler, E. Davidowitz, P. Tamburi, P. Rojas, and A. Grossman, "α2-Macroglobulin is a potential facilitator of prion protein transformation," *Amyloid*, vol. 14, no. 1, pp. 1–10, 2007.

[46] J. Meldolesi, "Neurotrophin receptors in the pathogenesis, diagnosis and therapy of neurodegenerative diseases," *Pharmacological Research*, vol. 121, pp. 129–137, 2017.

[47] W. Borth, A. Urbanski, R. Prohaska, M. Susanj, and T. A. Luger, "Binding of recombinant interleukin-1 beta to the third complement component and alpha 2-macroglobulin after activation of serum by immune complexes," *Blood*, vol. 75, no. 12, pp. 2388–2395, 1990.

[48] T. Matsuda, T. Hirano, S. Nagasawa, and T. Kishimoto, "Identification of alpha 2-macroglobulin as a carrier protein for IL-6," *The Journal of Immunology*, vol. 142, no. 1, pp. 148–152, 1989.

[49] G. K. Wollenberg, J. LaMarre, S. Rosendal, S. L. Gonias, and M. A. Hayes, "Binding of tumor necrosis factor alpha to activated forms of human plasma alpha 2 macroglobulin," *The American Journal of Pathology*, vol. 138, no. 2, pp. 265–272, 1991.

[50] S. Gellhaar, D. Sunnemark, H. Eriksson, L. Olson, and D. Galter, "Myeloperoxidase-immunoreactive cells are significantly increased in brain areas affected by neurodegeneration in Parkinson's and Alzheimer's disease," *Cell and Tissue Research*, vol. 369, no. 3, pp. 445–454, 2017.

[51] P. S. Green, A. J. Mendez, J. S. Jacob et al., "Neuronal expression of myeloperoxidase is increased in Alzheimer's disease," *Journal of Neurochemistry*, vol. 90, no. 3, pp. 724–733, 2004.

[52] L. K. Stamp, I. Khalilova, J. M. Tarr et al., "Myeloperoxidase and oxidative stress in rheumatoid arthritis," *Rheumatology*, vol. 51, no. 10, pp. 1796–1803, 2012.

[53] D. L. Lefkowitz and S. S. Lefkowitz, "Microglia and myeloperoxidase: a deadly partnership in neurodegenerative disease," *Free Radical Biology & Medicine*, vol. 45, no. 5, pp. 726–731, 2008.

[54] R. A. Maki, V. A. Tyurin, R. C. Lyon et al., "Aberrant expression of myeloperoxidase in astrocytes promotes phospholipid oxidation and memory deficits in a mouse model of Alzheimer disease," *The Journal of Biological Chemistry*, vol. 284, no. 5, pp. 3158–3169, 2009.

[55] Y. Matsuo, H. Onodera, Y. Shiga et al., "Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion," *Stroke*, vol. 25, no. 7, pp. 1469–1475, 1994.

[56] D. K. Choi, S. Pennathur, C. Perier et al., "Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice," *The Journal of Neuroscience*, vol. 25, no. 28, pp. 6594–6600, 2005.

[57] F. Lanza, "Clinical manifestation of myeloperoxidase deficiency," *Journal of Molecular Medicine (Berlin, Germany)*, vol. 76, no. 10, pp. 676–681, 1998.

[58] D. I. Pattison and M. J. Davies, "Absolute rate constants for the reaction of hypochlorous acid with protein side chains and peptide bonds," *Chemical Research in Toxicology*, vol. 14, no. 10, pp. 1453–1464, 2001.

[59] J. Winter, M. Ilbert, P. C. F. Graf, D. Özcelik, and U. Jakob, "Bleach activates a redox-regulated chaperone by oxidative protein unfolding," *Cell*, vol. 135, no. 4, pp. 691–701, 2008.

[60] L. J. Hazell, J. J. M. van den Berg, and R. Stocker, "Oxidation of low-density lipoprotein by hypochlorite causes aggregation that is mediated by modification of lysine residues rather than lipid oxidation," *The Biochemical Journal*, vol. 302, no. 1, pp. 297–304, 1994, Pt 1.

[61] E. Malle, C. Woenckhaus, G. Waeg, H. Esterbauer, E. F. Gröne, and H. J. Gröne, "Immunological evidence for hypochlorite-modified proteins in human kidney," *The American Journal of Pathology*, vol. 150, no. 2, pp. 603–615, 1997.

[62] G. Yu, S. Zheng, and H. Zhang, "Inhibition of myeloperoxidase by N-acetyl lysyltyrosylcysteine amide reduces experimental autoimmune encephalomyelitis-induced injury and promotes oligodendrocyte regeneration and neurogenesis in a murine model of progressive multiple sclerosis," *Neuroreport*, vol. 29, no. 3, pp. 208–213, 2018.

[63] I. Volf, A. Roth, J. Cooper, T. Moeslinger, and E. Koller, "Hypochlorite modified LDL are a stronger agonist for platelets than copper oxidized LDL," *FEBS Letters*, vol. 483, no. 2-3, pp. 155–159, 2000.

[64] G. Marsche, M. Semlitsch, A. Hammer et al., "Hypochlorite-modified albumin colocalizes with RAGE in the artery wall and promotes MCP-1 expression via the RAGE-Erk1/2 MAP-kinase pathway," *The FASEB Journal*, vol. 21, no. 4, pp. 1145–1152, 2007.

[65] D. D. Tang, H. X. Niu, F. F. Peng et al., "Hypochlorite-Modified Albumin Upregulates ICAM-1 Expressionviaa MAPK–NF-κB Signaling Cascade: Protective Effects of Apocynin," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 1852340, 14 pages, 2016.

[66] L. Li Zhou, F. F. Hou, G. B. Wang et al., "Accumulation of advanced oxidation protein products induces podocyte apoptosis and deletion through NADPH-dependent mechanisms," *Kidney International*, vol. 76, no. 11, pp. 1148–1160, 2009.

[67] V. Y. Reddy, P. E. Desorchers, S. V. Pizzo et al., "Oxidative dissociation of human alpha 2-macroglobulin tetramers into dysfunctional dimers," *The Journal of Biological Chemistry*, vol. 269, no. 6, pp. 4683–4691, 1994.

[68] T. Siddiqui, M. K. Zia, S. S. Ali, H. Ahsan, and F. H. Khan, "Insight into the interactions of proteinase inhibitor- alpha-2-macroglobulin with hypochlorite," *International Journal of Biological Macromolecules*, vol. 117, pp. 401–406, 2018.

[69] S. M. Wu and S. V. Pizzo, "Alpha(2)-macroglobulin from rheumatoid arthritis synovial fluid: functional analysis defines a role for oxidation in inflammation," *Archives of Biochemistry and Biophysics*, vol. 391, no. 1, pp. 119–126, 2001.

[70] S. M. Wu, C. M. Boyer, and S. V. Pizzo, "The binding of receptor-recognized alpha2-macroglobulin to the low density lipoprotein receptor-related protein and the alpha2M signaling receptor is decoupled by oxidation," *The Journal of Biological Chemistry*, vol. 272, no. 33, pp. 20627–20635, 1997.

[71] S. K. Moestrup and J. Gliemann, "Analysis of ligand recognition by the purified alpha 2-macroglobulin receptor (low density lipoprotein receptor-related protein) Evidence that high affinity of alpha 2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors," *The Journal of Biological Chemistry*, vol. 266, no. 21, pp. 14011–14017, 1991.

[72] J. J. Abbink, A. M. Kamp, E. J. Nieuwenhuys, J. H. Nuijens, A. J. G. Swaak, and C. E. Hack, "Predominant role of neutrophils in the inactivation of alpha 2-macroglobulin in arthritic joints," *Arthritis and Rheumatism*, vol. 34, no. 9, pp. 1139–1150, 1991.

[73] S. M. Wu and S. V. Pizzo, "Mechanism of hypochlorite-mediated inactivation of proteinase inhibition by alpha 2-macroglobulin," *Biochemistry*, vol. 38, no. 42, pp. 13983–13990, 1999.

[74] S. M. Wu, D. D. Patel, and S. V. Pizzo, "Oxidized α2-Macroglobulin (α2M) Differentially Regulates Receptor Binding by Cytokines/Growth Factors: Implications for Tissue Injury and Repair Mechanisms in Inflammation," *Journal of Immunology*, vol. 161, no. 8, pp. 4356–4365, 1998.

[75] M. Shingu, S. Nonaka, H. Nishimukai, M. Nobunaga, H. Kitamura, and K. Tomo-Oka, "Activation of complement in normal serum by hydrogen peroxide and hydrogen peroxide-related oxygen radicals produced by activated neutrophils," *Clinical and Experimental Immunology*, vol. 90, no. 1, pp. 72–78, 1992.

[76] W. Vogt, "Complement activation by myeloperoxidase products released from stimulated human polymorphonuclear leukocytes," *Immunobiology*, vol. 195, no. 3, pp. 334–346, 1996.

[77] S. J. Weiss, "Tissue destruction by neutrophils," *The New England Journal of Medicine*, vol. 320, no. 6, pp. 365–376, 1989.

[78] K. Devriendt, H. van den Berghe, J. J. Cassiman, and P. Marynen, "Primary structure of pregnancy zone protein. Molecular cloning of a full-length PZP cDNA clone by the polymerase chain reaction," *Biochimica et Biophysica Acta*, vol. 1088, no. 1, pp. 95–103, 1991.

[79] G. A. Chiabrando, M. C. Sánchez, E. L. Skornicka, and P. H. Koo, "Low-density lipoprotein receptor-related protein mediates in PC12 cell cultures the inhibition of nerve growth factor-promoted neurite outgrowth by pregnancy zone protein and alpha2-macroglobulin," *Journal of Neuroscience Research*, vol. 70, no. 1, pp. 57–64, 2002.

[80] A. Philip, L. Bostedt, T. Stigbrand, and M. D. O'connor-McCourt, "Binding of transforming growth factor-β (TGF-β) to pregnancy zone protein (PZP)," *European Journal of Biochemistry*, vol. 221, no. 2, pp. 687–693, 1994.

[81] E. L. Skornicka, N. Kiyatkina, M. C. Weber, M. L. Tykocinski, and P. H. Koo, "Pregnancy zone protein is a carrier and modulator of placental protein-14 in T-cell growth and cytokine production," *Cellular Immunology*, vol. 232, no. 1-2, pp. 144–156, 2004.

[82] L. Ekelund and C. . B. Laurell, "The pregnancy zone protein response during gestation: a metabolic challenge," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 54, no. 8, pp. 623–629, 1994.

[83] L. IJsselstijn, L. J. M. Dekker, C. Stingl et al., "Serum levels of pregnancy zone protein are elevated in presymptomatic Alzheimer's disease," *Journal of Proteome Research*, vol. 10, no. 11, pp. 4902–4910, 2011.

[84] D. A. T. Nijholt, L. Ijsselstijn, M. M. van der Weiden et al., "Pregnancy zone protein is increased in the Alzheimer's disease brain and associates with senile plaques," *Journal of Alzheimer's Disease*, vol. 46, no. 1, pp. 227–238, 2015.

[85] A. Henderson-Smith, J. J. Corneveaux, M. de Both et al., "Next-generation profiling to identify the molecular etiology of Parkinson dementia," *Neurology Genetics*, vol. 2, no. 3, p. e75, 2016.

[86] C. Horne, A. W. Thomson, C. B. Hunter, A. M. Tunstall, C. M. Towler, and M. E. Billingham, "Pregnancy-associated alpha 2-glycoprotein (alpha 2-PAG) and various acute phase reactants in rheumatoid arthritis and osteoarthritis," *Biomedicine*, vol. 30, no. 2, pp. 90–94, 1979.

[87] A. W. Thomson, T. Lehner, M. Adinolfi, and C. H. W. Horne, "Pregnancy-associated alpha-2-glycoprotein in recurrent oral ulceration and Behçet's syndrome," *International Archives of Allergy and Immunology*, vol. 66, no. 1, pp. 33–39, 1981.

[88] L. Beckman, K. Bergdahl, B. Cedergren et al., "Increased serum levels of the pregnancy zone protein in psoriasis," *Acta Dermato-Venereologica*, vol. 57, no. 5, pp. 403–406, 1977.

[89] L. Beckman, K. Bergdahl, B. Cedergren et al., "Association between Duffy blood groups and serum level of the pregnancy zone protein," *Human Heredity*, vol. 29, no. 5, pp. 257–260, 1979.

[90] A. Ramos et al., "Trypanosoma cruzi: cruzipain and membrane-bound cysteine proteinase isoform(s) interacts with human alpha(2)-macroglobulin and pregnancy zone protein," *Experimental Parasitology*, vol. 100, no. 2, pp. 121–130, 2002.

[91] E. J. Sarcione and W. C. Biddle, "Elevated serum pregnancy zone protein levels in HIV-1-infected men," *AIDS*, vol. 15, no. 18, pp. 2467–2469, 2001.

[92] J. A. Zarzur, M. Aldao, S. Sileoni, and M. A. Vides, "Serum pregnancy-associated alpha 2-glycoprotein levels in the evolution of hepatitis B virus infection," *Journal of Clinical Laboratory Analysis*, vol. 3, no. 2, pp. 73–77, 1989.

[93] E. Viennois, M. T. Baker, B. Xiao, L. Wang, H. Laroui, and D. Merlin, "Longitudinal study of circulating protein biomarkers in inflammatory bowel disease," *Journal of Proteomics*, vol. 112, pp. 166–179, 2015.

[94] W. H. Stimson, "Variations in the level of a pregnancy-associated alpha-macroglobulin in patients with cancer," *Journal of Clinical Pathology*, vol. 28, no. 11, pp. 868–871, 1975.

[95] F. E. Zalazar, G. A. Chiabrando, N. A. de Aldao, F. Ojeda, M. A. Vides, and M. A. J. Aldao, "Pregnancy-associated α2-glycoprotein in children with acute lymphocytic leukemia, Hodgkin's disease and non-Hodgkin's lymphomas," *Clinica Chimica Acta*, vol. 210, no. 1-2, pp. 133–138, 1992.

[96] A. Unger, A. Kay, A. J. Griffin, and G. S. Panayi, "Disease activity and pregnancy associated alpha 2-glycoprotein in rheumatoid arthritis during pregnancy," *British Medical Journal (Clinical Research Ed.)*, vol. 286, no. 6367, pp. 750–752, 1983.

[97] J. H. Cater, J. R. Kumita, R. Zeineddine Abdallah et al., "Human pregnancy zone protein stabilizes misfolded proteins including preeclampsia- and Alzheimer's-associated amyloid beta peptide," *Proceedings of the National Academy of Sciences*, vol. 116, no. 13, pp. 6101–6110, 2019.

[98] Y. Zhang, X. Wei, S. Browning, G. Scuderi, L. S. Hanna, and L. Wei, "Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection," *Arthritis Research & Therapy*, vol. 19, no. 1, pp. 175–175, 2017.

[99] J. Leipner and R. Saller, "Systemic enzyme therapy in oncology," *Drugs*, vol. 59, no. 4, pp. 769–780, 2000.

[100] G. Lorkowski, "Gastrointestinal absorption and biological activities of serine and cysteine proteases of animal and plant origin: review on absorption of serine and cysteine proteases," *International Journal of Physiology, Pathophysiology and Pharmacology*, vol. 4, no. 1, pp. 10–27, 2012.

[101] M. T. Heneka, M. J. Carson, J. E. Khoury et al., "Neuroinflammation in Alzheimer's disease," *The Lancet Neurology*, vol. 14, no. 4, pp. 388–405, 2015.