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Review

Sweet escape: Sialic acids in tumor immune evasion

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ABSTRACT

Sialic acids represent a family of sugar molecules derived from neuraminic acid that frequently terminate glycan chains and contribute to many biological processes. Already five decades ago, aberrantly high expression of sialic acids has been proposed to protect cancer cells from recognition and eradication by the immune system. Today, increased understanding at the molecular level demonstrates the broad immunomodulatory capacity of tumor-derived sialic acids that is, at least in part, mediated through interactions with immunoinhibitory Siglec receptors. Here we will review current studies from a sialic acid sugar perspective showing that tumor-derived sialic acids disable major killing mechanisms of effector immune cells, trigger production of immune suppressive cytokines and dampen activation of antigen-presenting cells and subsequent induction of anti-tumor immune responses. Furthermore, strategies to modulate sialic acid expression in cancer cells to improve cancer immunotherapy will be discussed.

Contents

1. Introduction

Every living cell is surrounded by a dense layer of glycans that are attached to cell surface glycoproteins and glycolipids. Glycans are composed of various monosaccharides and show an enormous, cell-specific structural diversity illustrating their importance in many biological processes at the molecular level [1]. Despite their abundance on the cell membrane, many physiological functions and effects of glycans are not yet understood. Nevertheless, it is known for decades that glycosylation changes reflect, and are causative for several pathological conditions. Upon malignant transformation, tumor cells present a significantly different glycosylation pattern relative to their normal counterparts, and several cancer-specific glycans have been identified

Abbreviations: $SLe^{A/X}$, sialyl Lewis antigen A and X; STn, sialyl Tn antigen; PSA, polysialic acid; SAMPs, self-associated molecular patterns; Siglecs, sialic acid-binding immunoglobulin-like lectins; ITIMs, immunoreceptor tyrosine-based inhibitory motifs; NK cell, natural killer cell; NKT cell, natural killer T cell; CTLs, cytotoxic T cells; DISC, death-inducing signaling complex; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell; DC, dendritic cell; Neu5Gc, N-glycolylneuraminic acid; TACA, tumor-associated carbohydrate antigen; GBM, glioblastoma multifore

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that promote tumor growth and progression. Among the glycosylation changes reported in cancer, aberrantly high expression of sialic acid sugar-carrying glycans (sialoglycans) is commonly found [2,3].

Sialic acids represent a family of about fifty derivates of neuraminic acids that share a common nine-carbon (C1-9) backbone. In general, sialic acids terminate glycan chains of all vertebrate and many invertebrate cells and contribute to protein stability and trafficking as well as cell–cell and cell–extracellular matrix interactions. Most cells possess a specific machinery to synthesize the different sialic acids from precursor carbohydrates in the cytoplasm. Following transport, Golgi-resident sialyltransferases incorporate these sialic acids into the glycans of glycoproteins and glycolipids. To date, more than 20 different sialyltransferases have been identified, each attaching sialic acids via different glycosidic linkages (α2,3; α2,6 or α2,8) to underlying sugars [4]. In cancer cells, overexpression of sialyltransferases leads to increased synthesis of sialoglycoconjugates that are deposited on the cell surface. Possibly, overexpression of sialyltransferases also leads to the neoformation of cancer sialoglycans, however this hypothesis remains to be proven [5,6]. Prominent cancer-associated sialoglycan structures include the sialogangliosides fucosyl-GM1, GD1a, GM2, GD2, GM3 and GD3, the sialyl Lewis antigens A and X ($SLe^{A/X}$), the sialyl Tn (STn) antigen, polysialic acid (PSA) and mucins [3,7–9]. Due to hypersialylation, cancer cells acquire distinct characteristics including resistance to apoptosis and enhanced migratory properties which correlate with tumor aggressiveness and a poor prognosis for patients [6,10,11]. An increasing amount of evidence advocates tumor sialoglycans as potent immune modulators acting at the tumor/immune interface. It becomes more and more apparent that hypersialylation provides a selective advantage for tumor cells to escape from anti-tumor immunity and is even involved in manipulating immune cell function to benefit tumor growth.

In this review, we examine recent findings emphasizing that tumor cells escape from host anti-tumor immunity through aberrant expression of sialic acids. In particular, the effect of tumor-derived sialoglycans on the function of different immune cell subsets involved in anti-tumor immunity will be discussed. An overview of the immunomodulatory events at the tumor sialoglycan/immune interface is provided in Fig. 1.

2. Sialic acids in immune regulation

The dense layer of glycans protruding from the cell surface is one of the first structures recognized by immune cells that constantly screen host cell surfaces for (malignant) aberrations or presence of pathogens. Indeed, immune cells express multiple distinct carbohydrate-binding receptor families that modulate their function and that have recently been reviewed elsewhere [12]. Among the various carbohydrates present in cell surface glycans, the diverse family of sialic acids is of particular interest [13]. Being vertebrate specific, expression of sialic acids allows discrimination of pathogens lacking sialic acid expression from sialylated host cells. Interestingly, pathogens have been identified that have evolved strategies to express host sialic acids as molecular mimicry to evade the host immune system [14,15].

A growing body of evidence suggests that sialic acids control immune homeostasis and dampen inappropriate immune activation in order to avoid or limit damage of sialylated host cells. The importance of sialic acids herein became apparent in a cohort of patients lacking the expression of 9-O acetylated sialic acids due to a defect in the sialic acid acetylesterase (SIAE) gene. These patients developed a broad spectrum of autoimmune diseases ranging from rheumatoid arthritis to type I diabetes [16]. In line with these findings, expression of sialoglycans on colonic epithelia cells or neurons has been indicated to prevent immune activation and to protect the gut mucosa and nervous system, respectively [17,18]. Together, the importance of sialic acids in the discrimination of *self* and *non-self* and as immune inhibitory signals preventing inappropriate immune activation has brought forth the idea that sialic acids act as *self-associated molecular patterns* (SAMPs, Ajit Varki 2011) [19]. This implies that there are sialic acid-recognizing receptors that transmit inhibitory signals to control immune activation. So far, three sialic acid-binding lectins have been identified including Selectins, factor H and the family of Sialic acid-binding immunoglobulin-like lectins (Siglecs). Selectins (P-, L-and E-Selectin) belong to the family of C-type lectins and are well-known for their binding to SLe^x and involvement in leukocyte trafficking, but also cancer metastasis. Factor H is a central regulatory protein in the alternative complement pathway discussed in more detail in Section 3.1 [4]. Siglecs comprise a family of more than 14 I-type lectins expressed by virtually all immune cells that specifically recognize diverse sialoglycans (Box 1). Siglecs are type I transmembrane proteins with an N-terminal sialic acid-binding site and most of them possess one or more immunoreceptor tyrosine-based inhibitory motifs (ITIMs) at their C-terminus [4,20]. In general, binding of sialic acid ligands to immune inhibitory Siglecs results in inhibition of immune cell activation and function. Therefore, the sialic acid/Siglec pathway has been proposed to signal self-recognition limiting immune activation and destruction of host cells [19].

3. Sialic acids in tumor immune evasion

Already fifty years ago, Barbara H. Sanford and others proposed that aberrantly high expression of sialic acids on tumor cells allows immune escape (Box 2). They suggested that the dense layer of sialic acids found on tumor cells masks surface antigens preventing recognition by the immune system (antigen masking). This concept was supported by the observation that removal of sialic acids from tumor cells using bacterial sialidases strongly increased their immunogenicity and hindered growth in immunocompetent mice [28,29]. Subsequently, sialidase-treated tumor cells were exploited as preventive and therapeutic vaccine in several animal tumor models. These vaccines showed impressive results in animal tumor models and culminated in clinical trials in men. However, efficacy of sialidase-treated tumor vaccines could not be demonstrated unequivocally in cancer patient cohorts between 1970 and 1990 [30,31]. Nevertheless, these early important studies indicated a vital role for sialic acids in tumor immunology and defined them as potential target for tumor immunotherapy.

Today, advances in glycobiology and immunology renewed the interest into tumor sialoglycan research and revealed their strong immunomodulatory potential beyond the concept of antigen masking. A growing body of evidence indicates that tumor sialoglycans affect numerous immune relevant processes and make a major contribution to immune evasion. Below, we review immunomodulatory processes at the tumor sialoglycan-immune cell interface in the context of tumor immune escape.

3.1. Sialic acids in complement system evasion

In vertebrates, sialoglycans were suggested to characterize host cells as *self* and have been reported to prevent activation of the complement system by recruitment of the complement control protein factor H to the cell surface [13,19]. Factor H has several polyanionic binding sites that bind to sialoglycans, glycosaminoglycans and other negatively charged molecules on the surface of host cells. Surface-bound factor H hinders deposition and amplification of the complement-activating protein C3b on the cell surface and downstream activation of the alternative complement pathway. This regulatory mechanism has been suggested to prevent inappropriate complement activation and killing of sialylated host cells [47]. Pathogens that lack sialic acid expression do not recruit factor H and activate the complement cascade [48]. Some pathogens like as *Neisseria meningitides*, have been found to utilize host sialic acids as molecular mimicry to recruit factor H to their surface and avoid lysis by the complement system [49,50]. Although the role of the complement system in tumorigenesis is not yet understood, it has been suggested that tumor cells escape from complement activation by covering their membrane with sialoglycans [51,52]. Removal of sialic

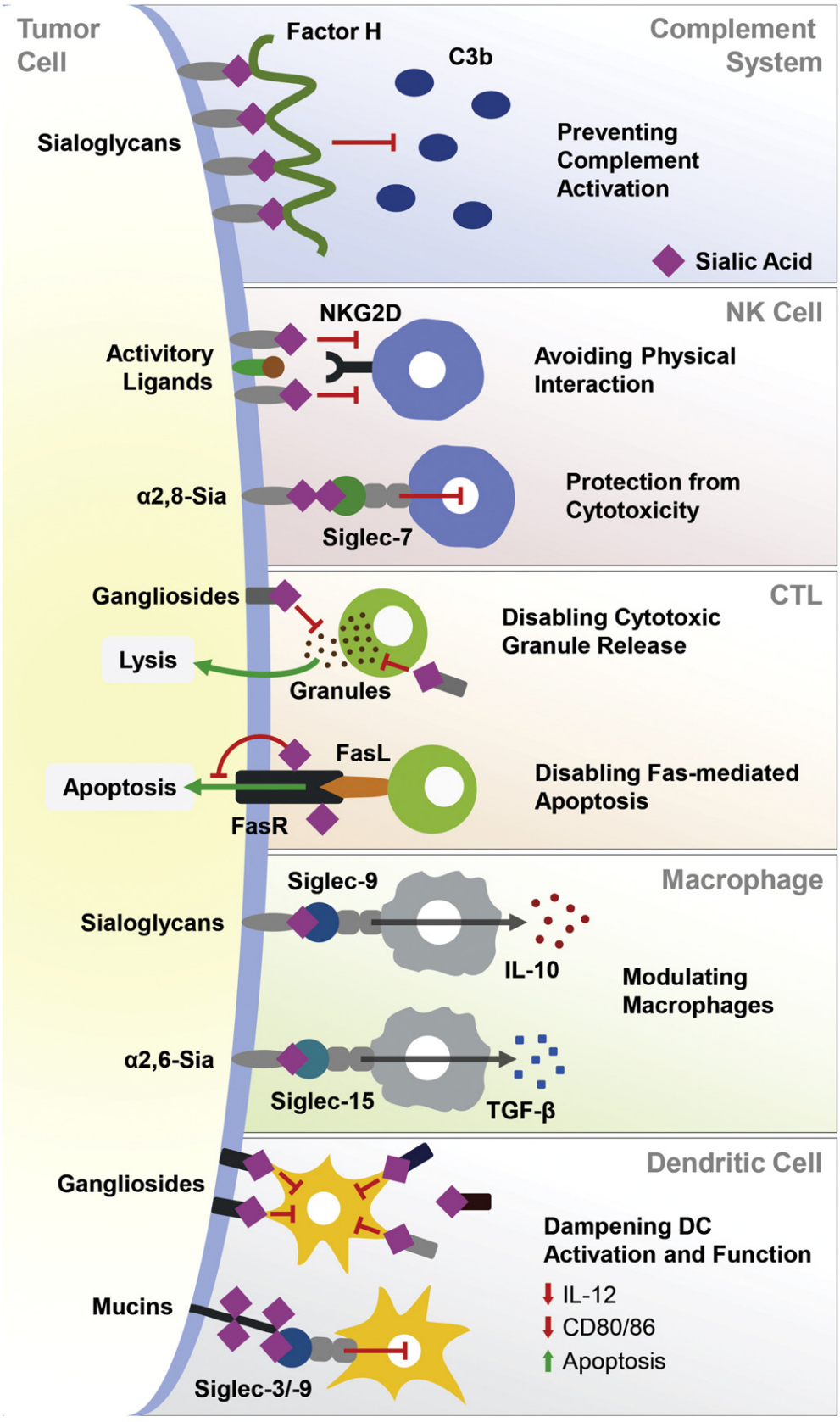

Fig. 1. Sialic acids in tumor immune evasion. Aberrant expression of sialic acids on cancer cells prevents complement activation (blue), protects from NK cell killing (red), disables major killing mechanisms of cytotoxic T cells (orange), modulates macrophage function (green) and dampens dendritic cell activation and function (gray).

Box 1
Siglecs in immune control.

The importance of sialic acid recognition in the immune system emerged with the discovery of sialic acid-containing ligands for lectins receptors. In the early 1990s, CD22 (Siglec-2) on B cells and sialoadhesin (Siglec-1) on macrophages were the first receptors shown to bind sialylated glycoproteins [21,22]. Their immunoglobulin-like domains distinguished them from other C-type or calcium-dependent lectins and formed the basis for a new, still growing family of I-type lectin receptors — the Siglecs. Next to their Ig-like domain, many Siglecs contain tyrosine-based signaling motifs, particularly *immunoreceptor tyrosine-based inhibitory motifs* (ITIMs) that are often implicated in attenuating cell signaling and endocytosis. Siglecs are almost exclusively expressed by cells of the immune system, and can be divided into two subsets based on their structural relatedness. The main subset mostly comprises ITIM-containing Siglecs which are closely related to CD33 (Siglec-3). This group shows important differences in repertoire between mammalian species and consists of Siglec-3, -5 to -12, -14 and -16 in humans and the mouse homologues Siglec-3, E–H. The second group contains Siglec-1, -2, -4 (MAG) and -15 that are conserved between humans and mice [20,23].

Various functions of Siglecs in immune cells have been described. Siglec-1 has been shown to play a role in the phagocytosis of pathogens covered with sialic acids. CD33-related Siglecs were shown to attenuate inflammatory responses by binding to ligands on the same cell-surface (*cis* interactions) or surfaces of other cells (*trans* interactions). Therefore, Siglecs have shown to be important in the attenuation of 'self'-inflammatory triggers, called damage-associated molecular patterns (DAMPs). Chen et al. demonstrated the existence of an inhibitory feedback mechanism that suppresses TLR signaling by DAMPs [24,25]. In their studies the DAMP *high-mobility group B1* (HMGB1) released from necrotic cells bound to CD24. This glycoprotein on dendritic cells consecutively interacted with the inhibitory Siglec-G on the same cell, which together inhibited HMGB1-driven TLR activation. In a more recent report the authors demonstrated the importance of this inhibitory mechanism in systemic sepsis [26]. Their data suggested that bacterial sialidases cause excessive inflammation-related damage by cleaving the sialic acids on CD24 required for binding by Siglec-G, or the human equivalent Siglec-10. Using various sialidase inhibitors they could attenuate the effects of bacterial sialidase, suggesting that these compounds can be used to dampen the morbidity associated with sepsis.

Finally, instead of modulating the levels of Siglec ligands, pathogens were also reported to modulate Siglec expression itself. Recently, RNA viruses were shown to specifically upregulate Siglec-G expression in macrophages by a RIG-I dependent mechanism [27]. This binding targeted RIG-I for proteasomal degradation, leading to less IFN production and resulting in a compromised protection against viral spreading.

The structure of Siglecs and the role they have in general immune regulation is more extensively described in a recent review [20].

acids from prostate, breast and ovarian carcinoma cells using sialidase has been reported to sensitize tumor cells to complement-mediated lysis [53]. In addition, tumor cells have been reported to produce factor H to acquire resistance to complement activation [54,55].

3.2. Sialic acids in natural killer cell evasion

Natural killer (NK) cells have the ability to recognize and kill tumor cells that lack expression of MHC class I molecules (*missing self-recognition*). Tumor cells can escape NK cell-mediated killing by expressing ligands for inhibitory NK cell receptors or by downregulating the expression of activating ligands, thereby limiting success of current

Box 2
Historical overview of sialic acid-focused tumor immunotherapy.

In 1967, Sanford, Currie and others independently reported that removal of sialic acids from tumor cells using sialidase causes loss of transplantability in mice [28,32–34]. This effect could be linked to altered recognition of the tumor cells by the immune system as sialidase-treated mouse and human tumor cells were shown to be more immunogenic in mixed lymphocyte reactions and only immunocompetent mice showed resistance to sialidase-treated tumors [29,35–38]. In addition, Currie reported that injection of sialidase-treated leukemia cells immunized mice against the non-immunogenic, untreated leukemia cells [29]. Sanford and others suggested that tumor cells hide surface antigens from the immune system by veiling their cell surface with a dense layer of sialoglycans. Consequently, the idea that sialidase-treated tumor cells can be used to therapeutically vaccinate hosts against tumors emerged. In 1971 Simmons and Rios provided first evidence for the feasibility of sialidase-treated tumor vaccines. They reported that established methylcholanthrene (MCA)-induced fibrosarcomas were rejected in mice injected with living, sialidase-treated tumor cells. After several sialidase-based tumor vaccines showed impressive results in animal tumor models, first non-randomized clinical trials in patients were conducted in the 1970s. Seigler and colleagues reported complete regression in a small cohort of melanoma patients after treatment with autologous, irradiated, sialidase-treated melanoma cells [39]. Rosato et al. showed beneficial effects using vaccinations with sialidase-treated tumor cells in patients with various solid tumors [40,41]. Based on these promising results, first randomized clinical trials were performed. In 1979 Bekesi and Holland reported significantly increased remission times in AML patients receiving vaccinations with irradiated, autologous sialidase-treated tumor cells [42]. However, findings in the late eighties by Urbanitz and colleagues were less encouraging. They reported only mild effects in patients treated with desialylated myeloblasts [43]. Moreover, Gray and colleagues evaluated the efficacy of sialidase-treated tumor cell vaccines in a large cohort of bowel cancer patients. Here, no improvement in five-year survival could be found [44]. Sedlacek and Seiler summarized the issues that might have complicated the use of sialidase-treated tumor cells as antitumor vaccine and offered an explanation for the contradictory results [30,31]. First, the tumor type, size, stage and pretreatment in the patients might be determinative for the efficacy. Next, no proper analysis has taken place regarding standardized treatment regimens including the number of tumor cells, the route of administration and the frequency of injections. These are important variables that need to be thoroughly determined in order to achieve successful vaccination against tumors in general, but have not been conducted at that time [45]. Notably, the molecular mechanisms linking removal of sialic acids from the tumor surface to increased immunogenicity were poorly understood. Conflicting results reported that sialidase remained bound to the tumor cells and eventually could be recognized as antigen [46]. The discrepancy between preclinical expectations and moderate results from clinical trials together with the lack of molecular and mechanistic understanding of the effects of sialidase treatment ended a hype that lasted more than 20 years. Nevertheless, these early studies have pointed out the detrimental effects of tumor sialic acids and founded the basis for recent therapeutic approaches to target sialic acids in cancer.

NK cell therapies [56]. More recently, two NK cell evasion strategies have been identified involving hypersialylation of tumor cells.

First, the dense layer of sialoglycans surrounding tumor cells has been reported to hinder physical interactions with NK cells and to mask activating ligands on the tumor cell surface. Cohen et al. showed that growth of desialylated fibrosarcoma cells was abrogated in immunocompetent mice, but could be restored by depletion of NK cells [57]. Furthermore, sialylation of tumor cells impaired the formation of immunological synapses between tumor cells and NK cells precluding cytotoxicity. This effect could be linked to impaired recognition of ligands for the activating NK cell receptor NKG2D on sialylated tumors most likely via charge repulsion due to the highly negative charge of sialylated membranes and/or hypersialylation of NKG2D ligands itself. Noteworthy, in the same study it was shown that immune reactivity selects for the outgrowth of hypersialylated tumor cell variants. Fibrosarcoma cells isolated from mice with impaired immunoediting capacity (IL-1$\alpha^{-/-}$ or IFN$\gamma^{-/-}$) exhibited lower expression of sialoglycans compared to wild type mice. Inoculation of these hyposialylated tumor cells into wild type mice showed reduced growth, indicating that high expression of sialic acids is beneficial for tumor immune evasion.

Second, several studies advocate that tumor sialoglycans interfere with NK cell function by triggering immune inhibitory signaling through Siglec receptors. Most human NK cells express Siglec-7 and about 40% of NK cells also express Siglec-9 [58,59]. Siglec-7 recognizes α2,8-linked sialic acids that are mainly expressed on NK cells themselves, cells of the central nervous system and tumor cells including melanoma, glioma or neuroblastoma [7,20,60]. Several studies indicate that binding of tumor α2,8-linked sialic acids (e.g. GD3) to Siglec-7 dampens NK cell activation and function allowing tumor cells to escape NK cell-mediated killing [60–63]. By loading tumor cell surfaces with synthetic sialic acid glycopolymers that serve as ligand for Siglec-7, Hudak et al. recently demonstrated their protection from NK cell-mediated cytotoxicity [64]. Increased Siglec-7 phosphorylation and recruitment of SHP-1 upon engagement with their sialic acid ligands on the surface of tumor cells inhibited NK cell degranulation and tumor killing. Less is known about the function of Siglec-9 on NK cells, but there is evidence that binding of α2,3-sialic acid-bearing mucin 16 (MUC16) to Siglec-9 prevents the formation of immunological synapses between tumor cells and NK cells [58,65]. In a recent study, Jandus et al. demonstrated expression of Siglec-7 and Siglec-9 ligands on tumor biopsies and confirmed that these ligands protect tumor cells from NK cell killing [59].

Next to NK cells, natural killer T (NKT) cells are important mediators in innate tumor immunity. While Siglec expression by NKT cells has not yet been investigated, it has been reported that the sialic acid-carrying melanoma antigen can bind to the NKT cell receptor CD1 [66]. Binding of GD3 isolated from ovarian cancer cells to CD1 suppressed NKT cells and inhibited NKT cell activation with α-galactosylceramide in vivo [67]. These findings indicate that tumor-derived sialogangliosides can affect NKT cell function and imply that it may be rewarding to further investigate the Sialic acid/Siglec axis in these innate lymphocytes.

3.3. Sialic acids in cytotoxic T cell evasion

In addition to NK cells, also adaptive immune cells are affected by tumor sialoglycans. Sialoglycans facilitate tumor immune evasion from cytotoxic T cells (CTLs) by inhibiting their activation and disabling their killing mechanisms. CTLs feature two powerful ways to eradicate tumor cells, granule- and Fas-mediated cytotoxicity, both of which can be affected by aberrant expression of sialoglycans on tumor cells. Lee et al. reported that tumor-derived sialic acid-containing gangliosides (GM1-3, GD1a) inhibit trafficking and exocytosis of lytic granules from CTLs [68]. They showed that gangliosides do not impair TCR engagement and immunological synapse formation, but affect trafficking of lytic granules to the immunological synapse and subsequent release of lytic proteins. Despite their potency as inhibitors of CTL function, it is still unclear yet how gangliosides affect trafficking and exocytosis of lytic granules.

Next, there is evidence that hypersialylation of the Fas receptor (FasR, CD95) desensitizes tumor cells to Fas-mediated cytotoxicity. CTLs express the Fas ligand (FasL, CD95L) and are capable of eliminating tumor cells that express FasR. Upon engagement of FasL, the death-inducing signaling complex (DISC) assembles at the cytoplasmic tail of FasR leading to internalization of the complex and downstream activation of caspases that lead to DNA and mitochondria disintegration and finally apoptosis [69]. Swindall and Bellis reported that upregulation of the sialyltransferase ST6Gal I in tumor cells leads to high α2,6-sialylation of FasR and reduced assembly and internalization of the DISC complex upon Fas ligand encounter [70]. Even though the relevance of this mechanism remains to be proven in vivo, the data suggest a novel strategy of tumor cells to disable major killing mechanisms of CTLs.

At the moment, it is neither clear which Siglecs are expressed on human T cells nor if Siglecs are involved in escape from CTL lysis [71]. In a Jurkat T cell overexpression model artificial introduction and stimulation of Siglec-7/-9 resulted in dephosphorylation of the ZAP-70 signaling molecule and subsequent inhibition of downstream TCR-signaling [72]. More recently, Bandala-Sanchez and colleagues provided evidence for Siglec-mediated regulation of T cell activation in humans [73]. They identified a novel $CD4^+$ T cell subset that expresses and releases high levels of the sialoglycoprotein CD52. These $CD4^+CD52^{high}$ T cells were capable of suppressing activation and function of $CD4^+CD52^{low}$ T cells via the interaction with Siglec-10. By suppressing other $CD4^+$ T cells, $CD4^+CD52^{high}$ T cells were suggested to be involved in immune homeostasis and control of autoimmunity as only low levels of this novel suppressive subset were found in type I diabetes patients. Noteworthy, CD52 expression has been reported on cells in malignant hematologic diseases and levels of circulating CD52 correlate with a poor prognosis in leukemia patients [74]. Future research is needed to elucidate if this recently identified immunosuppressive CD52/Siglec-10 axis is utilized by tumors in order to limit T-cell activation and function in the tumor microenvironment or even systemically.

Another outstanding question is whether tumor sialoglycans can affect the function of other T cell subsets like Th1, Th2, Th17 or regulatory T (Tregs) cells. It has been reported that *cis* sialic acids can regulate T cell polarization and activation [75,76]. Therefore, it would be interesting to investigate the influence of sialoglycans produced and secreted by tumor cells to affect the different T cell subsets *in trans*.

3.4. Sialic acids in myeloid cell function modulation

Myeloid cells have been reported to exhibit pro- and anti-tumorigenic roles depending on the different factors present in the tumor microenvironment [77]. Interestingly, also sialic acids expressed by tumor cells appear to have dual effects on myeloid cell. Tumor sialoglycans allow recognition and uptake by macrophages on one hand, but on the other hand tumor sialoglycans can skew macrophages towards a more pro-tumorigenic phenotype [20]. Macrophages commonly express Siglec-1 (sialoadhesin/CD169), a conserved Siglec without C-terminal ITIM motif that recognizes α2,3-linked sialic acids [78,79]. These Siglec-1 positive macrophages have been reported to be beneficial in anti-cancer immunity as they can phagocytose dead tumor cells and cross-present antigens to CTLs. Intriguingly, blockage of Siglec-1 hinders the activation of CTLs and subsequent antitumor immunity in mice [80].

Opposing the anti-tumorigenic effects, recent studies suggest that tumors can modulate the activation and secretion of tumor-promoting cytokines by macrophages via the interaction with Siglecs. Miyazaki et al. demonstrated that disialyl Lewis^a ligands on normal colonic epithelial cells serve as ligands for Siglec-7 and -9 on mucosal macrophages and suppress their activation possibly to avoid inflammation and tissue

damage. Notably, the authors showed that colon cancer cells change their sialic acid signature, expressing more of the selectin ligands sialyl Lewis^a and sialyl Lewis^x instead of disialyl Lewis^a. These findings suggest that Siglec-7/-9 control of macrophage activation might be abolished in colon cancer leading to production of inflammatory factors by macrophages that drive tumor progression [17]. In the aforementioned example loss of Siglec ligands on tumor cells favors activation of macrophages and production of pro-inflammatory cytokines. In a different study, Takamiya and colleagues reported that the expression of α2,6 sialic acids on lung tumor cells stimulated production of TGF-β by monocytes and macrophages in a Siglec-15-dependent manner [81]. This more recently discovered member of the Siglec family is expressed by tumor-associated macrophages in tumor tissue of patients. Binding of α2,6 sialic acid ligands on tumor cells induced recruitment of the activating adaptor protein DAP12 to the cytoplasmic tail of Siglec-15 and triggered downstream activating signaling via Syk-kinase resulting in increased production of the immunosuppressive cytokine TGF-β. In line with these findings, expression of Siglec-9 on macrophages has been reported to lower TNF-α production and increase IL-10 production [82]. Obviously, the varying sialic acid signatures on cancer cells of different origins and the expression pattern of Siglecs on myeloid cell subsets co-determine the context-dependent production of pro- or anti-inflammatory cytokines.

Apart from the discussed examples, the extent to which tumor sialoglycans influence myeloid cells is largely unknown. Regarding that myeloid cells broadly express siglecs, it is likely that they are highly sensitive to aberrant sialic acid expression on tumor cells. Recent evidence showing that Siglec-3 (CD33) drives myeloid-derived suppressor cell (MDSC) expansion allows us to speculate that tumor sialoglycans are involved in the recruitment of these potent immune suppressive cells in the tumor microenvironment [83,84].

3.5. Sialic acids in dendritic cell modulation

Dendritic cells (DCs) are the major antigen-presenting cells of the immune system capable of inducing T cell responses against tumor cells. To this aim, matured DCs need to present tumor antigens as well as co-stimulatory molecules to naïve T cells. Tumor-bound or secreted sialoglycans have been reported to modulate DC activation and maturation thereby hindering the initiation of antitumor T cell responses. GD1a, a disialoganglioside produced for instance by gastric, pancreatic or prostate cancer cells, was shown to counteract DC activation, upregulation of costimulatory molecules CD80/86 and IL-12 production. As a consequence, DCs pulsed with GD1a failed to induce Th1 effector cell development, but promoted differentiation into immunosuppressive Tregs [85]. Similarly, other tumor-derived gangliosides e.g. GD2 (neuroblastoma) or GM3/GD3 (melanoma) were reported to inhibit DC activation, IL-12 production and subsequent effector T cell activation [86,87]. Likewise, melanoma GM3/GD3 was reported to prevent activation and migratory function of tissue-residing Langerhans cells and induce to apoptosis [88]. How precisely tumor sialogangliosides mediate these immune suppressive effects are yet to be determined. Possibly, immune inhibitory Siglecs that are widely expressed on DCs recognize sialogangliosides and are responsible for the described effects. This assumption is in line with studies demonstrating that highly sialylated mucins bind to Siglecs on DCs and dampen their activation. For example, α2,6 sialic acid-carrying mucin 2 (MUC2) has been described to bind Siglec-3 and to induce apoptosis of monocyte-derived DCs [89]. Binding of tumor mucins and other sialoglycans to Siglec-9 on DCs was shown to dampen production of IL-12 while leaving IL-10 production unaffected [90]. Moreover, MUC1 has been described to attract immature DCs and induce maturation, but these DCs are functionally impaired and fail to produce IL-12 and to induce Th1 effector cells [91]. These data collectively suggest, that tumor sialoglycans dampen activation and function of DCs and prevent induction of anti-tumor immune responses [92].

4. Sialic acids as targets in cancer immunotherapy

Given the strong immunomodulatory capacity of sialic acids, hypersialylation appears to protect cancer cells from recognition and eradication by the immune system and limits the outcome of cancer immunotherapy. Therefore, sialic acids emerge as targets to enhance cancer immunotherapy. Removing cell surface sialic acids from cancer cells with bacterial sialidases increased their immunogenicity in mixed lymphocyte reactions and has been explored in the past to induce anti-tumor immune responses in cancer patients (Box 2). More recently, sialidase treatment was shown to render cancer cells immunoreactive in a mouse model suggesting that interfering with sialic expression could allow eradication of cancer cells by the immune system [57]. For decades, bacterial sialidases were the only way to efficiently remove sialic acids from cells, however, sialic acid expression restores quickly following enzymatic removal [93]. This issue could be solved by a novel fluorinated sialic acid analog (P-3F$_{ax}$-Neu5Ac), developed by Rillahan et al., that inhibits sialyltransferases and subsequent incorporation of sialic acids into glycans [94]. Our group could demonstrate that this inhibitor efficiently blocks sialic acid expression in cancer cells for several days [93]. Moreover, in vitro pre-treatment with P-3F$_{ax}$-Neu5Ac hampered outgrowth of melanoma in mice. Future research is needed to determine to what extent this effect is due to increased immunoreactivity against the melanoma cells and how this sialic acid analog can be utilized to block sialic acid expression in cancer cells in vivo.

Alternatively, synthetic sialic acids or sialic acid precursors carrying chemical modifications can be administered to cancer cells and enter the sialic acid biosynthesis pathway to affect anti-tumor immunity [10,95,96]. Surprisingly, incorporation of non-human, immunogenic sialic acids into cell surface sialoglycans of cancer cells can occur 'naturally'. Research led by Ajit Varki has identified expression of a non-human sialic acid, Neu5Gc (N-glycolylneuraminic acid), in several types of cancer. Neu5Gc cannot be synthesized by human cells, but is taken up from dietary sources, and introduced into cell surface sialoglycans of normal cells and especially cancer cells. Antibodies against this non-human sialic can be detected in most individuals and could potentially be used in cancer immunotherapy [97,98]. Future research is needed to explore safety and efficacy of such strategies in preclinical animal tumor models.

Instead of blocking or introducing synthetic sialic acids in cancer cells, also strategies to vaccinate patients against specific tumor sialoglycans are explored [7]. In cancer patients, antibodies against sialoglycans can be detected, suggesting that so called tumor-associated carbohydrate antigens (TACA) can be recognized as *non-self* by the immune system and induce an anti-tumor immune response. Therefore, sialoglycans that are almost exclusively expressed on cancer cells (e.g. fucosyl-GM1, GD1a, GM2, GD2, GM3, GD3, SLe$^{A/X}$, STn, PSA, mucins) are purified or synthesized and used to induce humoral and cellular anti-tumor immune responses in preclinical and in clinical studies [99–103]. The past, present and future of (sialo)carbohydrate-based anticancer vaccines was recently reviewed by others [104,105].

Next to these vaccines, therapeutic antibodies directed against specific tumor sialoglycans can be of high potency in cancer immunotherapy. For example, Lou et al. recently identified the α2,3 sialic acid-carrying sialoglycolipid SSEA-4 (stage-specific embryonic antigen-4) on glioblastoma multifore (GBM) cells as highly specific TACA that is not expressed in normal brain tissue. They showed that administration of a monoclonal antibody against SSEA-4 potently suppresses tumor growth in a GBM mouse model [106]. Overall, results obtained in preclinical animal models and clinical trials are encouraging, stimulating the further development of effective sialoglycan-based anti-cancer vaccines.

5. Concluding remarks

Almost five decades ago, the concept that tumor sialic acids allow immune escape was founded. At that time attempts were made to

translate this concept into the clinic and to induce tumor immune responses using sialic acid-depleted tumor cell vaccines. The absence of mechanistic insight and the lack of clear pre-clinical and clinical results obtained at that time, rebutted the concept that sialic acids facilitate tumor immune evasion. Since that time, a significant progress in the field of glycobiology and immunology was made and recent studies breathe new life into the early concept of tumor sialoglycan-mediated immune escape. So far, tumor sialoglycans were shown to hinder physical interactions with immune receptors and ligands on the tumor cell surface including antigens (i), to disguise them as *self* (ii), to disable major killing mechanisms of the immune system (iii) and to modulate immune cell function locally and systemically by forming the ligands for immune suppressive Siglec receptors (iv). Presumably, additional modes of action of sialic acids exist at the tumor-immune interface. Future research is required to understand the effect of the numerous tumor sialoglycans on immune cell function and to elucidate the role of sialic acid-recognizing lectins on immune cell subsets and in (tumor)immunology. For this purpose, high-throughput methods to decipher the large collection of sialoglycans on tumor cells (the *sialome)* and synthetic carbohydrates that resemble tumor sialoglycans are highly desired [107]. Finally, proper translation of strategies to block sialic acid expression in cancer cells, to incorporate unnatural, antigenic sialic acids into surface glycans or to vaccinate against tumor sialoglycans into the clinics could successfully follow up the "historical" attempts using sialidase-treated tumor cells and pave the way for tumor immunotherapy.

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