

## Gp-340/DMBT1 in mucosal innate immunity

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Deleted in Malignant Brain Tumour 1 (DMBT1) is a gene that encodes alternatively spliced proteins involved in mucosal innate immunity. It also encodes a glycoprotein with a molecular mass of 340 kDa, and is referred to as gp-340 (DMBT1<sup>gp340</sup>) and salivary agglutinin (DMBT1<sup>SAG</sup>). DMBT1<sup>gp340</sup> is secreted into broncho-alveolar surface lining fluid whereas DMBT<sup>SAG</sup> is present in the saliva. The two molecules were shown to be identical and both interact with and agglutinate several Gram-negative and Gram-positive bacteria including *Streptococcus mutans*, a bacterium responsible for caries in the oral cavity. DMBT1<sup>gp340</sup> interacts with surfactant proteins A and D (SP-D). DMBT1<sup>gp340</sup> and SP-D can individually and together interact and agglutinate influenza A virus. DMBT1<sup>gp340</sup> also binds to HIV-1 and facilitates transcytosis of the virus into epithelial cells. DMBT1 binds to a variety of other host proteins, including serum and secretory IgA, C1q, lactoferrin, MUC5B and trefoil factor 2 (TFF2), all molecules with involvement in innate immunity and/or wound-healing processes. Recent generation of Dmbt1-deficient mice has provided the research field of DMBT1 with a model that allows research to progress from *in vitro* studies to *in vivo* functional studies of the multifunctional proteins encoded by the DMBT1 gene.

**Keywords:** innate immunity, mucosal, deleted in malignant brain tumour 1, DMBT1, collectin, surfactant protein D

### INTRODUCTION

Glycoprotein 340 (DMBT1<sup>gp340</sup>) was discovered as an impurity during a SDS-PAGE analysis of a surfactant protein D (SP-D) purification from the 10,000-g supernatant from broncho-alveolar lavage (BAL) of a patient suffering from alveolar proteinosis.<sup>1</sup> Surfactant protein D is an oligomerised protein belonging to the collectin family due to the presence of a collagenous region and a calcium-dependent lectin activity. Characterization of the newly discovered protein showed a calcium-dependent binding to SP-D and the binding was not inhibited by maltose, showing the interaction was not being mediated through the lectin activity of SP-D but indicating a protein–protein interaction.<sup>1</sup> Amino acid

sequence analysis showed the protein contained a scavenger cysteine-rich domain (SRCR) and thereby belonged to the SRCR super family.<sup>1</sup> The number and spacing of the cysteine residues showed that DMBT1<sup>gp340</sup> belongs to group B of the SRCR super family where the SRCR domains have 8 cysteine residues, whereas group A members have 6 cysteine residues.<sup>2</sup> Most members of the group B SRCR are transmembrane proteins and many are located on immune cells such as B-lymphocytes, T-lymphocytes or macrophages. It is now well established that many of these molecules act as pattern recognition receptors.<sup>2</sup> At the same time, a gene was cloned that localised on chromosome 10 in a region that is deleted in malignant brain tumours (DMBT1). Sequence analysis showed that DMBT1 was the gene encoding

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DMBT1<sup>gp340</sup>.<sup>3,4</sup> Furthermore, salivary agglutinin (DMBT1<sup>SAG</sup>) was identified in 1983 as a 300–400-kDa glycoprotein that could be isolated by affinity adsorption of saliva to *Streptococcus mutans*.<sup>5</sup> Mass spectrometry of DMBT1<sup>SAG</sup> showed that the protein shared identical peptide sequences with DMBT1<sup>gp340</sup>.<sup>6,7</sup> Further characterization showed that DMBT1<sup>SAG</sup> bound to SP-D and DMBT1<sup>gp340</sup> agglutinated *S. mutans*. In addition, monoclonal antibodies raised against DMBT1<sup>gp340</sup> recognized DMBT1<sup>SAG</sup> and *vice versa*. When applying these antibodies to immunohistochemistry, an identical localization in the submandibular saliva gland was observed for DMBT1<sup>gp340</sup> and DMBT1<sup>SAG</sup>.<sup>6</sup> Furthermore, Western blotting of saliva using a monoclonal antibody raised against DMBT1<sup>gp340</sup> reacted with DMBT1<sup>SAG</sup>.<sup>7</sup> These results showed that DMBT1<sup>gp340</sup> and DMBT1<sup>SAG</sup> are proteins isolated from different tissues but with similar characteristics and encoded by the same gene, DMBT1.

#### Domain organization and expression

The gene encoding DMBT1 contains putative 55 exons and spans more than 80 kb of genomic DNA (Fig. 1A).<sup>8</sup> Of the 55 putative exons, 54 have been confirmed by their presence in various alternatively spliced mRNAs.<sup>3,8</sup> The six first exons encode the signal peptide and a motif of approximately 90 amino acids of unknown function. This is followed by a repeated pattern like 'pearls on a string' of SRCR domains separated by scavenger interspersed domains (SIDs). Each SRCR domain is encoded by a single exon while most SIDs are encoded by two exons.<sup>8</sup> The SRCR domains are followed by a CUB domain, another SRCR domain, a second CUB domain, and finally a zona pellucida (ZP) domain (Fig. 1A).

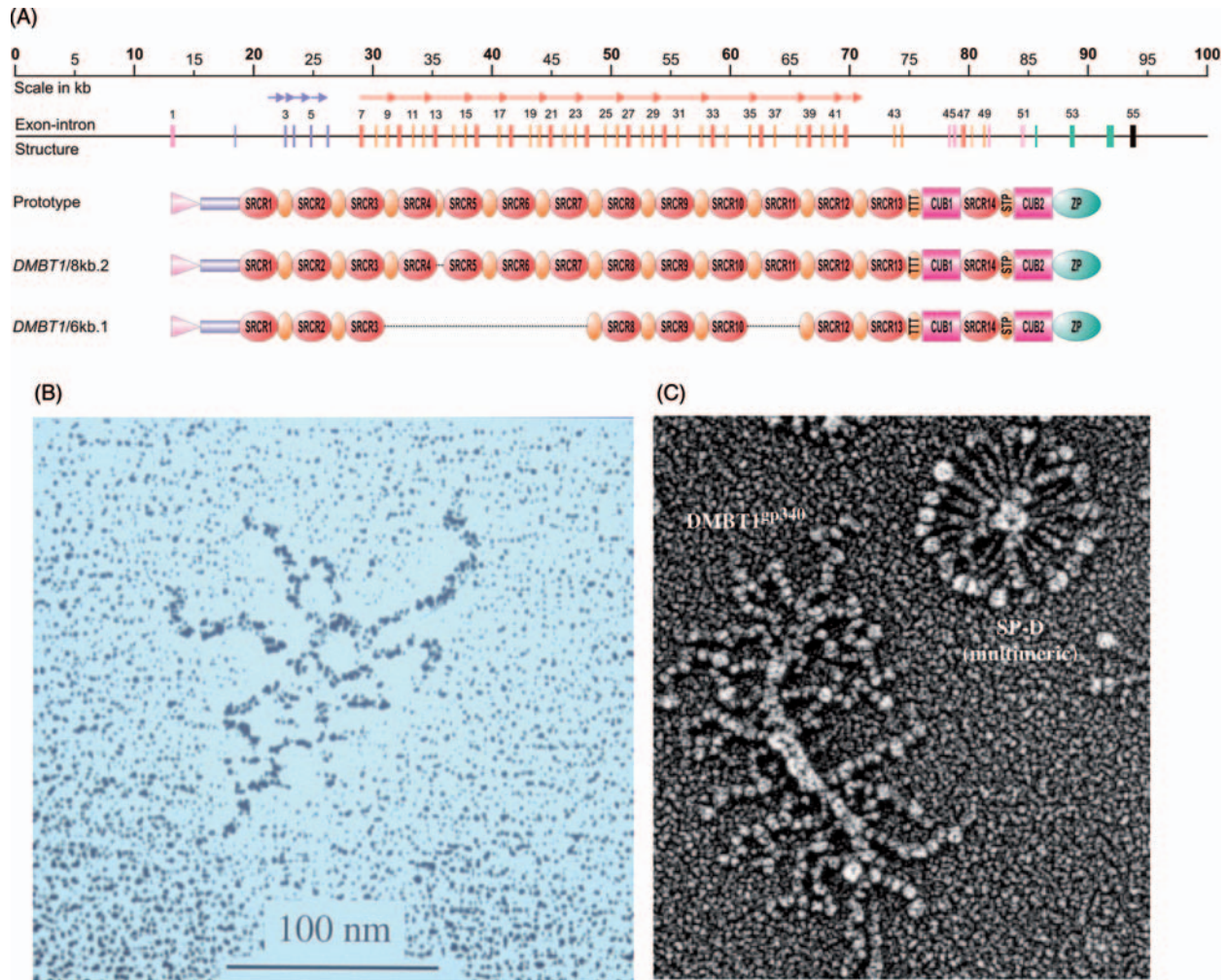
The DMBT1 gene undergoes extensive alternative splicing in the SRCR and SID region at the mRNA level and this gives rise to several differently sized mRNAs, of which the first one in Figure 1A is only the prototype that is derived from assembling all genomic exons, but it is not a transcript that has been verified. The longest form of 8 kb encodes DMBT1<sup>gp340</sup> with 13 SRCR domains in a row while the shortest DMBT1 form of 6 kb has 8 SRCR domains (Fig. 1A).<sup>3,4</sup> The difference between the prototype shown in Figure 1A and DMBT1<sup>gp340</sup> is one exon encoding half a SID between SRCR4 and SRCR5, which is not present in DMBT1<sup>gp340</sup>.<sup>3,8</sup>

Approximately 10% of the molecular mass of DMBT1<sup>gp340</sup> is due to *N*-glycosylation.<sup>1</sup> Nucleotide sequence analysis of the mRNA encoding DMBT1<sup>gp340</sup> showed that potential *N*-linked glycosylation sites were located in the ZP, CUB, and SRCR domains.<sup>3</sup> Another study found approximately 25% of the molecular mass

of DMBT1<sup>SAG</sup> to be due to glycosylations<sup>9</sup> and 15% of the molecular mass could, therefore, be due to *O*-linked glycosylations. *O*-Linked glycosylation is found on serine and threonine amino residues and the SIDs have a high density of these.<sup>3,8</sup> The potential extensive *O*-linked glycosylation of the serine and threonine residues in the SID domains will most likely force these regions into an extended conformation as has previously been shown for mucins.<sup>10</sup> Threonine and serine residues are also found in the SRCR domains but mass spectrometry analysis of DMBT1 from tear-fluid showed that some of these residues were not fully glycosylated as they were detected as unmodified peptides.<sup>11</sup>

Protein glycosylation is regulated via a complex genetically determined regulation by glycosyltransferases (recently reviewed by Lauc *et al.*<sup>12</sup>). Blood group and secretor status determines the ABH blood group antigens and the Lewis (Le) blood group antigen found on DMBT1<sup>SAG</sup>.<sup>13,14</sup> Secretors Se(+) had DMBT1<sup>SAG</sup> containing ABH, Le<sup>a</sup>, Le<sup>b</sup>, Le<sup>x</sup> and Le<sup>y</sup> epitopes whereas DMBT1<sup>SAG</sup> from non-secretors Se(−) displayed Le<sup>a</sup> and Le<sup>x</sup> and no ABH antigens.<sup>13</sup> DMBT1<sup>gp340</sup> was found not to contain either ABH or Le antigens and the two forms of DMBT1 proteins found in tear-fluid were found not to contain Le<sup>x</sup> but the Le<sup>a</sup> epitope.<sup>11,13</sup> In-depth mass spectrometry analysis of the two forms of DMBT1 found in tear-fluid showed that the *O*-linked oligosaccharides were comprised mainly of branched, highly sialylated oligosaccharides with up to 16 monosaccharide units and up to four sialic residues on them.<sup>11</sup> Analysis of DMBT1<sup>SAG</sup> and DMBT1<sup>gp340</sup> from different donors showed that they both contain  $\alpha$ -(2→6)-linkage of the sialic acid residues but DMBT1<sup>SAG</sup> had significantly more  $\alpha$ -(2→3)-linked sialic acid residues than DMBT1<sup>gp340</sup>.<sup>15</sup> This indicates that there might be tissue-specific glycosylation of the various DMBT molecules. As mentioned above, a single transcript of approximately 8 kb has been reported for adult lung.<sup>4</sup> We have seen a similar single transcript in saliva glands (unpublished results, Madsen and Holmskov). This indicates that the size differences seen in the DMBT1<sup>gp340</sup> and DMBT1<sup>SAG</sup> is mainly due to post-translational modifications. However, no single study has examined DMBT1 from broncho-alveolar lavage and saliva (or tear-fluid) from the same person and this still leaves the question open as to whether the reported post-translational modifications of the DMBT1 proteins differ between tissues and/or individuals.

Preliminary transmission and scanning electron microscopic images of DMBT1<sup>gp340</sup> showed the molecule with a central core from which 'pearls on a string'-like extrusions appear (Fig 1B,C). The crystal structure of the CUB1–EGF (epidermal growth factor)–CUB2 domain of human MASP-1/3 shows a dimeric



**Fig. 1.** Structural overview of DMBT1. (A) Structure of the genomic DMBT1 locus. Top: scale in kb. Next lines: distribution of exons over the genomic locus. Repeating units are indicated by arrows/arrowheads. Only odd exons are numbered. Note that the small exons (e.g. those coding for SIDs) are not drawn to scale. Exon colours are selected according to the domains they are coding for. The prototype protein is assembled from all exons present in the genomic DNA except for exon 55 (black square), which has coding potential for a transmembrane domain, but to this end has not been recovered from any transcript variant. DMBT1/8kb.2 and DMBT1/6kb.1 represent the largest and the smallest human variant, respectively, which have been recovered so far. The DMBT1 prototype features 13 scavenger receptor cysteine-rich (SRCR) domains, separated by SIDs. The SRCR domains are followed by a short Thr-rich region (TTT), a CUB domain, a 14th SRCR domain, a Ser-Thr-Pro-rich region (STP), a second CUB domain and a zona pellucida (ZP) domain. Figure from Ligtenberg *et al.*<sup>62</sup> (B) Transmission electron microscopy picture of purified DMBT1<sup>ep340</sup> from BAL. (C) Scanning electron microscopy picture of DMBT1<sup>ep340</sup> from BAL. Note that the picture also shows the multimeric form of SP-D seen in BAL from patients with alveolar proteinosis as previously reported.<sup>64</sup> Electron microscopy pictures by courtesy of Rupert Timpl and Hanna Widemann.

head-to-tail interaction where the CUB domain interacts with the EGF domain.<sup>16</sup> DMBT1 does not have an EGF domain but a similar domain structure with an interspersed SRCR domain: CUB1–SRCR–CUB2.<sup>3,8</sup> However, no reports have so far identified a potential interaction between CUB and SRCR domains. In general, the ZP domain is found at the C-terminus of secreted multidomain proteins and has been suggested as a conserved module for polymerization of extracellular proteins.<sup>17</sup> Therefore, it is tempting to speculate that CUB and ZP domains form the central core through

various protein–protein interactions and that the extensions stems from the repeating SRCR and SID domains (Fig. 1B,C). However, more data are required to verify these potential interactions and explain the quaternary structure of DMBT1 seen in the preliminary electron microscopy pictures.

Orthologues of DMBT1 have also been identified in and cloned from several species such as mouse *Dmbt1* (CRP-ductin, Vomeroglandin, Muclin<sup>18–20</sup>), rat *dmbt1* (Ebnerin<sup>21</sup>), rabbit (Hensin<sup>22</sup>), porcine,<sup>23</sup> bovine (bovine gall bladder mucin<sup>24</sup>), and monkey *DMBT1* (H3<sup>25</sup>).

These all show different numbers of SRCR and CUB domains between the different species but they all have a similar domain organization as human DMBT1.<sup>3,8</sup> Furthermore, an exon encoding for a transmembrane region has been identified in rodent and porcine DMBT1, where mice express two alternative spliced forms of DMBT1<sup>mouse</sup>, one form with and one form without the transmembrane region while in rats only a DMBT1<sup>rat</sup> mRNA encoding the transmembrane region has been identified.<sup>18,21</sup> A similar transmembrane region is encoded by exon 55 in the human gene, but it has not been identified at the mRNA level despite extensive investigation.<sup>1,3,4,8</sup>

Reverse transcriptase-polymerase chain reaction and Northern blotting have shown major sites of expression being the respiratory and gastrointestinal tracts.<sup>3,4</sup> Immunohistochemistry showed localization mainly to mucosal epithelial cells in these organs.<sup>1,26</sup> DMBT1 is, in general, expressed and localized to epithelial cells on surfaces that have contact to the environment (such as skin) and mucosal tissues (such as the respiratory tract, gastrointestinal channel and associated organs), and the urogenital organs.<sup>1,26,27</sup>

#### *Gp-340/agglutinin/DMBT1 and innate immune functions*

The localisation of DMBT1 in skin and mucosal surfaces is ideal for a protein with immune functions in first-line defence. Furthermore, DMBT1 is a secreted molecule and has been detected in several human body fluids such as broncho-alveolar lavage, saliva, pancreatic juice, and tear film.<sup>1,5,11,28</sup> As mentioned above, DMBT1<sup>SAG</sup> was first described as an agglutinating agent for *S. mutans* and other streptococci.<sup>5</sup> The binding site for *S. mutans* has been mapped to a specific region within the SRCR domain and the binding is calcium dependent.<sup>29</sup> By using non-overlapping amino-peptides based on a consensus SRCR sequence the binding site was found to contain the sequence: QGRVEVLYRGSWGTVVC,<sup>29</sup> where the hydrophobic amino acids (V4, V6, Y8 and W12) were involved in binding to several bacteria whereas other residues (R3, E5, L7 and S11) were shown to have some effect on the binding, dependent on the bacterial species being tested.<sup>30</sup> Computer modelling based on the crystal structure of the SRCR domain from the Mac-2 binding protein indicated that the region is located in a surface loop structure with a  $\beta$ -turn,<sup>29</sup> which makes it ideal for interaction with ligands. This region bound to the Gram-positive bacterium *Streptococcus gordonii* as well as the Gram-negative bacteria *Escherichia coli* and *Helicobacter pylori*.<sup>31</sup> *Streptococcus mutans* was also bound and agglutinated by the mouse orthologue CRP-ductin and, in addition, bound to *Haemophilus*

*influenzae*, *Klebsiella oxytoca*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.<sup>32</sup> The region responsible for binding to bacteria in CRP-ductin was shown to correspond to the region identified in the SRCR domains of DMBT1<sup>SAG</sup>.<sup>31</sup> Recently, a more functional model using the intestinal epithelial cell line SW480 showed that DMBT1 confers resistance to bacterial invasion of *Salmonella enterica* highlighting the potential importance of DMBT1 in mucosal innate immunity.<sup>33</sup> The broad bacterial-binding specificity of DMBT1 was recently shown by competition and ELISA studies to be related to poly-phosphorylated ligands such as LPS for Gram-negative bacteria and LTA for Gram-positive bacteria.<sup>34</sup> The bacterial binding efficacy of DMBT1 depended on the accessibility and availability of the phosphorylated structures.<sup>34</sup>

Besides having the capability to bind potential harmful bacteria, DMBT1 also binds to viruses like influenza A virus (IAV) and human immunodeficiency virus type I (HIV-I) and inhibits their infectivity. Opposite to the binding of bacteria, the binding to IAV is not calcium dependent and the anti-IAV effect is mediated by the virus binding to sialic acid-bearing carbohydrates on DMBT1<sup>gp340</sup>.<sup>35</sup> The sialylation of DMBT1<sup>gp340</sup> varies between individuals and this is also observed for the anti-IAV activity of DMBT1<sup>gp340</sup>, where a higher degree of sialylation corresponds to a higher IAV activity.<sup>15</sup> Human saliva inhibits HIV-I infection *in vitro* and DMBT1<sup>SAG</sup> was shown to be one inhibitory component.<sup>36,37</sup> Native DMBT1<sup>SAG</sup> and recombinant DMBT1<sup>gp340</sup> transiently expressed in human endothelial kidney (HEK) 293T cells interacts with gp120 from the envelope of HIV-1 and, contrary to the interaction with IAV, the binding to gp120 is calcium dependent.<sup>38–40</sup> The binding to gp120 was narrowed down to a highly conserved stem region near the loop in the V3 region of gp120.<sup>38</sup>

A recombinant fragment of DMBT1 consisting of the first SRCR and one half of the following SID domain showed interaction with gp120.<sup>39</sup> The binding to gp120 was diminished compared to purified, native, full-length DMBT1<sup>SAG</sup> and this probably reflects the overall increased avidity of the multiple SRCR domains in native DMBT1<sup>SAG</sup> compared to a single SRCR domain. This was reflected in the observation that the interaction between the peptide V3 loop sequence of gp120 and the recombinant DMBT1<sup>gp340</sup> and the recombinant SRCR domain, respectively, showed the same level of relative binding properties.<sup>39</sup> A scan of synthetic overlapping peptides of gp120 revealed that recombinant DMBT1<sup>gp340</sup> and the single SRCR domain could interact with nine different regions of gp120.<sup>39</sup> The crystal structure of gp120 predicts that the different regions of the binding peptides are in close proximity to each other.<sup>41</sup>

However, it is still not clear if a single SRCR molecule interacts with several of these regions in a gp120 molecule or if multiple SRCR domains can interact with a single gp120 molecule. This implies the benefit of having multiple binding sites with a higher combined avidity than the added values of multiple affinities and opens up the possibility of DMBT1 proteins to cross-link or agglutinate HIV-1 for facilitated clearance. This agglutination effect will increase the local concentration of the virus and this might not always be a beneficial effect. Weissman and colleagues<sup>42</sup> recently used genital tract derived cell lines and primary endocervical tissue to show that DMBT1<sup>gp340</sup> can facilitate direct transcytosis of cell free virus from the apical to the basolateral side of these cells. This result highlights the fact that proteins in innate immunity that normally protect the host from infection can be used by the invading pathogen as an entry point.

In addition to binding bacteria and viruses, DMBT1<sup>SAG</sup> and DMBT1<sup>gp340</sup> bind to several endogenous protein ligands, all being involved in innate immunity, such as SP-D<sup>1</sup> and SP-A,<sup>43</sup> secretory IgA,<sup>44</sup> trefoil factors (TFFs),<sup>45</sup> MUC5B,<sup>46</sup> complement factor C1q,<sup>47</sup> and lactoferrin.<sup>48</sup> DMBT1 also scavenges endogenous ligands like DNA.<sup>34</sup>

#### *DMBT1<sup>gp340</sup> and its interaction with SP-D*

Of all the interactions between DMBT1 and other endogenous protein ligands the interaction between DMBT1 and SP-D has been characterized in most detail. DMBT1<sup>gp340</sup> binds to SP-D via a calcium-dependent, protein-protein interaction.<sup>1</sup> DMBT1<sup>gp340</sup> also binds to SP-A and this binding is also calcium-dependent and not mediated through the lectin activity of SP-A.<sup>43</sup> Both SP-A and SP-D have important roles in innate immunity by binding to and aggregating bacteria and viruses.<sup>49</sup> As DMBT1<sup>gp340</sup>, SP-A, and SP-D interact with several bacteria and viruses, Hartshorn and colleagues<sup>50</sup> have been investigating the potential co-operative effects of these molecules on invading micro-organisms using IAV as a model. They showed a co-operative effect between these molecules, which inhibited IAV infection in the following order: SP-D > DMBT1<sup>gp340</sup> > SP-A.<sup>50</sup> The co-operative effect was most evident in viral aggregation but was also observed in haemagglutinin inhibition and viral neutralization assays.<sup>50</sup> However, the co-operative effect between SP-D and DMBT1<sup>gp340</sup> was not mediated through SP-D binding to DMBT1<sup>gp340</sup> but more likely due to independent aggregation activities of the individual proteins.<sup>50</sup> The binding sites on SP-D for carbohydrates and DMBT1<sup>gp340</sup> are both located in the carbohydrate recognition domain (CRD) of SP-D but they do not

overlap.<sup>1,50</sup> However, if the affinity for SP-D and DMBT1<sup>gp340</sup> becomes higher than the affinity between SP-D and IAV or between DMBT1<sup>gp340</sup> and IAV, respectively, the two proteins will reciprocally inhibit each other's antiviral activities by binding to each other and thereby block binding to IAV due to steric hindrance of the non-overlapping binding sites in the CRD of SP-D.<sup>15</sup> Sialylation of DMBT1<sup>gp340</sup> varies from donor to donor with higher degree of sialylation of DMBT1<sup>gp340</sup> showing higher antiviral activity against avian-like IAV strains and differences were also seen with DMBT1<sup>gp340</sup> from different donors and the interaction with SP-D.<sup>15</sup>

The co-operative effect of SP-D and DMBT1<sup>gp340</sup> also influences the interaction between IAV and neutrophils. Pre-incubation of IAV with SP-D strongly increases neutrophil respiratory burst response to the virus *in vitro*.<sup>51</sup> However, when DMBT1<sup>gp340</sup> was added, a significant reduction in the neutrophil respiratory burst response was observed.<sup>51</sup> This shows that the interaction of these proteins increased the neutrophil uptake of IAV while reducing the respiratory burst to the virus, thereby limiting the potential harmful effect of this burst.

#### *DMBT1 and its interaction with other host molecules*

DMBT1 binds to a variety of other host proteins, including serum and secretory IgA, C1q, lactoferrin, trefoil factor 2 (TFF2), MUC5B and albumin. DMBT1<sup>SAG</sup> is naturally found associated with secretory IgA.<sup>44,52</sup> The interaction between DMBT1<sup>SAG</sup> and sIgA is calcium dependent.<sup>44,52</sup> The binding can be inhibited by high concentrations of salt indicating that electrostatic interactions are involved.<sup>53</sup> The interaction with sIgA was abolished after reduction of DMBT1<sup>SAG</sup>, suggesting that a protein moiety depending on disulphide bridges was involved in the binding.<sup>53</sup> Experiments using antibodies against IgA or DMBT1<sup>SAG</sup> showed that it is mainly DMBT1 that agglutinates *S. mutans* and *S. enterica* sv. Typhimurium and not sIgA.<sup>44</sup> The interaction between DMBT1<sup>SAG</sup> and IgA in agglutinating *S. mutans* was found to be additive and the calcium-dependent properties of the DMBT1<sup>SAG</sup>-sIgA complex favoured the enhancement of their respective activities.<sup>44</sup> The same effect was found when only using the surface protein antigen (Pac) of *S. mutans*.<sup>9</sup> A number of consensus-based peptides of the SRCR domains and SRCR interspersed domains were designed and synthesized to pinpoint further the binding domain for IgA on DMBT1<sup>SAG</sup>. ELISA binding studies with IgA indicated that only one of the peptides tested, comprising amino acids 18–33 (QGRVEVLYRGSWGTVTC) of the 109-amino-acid SRCR domain, exhibited binding to IgA.<sup>53</sup> This domain is identical to the domain of DMBT1<sup>SAG</sup> that is involved in binding to bacteria.<sup>29</sup>

Despite this similar binding site, IgA did not inhibit binding of *S. mutans* to SAG or peptide.<sup>53</sup>

DMBT1 also binds to bovine lactoferrin and this binding inhibits the binding of *S. mutans* to DMBT1<sup>SAG</sup>.<sup>48,54</sup> Lactoferrin is a non-haem iron binding protein widely localized in external fluids (such as milk and mucosal secretions), with a role in host protection against microbial infections (reviewed by Ward and Conneely<sup>55</sup>). The peptide domain of bovine lactoferrin, inhibiting the interaction between DMBT1<sup>SAG</sup> and *S. mutans*, was shown to bind to the same surface protein antigen on *S. mutans* as DMBT1<sup>SAG</sup>.<sup>48</sup>

DMBT1<sup>SAG</sup> has been shown to bind to the C1q globular heads and activate the classic complement pathway through native C1 in freshly isolated normal human serum *in vitro*.<sup>47,56</sup> Although DMBT1<sup>SAG</sup> and DMBT1<sup>gp340</sup> mainly are present on mucosal surfaces and C1q is a serum protein, these proteins could come in contact with each other during local inflammatory reactions and, thereby, provide an additional way of local complement activation.

DMBT1<sup>gp340</sup> is associated with the mucin MUC5B *in vivo*.<sup>46,57</sup> Mucins are large, oligomeric, gel-forming glycoproteins and when cross-linking through their cysteine residues, they make viscous mucus gel. Mucus gel performs a critical function in defending every mucosal surface against pathogenic and environmental challenges. Using respiratory mucus or whole saliva, DMBT1<sup>SAG</sup>/gp340 was found to be associated with MUC5B in both secretions.<sup>46,57</sup>

Porcine DMBT1 was found to bind to porcine trefoil factor 2 (TFF2).<sup>45</sup> There are three known TFF proteins (TFF1, TFF2 and TFF3) and they are (like DMBT1) associated with mucosal surfaces,<sup>58</sup> where they are involved in tissue homeostasis and maintenance (reviewed by Taupin and Podolsky<sup>59</sup>). As mentioned above, DMBT1 has been found to bind to phosphorylated structures in LPS and LTA.<sup>34</sup> This was found not only to be related to pathogens but was also seen in host molecules such as DNA and, in addition, to poly-sulphated molecules such as heparan sulphate.<sup>34</sup> This expands the role of DMBT1 from a molecule involved in protection against invading pathogens to also be involved in the process of inflammation.

## FUTURE DIRECTIONS

It is now clear that DMBT1, alone and through its interactions with other molecules, plays an important role as an innate immune defence molecule. Direct evidence indicating that DMBT1 also play a role in protection and prevention of inflammation came from studies in mice deficient for *Dmbt1* (*Dmbt1*<sup>-/-</sup>), which displayed enhanced susceptibility to dextran sulphate

sodium (DSS)-induced colitis and this effect was dependent on the DSS concentration used *in vivo*.<sup>34,60</sup> The induced colitis resulted in elevated tumor necrosis factor- $\alpha$ , interleukin-6, and nucleotide-binding oligomerization domain containing 2 expression levels during inflammation in *Dmbt1*<sup>-/-</sup> mice.<sup>60</sup> DMBT1 is up-regulated in the intestinal epithelial surface cells and Paneth cells in inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis.<sup>33,60</sup> Furthermore, the deletion polymorphism *DMBT1*<sup>SR47</sup>, which has a reduced number of SRCR domains, was found to be associated with Crohn's disease.<sup>60</sup> Other studies showed up-regulation in neonatal lung infections.<sup>60,61</sup> These studies show the potential importance of DMBT1 in mucosal immunology but it is still unclear if the observed up-regulation is a part of a 'general inflammation response' or a part of a 'specific response' according to the on-going event.

The *Dmbt1*<sup>-/-</sup> mice now make it possible to answer many of the remaining questions in relation to the physiological relevance of DMBT1 in innate immunity. For example, what is the relative importance of DMBT1 in relation to different bacterial and viral infections? As DMBT1<sup>gp340/SAG</sup> and SP-D have been shown to work co-operatively *in vitro*, would *Dmbt1*<sup>-/-</sup>/SP-D<sup>-/-</sup> double knock-out mice be more susceptible to infection or inflammatory damage than the corresponding single gene deficient mice? It is well documented that DMBT1 also plays an important role in cell differentiation and cancer (recently reviewed by Ligtenberg and colleagues<sup>62</sup>) and one obvious question is, therefore, if *Dmbt1*<sup>-/-</sup> mice might be more susceptible to epithelial damage and cancer than the corresponding wild-type mice?

There is a huge variety in the isolated forms of DMBT1<sup>gp340</sup>, DMBT1<sup>SAG</sup> and DMBT1 from tear-fluids and this heterogeneity is reflected in the function and interaction with bacteria, IAV and SP-D.<sup>11,13,15</sup> A way forward to overcome this heterogeneity observed for native purified DMBT1 proteins represents recombinant expression of defined variants and to apply a systematic approach to identify the role(s) of DMBT1 in innate immunity and the therapeutic potential of DMBT1. Recently, a vector system for expressing recombinant full-length DMBT1 in Chinese hamster ovary (CHO) cells has been established.<sup>63</sup> Differences were seen in the glycosylation moieties of the recombinant protein compared to DMBT1<sup>SAG</sup> from two different donors but the recombinant protein showed similar properties to native DMBT1<sup>SAG</sup> in terms of binding to C1q and lactoferrin and agglutinated the Gram-positive bacterium *S. gordonii* and the Gram-negative bacterium *E. coli*.<sup>63</sup> The recombinant protein also agglutinated *S. enterica* in an intestinal epithelial cell line model and inhibited cyto-invasion of the cells by the bacterium.<sup>33</sup> The two recombinant DMBT1 proteins that now are available are expressed in different cell lines, one in HEK 293T

cells,<sup>39</sup> which is a human cell line, and the other in CHO cells, which is a hamster cell line.<sup>63</sup> No comparison has been made between the two recombinant forms of the DMBT1 protein and how this is reflected in their glycosylation patterns and functions. However, these recombinant proteins could be used as a systematic screening tools for potential interaction partners and in combination with the *Dmbt1*<sup>-/-</sup> mice allow the research field to move from a basic characterization of the gene and molecule(s) into more functional based studies to identify and clarify the functions of the many heterogeneous DMBT1 proteins; not only in innate immunity, but also in epithelial differentiation and cancer. The dual functions of DMBT1 in innate immunity and epithelial differentiation/cancer indicates an important role in protection and homeostasis of mucosal surfaces with an involvement in the high turnover of cells at these surfaces. DMBT1 might, therefore, be in a unique position for protection and surveillance at the mucosal surface, keeping the epithelial layer intact by protecting the cells from potential pathogens and trauma from both the inside and the outside by co-ordinating the cell differentiating/cancer-related functions of the DMBT1 molecules with the innate immunity functions.

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