

Soluble CD21: From Shedding to Autoimmunity

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Keywords

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Abstract

Background: Soluble CD21 (sCD21) is the product of metalloprotease-mediated proteolysis of CD21, a mechanism in which the entire extracellular domain of CD21 is shed from the cell surface. Through its retained ligand-binding ability and presence in human serum, sCD21 joins the growing list of surface proteins shed from the leukocyte cell surface which allows modulation of the immune response. **Summary:** sCD21 plays a multifaceted role in the body, including the promotion of inflammatory responses through receptor-ligand interactions with monocyte CD23, acting as a decoy receptor during Epstein-Barr virus infection preventing lymphoproliferation, and suppression of IgG and IgE responses by competitively inhibiting cell surface CD21. Clinical studies have shown that in comparison with healthy individuals, levels of sCD21 in serum are significantly altered in various diseases, highlighted by diverse viral infections, B-cell leukemias, and autoimmune disorders. **Key Messages:** Although findings of prevalence and functionality suggest sCD21 to be a key modulator of cellular and humoral immunity, questions remain about its origins and the regulation of its responses. Here, we aim to clarify and connect the advances in understanding sCD21

over time with emphasis on its generation by surface cleavage, binding partners, and functional roles. We also provide an outlook on its clinical significance and usage as a diagnostic target and therapeutic biomarker to monitor treatment efficacy in the context of chronic autoimmune disorders.

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Introduction

Complement receptor 2 or CD21 is a 145 kDa membrane glycoprotein that acts as the primary cellular receptor for C3d and other complement C3 fragments [1] alongside the gp350/220 entry glycoprotein of the Epstein-Barr virus (EBV) [2]. Similar to other complement-associated proteins, CD21 is composed of either 19 or 20 short consensus repeats (SCRs), based on the alternative usage of exon 11 encoding SCR11 [3]. CD21 is composed of three distinct regions, a large extracellular domain made up almost fully of SCRs, and a transmembrane domain and cytoplasmic domain [4]. On the surface of B lymphocytes, CD21 associates with CD19, CD81, and Leu-13 to form the B-cell coreceptor complex [5] which aids in decreasing the B-cell receptor (BCR) signal threshold and the processing of antigens [6]. On dendritic cells, CD21 alongside CD35 aids in the maintenance of immune complexes on the cell surface in

the germinal center [7]. Expression of surface CD21 has also been suggested on thymocytes [8], astrocytes [9], basophils [10], and peripheral T lymphocytes [11]. Alongside complement C3 fragments and EBV, CD21 also recognizes the low-affinity IgE receptor CD23 [12] and the type I cytokine IFN- α [13]. Like several other leukocyte surface molecules, CD21 can be shed from the cell surface to yield soluble CD21 (sCD21), a molecule found in lymphocyte cell supernatants [14] and circulating in human blood [15]. Since its discovery as a serum protein that is regularly shed from the cell surface, sCD21 has been associated with the modulation of the normal immune response but also in the pathogenesis of multiple infectious and autoimmune diseases. Especially in viral and chronic inflammatory autoimmune disorders, sCD21 levels in serum are dysregulated and can be linked to disease pathophysiology. Here, we discuss the cellular mechanisms that govern the generation of sCD21, its potential functions in the normal immune response and disease, and lastly its application as a clinical biomarker for diagnosis and monitoring.

Generation of sCD21

The generation of soluble forms of cell surface molecules is usually subject to two forms of regulation: posttranscriptional regulation through the removal of the transmembrane regions or posttranslational regulation through protease cleavage from the cell surface [16]. In the generation of B-cell immunoglobulin receptors, transcripts encoding both the membrane-bound form and secreted form are generated by alternative mRNA splicing. Transcripts contain a hydrophobic transmembrane domain or a hydrophilic secretory tail, depending on which exon is utilized [17]. In numerous extracellular proteins and receptors such as CD14, CD23, and CD35, the soluble fragment is generated by enzymatic cleavage of the surface-bound molecule [18] resulting in the release of the extracellular domain termed the “ectodomain” [19]. Unlike most other molecules where a single mechanism is employed, the receptor for IL-6 can be generated by shedding from the cell surface [20] and via an alternatively spliced mRNA transcript lacking the transmembrane domain [21].

Alternative splicing was excluded as a mechanism to generate sCD21 as reverse-transcription PCR analysis of CD21 transcription did not reveal transcripts lacking the exons encoding the transmembrane domain [22]. This initial finding suggests that similar to other leukocyte receptors, sCD21 is released from the cell surface by

protease cleavage. While the exact protease responsible for cleavage of membrane CD21 has yet to be identified, it has been suggested to be ADAM17, a member of the a disintegrin and metalloproteinase (ADAM) family [23]. These transmembrane metalloproteases are specialized in the cleavage near the plasma membrane to allow for the release of the soluble ectodomain of their substrate protein. Most metalloproteases including the ADAM family are synthesized as inactive precursors, where proteolytic cleavage of the prodomain by serine endoproteases [24] results in their activation [19]. Regulation of activity can be attributed to a conserved cysteine residue present in the prodomain which interacts with the Zn²⁺ ion present in the catalytic domain and blocks the active site. Upon proteolysis of the prodomain by serine endoproteases, the cysteine dissociates from the Zn²⁺ ion, a mechanism termed the “cysteine switch” allowing the metalloprotease to become active [25].

A commonly associated enzyme in the recruitment of metalloproteases and signal transduction in shedding pathways is protein kinase C (PKC), where stimulation of PKC has been shown to increase shedding processes. Activation of PKC is often achieved through the mitogen phorbol myristate acetate (PMA) or Ca²⁺ ionophores such as ionomycin (Cal) which activate the kinase [19]. Stimulation of peripheral B lymphocytes and the Burkitt lymphoma (BL) cell line Raji with either PMA or Cal has shown to increase the amounts of sCD21 present in the culture supernatant [26]. Despite PMA/Cal being an unspecific stimulus, PKC is likely directly involved in the signal transduction pathway leading to CD21 shedding. Using the oxidant pervanadate, similar effects on sCD21 levels were seen in the BL cell line Daudi, peripheral B lymphocytes, and even the human embryonic kidney cell line 293 transfected to express full-length CD21 [23]. Interestingly, pervanadate mimics stimulation of the BCR activating similar signal transduction pathways leading to tyrosine phosphorylation [27], suggesting B-cell activation via the BCR may lead to CD21 shedding. This hypothesis was experimentally verified, as stimulation of IgM and CD40 by monoclonal antibodies (mAbs) on peripheral B lymphocytes induced CD21 shedding [26]. This activation-mediated shedding suggests a physiological role of sCD21 in the immune response upon B-cell recognition of an antigen. As pervanadate-mediated shedding can be inhibited by using the PKC inhibitor BIM [23], the signal transduction pathway leading to CD21 shedding likely involves PKC and similar tyrosine kinases as those active in BCR signaling.

The antioxidants and reducing agents N-acetylcysteine (NAC) and glutathione have also been shown to increase

Table 1. List of experimental stimuli which induce CD21 shedding

Type	Stimulus	Proposed target	Reference
Mitogen	PMA	PKC	[26]
Ca ²⁺ ionophore	Cal	PKC	[26]
Oxidant	Pervanadate	Tyrosine kinases	[23]
mAbs	Anti-IgM/anti-CD40	BCR signaling pathway	[26]
Thiol antioxidant	NAC ^a	Metalloprotease	[23, 28]
Thiol antioxidant	GSH	Metalloprotease	[23]
Nucleoside	ATP	P2X7R	[30]

GSH, glutathione. ^aReports are contradictory about the effectiveness of NAC in the induction of CD21 shedding.

the amount of shed sCD21 in the cell supernatant, coupled with a decrease of membrane-bound CD21 [23]. While previous studies have also found decreases in membrane-bound CD21 upon treatment with NAC, no associated increase in the amount of shed sCD21 was seen [28]. Both studies taken together raise questions of if and how reducing agents affect the shedding of CD21. Aichele et al. [23] showed that NAC-induced CD21 shedding was independent of tyrosine phosphorylation, suggesting reducing agents affect a different part of the signal transduction pathway than pervanadate or PMA/Cal [23]. While metalloprotease inhibitors blocked NAC-induced shedding, no effect was seen if serine endoprotease inhibitors were used, suggesting NAC may directly activate the metalloprotease causing CD21 shedding. While this mechanism is still unclear, a potential hypothesis suggests NAC can cause dissociation of the cysteine residue from the zinc ion of the prodomain by reduction to cause metalloprotease activation.

Another molecule shown to induce shedding of CD21 to generate sCD21 is adenosine triphosphate (ATP), a mechanism which has been observed for numerous cellular receptors including CD23 [29]. Using the ATP analog benzoyl-benzoyl triphosphate, CD21 shedding and sCD21 generation were inducible in human PBMCs [30]. This effect was pinpointed to the ATP receptor P2X7R, as the effect on CD21 was abolished upon usage of a P2X7R inhibitor. As PKC and metalloproteases [31] such as ADAM17 [32] are one downstream target of P2X7R, this ATP-sensitive mechanism may be yet another stimulus of CD21 shedding similar to exposure to mitogens or BCR stimulation. Taken together, all stimuli that can induce CD21 shedding (shown in Table 1) likely affect different parts of the signal transduction pathway. Through stimulation, the activity of PKC and tyrosine kinases is triggered activating the metalloprotease to shed

CD21, either through a serine endoprotease or directly by a reducing mechanism.

Instead of a specific shared sequence, proteases especially those of the ADAM family require a specific length of amino acids to be present as the juxtamembrane region or “stalk” to mediate cleavage. The cleavage site of sCD21 has been proposed to lie inside SCR16, the SCR closest to the cell surface and adjacent to the transmembrane domain [23]. SCR16, like the other SCRs of CD21, contains four cysteine residues that are involved in two disulfide bonds where the first cysteine of each SCR (Cys-1) bonds to the third (Cys-3), and the second (Cys-2) to the fourth (Cys-4) [33]. Interestingly, in the normal oxidized form of membrane CD21, two amino acids separate the transmembrane domain and the disulfide bridge involving Cys-4 of SCR16. As ADAM metalloproteases require a stalk region of at least 11 amino acids, cleavage of CD21 is likely not possible in its oxidized state [19]. Reduction of the Cys-4 to Cys-2 bridge would extend the stalk region to 14 amino acids and likely would be sufficient to allow protease cleavage [23]. The impact of the Cys-2 to Cys-4 disulfide bridge in SCR16 was experimentally verified through two systems: replacing both cysteines with selenocysteines to form a non-reducible diselenide bridge or replacing them with two methionine residues preventing bridge formation. NAC treatment of Daudi and human embryonic kidney cell line 293 transfected with constructs encoding both modified variants, and normal CD21 revealed shedding was inhibited by the diselenide bridge and further increased in the methionine system [23]. As CD21 shedding was drastically reduced if the Cys-2 to Cys-4 disulfide bridge is not reduced, it can be suggested that reduction of this disulfide bridge forms the stalk region for ADAM cleavage and is a prerequisite to shedding. Supporting the hypothesis that cleavage occurs in SCR16, the isolation of

human sCD21 reveals a protein of 126 kDa [34], which corresponds to the full ectodomain if cleavage occurs in SCR16. All in all, shedding of CD21 is likely controlled by a complex interconnected signaling pathway involving PKC, activation of metalloproteases by the “cysteine switch” mechanism and reduction of the disulfide bridge present in SCR16. More research on the exact signaling cascade and the specific ADAM metalloprotease is required to gain a full understanding of the cellular mechanisms which lead to sCD21 generation.

Function of sCD21 and Presence in Human Sera

Downmodulation of surface proteins by ectodomain shedding is capable of accomplishing two main goals, either the decrease of target surface protein density or the generation of a soluble ectodomain [19]. Given the specific cleavage of CD21 to release the entire extracellular domain and its retained ligand-binding ability, CD21 shedding is highly likely to occur as a mechanism to generate sCD21 as a soluble form of the membrane-bound receptor.

The presence of a soluble fragment of CD21 was first reported in 1981, as a 72 kDa glycoprotein (gp72) which was capable of binding C3d was identified to be present in the supernatant of Raji and Daudi cell lines [14]. gp72 was thought to be a soluble form of membrane-bound CD21; however, after discovery that the molecular weight of CD21 was around 140 kDa [35], gp72 either represented a degradation product or a second cellular C3d-receptor. The relationship of gp72 to membrane-bound CD21 was clarified in 1987, showing that gp72 is a soluble molecule generated by CD21 proteolysis, and its generation could be inhibited using protease inhibitors [36]. Initial functional suggestions included that sCD21 might act as a competitive inhibitor to B cells for CD21-associated ligands. As the C3d- and EBV-binding sites were present on gp72, it might compete with membrane CD21 for binding to C3d or block B-cell infection by EBV through binding of its outer glycoproteins [36].

The hypothesis that the generation of sCD21 may play a role in B-cell function was strengthened by the discovery of a soluble form of CD21 in the serum of healthy patients as well as those with B-cell chronic lymphocytic leukemia (B-CLL) in 1989. Using two mAbs which allowed for the cross-linking of CD21 between sheep erythrocytes, sCD21 was detected to circulate in human serum. In the same study, a soluble form of CD23 (sCD23) was also discovered to be present in serum and even urine [15]. A potential connection between sCD21

and infection of B cells by EBV was experimentally verified as recombinant sCD21 inhibited *in vitro* infection of peripheral B lymphocytes by EBV [37] and blocked *in vivo* EBV-mediated proliferation of B cells [38]. Results of Nemerow et al. [37] and Moore et al. [38] provide further evidence which suggests that serum sCD21 might play a similar role *in vivo* to prevent EBV infection by acting as a decoy receptor for EBV. If sCD21 occupies all EBV gp350/220 sites, the binding to membrane-bound CD21 on the B cell is inhibited, preventing infection.

Given the presence of sCD21 in human serum, its cellular origins were initially suggested to be a result of shedding by peripheral B lymphocytes. In 1991, the origins of sCD21 were analyzed showing sCD21 to be present in supernatant of cultured tonsillar and peripheral B cells [39]. sCD21 was found as a 67 kDa molecule found at concentrations ranging between 30 and 90 ng/mL in the serum of healthy donors and was capable of binding to immobilized EBV and C3d. Ling et al. [39] further analyzed serum levels showing similar sCD21 levels in patients with common variable immunodeficiency and X-linked agammaglobulinemia, genetic disorders in which mature B-cell numbers are reduced or completely absent. The lack of change to sCD21 levels suggests B cells may not be the primary source of serum sCD21. Follicular dendritic cells (FDCs) or T cells were suggested as potential sources, supported by the high density of CD21 expressed on the surface of FDCs [7] and low expression on the surface of T cells [11] which supported increased shedding to generate the serum pool.

Provided the differences in molecular weight and identity of reported sCD21 molecules in human serum and cell supernatants, its properties were further analyzed in 1992 to elucidate its origins and functions. sCD21 which was affinity-purified from several B-cell lines was shown to have a molecular weight of around 130 kDa, which was reduced to 115 kDa upon treatment with an N-glycosidase that removes glycosylation. Alongside the 130 kDa molecule, several smaller proteins of around 20–40 and 60 kDa were also identified [40]. The 130 kDa molecule would approximately match the entire extracellular domain which Ling and Brown [39] hypothesized was shed from the cell surface, and smaller products may be side effects of metabolic labeling or products of degradation. Using gel filtration, cell line and serum CD21 were shown to have a molecular weight of 320 kDa, suggesting it may circulate as a complex with other molecules or be produced during filtration due to its rod-like shape [40]. Supporting both the hypothesis of Ling and Brown [39] that sCD21 represents the full

extracellular domain and that CD21 is subject to cleavage, a 135 kDa sCD21 was experimentally demonstrated to be shed from the cellular surface of both peripheral B and T lymphocytes [41]. The 135 kDa sCD21 was directly related to a decrease in membrane-bound CD21, proving its generation by protease cleavage of membrane CD21 and retained its ability to bind iC3b and CD23. While its properties and size were identified, its role in the immune response was still unclear.

Potential binding partners of sCD21 besides complement proteins include immune complexes and even the low-affinity IgE receptor CD23. Interactions between CD21 and CD23 have been mapped to specific sites on CD21, showing a lectin-sugar interaction in SCRs 5–8 of CD21 and an accessory protein-protein interaction in SCRs 1–2 [42]. CD23 has also been shown to naturally undergo proteolytic cleavage to smaller sCD23 fragments which can form soluble oligomers. Based on the presence of the lectin-head domain in each fragment, sCD23 fragments were hypothesized to either bind one IgE and one sCD21, or up to three sCD21 molecules [43]. Experimental evidence for such interactions between sCD21 and sCD23 in human serum was shown in 1998, in which SDS-PAGE separation of affinity-purified sCD21 revealed the presence of several proteins between 25 and 75 kDa alongside the 135 kDa main form of sCD21 [44]. Further immunoblot analysis of these proteins revealed reactions with antibodies against CD23, complement C3 and iC3b, and the immunoglobulins IgG and even IgE. Frémeaux-Bacchi et al. [41] were further able to determine that 75 kDa CD23 present in sCD21 complexes is in a trimer of three 25 kDa sCD23 molecules and, given the reactivity of an anti-IgE mAb, supports the hypothesis that sCD23 is capable of binding IgE and CD21 with its three lectin heads to form a complex.

A role of CD21 in IgE responses was initially proposed in 1992, where it was shown that ligation of membrane CD21 by anti-CD21 mAbs or sCD23 increased the production of IgE in IL-4-stimulated B cells [12]. A suppressive effect of sCD21 on this interaction was shown in 1998, as the stimulatory effect on IgE generation by sCD23 binding to membrane CD21 was completely inhibited by the addition of sCD21. This suppression is likely due to sCD21 acting as a competitive inhibitor to membrane CD21 for binding to sCD23 [44]. This active inhibition of IgE responses suggests sCD21 may play a role in dampening allergic responses or as a regulatory mechanism to curb the stimulatory effect on IgE production upon CD21 ligation. Contrasting the binding of sCD23 to membrane CD21, the binding of sCD21 to membrane CD23 was shown as the first biological

function of sCD21, where the interaction on IL-4-cultured human monocytes resulted in their activation, proliferation, and release of inflammatory mediators [45]. Purified serum sCD21 was shown to directly interact with surface CD23 on IL-4-cultured monocytes, resulting in the activation of nitric oxide synthase signaling pathways causing the release of IL-6 and TNF, and the upregulation of CD40 and MHC II. Based on the ability of sCD21 to modulate monocyte responses, Frémeaux-Bacchi et al. [44] proposed a “cytokine-like” role of sCD21. Monocytes either in serum or tissue could potentially interact with sCD21 via CD23 and potentially be polarized toward a pro-inflammatory phenotype or differentiation.

The importance of CD21 in T-cell-dependent responses was demonstrated in mice using a recombinant fusion protein of the N-terminal C3d-binding SCRs of CD21 and IgG1, capable of acting as a competitive inhibitor to the B-cell membrane-bound CD21. Immunization of mice in the presence of the soluble molecule suppressed the humoral immune response, with a decreased primary response, impaired class switching, and decreased IgG titers in response to T-cell-dependent antigens [46]. The effects of a recombinant sCD21 show potential for an additional immunosuppressive role *in vivo* where cellular responses to T-cell-dependent antigens can be downregulated. Using the same IgG-CD21 chimera, mouse IgG responses were able to be suppressed *in vitro* by blocking the interaction of C3 fragments deposited on FDCs with B-cell membrane-bound CD21 [47]. Inhibition of this interaction likely directly impacts the costimulatory signal delivered by the CD19/CD21 coreceptor complex needed to lower the activation threshold of B cells. Given its hypothesized high shedding from FDCs in germinal centers, sCD21 may allow for local immune suppression of humoral responses and downregulation of responses to T-cell-dependent antigens.

Despite the characterization that the two splice isoforms of CD21 based on the inclusion or removal of exon 11 are expressed simultaneously [22], mainly the full-length form makes up sCD21 found in human serum [48]. In this study, sCD21 protein isoforms were measured by cross-linking sCD21 between sheep erythrocytes coated with an mAb specific for the full-length isoform. Given the finding that FDC mainly expresses the full-length isoform [49], it was suggested that the serum pool of sCD21 is mainly generated by FDCs. Contrary to this hypothesis, later mass spectroscopic analysis of sCD21 isolated from human serum indicated the opposite that the alternatively spliced exon 11-lacking form makes up sCD21 found in human serum [26]. In an earlier analysis,

Masilamani et al. [26] were also able to further characterize and isolate human serum sCD21 using a novel two-step process of affinity chromatography and density gradient centrifugation and described a 126 kDa molecule which corresponds to the entire extracellular domain, if cleaved in the immediate proximity of the plasma membrane [34]. Given the differences in reported splice isoform present in human sera, further research is required to fully elucidate which cells contribute to the serum pool of sCD21 and which intracellular pathways are involved in the splicing process of CD21 mRNA. As B-cell activation has been shown to be a stimulus which increases shed CD21, perhaps activation or pro-inflammatory stimulation may also alter the splice behavior of CD21; however, this remains fully unexplored.

While sCD21 has been demonstrated to be involved in several *in vitro* and *in vivo* interactions, its role in the human body is likely a combination of roles in pro-inflammatory, protective, and regulatory responses. In response to EBV infection, sCD21 may act as a decoy receptor to prevent viral binding and entry, while its capability of binding complement C3 ligands and surface receptors such as CD23 allows for formation of larger serum complexes. Whether these complexes are associated with complement proteins or CD23/IgE, sCD21 likely plays a role in the transport of immune complexes in serum. The formation of immune complexes containing IgE and inhibition of IgE formation may hint at sCD21 modulating allergic responses by facilitating serum clearance of IgE. Given its capability to actively affect monocyte activity and promote a pro-inflammatory response, sCD21 may also modulate other immune cells in similar fashions to alter their phenotypes and associated functions.

sCD21 in Human Disease

A clinical importance for sCD21 was proposed alongside its initial characterization of its presence inside normal human serum, due to the finding that B-CLL patients had elevated levels of the soluble receptor in their serum. The association of sCD21 and various diseases has become more apparent over the years, as serum levels of sCD21 and its expression on the B-cell surface have continually been shown to be dysregulated in chronic viral and autoimmune diseases. In these disorders, sCD21 serves as a valuable serum biomarker for not only clinical diagnosis but also for monitoring disease progression and enabling therapeutic developments.

The elevated sCD21 levels found in B-CLL were initially attributed to the large number of leukemic B-CLL

cells in circulation capable of generating sCD21 [15]. Despite higher levels of sCD21 in serum, individual B-CLL cells were shown to have reduced surface expression of CD21 [50]. No evidence of autoantibodies against CD21 or other complement proteins was found which might decrease surface levels and B-CLL survival [51]. Given the upregulated expression of metalloproteases in early B-CLL, shedding of sCD21 may be promoted from B-CLL cells to generate an elevated serum pool [52]. In B-CLL, the elevated levels of sCD21 were also shown to correlate with high expression of ZAP70 and the presence of unmutated heavy-chain immunoglobulin genes.

Elevated serum sCD21 was also detected in several viral infections that induce B-cell proliferation, namely, EBV and rubella virus [53]. Experimental evidence connecting EBV infection and CD21 expression was shown as early as 1987, where *in vitro* EBV infection of BL cell lines was shown to upregulate CD21 and CD23 [54]. The importance of Epstein-Barr nuclear antigen 2 (EBNA2) to this upregulation was demonstrated as no changes were observed upon infection with an EBNA2-lacking EBV strain [54]. EBNA2-transfected BL cells were also shown to express higher levels of CD21 and CD23 mRNA alongside CD21 [55]. The generation of sCD21 by EBV-infected B cells can also be directly attributed to EBNA2, as *in vitro* transfection of BL cell lines resulted in upregulation of CD21 mRNA, CD21 surface receptors, and amounts of sCD21 found in the culture supernatant of the transfected BL cell lines [56]. Furthermore, this was pinpointed to type 2 EBNA2 produced by EBV-2, highlighting a specific interaction of EBNA2 with the CD21 to alter B-cell gene expression after infection [57]. Increased shedding of sCD21 as a result of EBV infection and EBNA2 activity opens up an intriguing question as to how EBV benefits from sCD21 shedding to EBV, despite CD21 being its viral entry receptor to allow infection of cells. This question raised by Larcher et al. [56] is supported by the findings of Huemer et al. [53] that serum levels of sCD21 are increased upon EBV infection, suggesting that EBV infection leads to *in vivo* release of sCD21. This supports the hypothesis that increased sCD21 release might confer a protective role where sCD21 acts as a decoy receptor to slow viral entry and B-cell infection. Further research into EBV-mediated sCD21 generation is required, but other hypotheses suggested a potential virus-mediated release of sCD21 to inhibit local B-cell presentation of EBV antigens upon infection. As membrane CD21 as part of the coreceptor complex is crucial in enhancing antigen processing and presentation [6], this can promote viral survival and favor

Table 2. Comparison of serum sCD21 levels in various diseases and disorders

Disease type	Condition	Effect on sCD21 levels	Reference
Leukemia	B-CLL	Increased	[15]
Viral infection	EBV	Increased	[53]
	Rubella virus	Increased	[53]
	Dengue virus	Decreased	[58]
Autoimmune disorder	RA	Decreased	[59]
	SLE	Decreased	[60]
	Sjögren's syndrome	Decreased	[60]
	JA ^a	Decreased	[61]
	APS	Decreased	[62]
	SSc	Decreased ^b	[63, 64]
	Myasthenia gravis	Decreased	[65]
MS	Decreased	[66, 67]	

SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome. ^aDecreased serum sCD21 levels were only seen in pauciarticular, polyarticular, and systemic JA. ^bReports are contradictory about the levels of sCD21 in serum of SSc patients.

chronic infection. While viral infections seem to increase the levels of sCD21 in serum, more recent research reveals both sCD21 and sCD35 are reduced in patients with dengue fever as a result of dengue virus infection [58]. The cause for the lack of correlation with other viral diseases remains unclear; however, in dengue fevers, sCD21 could also directly be linked to disease progression with sCD21 levels decreasing as severity increases. Given the potent immune and complement activation that occurs during dengue virus infection and the effects on sCD21 levels, the loss of the regulatory functions of sCD21 may play a critical role in disease progression over time.

While previous clinical instances of altered sCD21 levels in human disease have involved elevated levels in viral infection and leukemia, serum levels have been reduced in all autoimmune disorders analyzed to this date (summarized in Table 2). The first link between sCD21 and autoimmunity was found in patients with rheumatoid arthritis (RA). While levels of serum sCD21 in healthy patients were found to decrease with age, using an ELISA-based approach, patients with RA were found to have significantly decreased levels of serum sCD21 irrespective of age [59]. Further correlation between reduced serum sCD21 and autoimmunity was established based on the findings that similar reduced sCD21 levels were also present in other notable autoimmune conditions, namely, systemic lupus erythematosus and Sjögren's syndrome [60]. In both conditions, serum levels of sCD21 were found to be decreased when compared to healthy donors and were similar to levels found earlier in

RA. In all patients, levels of sCD21 in synovial fluid were also found to be further reduced compared to serum levels, but concentrations were independent of one another.

Decreased sCD21 in the synovial fluid is potentially due to the cellular makeup of the fluid, as B lymphocytes in synovial fluid of patients with arthritic diseases have been shown to express lower levels of surface CD21 when compared to peripheral blood B lymphocytes [68]. These B cells expressing lower surface levels of CD21 (CD21^{lo} B cells) have also been characterized in numerous autoimmune and chronic infections, recently reviewed extensively by Gjertsson et al. [69]. Reduced levels of sCD21 in synovial fluid were also found in patients with several arthritic diseases including RA, indicating reduced shedding of B cells in the rheumatoid joints is possibly linked to their low surface expression of CD21 [70]. While levels of sCD21 in serum from juvenile arthritis (JA) patients were reported to be normal, a deeper analysis of JA subtypes shows a similar decrease as seen in other arthritic diseases in pauciarticular JA, polyarticular JA, and systemic JA [61]. Similar decreases were also seen with sCD23 in JA subtypes, further highlighting the important interactions and relationships between sCD21 and sCD23 in human serum where impairment might contribute to disease progression. Even in antiphospholipid syndrome, low serum levels of sCD21 were observed, however without any correlation to anti-β₂-glycoprotein 1 antibodies which are markers of antiphospholipid syndrome [62]. As high levels of anti-β₂-glycoprotein 1 antibodies would have the possibility to

form immune complexes capable of binding C3d and sCD21, these could mediate its clearance from serum and explain the reduction. However, the lack of correlation to anti- β 2-glycoprotein 1 antibody presence suggests immune complexes are not directly involved in the reduction of sCD21 levels in serum. Similar to the synovial fluid of arthritic patients, CD21^{lo} B cells were also detectable in myasthenia gravis and patients had markedly decreased serum levels of sCD21 [65]. Either increased clearance due to elevated levels of complement proteins or low shedding from the cell surface might be the cause of this decrease. Interestingly, analysis of serum sCD21 levels during and after pregnancy also revealed a sharp decline during pregnancy and stabilization of sCD21 levels postpartum [71].

In systemic sclerosis (SSc), a similar effect on serum sCD21 was observed, where it was hypothesized to be the result of enhanced serum clearance by overexpressed complement proteins or in contrast to B-CLL, reduced shedding through higher expression of metalloprotease inhibitors [63]. Despite this finding, more recent research found no significant difference in serum sCD21 levels when comparing SSc patients to healthy controls [64]. In the same study, sCD21 was analyzed as a soluble marker for B-cell activation, highlighting the potential of sCD21 to function as a diagnostic marker for specific disorders where its serum levels may be altered. As elevated CD21^{lo} B cells have now also been detected in SSc [72] and shown to have enhanced activation profiles [73], a potential association between increased CD21^{lo} B cells and decreases to sCD21 levels continues to be a relevant characteristic in the diagnosis and monitoring of rheumatic diseases.

Besides rheumatic autoimmune conditions, altered sCD21 levels have also been observed in chronic neuroinflammatory disorders with a heavy focus on multiple sclerosis (MS). In line with the pathophysiology of MS as an inflammatory autoimmune disorder in which the immune system targets myelin and the central nervous system, lowered levels of sCD21 have been detected in patient serum [66]. As EBV is implicated in the development of MS, sCD21 might also function as a decoy receptor in MS to bind EBV and potentially inhibit further lymphoproliferation. Further studies of MS patients confirmed a reduction of serum sCD21, but the elevation of sCD83 where a correlation was seen with the amount of anti-EBNA-1 antibodies was present [67]. As chronic EBV infection has previously been shown to cause lymphoproliferation [53] and elevate serum sCD21 potentially due to increased shedding [56], EBV may only be involved in MS development and not its progression.

While viral infection with EBV is believed to be one of the causative agents of MS, latest research shows that immune memory against cytomegalovirus may confer a protective role in the onset of MS [74]. In CMV-immune early-onset MS patients, elevated levels of serum sCD21 were identified in contrast to the decreased levels typically seen as MS progresses [66]. The association between high sCD21 levels alongside lower MS progression markers such as neurofilament light chain suggests this shift might occur due to some aspect of sCD21's role as a protective immunomodulator preventing MS progression.

While alterations of serum sCD21 open possibilities to use sCD21 as a clinical marker for disease diagnosis and progression, it can also function as a viable clinical biomarker monitoring disease treatment efficacy. In the search for an effective treatment against primary progressive multiple sclerosis (PPMS), sCD21 was used as a soluble marker present in cerebrospinal fluid (CSF) in clinical trials of rituximab: an mAb against CD20 aiming for B-cell depletion in central nervous tissue to alleviate inflammation [75]. The levels of sCD21 in CSF as well as the ratio of sCD21 to the total number of CNS B cells were both used to measure rituximab effectiveness. In a later clinical trial also analyzing the efficacy of rituximab, the same two measures were used as markers revealing rituximab mainly depletes CSF B cells and fails to effectively target the CNS-resident B cells which are implicated in PPMS [76]. In trials of the mAb natalizumab against leukocyte α 4 β 1 integrin and the corticosteroid methylprednisolone, sCD21 was again used to measure treatment efficacy and gain insight on the effect of treatment on the B-cell compartment [77]. Together with other markers such as sCD27 [78], sCD14 or sCD163 [79], sCD21 proves to be an extremely valuable clinical biomarker extending beyond solely disease monitoring to rapid screening of candidate drugs in development of new PPMS therapies. Despite its implicated functionality in inhibiting IgE responses, the role of sCD21 in allergic diseases has not been explored but it could potentially be used to the same effect for both diagnosis and disease monitoring.

Conclusions

The properties and occurrences of sCD21 in the human body have been well characterized over the years; however, many questions remain about which mechanisms govern its generation and function. Experimental work has demonstrated a combination of roles including competition with membrane-bound CD21 for ligand availability, local immune suppression through inhibition

of IgE and IgG responses, and modulation of monocyte activation by pro-inflammatory polarization. Its shedding from the cell surface by protease cleavage opens numerous possibilities for both intracellular and external regulation which in turn modulate its serum availability and functions in the immune response and disease. While the exact mechanisms of its generation and involvement in disease remain to be explored, it is clear that sCD21 plays a multifaceted role as a modulator of immunity. Considering its well-documented association with chronic viral, leukemic, and autoimmune diseases, sCD21 continues to be a useful clinical indicator for disease diagnosis and the evaluation of new therapeutic approaches.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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