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CD5L is a canonical component of circulatory IgM

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Immunoglobulin M (IgM) is an evolutionary conserved key component of humoral immunity, and the first antibody isotype to emerge during an immune response. IgM is a large (1 MDa), multimeric protein, for which both hexameric and pentameric structures have been described, the latter additionally containing a joining (J) chain. Using a combination of single-particle mass spectrometry and mass photometry, proteomics, and immunochemical assays, we here demonstrate that circulatory (serum) IgM exclusively exists as a complex of J-chain-containing pentamers covalently bound to the small (36 kDa) protein CD5 antigen-like (CD5L, also called apoptosis inhibitor of macrophage). In sharp contrast, secretory IgM in saliva and milk is principally devoid of CD5L. Unlike IgM itself, CD5L is not produced by B cells, implying that it associates with IgM in the extracellular space. We demonstrate that CD5L integration has functional implications, i.e., it diminishes IgM binding to two of its receptors, the FcαμR and the polymeric Immunoglobulin receptor. On the other hand, binding to FcμR as well as complement activation via C1q seem unaffected by CD5L integration. Taken together, we redefine the composition of circulatory IgM as a J-chain containing pentamer, always in complex with CD5L.

IgM | CD5L | mass spectrometry | immunoglobulin | structure

Significance

Immunoglobulin M (IgM) is in high concentrations present in human body fluids, notably in serum, milk, and saliva. IgM also represents the first antibody isotype in our immune response. Current knowledge describes IgM as a multimeric protein assembly, displaying both hexameric and pentameric structures, the latter additionally enclosing a joining (J) chain. In contrast, here we show that all serum IgM contains an additional protein, namely CD5L, bound exclusively to IgM pentamers that have incorporated the J-chain. In milk and saliva, of the same donors, IgM is completely devoid of CD5L and binds instead to the secretory component of pIgR (polymeric Immunoglobulin receptor). We demonstrate that CD5L incorporation affects binding to explicit IgM receptors.

Immunoglobulin M (IgM) is the first antibody isotype to emerge in ontology and during an immune response. IgM is integral to the initiation of the humoral immune response but also for maintaining immune homeostasis through the induction of tolerance and for instance the clearance of apoptotic cells (1, 2). It is furthermore a potent activator of the classical pathway of the complement system and regulates antigen presentation and B-cell maturation through interactions with the IgM-specific receptors FcμR (3) and FcαμR (4). Additionally, secretory IgM plays an important role in mucosal immunity, as the integration of a small joining (J) chain allows it to be transported to mucosal surfaces via the polymeric immunoglobulin receptor (pIgR) (5). IgM is present throughout our body, with concentrations in serum, human milk, and saliva of ca. 1,500 mg/L (6), 2.8 mg/L (7) and 1.2 mg/L (8), respectively.

IgM is expressed by B cells as a precursory monomeric (H2L2), membrane-bound B cell receptor, which recognizes antigen and relays survival and proliferation signals for maturing B cells. When a B cell switches to IgM secretion through alternative splicing of the μ heavy chains (HC), it can concomitantly coexpress the J-chain. Five IgM protomers, each consisting of two HC coupled to two light chains, can combine with a J-chain to form one pentameric molecule, which has for decades been assumed to be the principal arrangement of IgM in circulation (1, 9). Furthermore, it has been shown that if IgM is expressed without J-chain it can assemble into hexamers (10–12). However, (monoclonal) hexameric IgM observed in circulation seems invariably linked to pathologies such as Waldenström macroglobulinemia or cold agglutinin disease (10, 11). Despite persistent speculation in literature, it is currently unclear what fraction, if any, of normal human circulatory IgM is hexameric (12–14).

Recently, several cryogenic electron microscopy studies have shed more light on the detailed molecular structure of pentameric IgM. Rather than the previously assumed symmetrical pentameric arrangement, recombinant IgM with J-chain forms an asymmetrical pentamer resembling a hexameric structure, wherein the J-chain bridges the gap in place of a sixth IgM subunit (15–19). The core of these structures is composed of an amyloid-like assembly of the J-chain with the C-terminal IgM tailpieces, which are responsible for this isotype's propensity to oligomerize. On top of this assembly, the secretory component (SC) of pIgR can bind IgM through interaction with the J-chain and IgM HC (16, 19).

Intriguingly, by using in vitro reconstitution, using negative stain EM, it was reported that a protein called CD5 antigen-like (CD5L) can also associate into the gap of murine J-chain-linked pentameric IgM and form a covalent attachment through a disulfide bond

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(18). CD5L is a member of the scavenger receptor cysteine-rich (SRCR) family and consists of three SRCR domains in both murine and human CD5L (20). It was originally discovered as Spα or apoptosis inhibitor of macrophage, being expressed predominantly by macrophages (21). In circulation, it has been speculated that CD5L binds to IgM to avoid renal excretion (22), whereby the protein is released under certain conditions. However, it is unclear when, where, and how frequently CD5L incorporation happens. Disconcertingly, reported circulatory CD5L concentrations differ wildly, ranging from 0.1 to 60 mg/L (i.e., ~500-fold) (20, 22–24), possibly reflecting limitations to detect either the free or putatively IgM-bound form. While free CD5L has been reported to induce a plethora of immunomodulatory effects, the exact functions of this protein remain largely unknown (25–27). Above all, the incidence and significance of the IgM-bound form of CD5L are currently undetermined. Here, we characterize human circulatory IgM for its structural composition and association with CD5L. Combining molecular biology and mass spectrometry (MS)-based techniques, we redefine the circulatory IgM complex and explore the role of CD5L in complement activation and Fc receptor binding.

Results

CD5L Occurs Synchronously with IgM in Circulation. We started our characterization of IgM and CD5L by tracking the abundances and associations of these proteins in serum from healthy donors (n = 42). To investigate the abundance of the individual protein chains (Igμ, J, CD5L) in a way that is unbiased to structure and complex formation, we used bottom–up proteomics with label-free quantitation (Fig. 1*A* and *SI Appendix*, Fig. S1). Using this approach, we detected an average total CD5L serum concentration of 1.7 μM or 60 mg/L, consistent with the higher end of literature values (20). Remarkably, we found that CD5L levels correlated

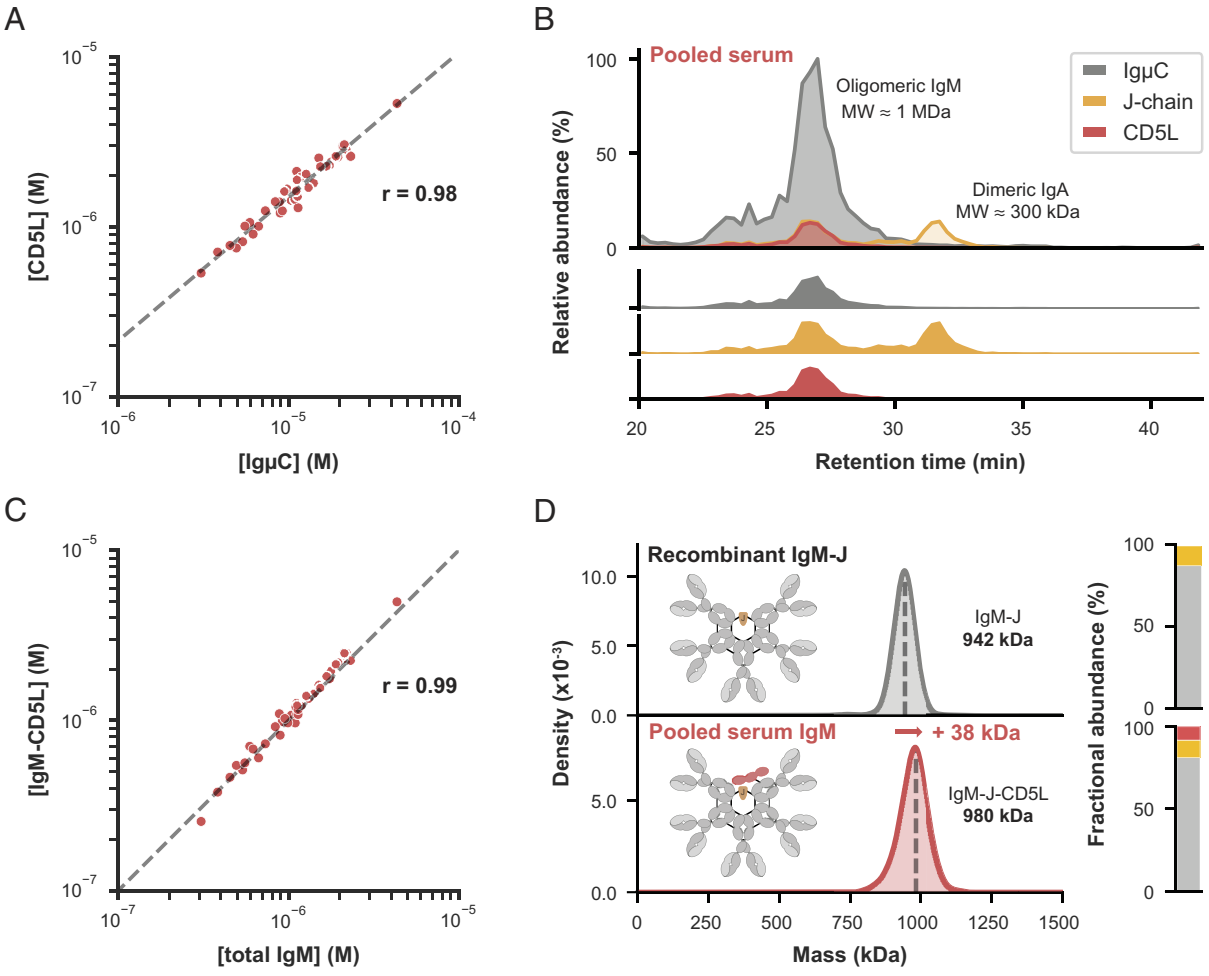

Fig. 1. All circulatory IgM is pentameric with J-chain and CD5L incorporated. (*A*) Total levels of CD5L and IgM heavy chain (IgμC) show a strong correlation in serum (r = 0.98). Absolute concentrations were assessed by label-free quantitation by proteomics. The molecular ratio between CD5L and IgμC was approximately 1:7. Considering that each IgM pentamer holds 10 copies of IgμC, this advocates for the incorporation of one CD5L molecule per IgM pentamer. The dotted line indicates a linear regression model fitted to logarithmically scaled data. (*B*) In complex-centric protein profiling of pooled serum, CD5L elutes exclusively (>99%) with IgμC and J-chain as a single complex of ca. 1 MDa. Shown are the elution profiles of the individual protein chains obtained from proteomics on individual size-exclusion chromatography (SEC) fractions. The secondary elution peak of the J-chain corresponds to incorporation in dimeric IgA. (*C*) Levels of IgM-CD5L complexes and total IgM ELISA similarly show a high correlation in serum (r = 0.99). The ELISA makes use of a mAb (5B5) that can recognize CD5L when bound to IgM. Quantification was based on a recombinantly produced IgM-J-CD5L complex standard (Fig. 2 and *SI Appendix*, Fig. S5). The ratio between IgM-CD5L complexes and total IgM was approximately 1:1, implying that all serum IgM incorporates a CD5L molecule. The grey line indicates this 1:1 molecular ratio. (*D*) The principal configuration of circulatory IgM is a pentamer with J-chain and CD5L. Combining mass measurement by CDMS (*Left*) with proteomics (*Right*) confirmed that recombinant IgM-J (targeting wall teichoic acid of *Staphylococcus aureus*) is a pentamer with J-chain [$(IgM)_5$:$(J)_1$] (*Top*). IgM purified from pooled serum was similarly homogeneous (*Bottom*), though the average mass was shifted by +38 kDa and CD5L was detected by proteomics. This matches the mass increase expected for the incorporation of one CD5L molecule to form [$(IgM)_5$:$(J)_1$:$(CD5L)_1$].

very tightly with those of the IgM heavy chain constant region (IgμC, r = 0.98), hinting at high levels of circulating IgM–CD5L complexes. Furthermore, the observed CD5L/IgμC molecular ratio was consistently ca. 0.15. Considering that each IgM pentamer holds 10 copies of IgμC, this translates to the presence of close to one CD5L molecule per IgM pentamer.

To further investigate a putative structural relationship between IgM and CD5L, we next subjected pooled sera to size-exclusion chromatography (SEC) MS. In this approach, bottom–up proteomics was applied to SEC fractions to generate chromatograms of individual proteins, revealing complexation based on synchronous elution. Strikingly, the chromatogram of CD5L revealed essentially complete (>99%) synchronous elution with IgM as well as with the J-chain in the higher MW fractions around 1 MDa (Fig. 1*B*). No secondary CD5L elution peak was observed in lower MW fractions, indicating that the bulk of CD5L is in association with IgM and the J-chain. Also in SEC MS, we observed an intensity ratio of about 1:1:10 between CD5L, J-chain, and IgμC, again hinting toward a 1:1:1 molecular ratio of pentameric IgM, J-chain, and CD5L in circulation.

IgM in Circulation Is Principally a J-Chain-Linked Pentamer with CD5L. Having demonstrated that CD5L is tightly connected to IgM in circulation, we next proceeded to characterize the exact molecular composition of circulatory IgM. First, to directly measure and quantify levels of CD5L-IgM complexes, we set up an ELISA that specifically measures these complexes. For this, we generated a dedicated mAb (5B5) that recognizes CD5L when it is bound to IgM (*SI Appendix*, Fig. S2). Using this approach, we found that in the serum of 42 healthy donors, the levels of IgM-bound CD5L and total IgM also showed a near-perfect correlation (r = 0.99) and a molecular ratio of 1 (Fig. 1*C*), further implying that IgM exists mainly as a CD5L-containing complex in circulation. In contrast, employing a setup that solely detects unbound CD5L, we found those levels averaged 0.7 mg/L, thus making up only a small fraction (~1%) of the total circulating CD5L population (*SI Appendix*, Fig. S2*D*).

To elucidate the exact molecular composition of circulatory IgM, we next subjected IgM purified from pooled serum and the plasma of individual donors to two single-particle mass measurement techniques, native charge detection (CD) MS (28) and mass photometry (29). Combining these precise intact molecular weight measurements with bottom–up proteomics to identify constituent proteins, allowed us to establish the exact stoichiometries and possible cooccurrences of different oligomeric states (Fig. 1*D* and *SI Appendix*, Fig. S3). For comparison, we also analyzed a recombinant IgM-J and observed it to be exclusively a J-chain-linked pentamer with a measured mass of 942 kDa [$(IgM)_5$:$(J)_1$]. For each serum or plasma IgM sample, we consistently observed a mass of about 40 kDa higher, a shift that closely matches the incorporation of one 36 kDa CD5L molecule. Simultaneously, proteomics analyses on the same circulatory IgM samples consistently revealed the presence of CD5L at a molecular ratio of about 1:1:10 (CD5L:J-chain:IgμC). Notably, circulatory IgM was also highly homogeneous in mass and therefore oligomeric state, without evidence for the existence of IgM hexamers in any of the samples analyzed (abundance < 1%) (Fig. 1). We thus conclude that the canonical form of human circulatory IgM is a J-chain-linked pentamer with one CD5L molecule [$(IgM)_5$:$(J)_1$:$(CD5L)_1$].

CD5L Is Not Expressed by B Cells and Is Covalently Integrated in IgM via CD5L-Cys191. The near-complete integration of CD5L into IgM as observed here would be best explained by secretion from B cells as a fully formed complex. However, the expression of CD5L is reported to be mostly restricted to macrophages (21, 30). To investigate the possibility of coexpression of CD5L and IgM in B cells, we cultured naive and memory B cells, both in a T cell-dependent and T cell-independent manner and analyzed IgM in the supernatants (Fig. 2*A*). The secreted IgM was completely devoid of CD5L, demonstrating that B cells do not produce CD5L. Recombinant cotransfection of IgM-J and CD5L within the same cell proved to be challenging and did not lead to efficient complex formation. In contrast, coculture of IgM-J-expressing and CD5L-expressing cells reliably produced homogenous IgM-J–CD5L (*SI Appendix*, Fig. S4). This implies that complex formation of B cell-derived IgM with CD5L occurs in the extracellular space after both IgM-J and CD5L have been expressed independently.

To further clarify the binding mechanism of CD5L to IgM, we recombinantly produced CD5L (rCD5L) and pentameric IgM-J and studied CD5L incorporation in vitro (Fig. 2*B* and *SI Appendix*, Fig. S5). We observed an efficient association of rCD5L with IgM under mild reducing conditions, implicating disulfide bond formation between IgM and CD5L to be an integral part of complex formation. Saturation of IgM binding was reached upon incubation with an excess of CD5L. These binding experiments were repeated with CD5L purified from serum IgM (sIgM–CD5L) and with recombinant IgM-Fc, producing similar results (*SI Appendix*, Fig. S5). Integral complex formation was confirmed by shielding of CD5L epitopes for two distinct anti-CD5L mAbs (10D11, 7E4) that do not bind serum-derived IgM (*SI Appendix*, Fig. S2*E*).

Based on a structure prediction by Alphafold2 (32), human CD5L contains two unpaired cysteines, C191 and C300. To assess their role in complex formation with IgM, which itself has two unpaired cysteines at position C414 in the chains adjacent to the gap, we produced recombinant hCD5L variants with these cysteines mutated to serines and tested their ability to associate with IgM-J (Fig. 2*C*). Complex formation was severely reduced by a C191S mutation but essentially unaffected by a C300S mutation. This suggests that only C191 could be disulfide-linked to IgM. These data are in line with the earlier proposed IgM–CD5L linkage via the homologous C194 in experiments wherein murine IgM was reconstituted in vitro with murine CD5L (18) [Fig. 2*D* and (18)]. To further elucidate the interaction, we next subjected CD5L, J-chain, and two IgM-Fc regions to structure prediction by Alphafold2 multimer (32). The model positioned CD5L in the gap of the IgM-Fc pentamer but somewhat sticking out of the IgM-Fc plane (Fig. 2 *E* and *F*). The SRC3 domain is predicted to be positioned closest to the junction formed by the J-chain, in between the N- and C-terminal loops of the J-chain. The SRC2 domain is located above the cleft between the Fc-Cμ4 and Fc-Cμ3 domains and the N-terminal loop of the J-chain, positioning C191 in close proximity to an unpaired C414 of IgM. The SRC1 domain is expected to be flexible. Taken together, CD5L thus binds the gap of IgM-J and involves disulfide bond formation requiring C191.

IgM-J-CD5L Equally Activates Complement But Is Less Potent in pIgR and FcαμR Binding. We next assessed the putative functional consequences of CD5L incorporation into IgM. First, we investigated a potential effect in the activation of the classical pathway of the complement system, for which IgM can be a potent activator (Fig. 3*A* and *SI Appendix*, Fig. S6). We used a recombinant monoclonal IgM antibody specific for biotin with and without CD5L and assessed its ability to induce C3b deposition in ELISA and cell lysis of biotinylated human red blood cells (14). Both rIgM-J and rIgM-J-CD5L equally induced C3b deposition and

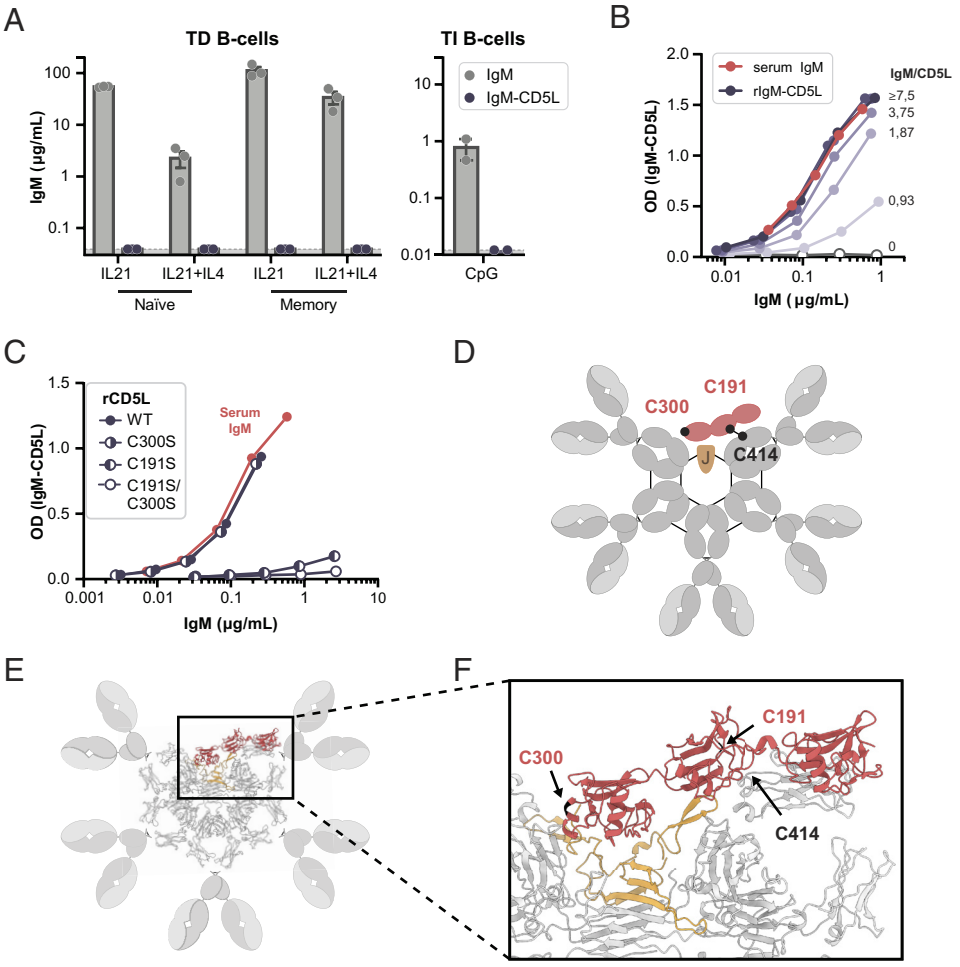

Fig. 2. Formation of IgM–CD5L complexes. (*A*) Isolated human peripheral blood B cells were cultured in vitro with both T-dependent (TD) and independent (TI) stimuli, after which supernatants were analyzed for secreted IgM and IgM–CD5L complexes by ELISA. For the T-dependent cultures, plasma cell differentiation was previously demonstrated (31). Only IgM devoid of CD5L was detected, irrespective of culture conditions. Data shown are from 2 and 3 different donors. (*B*) In vitro generation of recombinant IgM–CD5L complexes. Different molar ratios of CD5L to IgM [clone 2D5 (14)] were incubated in the presence of 0.1 mM reduced/oxidized glutathione (GSH/GSSG). Complex formation was assessed by ELISA. With a molar excess of CD5L, saturation was observed. Representative plots of n = 2 experiments. (*C*) Human CD5L contains two predicted unpaired cysteines, C191 and C300, that could potentially interact with IgM. Recombinant CD5L was produced with C191S or C300S mutations or both and was tested for their ability to form complexes with IgM as determined by ELISA. C191S disrupts complex formation, whereas C300S does not. Representative plots of n = 2 experiments. (*D*) Schematic representation of IgM–CD5L complex with highlighted C191 and C300 of CD5L and C414 of IgM-Fc. (*E*) Proposed structural model of IgM–CD5L complex. AlphaFold2 model of CD5L and J-chain was fitted into the structure of IgM core/Fc region with J-chain (PDB: 8ADY). (*F*) Detailed view on CD5L and J-chain within IgM.

cell lysis. We likewise investigated a potential effect of CD5L on C3b deposition on bacteria, using a monoclonal antibody that binds the wall teichoic acid glycopolymer on *Staphylococcus aureus* (33) but again found no differences in activities (*SI Appendix*, Fig. S6*D*).

We next proceeded to characterize the interaction of IgM-J-CD5L with IgM Fc receptor proteins (FcRs) in ELISA. These included pIgR, FcμR, and FcαμR, which are involved in mucosal transport (5), regulation of lymphocyte responses (3), and immune complex uptake by antigen-presenting cells (4), respectively. To this end, we measured the binding of recombinant and serum-derived IgM complexes to FcRs (Fig. 3*B*). We found that the binding of rIgM-J-CD5L to both pIgR and FcαμR was reduced compared to rIgM-J, while binding to FcμR was unaffected. Also, serum-derived IgM (containing CD5L) displayed reduced binding. Binding to pIgR was also assessed using rIgM-Fc and in a reverse binding experiment, both with results corroborating our initial findings (*SI Appendix*, Fig. S7*A*). To confirm that these effects were caused by CD5L, we also tested whether the release of CD5L from serum IgM would enhance binding to pIgR and FcαμR. Selective release of CD5L from serum IgM upon limited reducing conditions proved feasible (Fig. 3*C* and *SI Appendix*, Fig. S7*B*). This resulted in increased binding to the pIgR and FcαμR, while binding to the FcμR was not affected (*SI Appendix*, Fig. S7*C*). Combined, CD5L incorporation reduces the binding of IgM-J to pIgR and FcαμR, but has no substantial effects on binding to FcμR or complement activation by IgM-J.

Secretory IgM Is Principally Devoid of CD5L. Secretory IgM is known to be associated with the SC, the extracellular portion of the pIgR, which is cleaved from the cells enzymatically after transcytosis to mucosal apical sides. Based on the reduced reactivity of IgM-J-CD5L with pIgR, we hypothesized that unlike circulatory IgM, secretory IgM may be devoid of CD5L. In stark contrast to serum, we found that CD5L levels in saliva and milk determined by proteomics were much lower and did not correlate with IgμC (Fig. 4). In agreement, ELISA detection of IgM-bound CD5L in secretory fluids was also much lower than in serum and did not match that of total IgM. Instead, we found a high abundance of SC and a 1:1 molecular ratio between IgM-bound

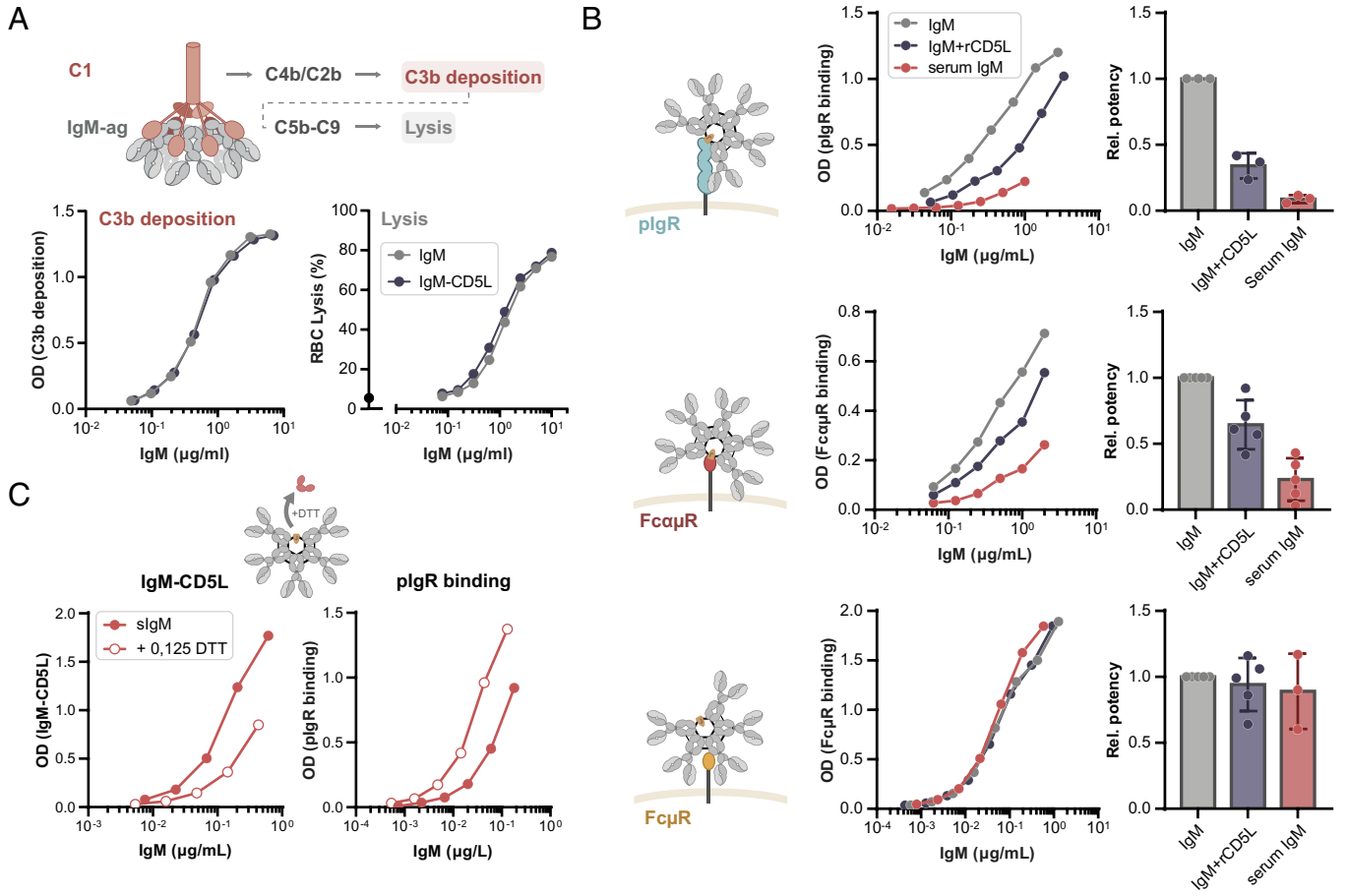

Fig. 3. CD5L in IgM decreases pIgR and FcαµR binding; complement activation and FcµR binding unaltered. (*A*) Complement activation by IgM was assessed by measuring C3b deposition or red blood cell (RBC) lysis upon binding of recombinant anti-biotin IgM ± CD5L to plates coated with biotinylated albumin, or biotinylated RBCs, respectively. Representative plots of n = 3 experiments. (*B*) Binding of recombinant IgM (2D5) ± CD5L and serum IgM to different IgM-binding receptors. Whereas binding to FcµR is unaffected by CD5L, binding to both pIgR and FcαµR is reduced for CD5L-containing IgM. *Right* panels: relative binding strength calculated in comparison to IgM without CD5L, data from n = 3 to 5 experiments. (*C*) Upon limited reduction of serum IgM with DTT, selective release of CD5L proved feasible (*Left*) which resulted in increased pIgR binding (*Right*). Data representative of n = 3 experiments. Bars and error bars represent mean and SE, respectively.

SC and total IgM. In contrast to circulatory IgM, secretory IgM is thus mostly devoid of CD5L and contains the SC instead.

Discussion

In this study, we redefined the molecular composition of IgM and revealed its intimate link with the scavenger protein CD5L in circulation. Strikingly, we have now shown that the principal form of circulatory IgM is a J-chain-linked pentamer in complex with CD5L. Conversely, while secretory IgM also consists of J-chain linked pentamer, it is mostly devoid of CD5L but associated with the SC. We speculate that this distinction has eluded discovery due to relatively small size of CD5L, because it is assembled after secretion, and the lack of dedicated assays.

While CD5L has been known to copurify with IgM (20), its relatively small size (about 4% of the IgM mass) makes it easy to overlook its presence and quantify its abundance. Equally, immunoassays are likely to have underestimated CD5L levels since they were not optimized to include the detection of the IgM-bound form. Nonetheless, several recent plasma proteomics studies revealed high concentrations of total CD5L and a tight correlation with IgM abundance (34–36), in line with our data and an earlier study by Arai et al. (22). In contrast to the J-chain, CD5L is not B-cell derived and is thus integrated into IgM after its expression by a different cell type. Many studies on antibodies have traditionally been performed using immortalized hybridoma B cells and may have wrongly inferred that the resulting CD5L-devoid IgM molecules are representative of circulatory IgM.

While several impressive high-resolution cryo-EM structures of IgM have been published in recent years (15–17, 19), the authentic picture of circulatory IgM is thus still incomplete. Most of these studies used in vitro-produced IgM without CD5L, with only one negative-stain EM study describing the putative binding of human CD5L to human IgM-Fc, when reconstituted in vitro (18). A very recent cryo-EM study resolved a structure of serum-derived IgM (17), but surprisingly, also here, CD5L is lacking. We speculate that this may be due to the use of a myeloma-derived sample in this study, wherein IgM may be overproduced to a level where CD5L incorporation is much lower. Further structural studies on healthy human IgM that does include CD5L are thus highly encouraged.

Also, the role of (free) CD5L may need some rethinking. Both anti- and proinflammatory effects have been attributed to CD5L (25, 26), but often such experiments were done in the absence of IgM. High concentrations of CD5L may be produced locally within tissues, but once bound to IgM, CD5L likely needs to dissociate in order to exert its functions (37). Of note, mice lacking IgM or J-chain show markedly reduced levels of CD5L,

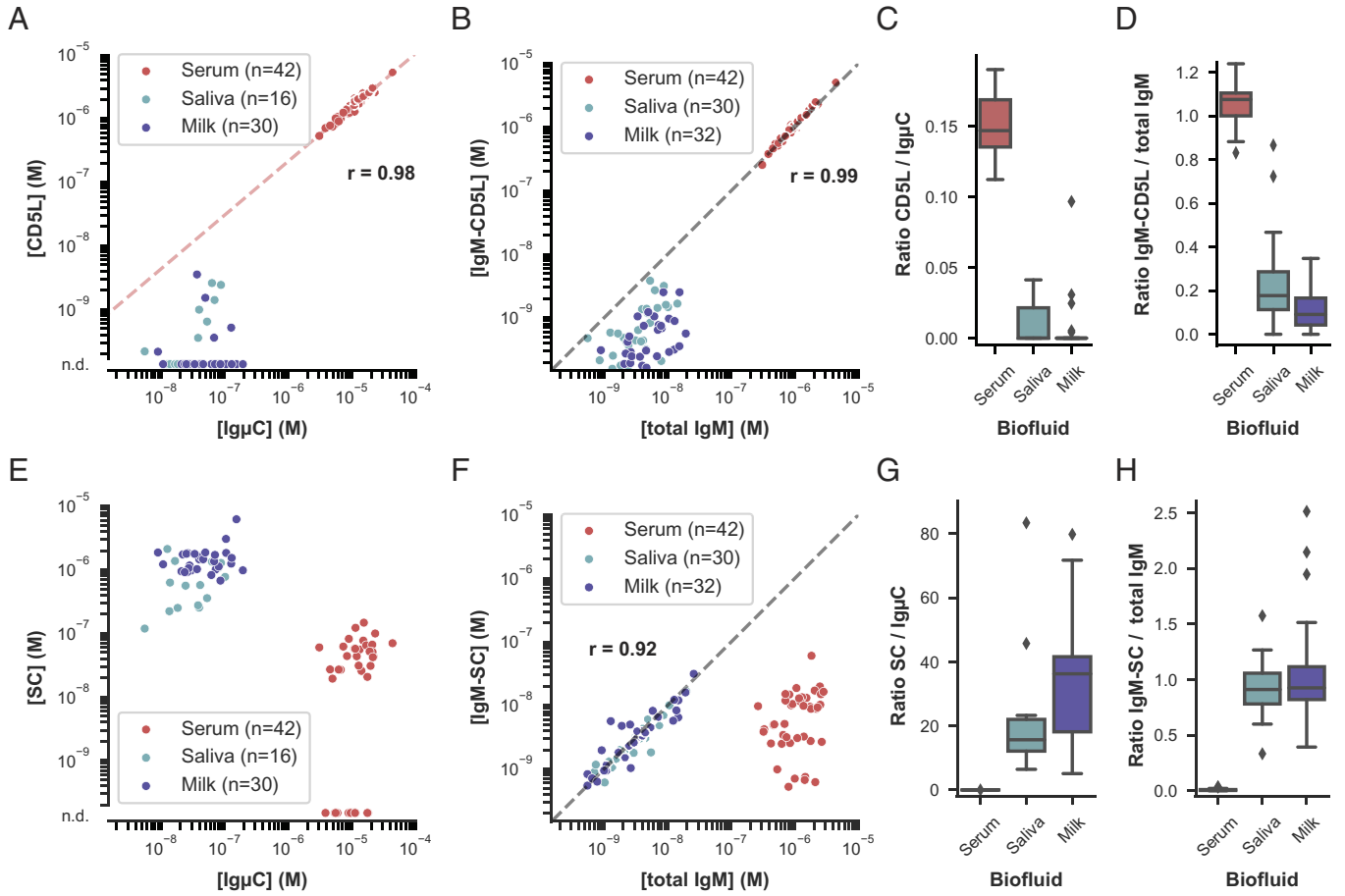

Fig. 4. Secretory IgM is largely devoid of CD5L and contains instead the SC of pIgR. (*A*) Total levels of CD5L and IgM heavy chain (IgμC) show a remarkably strong correlation in serum (r = 0.98) but not in saliva and milk, as determined by label-free proteomics. CD5L levels in saliva and milk were very low, frequently even below the detection limit. These values are indicated at the *Bottom* as not determined (n.d.). The red line indicates a linear regression model fitted to logarithmically scaled serum data. (*B*) Levels of IgM–CD5L complexes and total IgM similarly show a high correlation in serum (r = 0.99) but not in saliva and milk, as determined by ELISA (Fig. 1). The gray line indicates a 1:1 molecular ratio. (*C*) The molecular ratio between CD5L and IgμC was approximately 1:7 in serum, closely matching the incorporation of one CD5L molecule per IgM pentamer. In saliva and milk, this ratio is much lower. (*D*) Similarly, while the ratio between IgM-bound CD5L and total IgM was approximately 1:1 in serum, IgM from saliva and milk was nearly devoid of CD5L. (*E*) Total levels of pIgR were very high in saliva and milk, frequently over an order of magnitude higher than IgμC levels. In serum, pIgR levels were much lower, sometimes even below the detection limit. These values are indicated as not determined (n.d.). (*F*) Consequently, levels of IgM-pIgR complexes and total IgM ELISA similarly show a good correlation in saliva and milk (r = 0.92) but not in serum. The gray line indicates a 1:1 molecular ratio. (*G*) The molecular ratio between pIgR and IgμC was in the order of 10:1 to 40:1 in saliva and milk, highlighting a large excess. In contrast, the ratio in serum was only 0.002:1. (*H*) While the ratio between IgM-bound pIgR and total IgM was approximately 1:1 in saliva in milk, serum IgM was principally devoid of pIgR.

emphasizing the tight link between the two (22). We hypothesize that dissociation of CD5L from IgM may occur for instance at inflamed sites, possibly through released glutathione or thioredoxin. Indeed, CD5L levels have been reported to be increased in a number of inflammatory disorders (23, 24, 38–43). These dynamics may resemble redox-dependent association/dissociation seen for IgG4 Fab arm exchange (44). Alternatively, inflammation may induce increased expression of CD5L by macrophages. Still, the findings of these studies need to be rethought based on our finding that CD5L preliminary associates with IgM in circulation.

The striking difference in CD5L incorporation between circulatory and secretory IgM also inspires many exciting questions to be addressed. In particular, how and where in the body IgM unites with CD5L during homeostasis as well as during an active (humoral) immune response, and how the complex finds its way into circulation or mucosa? Also, the role of CD5L produced locally in, e.g., lung tissue is as yet unknown. (45) The lack of CD5L in secretions suggests that the protein may have a regulatory role in transport, that it may be removed, or perhaps even that secretory IgM is an entirely separate compartment that is produced through a different route that does not involve CD5L. It would therefore be of key interest to study differences and commonalities in the clonal repertoires of IgM in these compartments, and how this may relate to CD5L incorporation. In theory, this might be akin to the distinction seen in circulatory monomeric IgA and secretory dimeric/polymeric IgA which contains J-chain (46).

Regardless, functional separation of IgM with or without CD5L in the circulation or mucosa, respectively, might establish as either a function of the observational decreased affinity to pIgR, and/or less time for IgM produced in local mucosal tissues to acquire CD5L. In addition, these molecules are likely to be functionally distinct due to altered FcαμR binding, previously also shown by Arai et al. (22), which is thought to play a role in immune complex uptake and antigen presentation thereby shaping the adaptive immune response. Therefore, it will be highly important that studies towards the role of IgM in modulating immune responses will explicitly take CD5L into consideration. Also, IgM is more and more considered as an alternative format for therapeutic antibodies, for which incorporation of CD5L might be a relevant

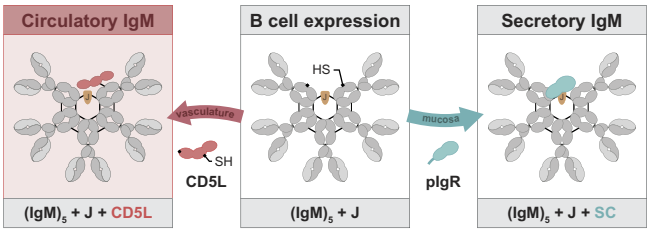

Fig. 5. Graphical summary. J-chain coupled IgM pentamers produced in B cells exclusively engage with CD5L when destined to be secreted into the bloodstream, whereas they attach to the SC of pIgR en route into secretory biofluids such as milk and saliva.

consideration. We conclude that these results show that the molecular structure of IgM needs to be redrafted (Fig. 5), either containing CD5L (in circulation) or SC (in mucosa).

Materials and Methods

All liquid biopsy samples (serum, saliva, and breastmilk) were obtained upon approval of the institutes' ethical advisory boards and when needed with consent of the donors, as described in detail in *SI Appendix, Supplementary Materials and Methods*. *SI Appendix, Supplementary Materials and Methods* section also contains a detailed description on the methods used for the generation of antibodies and the *Production of the Recombinant Proteins*, *Affinity Purification of Serum IgM*. It also provides a description of all materials and methods used for the MS and mass photometry-based analyses, the biochemical assays and the B cell cultures used.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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