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The potential role of sialoadhesin as a macrophage recognition molecule in health and disease

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Sialoadhesin is a macrophage-restricted transmembrane glycoprotein of 185 kDa that mediates cell–cell interactions through recognition of Neu5Acα2,3Gal in glycoconjugates. The extracellular region of sialoadhesin is composed of seventeen immunoglobulin-like domains, of which the amino-terminal two are highly-related structurally and functionally to the amino-terminal domains of CD22, myelin associated glycoprotein and CD33. These proteins, collectively known as the sialoadhesin family, are able to mediate sialic acid-dependent binding with distinct specificities for both the type of sialic acid and its linkage to subterminal sugars. In this review we discuss our recent studies on sialoadhesin and suggest how this molecule may contribute to a range of macrophage functions, both under normal conditions as well as during inflammatory reactions.

***Keywords*: sialic acid, cell adhesion, immunoglobulin, inflammation, macrophage**

***Abbreviations*: Ig, immunoglobulin; CEA, carcinoembryonic antigen; MAG, myelin associated glycoprotein; SMP Schwann cell myelin protein; mAb, monoclonal antibody; Chinese hamster ovary (CHO); UTR, untranslated region**

Introduction

Macrophages are highly versatile cells, present in virtually every tissue of the body and potentially involved in a wide range of functions (reviewed in [1]). The pioneering studies of Elie Metchnikoff [2] were amongst the first to reveal the important role of macrophages in anti-microbial host defence, a function for which these cells have subsequently become best-understood, both during innate and acquired immune responses. Implicit in our understanding of the host defence functions of macrophages is their ability to discriminate 'self' from 'non-self'. This is achieved through expression of a diverse array of cell surface receptors that either mediate direct recognition (*eg* mannose receptor) or indirect recognition, via specific opsonins (*eg* antibody and complement). Besides host defence, recognition and phagocytic receptors of macrophages play a key role in homeostasis, for instance in mediating the efficient clearance of effete and apoptotic cells throughout the body and of erythroblast nuclei extruded in haemopoietic tissues. During the apoptotic process, cells are known to display a range of surface changes that lead to specific recognition by macrophage receptors involved in scavenging functions (reviewed in [3]). This type of recognition can be considered as that of 'altered-self', a process which also applies to the interaction of macrophages with tumour cells displaying altered cell surface structures over their non-tumorigenic counter-parts. In addition to receptors that recognize 'non-self' and 'altered self', macrophages also express receptors that specifically recognize 'self'. These receptors are typically involved in adhesive, non-phagocytic functions that are important in various aspects of cellular behaviour, such as localization and migration within tissues. Such receptors may also act as accessory molecules to increase the efficiency of receptors involved in 'altered self' or even 'non-self' recognition.

An increasing number of macrophage cell surface receptors have been found to mediate recognition by binding to specific carbohydrate ligands [4]. The enormous diversity of exposed oligosaccharide structures found on the surface of microbes, host cells and within extracellular matrix allows them to function as highly effective ligands in all aspects of macrophage recognition phenomena. For a number of years our laboratory has studied the structure and properties of one such macrophage receptor, sialoadhesin. In this review we will discuss our recent results and consider how this receptor may be used in macrophage functions relating both to homeostasis and to host defence. Although much of the

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discussion will be speculative, it is hoped that the future generation of mutant mice, either lacking sialoadhesin or expressing it ectopically, will allow some of the ideas presented here to be tested experimentally.

Properties of sialoadhesin

Sialoadhesin was originally discovered by serendipity as a sheep erythrocyte receptor (SER) on isolated murine resident bone marrow macrophages. It was shown to be a macrophage-restricted non-phagocytic receptor that recognized sialylated components on the sheep erythrocytes [5]. In addition to bone marrow macrophages, erythrocyte rosetting activity could be demonstrated on subpopulations of other tissue macrophages, especially those isolated by collagenase digestion from lymph nodes and spleen, whereas their monocyte precursors and macrophages isolated by lavage from the peritoneal and pleural cavities expressed low or undetectable levels of binding activity [5].

The isolation of an inhibitory mAb, SER-4, to sialoadhesin [6] was a crucial step in its subsequent purification [7] and molecular cloning [8]. As a result, sialoadhesin was predicted to be a type I membrane protein and a new member of the Ig superfamily, with a large extracellular region of 1619 amino acids, a transmembrane region and a relatively short cytoplasmic tail of 35 amino acids with several potential serine and threonine phosphorylation sites (Figure 1). When a full-length sialoadhesin cDNA was transfected into COS cells, the transfected cells were able to bind enzymatically-modified human RBC in a manner similar to that of the native molecule, binding preferentially to terminally exposed Neu5Acα2,3Gal in either N- or O-glycans over Neu5Acα2,6Gal in N-glycans [7, 8]. In addition to the cDNA clones encoding the expected transmembrane form of sialoadhesin, several clones also predicted alternatively spliced, secreted forms of sialoadhesin containing the first three or sixteen Ig domains [8]. As yet, however, there is no direct evidence that sialoadhesin is normally secreted by macrophages and it is possible that the alternatively spliced cDNAs represent cloning artefacts.

As illustrated in Figure 1, the extracellular region of sialoadhesin is made up entirely of 17 consecutive Ig-like domains which can be further categorized into an amino-terminal V-set Ig domain and 16 C2-set Ig domains [8]. Domains 4–17 (numbering from the amino-terminus) are composed of seven tandemly-arranged homologous pairs of domains, each pair consisting of a short and a long domain. Statistical analyses show that all of the short domains in each pair are highly related to each other, as are the long domains, whereas none of the short domains are significantly related to any of the long domains [9]. This region of the molecule may therefore have evolved by seven-fold replication of a two-exon module, consisting of a short and a long domain. Interestingly, members of the carcinoembryonic antigen (CEA) family share a similar pattern of repeating short/long Ig domains [10] but statistical comparison of sialoadhesin and members of the CEA family show that these repeat regions almost certainly evolved independently of each other [9].

The sialoadhesin family of cell–cell interaction molecules and sialic acid recognition

The cloning of sialoadhesin led directly to the characterization of the sialoadhesin family of I-type lectins [11, 12], currently comprised of sialoadhesin, CD22, myelin-associated glycoprotein) (MAG), Schwann cell myelin protein (SMP, only detected so far in avian species) and CD33 (reviewed in [13, 14]). Although these proteins mediate diverse biological functions [14], their extracellular regions share striking sequence similarity, especially over the V-set Ig domain 1 and C2-set domain 2 [8]. In addition, members of the sialoadhesin family have an unusual pattern of conserved cysteines, resulting in a predicted intra-β-sheet

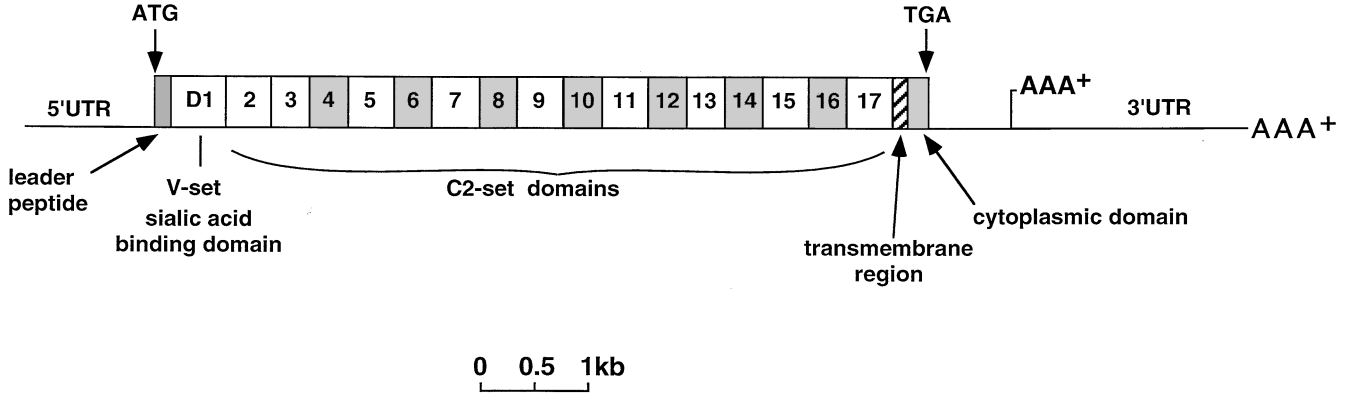

Figure 1. Schematic diagram showing organization of sialoadhesin mRNA. The short domains in the ''stem'' region of sialoadhesin (domains 4–17) are shaded. There are two alternative polyadenylation signals in the 3′ untranslated region (3′UTR), giving rise to two transcripts of 6.5 and $\sim$ 8.0 kb (see Figure 2). For further information see [8].

disulfide bridge in domain 1 and an inter-domain disulphide bond between domains 1 and 2 [8, 15].

Experiments conducted in our laboratory [7, 8, 11, 16–19] and elsewhere [20–29] have now clearly established that these proteins can all function as sialic acid-dependent lectins but with distinct specificities for both the type of sialic acid and its linkage to subterminal sugars [18, 25, 27, 29]. Recently, considerable progress has been made in our understanding of the molecular basis for sialic acid recognition by the sialoadhesins. A combination of approaches, including the generation of truncation mutants [19], site-directed mutagenesis [30, 31], X-ray crystallography and NMR (unpublished observations) have established unequivocally that the GFCC′C″ face of the amino-terminal V-set domain interacts with sialic acid. Although, for sialoadhesin, the V-set domain is sufficient for sialic acid-dependent binding to cells [19], in the case of CD22 [19, 22, 32], CD33 and probably MAG (unpublished observations), the first two domains appear to constitute a minimal unit for sialic acid-dependent binding, possibly because, for these proteins but not for sialoadhesin, the presence of the second domain is a prerequisite for correct folding of the V-set domain.

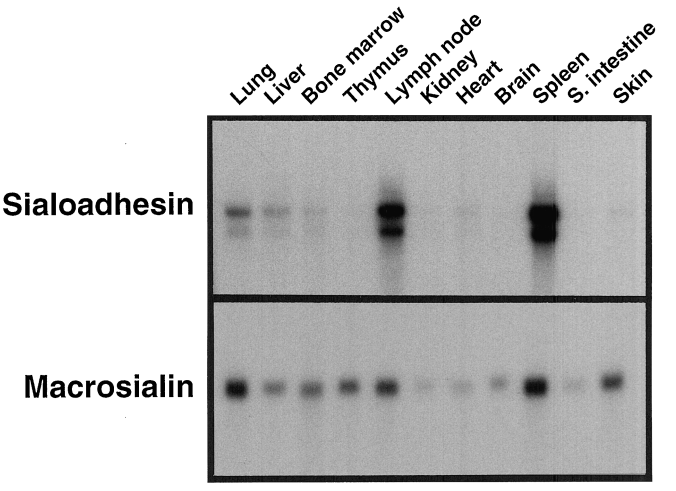

Figure 2. Northern blot showing levels of sialoadhesin mRNA in various mouse tissues compared with macrosialin mRNA. Because macrosialin (mouse CD68) is a good pan-macrophage-specific marker, the level of macrosialin mRNA reflects the number of macrophages present in each tissue. It can be seen that sialoadhesin mRNA is especially abundant in spleen and lymph node. Undetectable levels are present in thymus and brain, in contrast to macrosialin mRNA which can be readily detected in both organs. 10 μg mRNA were loaded on each track and probed with P^{32}-labelled cDNAs as described [8]

Expression of sialoadhesin in tissues

In considering the possible cell–cell and/or cell-substrate interactions mediated by sialoadhesin *in vivo*, it is important to discuss the distribution of the molecule on macrophage subsets and how this might be regulated.

Non-inflammatory conditions

Even in the absence of inflammation, macrophages are found in virtually every organ of the body and can therefore interact with many different cell types (reviewed in [1]; also see Figure 2). However, immunocytochemical studies in rodents have shown that high levels of sialoadhesin are only present on subsets of macrophages and therefore sialoadhesin-mediated adhesive interactions are more likely to be restricted to these subpopulations. In general, the levels of sialoadhesin detected in different tissues correlates well with levels of mRNA, suggesting that protein expression is determined primarily at the level of gene transcription [8]. The heterogeneity of sialoadhesin mRNA levels in different tissues is shown in Figure 2, in comparison with mRNA levels for macrosialin (mouse CD68), currently the best pan-macrophage marker available in mice [33]. In normal, non-inflamed tissues, for example, resident macrophages in the subcapsular sinus and medullary cords of lymph nodes and the marginal metallophils of the spleen express very high levels of the receptor and in the case of resident bone marrow macrophages, intermediate levels are present but the receptor appears to be selectively concentrated at contact sites of macrophages with developing granulocytes [6, 34]. Interestingly, sialoadhesin is expressed at low, though detectable, levels on macrophages in direct contact with circulating blood cells, for example Kupffer cells in the liver and splenic red pulp macrophages [6]. At present it is unknown if the lower expression on these macrophages is due to reduced levels of gene transcription or whether it reflects increased degradation of the protein, perhaps as a result of the high phagocytic activity of these cells. At the other end of the spectrum, the resident macrophages of the brain, microglia, do not express detectable sialoadhesin, even with the most sensitive staining procedures available [35]. This correlates well with the undetectable levels of sialoadhesin mRNA in the brain (Figure 2).

Recently, the cloning of human sialoadhesin and production of specific antibodies have allowed an assessment of the extent to which the expression pattern is conserved between rodents and humans. This, in turn, has provided useful clues regarding potential conserved functions. Where examined to date, the overall expression pattern of sialoadhesin in uninflamed tissues taken from humans and mice is remarkably similar, being restricted to macrophage-like cells, but absent from blood monocytes, with high levels of expression on resident stromal macrophages in lymphoid and haemopoietic tissues (manuscript in preparation). Regarding possible functional conservation, the expression pattern in the spleen has proven to be of great interest (manuscript submitted). In rodents, blood enters the spleen in the marginal sinus within the inner marginal zone, a region that separates

the red pulp from the white pulp (reviewed in [36]). Positioned underneath the marginal sinus, adjacent to lymphoid follicles is a concentric ring of intensely sialoadhesin-positive macrophages called marginal metallophils [37]. In human spleens the marginal sinus appears to be absent and blood is thought to enter the spleen via sheathed capillaries present in the perifollicular zone, positioned between the marginal zone and the red pulp. Intriguingly, strongly sialoadhesin-positive macrophages in human spleen are only found in the perifollicular region, often forming part of the sheath of the sheathed capillaries. Thus, the strongly positive macrophages are in distinct anatomical compartments in rodents and humans, yet there appears to be a functional conservation, such that in both species they are prominent at portals of blood entry.

Inflammatory conditions

In experimental autoimmune encephalomyelitis of rats, a model for multiple sclerosis, inflammatory macrophages stain strongly with mAb ED3, thought to recognize the rat sialoadhesin homologue [38, 39]. Preliminary examination of sialoadhesin expression in certain inflammatory human diseases has also shown that the receptor can be expressed at high levels, especially on macrophages present in chronic inflammatory conditions such as atherosclerosis and rheumatoid arthritis (unpublished observations). Furthermore, sialoadhesin can be readily detected on macrophages that infiltrate human breast tumours (unpublished observations). Thus, it is likely that sialoadhesin mediates cell–cell and/or cell-substrate interactions in a range of pathological conditions as well as under normal conditions.

Regulation of sialoadhesin expression

The heterogeneity in sialoadhesin expression on macrophage populations in non-inflamed tissues and its potentially high expression in chronic inflammatory diseases raises the interesting issue of which regulatory factors are involved (Figure 3). In an early study it was shown that sialoadhesin could be induced on macrophages that do not normally express it by an unidentified 'factor' in mouse plasma or serum that was absent from a range of other species' sera [40]. By gel filtration, the activity co-eluted with albumin and on the basis of this it was proposed that the degree of tissue permeability to this inducing factor could play a role in regulating expression of sialoadhesin *in vivo*. Support for this idea came from the observation that within the brain, sialoadhesin was expressed by macrophage subsets outside the blood-brain barrier (*eg* choroid plexus)

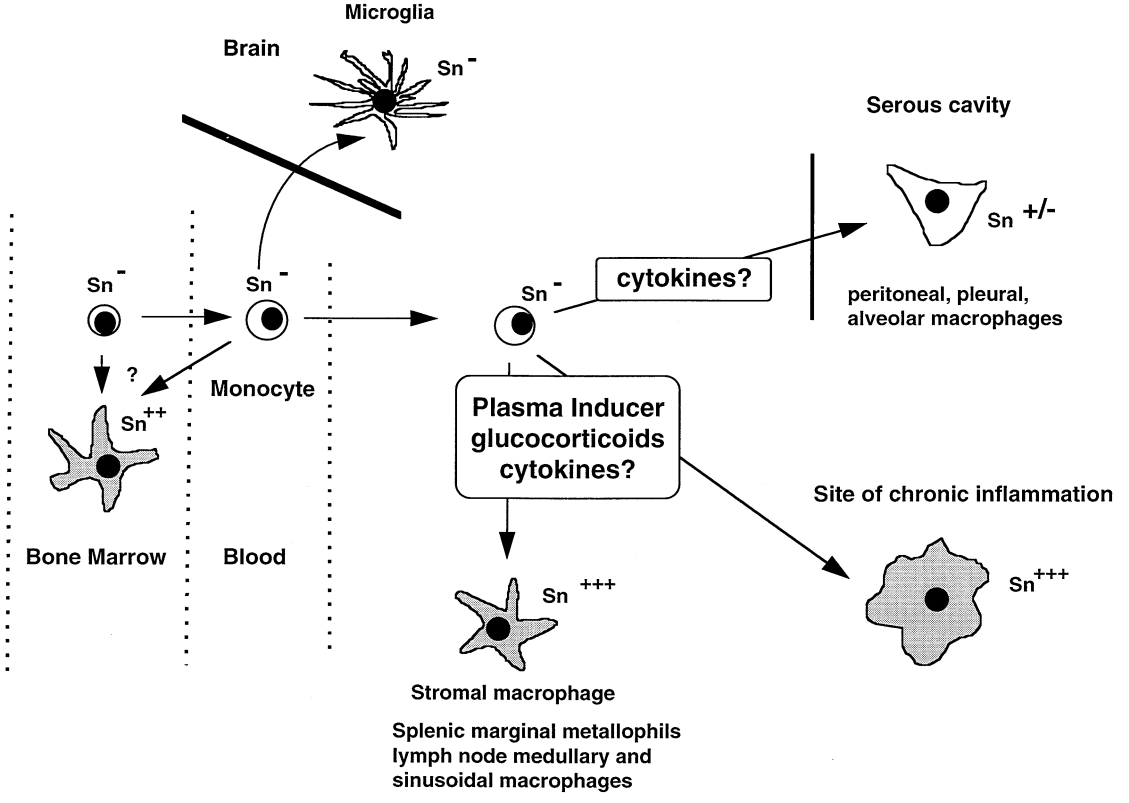

Figure 3. Diagram illustrating expression patterns of sialoadhesin on different macrophage subpopulations *in vivo* and the possible regulatory factors involved.

whereas it was entirely absent from the resident microglia within. Furthermore, when the blood-brain barrier was rendered leaky following experimental injury, a subpopulation of recruited macrophages were induced to express sialoadhesin which was then down-regulated after reformation of the blood-brain barrier [35].

In attempts to identify factors able to influence the serum-dependent expression of sialoadhesin by mouse peritoneal macrophages it was found that interferon-γ, IL-4 and IL-13 had an inhibitory effect [40–42]. In a recent study of the rat sialoadhesin homologue, however, it was shown that glucocorticoids like dexamethasone in the presence of foetal calf serum could induce expression of sialoadhesin mRNA and protein on a macrophage-like cell line [39]. Furthermore, this could be enhanced by interferon-β, interferon-γ, IL-4 and lipopolysaccharide when added together with dexamethasone, but none of these reagents alone could induce sialoadhesin expression. These apparently contradictory results found in the mouse and rat may reflect species-specific differences in regulation, despite the fact that sialoadhesin shows a very similar overall pattern of expression on macrophage subsets in the two species. Further experiments are required to determine the *in vivo* significance of these *in vitro* observations, whether other inductive/suppressive factors are involved and whether the regulatory mechanisms that operate under inflammatory and non-inflammatory conditions are distinct. The future characterization of the sialoadhesin gene promotor and associated regulatory regions may shed light on these issues.

Sialoadhesin as a macrophage 'self' recognition molecule – general considerations

Oligosaccharide ligands that can be recognized by sialoadhesin are abundantly expressed on cell surfaces, in the extracellular matrix and in extracellular fluids, being attached to a diverse array of glycoprotein and glycolipid carriers. Sialoadhesin therefore has the potential to interact with many different cell types, substrata and soluble molecules encountered by macrophages. Experimental studies have shown, however, that sialoadhesin expressed by macrophages, monkey COS cells or as a purified molecule can mediate surprisingly selective cell–cell interactions [7, 8, 11, 16]. In addition, recent experiments with stably-transfected Chinese hamster ovary (CHO) cells have suggested that sialoadhesin can function in anchoring cells to the substratum, possibly through sialic acid recognition (unpublished observations).

Although cell–cell and cell-substrate interactions mediated by sialoadhesin have so far only been demonstrated *in vitro*, it seems likely from a number of considerations that they can also occur *in vivo*. Importantly, it was shown that addition of undiluted mouse serum to macrophages did not inhibit their sialoadhesin-dependent binding to RBC [5]. The concentration of α2,3-linked sialic acid in plasma was found to be ~ 0.5 mM [43] and the K_d of monomeric sialoadhesin for a simple sialylated oligosaccharide ligand such as 3′ sialyllactose is ~ 2 mM (unpublished observations). Therefore the ability of sialoadhesin to mediate cellular interactions in the presence of serum/plasma glycoproteins is likely to be a consequence of the very low affinity of its binding site for carbohydrate ligands, a feature shared with other mammalian lectins [4]. Stable, measurable interactions would be expected to occur only under conditions where both receptor and ligand molecules are highly clustered, to create high-avidity binding interactions. During cell-cell contacts, this could take place as a result of co-clustering of receptors and counter-receptors within the plasma membranes of apposing cells. For soluble glycoproteins, however, stable interactions would only be expected to occur with molecules such as mucins that display a high density of sialylated oligosaccharides.

Importance of cis-interactions with ligands

A logical consequence of the fact that ligands for sialoadhesin are commonly expressed on the surface of most cells, including macrophages, is that the lectin binding site of sialoadhesin can be potentially occupied *in a cis* configuration by sialo-oligosaccharides within the glycocalyx. Although the concentration of α2,3-linked sialic acid in the macrophage glycocalyx is unknown, by comparison with calculations made in yeast for galactose [44], it is likely to be several-fold higher than the K_d of sialoadhesin for its ligand. In order for a lectin-like cell interaction molecule such as sialoadhesin to escape the potentially inhibitory influence of the glycocalyx, one evolutionary strategy would be to project its binding site beyond the limits of the glycocalyx which is usually estimated to be in the order of 20–80 nm depending on the cell type [45, 46]. Consistent with this notion, purified, native sialoadhesin molecules have been visualized by electron microscopy as relatively extended structures of around 50 nm in length [7].

The rainforest model and evolution

With 17 Ig domains, sialoadhesin is the largest cell surface member of the Ig superfamily so far characterized. Furthermore, this unusual number of Ig domains is precisely conserved in the human homologue of sialoadhesin (unpublished observations), suggesting it to be an important functional requirement. We therefore hypothesize that sialoadhesin has evolved 17 Ig domains to reduce the inhibitory potential of the glycocalyx and allow ahesive interactions to occur between macrophages and cells bearing appropriate counter-receptors. This evolutionary explanation has been termed the 'rainforest model' because, in certain respects, it is analagous to the evolution of unusually tall trees in tropical rainforests which need to project their leaves above the underlying canopy for optimal access to sunlight (Figure 4). Experimental support for the rainforest

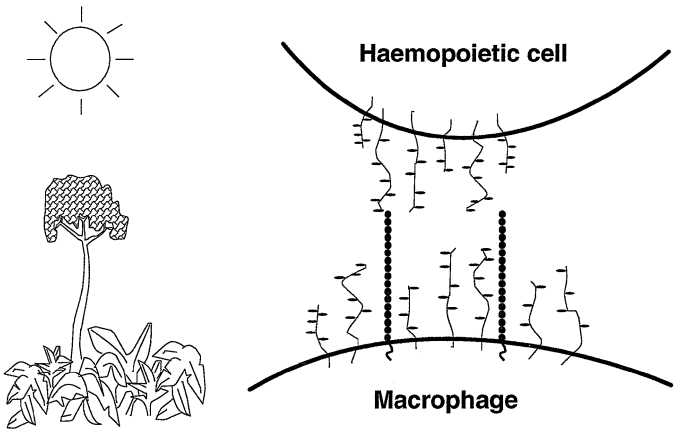

Figure 4. The rainforest model. By analogy with tall trees in tropical rainforests which need to project their leaves above the underlying canopy for optimal access to sunlight, we propose that sialoadhesin has evolved 17 Ig domains to project its binding site above the glycocalyx. This would reduce the inhbitory potential of the glycocalyx and allow adhesive interactions to occur between macrophages and cells (*eg* haemopoietic cells) bearing appropriate counter-receptors.

model has recently been obtained using stable clones of CHO cells expressing truncated forms of sialoadhesin. We have found that reducing the number of Ig domains to 10 or less leads to greatly decreased binding activity despite high levels of protein expression (unpublished observations).

Similar observations for the importance of receptor length in cell–cell adhesion have been reported for P-selectin which, when expressed in CHO cells, has been shown to require at least five complement regulatory domains to mediate efficient attachment to neutrophils under conditions of shear stress [47]. In this case, however, it was proposed that a critical length was required, not to reduce the potential for inhibitory cis-interactions, but to counterbalance the electrostatic repulsive effects of the glycocalyx. Although similar considerations could also apply to sialoadhesin, it is important to point out that the 3′sialyl ligands recognized by sialoadhesin are commonly found on all mammalian cells and hence likely to mediate cis-interactions, whereas the sialy-Lewis^x-related ligands recognized by selectins show a much more restricted cellular distribution [48].

Threshold effects on binding activity

The low affinity of sialoadhesin for 3′sialyl ligands, together with the potential for inhibitory *cis*-interactions discussed above, implies that stable cellular interactions are only likely to occur above a certain threshold level of receptor expression. Experimentally, this has been observed when sialoadhesin is expressed at low to moderate levels on macrophages or stably-transfected CHO cells, in which cases it is unable to mediate cell binding. However, a relatively small additional increase in expression can lead to dramatically enhanced binding activity [6, 7, 16]. This phenomenon has also been observed in cell binding assays where recombinant forms of sialoadhesin have been coated on plastic at different densities [11]. These threshold effects should be taken into account when considering the potential cellular binding functions of sialoadhesin *in vivo*, as only strongly-positive cells are likely to mediate stable cell–cell interactions.

Sialoadhesin-granulocyte interactions

Two independent findings indicate that granulocytes are an important cellular target for sialoadhesin. First, in haemopoietic tissues the receptor is selectively localized at sites of contact between mouse resident bone marrow macrophages and developing granulocytes [34]. Second, in a range of binding assays with haemopoietic cell populations, granulocytes were consistently found to bind at the highest levels to sialoadhesin in both mouse [11, 16] and man (manuscript in preparation). In contrast, resting T and B lymphocytes bound very poorly [11, 16] although in another study activated lymphocytes were more strongly recognized by sialoadhesin, as was a T cell lymphoma, TK1 [49].

Role in clearance?

The physiological significance of sialoadhesin-neutrophil interactions is currently unclear but an attractive possibility is that sialoadhesin plays a role in determining the fate of neutrophils in tissues. Neutrophils are continuously produced in the bone marrow and a recent study in mice [50] has provided evidence that the majority die *in situ*, with only a small fraction entering the bloodstream where they circulate with a half-life of $\sim$8 h [51]. In the absence of inflammatory signals, it has been demonstrated that neutrophils can be taken up by tissues such as liver, spleen and bone marrow [52] where they are presumably cleared by resident macrophages. It is widely assumed, but not yet proven, that apoptosis of neutrophils is a key factor leading to their constitutive clearance in tissues by macrophages, via receptors such as CD36 which recognize apoptosis-related changes on the cell surface [3]. While this is a likely scenario at inflammatory sites where activation *per se* can accelerate the apoptotic process [53], it is a less plausible mechanism for the constitutive clearance of non-activated, circulating neutrophils since these cells are continuously exposed to survival factors in plasma which would be expected to delay apoptosis well beyond the observed life-span [54]. This notion is also supported by studies of transgenic mice whose neutrophils were prevented from undergoing programmed cell death by overexpression of Bcl-2 yet displayed normal kinetics of production and clearance *in vivo* [55].

Since macrophages can bind, but not phagocytose, both non-apoptotic and apoptotic neutrophils in a sialoadhesin-dependent manner ([16] and unpublished observations), it is conceivable that this initial interaction might potentiate the functions of phagocytic receptors like CD36 and the vitronectin receptor. As discussed above, this type of sialoadhesin-dependent binding of neutrophils to macrophages would be expected to only take place with strongly-positive macrophages such as those found in lymph nodes and at sites of chronic inflammation and would be less likely to occur on Kupffer cells and other blood-exposed macrophages which express much lower levels of sialoadhesin. Could the general absence of neutrophils in chronic inflammatory disorders like atherosclerosis and rheumatoid arthritis be causally related to the high levels of sialoadhesin on the inflammatory macrophages?

Modulation of neutrophil function?

Although granulocytes are continuously released into the bloodstream, only a minority are likely to be recruited to sites of inflammation in a normal healthy individual [52]. The presence of large numbers of recirculating granulocytes poses a potential threat to the body because these cells, if activated, could release oxygen radicals or other toxic substances and cause undesired tissue damage. It is likely, therefore, that regulatory mechanisms exist to suppress granulocyte activation under non-inflammatory conditions. Interestingly, a variety of sialic acid binding proteins have been shown capable of modulating the respiratory burst activity of neutrophils. These include influenza virus haemagglutinin [56], *Limulus polyphemus* or *Limax flavus* agglutinin [56], P-selectin [57, 58] and antibodies to the sialomucin, CD43 [59]. Future studies will be needed to address the possibility that sialoadhesin is able to modulate neutrophil function in similar ways.

Sialoadhesin-lymphocyte interactions

Although resting lymphocytes bind poorly to sialoadhesin, it is striking that under non-inflammatory conditions, the highest levels of sialoadhesin expression are found in secondary lymphoid tissues, in close association with lymphocytes, especially B lymphocytes. It is therefore tempting to speculate that sialoadhesin may play a role in some aspect of lymphocyte behaviour, such as localization or trafficking. Both T and B lymphocytes are known to undergo major changes in cell surface glycosylation during differentiation and activation which could have an influence on interactions with sialoadhesin [49]. Lymphocytes express a variety of abundant glycoproteins displaying clustered sialic acid, in particular CD45, CD43 and PSGL-1. All of these molecules show altered glycosylation patterns upon T cell activation. Alternative splicing of CD45 exons can result in striking changes in the display of N- and O-linked glycans [60]. In the case of CD43, enhanced expression of the β1-6 GlcNAc transferase (known as the core 2 enzyme) leads to a more heavily glycosylated form of CD43 and generation of neo-epitopes [61]. Increased expression of the core 2 enzyme during T cell activation is also crucial in converting PSGL-1 into a form that can be recognized by P-selectin [62]. Recent studies have also demonstrated that TH1 and TH2 T cell subsets can be differentially recognized by P-selectin and E-selectin, presumably reflecting altered glycosylation patterns between these subsets [63]. Although less-studied, activated B cells and plasma cells also show striking changes in cell surface glycosylation, one of the best examples being expression of the mucin, CD43 which is initially absent on resting B cells but is strongly induced on activation and retained on plasma cells [64, 65]. It is noteworthy in this respect that plasma cells are often localized in sialoadhesin-rich environments such as the medullary cords of lymph nodes and in bone marrow but little is known of the molecular interactions involved.

Possible role of sialoadhesin in 'altered self' recognition

Macrophage recognition of altered self is of established importance in the clearance of apoptotic cells and is also likely to be important for recognition and clearance of extruded erythroblast nuclei by macrophages [66]. To date, there is no evidence that either of these processes directly involves changes in sialoadhesin ligand expression (unpublished observations). However, an interesting example of where altered self may be of direct relevance to sialoadhesin is in the interaction with tumour cells, since it is well-known that tumour cells can undergo major changes in glycosylation patterns compared with their non-tumorigenic counterparts [67]. For example, in human breast cancer, the mucin MUC-1, normally expressed by epithelial cells, shows altered glycosylation patterns involving a reduction in levels of the branched, core 2 O-linked glycans, changes which can be detected with specific monoclonal antibodies [68]. As discussed above, large numbers of sialoadhesin-positive macrophages can infiltrate human breast tumours. Whether sialoadhesin is involved in macrophage-tumour cell interactions clearly requires further study, but it is of interest that MUC-1 expressed by breast cancer cell lines can be specifically recognized by recombinant sialoadhesin in precipitation assays (unpublished observations). In addition, during breast and ovarian cancers levels of MUC-1 in the serum can rise above normal [69, 70]. The possibility that such soluble mucins can inhibit the binding activity of sialoadhesin or, through interacting with sialoadhesin, alter macrophage functions merits further investigation.

Possible role of sialoadhesin in 'non-self' recognition

Although this review has focused on the self-recognition properties of sialoadhesin, there may be circumstances when

sialoadhesin could play a role in the interaction of macrophages with pathogenic organisms. In general, sialic acids are only synthesized and expressed by higher animal forms but certain viruses, bacteria and parasites have acquired the capacity to synthesize sialic acid or capture it from their hosts (reviewed in [71]) thereby leading to a form of molecular disguise which in some cases has been shown to function as an important determinant in pathogenesis. For example, trypanosomes [72, 73], certain pathogenic strains of meningococci [74], gonococci [75] and streptococci [76] can all express α2,3-linked terminal sialic acids, potential ligands for sialoadhesin. Further studies are required to determine whether the presence of sialic acid on these organisms has any influence on the ability of macrophages to carry out their normal host defence functions.

Conclusions

In the absence of inflammation, the highly-restricted expression pattern of sialoadhesin on macrophage subsets in tissues indicates a specialized role in 'self' recognition functions of macrophages. The sialylated ligands for sialoadhesin are present in abundance on other cells, in the extracellular matrix and in extracellular fluids, but as a consequence of the low affinity of sialoadhesin for these ligands and its extended structure, sialoadhesin can mediate surprisingly selective interactions between macrophages and other cells. The high expression of sialoadhesin in a range of inflammatory conditions indicates a role in host defence and pathology. Challenges for the future include determining in which physiological situations sialoadhesin-dependent adhesion is important and whether manipulating its expression or activity could contribute in some way to the treatment of human pathologies, especially in situations where macrophages play an important role.

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References

1 Gordon S (1995) *BioEssays* **17**: 977–86.
2 Metchnikoff E (1893) *Lectures on the Comparitive Pathology of Inflammation*; London, Kegan Paul, Trench, Truber & Co.
3 Savill J, Fadok V, Henson P, Haslett C (1993) *Immunol Today* **14**: 131–36.
4 Drickamer K, Taylor ME (1993) *Annu Rev Cell Biol* **9**: 237–64.
5 Crocker PR, Gordon S (1986) *J Exp Med* **164**: 1862–75.
6 Crocker PR, Gordon S (1989) *J Exp Med* **169**: 1333–46.
7 Crocker PR, Kelm S, Dubois C, Martin B, McWilliam AS, Shotton DM, Paulson JC, Gordon S (1991) *EMBO J* **10**: 1661–69.
8 Crocker PR, Mucklow S, Bouckson V, McWilliam A, Willis AC, Gordon S, Milon G, Kelm S, Bradfield P (1994) *EMBO J* **13**: 4490–503.
9 Mucklow S (1996) D Phil Thesis, Oxford University.
10 Rudert F, Saunders AM, Rebstock S, Thompson JA, Zimmermann W (1992) *Mammal Gen* **3**: 262–73.
11 Kelm S, Pelz A, Schauer R, Filbin MT, Tang S, Bellard M-Ed, Schnaar RL, Mahoney JA, Hartnell A, Bradfield P, Crocker PR (1994) *Curr Biol* **4**: 965–72.
12 Powell LD, Varki A (1995) *J Biol Chem* **270**: 14243–46.
13 Crocker PR, Kelm S, Hartnell A, Freeman S, Nath D, Vinson M, Mucklow S (1996) *Biochem Soc Trans* **24**: 149–56.
14 Kelm S, Schauer R, Crocker PR (1996) *Glycoconj J* **13**: 913–26.
15 Williams AF, Davis SJ, He Q, Barclay AN (1989) *Cold Spring Harbor Symp Quant Biol* **54**: 637–47.
16 Crocker PR, Freeman S, Gordon S, Kelm S (1995) *J Clin Invest* **95**: 635–43.
17 Freeman SD, Kelm S, Barber EK, Crocker PR (1995) *Blood* **85**: 2005–12.
18 Kelm S, Schauer R, Manuguerra J-C, Gross H-J, Crocker PR (1994) *Glycoconj J* **11**: 576–85.
19 Nath D, van der Merwe PA, Kelm S, Crocker PR (1995) *J Biol Chem* **270**: 26184–91.
20 Braesch-Andersen S, Stamenkovic I (1994) *J Biol Chem* **269**: 11783–86.
21 Engel P, Nojima Y, Rothstein D, Zhou L, Wilson GL, Kehrl JH, Tedder TF (1993) *J Immunol* **150**: 4719–32.
22 Engel P, Wagner N, Miller AS, Tedder TF (1995) *J Exp Med* **181**: 1581–86.
23 Powell LD, Sgroi D, Sjoberg ER, Stamenkovic I, and Varki A (1993) *J Biol Chem* **268**: 7019–27.
24 Powell LD, Varki A (1994) *J Biol Chem* **269**: 10628–36.
25 Powell LD, Jain RK, Matta KL, Sabesan S, Varki A (1995) *J. Biol Chem* **270**: 7523–32.
26 Sgroi D, Varki A, Braesch-Anderson S, Stamenkovic I (1993) *J Biol Chem* **268**: 7011–18.
27 Sjoberg E, Powell L, Klein A, Varki A (1994) *J Cell Biol* **126**: 549–62.
28 Yang LJ, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, Schnaar RL (1996) *Proc Natl Acad Sci USA* **93**: 814–18.
29 Collins BE, Yang LJ-S, Mukhopadhyay G, Filbin MT, Kiso M, Hasegawa A, Schnaar RL (1997) *J Biol Chem* **272**: 1248–55.
30 Van der Merwe PA, Crocker PR, Vinson M, Barclay AN, Schauer R, Kelm S (1996) *J Biol Chem* **271**: 9273–80.
31 Vinson M, van der Merwe PA, Kelm S, May A, Jones EY, Crocker PR (1996) *J Biol Chem* **271**: 9267–72.
32 Law CL, Aruffo A, Chandran KA, Doty RT, Clark EA (1995) *J Immunol* **155**: 3368–76.
33 Holness CL, da Silva RP, Fawcett J, Gordon S, Simmons DL (1993) *J Biol Chem* **268**: 9661–66.
34 Crocker PR, Werb Z, Gordon S, Bainton DF (1990) *Blood* **76**: 1131–38.
35 Perry VH, Crocker PR, Gordon S (1992) *J Cell Sci* **101**: 201–7.
36 Kraal G (1992) *Int Rev Cytol* **132**: 31–74.
37 Snook T (1964) *Anat Rec* **148**: 149.
38 Damoiseaux JG, Huitinga I, Dopp EA, Dijkstra CD (1992) *J Exp Med* **176**: 647–55.

39 Van den Berg TVd, van Die IV, Delavalette CR, Dopp AE, Smit LD, van der Meide PH, Tilders FJH, Crocker PR, Dijkstra CD (1996) *J Immunol* **157**: 3130–38.
40 Crocker PR, Hill M, Gordon S (1988) *Immunology* **65**: 515–22.
41 McWilliam AS, Tree P, Gordon S (1992) *Proc Natl Acad Sci USA* **89**: 10522–26.
42 Doyle AG, Herbein G, Montaner LJ, Minty AJ, Caput D, Ferrara P, Gordon S (1994) *Eur J Immunol* **24**: 1441–45.
43 Hanasaki K, Powell LD, Varki A (1995) *J Biol Chem* **270**: 7543–50.
44 Chappell T, Warren G (1989) *J Cell Biol* **109**: 2693–702.
45 Simionescu N, Simionescu M, Palade GE (1981) *J Cell Biol* **90**: 605–13.
46 Haldenby KA, Chappell DC, Winlove CP, Parker KH, Firth JA (1994) *J Vasc Res* **31**: 2–9.
47 Patel KD, Nollert MU, McEver RP (1995) *J Cell Biol* **131**: 1893–902.
48 Crocker PR, Feizi T (1996) *Curr Opin Struct Biol* **6**: 679–91.
49 Van den Berg TK, Breve JP, Damoiseaux JGMC, Dopp EA, Kelm S, Crocker PR, Dijkstra CD, Kraal G (1992) *J Exp Med* **176**: 647–55.
50 Metcalf D, Lindeman GJ, Nicola NA (1995) *Blood* **85**: 2364–70.
51 Lord BI, Molineux G, Pojda Z, Souza LM, Mermod JJ, Dexter TM (1991) *Blood* **77**: 2154–59.
52 Lovas K, Knudsen E, Iversen PO, Benestad HB (1996) *Eur J Haematol* **56**: 221–29.
53 Coxon A, Rieu P, Barkalow FJ, Askari S, Sharpe AH, Andrian UHv, Arnaout MA, Mayadas TN (1996) *Immunity* **5**: 653–66.
54 Liles WC, Kiener PA, Ledbetter JA, Aruffo A, Klebanoff SJ (1996) *J Exp Med* **184**: 429–40.
55 Lagasse E, Weissman IL (1994) *J Exp Med* **179**: 1047–52.
56 Cassidy LF, Lyles DS, Abramson JS (1989) *J Immunol* **142**: 4401–06.
57 Tsuji T, Nagata K, Koike J, Todoroki N, Irimura T (1994) *J Leukoc Biol* **56**: 583–87.
58 Wong CS, Gamble JR, Skinner MP, Lucas CM, Berndt MC, Vadas MA (1991) *Proc Natl Acad Sci USA* **88**: 2397–401.
59 Abramson JS, Hudnor HR (1995) *Blood* **85**: 1615–19.
60 Trowbridge IS, Thomas ML (1994) *Annu Rev Immunol* **12**: 85–116.
61 Ellies LG, Tao W, Fellinger W, Teh HS, Ziltener HJ (1996) *Blood* **88**: 1725–32.
62 Vachino G, Chang XJ, Veldman GM, Kumar R, Sako D, Fouser LA, Berndt MC, Cumming DA (1995) *J Biol Chem* **270**: 21966–74.
63 Austrup F, Vestweber D, Borges E, Lohning M, Brauer R, Herz U, Renz H, Hallmann R, Scheffold A, Radbruch A, Hamann A (1997) *Nature* **385**: 81–83.
64 Dustin LB, Bullock ED, Hamada Y, Azuma T, Loh DY (1995) *J Immunol* **154**: 4936–49.
65 Bjorck P, Axelsson B, Paulie S (1991) *Scand J Immunol* **33**: 211–18
66 Qiu L-B, Dickson H, Hajibagheri MAN, Crocker PR (1995) *Blood* **85**: 1630–1640.
67 Hakomori S (1989) *Adv Canc Res* **52**: 257–331.
68 Brockhausen I, Yang JM, Burchell J, Whitehouse C, Taylor-Papadimitriou J (1995) *Eur J Biochem* **233**: 607–17.
69 Burchell J, Wang D, Taylor-Papadimitriou J (1984) *Int J Cancer* **34**: 763–68.
70 McGuckin MA, Devine PL, Ramm LE, Ward BG (1994) *Tumour Biol* **15**: 33–44.
71 Schauer R (1982) *Sialic acids*: *Chemistry*, *Metabolism and Function*. New York: Springer Verlag.
72 Schenkman S, Eichinger D (1993) *Parasitol Today* **9**: 218–22.
73 Engstler M, Schauer R (1993) *Parasitol Today* **9**: 222–25.
74 Hammerschmidt S, Hilse R, van Putten JP, Gerardy Schahn R, Unkmeir A, Frosch M (1996) *EMBO J* **15**: 192–98.
75 van Putten JP, Grassme HU, Robertson BD, Schwan ET (1995) *Prog Clin Biol Res* **392**: 49–58.
76 Haft RF, Wessels MR, Mebane MF, Conaty N, Rubens CE (1996) *Mol Microbiol* **19**: 555–63.

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