Published in final edited form as:

Front Biosci (Elite Ed). ; 4: 631–650.

The immunomodulating roles of glycoproteins in epithelial ovarian cancer

Manish S. Patankar¹, Jennifer A.A. Gubbels², Mildred Felder¹, and Joseph P. Connor¹

¹Department of Obstetrics and Gynecology, University of Wisconsin-Madison, WI-53792-6188

²Department of Biology, Augustana College, Sioux Falls SD

Abstract

The complexity of the immune system demands an intricate defense mechanism by tumors. Ovarian and other tumors employ specific glycoproteins and the associated glycan sequences to modulate immune responses. Glycoproteins enable tumor cells that express or secrete these molecules to evade immune cell attack and induce the immune system to promote tumor growth. This review focuses first on the immune environment in ovarian cancer, and the mechanisms of activation and inhibition that immune cells undergo in order to either attack or ignore a target cell. Next we illustrate the immunomodulatory roles of ovarian cancer-associated glycans and glycoproteins in 1. preventing immune synapse formation, 2. serving as ligands of immune cell receptors, 3. scavenging cytokines and chemokines, and 4. participating in the formation of autoantibodies against the tumor. The importance of these immunomodulating strategies from the view points of understanding the tumor immunology of ovarian tumors, potential origin of such mechanisms, and specific strategies to circumvent the glycoconjugate-mediated suppression of immune responses is discussed in this review.

Keywords

Glycoproteins; T cells; NK cells; Epithelia Ovarian Cancer; MUC16; Glycodelin; MUC1; Mucins; Immune Synapse; Review

2. INTRODUCTION

Ovarian cancer is a highly insidious disease that is usually detected at a very late stage when the possibility of an efficient therapeutic management of the cancer is relatively low (1-3). Therefore, in most women with advanced ovarian cancer the five year survival rate is around 30-55% (4). Specific biomarkers that can detect the cancer at an early stage (when the survival rate after treatment is 80%) are not available (5, 6). In women with advanced disease, the standard of care includes an initial surgical debulking of the tumor followed by an intense chemotherapy with platinum or taxol based compounds. Under these treatment conditions the cancer regresses and the low level of the tumor is monitored by measuring the

serum concentration of the biomarker CA125 (7-10). A steady elevation in serum CA125 levels from this nadir is indicative of recurrent disease (10).

The progression of ovarian cancer requires the cancer cells to first develop on the surface of the ovary or along the walls of the fallopian tubes and then metastasize to other sites within the peritoneum (11-18). Starting with the initiation of the cancer to metastasis, the tumor cells encounter distinct immunologic environments and therefore have to adapt at each site to not only overcome immune recognition but also actively suppress cytotoxic immune responses. Modulation of the immune responses is achieved via various strategies, including downregulation of MHC Class I molecules, expression of soluble MICA, MICB and other ligands of the immune activating receptor NKG2D (19, 20-23), expression of immunosuppressive cytokines, induction of regulatory T cells (24-27), and others. Another important mechanism displayed by ovarian (and other tumors) involves the selective expression of immunomodulating glycoconjugates. In this review, we will discuss the biological properties of well characterized glycoconjugates expressed by ovarian tumors and their effects on immune cells that likely lead to the generation of a diverse array of redundant mechanisms that allow protection of ovarian cancer cells from immune attack. While a major emphasis will be on the discussion of the effects of specific glycoproteins on cellular immunity, we will also briefly discuss auto-antibody responses against glycoproteins and the disease-specific changes in glycosylation occurring on IgGs that may reduce their ability to trigger humoral immune responses via the Fc receptors. An overview of the literature on tumor immune surveillance and the immune environment associated with ovarian tumors is initially provided to set the stage for the discussion of the immunological relationships with the glycoproteins expressed by ovarian tumors.

3. IMMUNE SURVEILLANCE AND IMMUNE EDITING

Early reports of active immune surveillance have now been validated by several investigations and explained by the immunoediting model described by Robert Schreiber and colleagues (28, 29). According to this model, the immune system is continuously encountering and eliminating aberrant tumorigenic cells (immune surveillance) (30-34). While successful elimination occurs in the majority of the cases, in some instances the immune system is unable to completely cytolyse the aberrant lesions. In such cases, a stasis is reached where the immune cells co-exist with the aberrant lesion. It is likely that at this stage the immune system is also undertaking immune surveillance and eliminating some aberrant cells but is unable to completely eliminate the lesion. On the other hand, the aberrant cells that are not being eliminated have likely developed mechanisms that allow them to escape immune recognition. Certain genetic and other molecular events trigger these pre-cancer lesions to break through the equilibrium with the immune cells as they become highly malignant while retaining or adding to the mechanisms of immune evasion and immune suppression that they have already developed during the equilibrium stage (30, 35, 36). The exact nature of these immune evasion or immune suppression strategies are currently under intense investigation and are likely to be cancer-specific. The discussion provided in this review will indicate that specific glycoproteins likely mediate an immunomodulating role during the entire lifecycle of the tumor that enables the cancer cells to escape immune recognition in diverse immune environments.

4. IMMUNE ENVIRONMENT IN OVARIAN CANCER

Ovarian tumors are primarily confined to the peritoneum. Cancer cells sloughing off from the primary tumor metastasize to the diaphragm, omentum and other sites within the peritoneal cavity. Advanced stages of the cancer are associated with significant accumulation of ascites (37, 38). As a part of standard care to relieve discomfort and bloating, this peritoneal exudate is surgically extracted from patients. An analysis of the total cellular component of the ascites indicates approximately 1-5% of the cells are tumor cells and 40-60% are likely of stromal or mesothelial origin (Felder, Belisle and Patankar unpublished observations). The remaining proportion of the cells are predominantly CD45^{pos}, indicating that they are of immune origin. These peritoneal immune cells are continuously exposed to tumor antigens that affect their phenotype and function (24, 39-41). Thus ovarian cancer affords an opportunity to simultaneously analyze immune cells in peripheral circulation that are relatively less affected by the tumor, the peritoneal immune cells that are in direct contact with tumor derived factors, and finally also the immune cells that infiltrate into the ovarian tumor mass (25, 41-45).

The effect of immune response on patient survival has been extensively studied in ovarian cancer. The infiltration of CD4^{pos} cytotoxic T cells is associated with increased survival whereas negative correlation with disease free survival is observed in patients with increased numbers of regulatory T cells (T_{reg}) within the tumor (25, 44, 45). Lymphocytes in the peritoneal fluid of ovarian cancer patients include T cells, B cells, NK cells and monocytes in roughly the same proportion as those found in the peripheral circulation of the patients (42, 46). However, major phenotypic differences, especially in the NK cells, are observed among the peritoneal cells as compared to the peripheral blood derived immune cells (42). A higher percentage (60-70%) of the peritoneal NK cells express low levels of the Fc receptor CD16 and higher levels of CD56, a phenotype strongly associated with low cytolytic function (47-49). Our subsequent discussions will show that such changes in immune cell phenotype and the associated differences in immune function are likely due to a contribution from ovarian cancer associated glycoconjugates.

5. ANTI-TUMOR IMMUNE RESPONSE

Immune reaction against tumors may occur via humoral responses as well as cellular cytotoxicity. In subsequent discussion, we will review data on the generation of autoantibodies, especially against glycoprotein antigens, in ovarian cancer patients. Cellular cytotoxic responses occur via the activation of the adaptive as well as the innate immune system. Typically the cytotoxic T cells (adaptive immune response) and NK cells (innate immune response) are prominent mediators of anti-cancer cytotoxicity. The decision by the T cells and NK cells to kill the target cells or not is made depending on the interaction of the activating or the inhibitory receptors of the immune cells with their corresponding ligands on the tumor targets via the formation of an immune synapse (Figure 1) (50-52). Thus, an increase in expression and recognition of inhibitory ligands on the tumor cells by the immune cells results in inhibition of the cytotoxic immune response. This ultimately leads to survival of the targets. This inhibitory response is essential for the normal regulation of NK and cytotoxic T cells to prevent them from attacking normal self cells. On the other hand,

high expression of activating ligands on the target cells results in the engagement of activating receptors of the immune cells (53). Specific cell signaling events triggered through these interactions between the activating receptors and their ligands result in the lysis of the tumor cells via recruitment of perforin and granzymes to the immune synapse (Figure 2).

One major class of inhibitory immune receptors includes the Killer Immunoglobulin-like Receptors (KIR) (54-58). Prominent examples of the KIR family of receptors expressed on NK cells and T cells include the KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1. Specific allotypes of MHC class I molecules are ligands of the KIRs. KIR2DL1 recognizes HLA-C2, KIR2DL2 and KIR2DL3 recognize HLA-C1, and KIR3DL1 recognize HLA-Bw4. Cancer cells expressing these MHC class I molecules are protected from cytolysis by NK or T cell clones that express the corresponding KIRs.

Other inhibitory immune cell receptors that are important in our discussion of tumor-immune interactions include CD94/NKG2A (recognizes the leader sequence of HLA-E as ligand) (59, 60), LIR-1 (Leukocyte Immunoglobulin-like Receptor-1; recognize HLA-G as ligands) and other LIRs (61, 62), Siglecs (I-type lectins that recognize terminal sialic acids residues) (63-66). Engagement of the inhibitory receptors mediates strong attenuation of the immune response via recruitment of the phosphatases SHP-1, SHP-2 and SHIP (67-70).

Several activating receptors are also expressed by cytotoxic immune cells and their engagement is vitally important for cell mediated cytotoxicity against tumor cells. Fc receptors constitute a potent activating receptor system that binds to antigen bound antibodies and causes a high level of activation of the cytotoxic cellular immune response. Other activating receptors include NKG2D, DNAM-1, NKp30, NKp44, NKp46, and others. From the perspective of ovarian cancer, the NKG2D and DNAM-1 receptor systems are of importance because ovarian tumors express ligands for these two receptors (23, 71-75). It has been proposed that naïve or activated autologous NK cells can mediate cytolysis of ovarian tumors because of the expression of NKG2D and DNAM-1 ligands by ovarian tumors (76). However, it should be noted that the killing of ovarian tumor cells by NK cells is significantly lower than the killing observed with NK cell susceptible targets (Figure 3). This is likely because ovarian tumor cells have developed elaborate mechanisms to circumvent NK cell killing.

6. THE DECISION TO KILL

The expression of the array of inhibitory and activating receptors implies that the decision by the T cells and NK cells to kill their targets is made via a complex series of molecular events (51, 77). This decision is the net result of the interactions of the inhibitory and the activating receptors with their corresponding ligands on the target cells. The strength (and number) of the receptor-ligand interaction appears to be a critical factor in deciding whether the immune cells will be inhibited or activated. Inhibitory KIRs generate strong negative responses. Indeed blocking of the KIR-MHC interactions in ovarian cancer cells (Felder, Gubbels and Patankar, unpublished observations) results in increased cytolysis of ovarian tumor cells by naïve healthy donor derived NK cells. The strong inhibition of the cytotoxic

responses via the KIRs likely ensures a non-response to MHC class I antigen expressing normal self cells.

From the perspective of the tumor, an over reliance of the MHC class I-KIR inhibition may result in presentation of aberrant peptide antigens on its MHC molecules that can be identified by the T cell receptor (TCR) system leading to a strong adaptive response. Thus the tumor cells cannot rely completely on the KIR-MHC recognition for protection from the immune response. Redundant mechanisms must be displayed by the tumor that collectively results in immune protection. It is in this context that we should consider the potential immunomodulatory properties of specific glycan-based strategies displayed by ovarian and other cancers to protect themselves from immune attack.

Another important point to consider is that the tumor is displaying such redundant mechanisms not only to evade the immune response but also to mediate immune suppression and to co-opt the immune cells to produce trophic factors that promote tumor angiogenesis and hence tumor growth. In other words, the effect from the immune system's perspective is akin to the "Stockholm syndrome" experienced by kidnapping victims who through severe psychological manipulation are made to help their kidnapper.

7. GLYCOPROTEINS AND IMMUNE PROTECTION

Glycoconjugates expressed by cancer cells play important roles in promoting metastasis and angiogenesis. For example, binding of the ovarian tumor mucin MUC16 to mesothelin, a binding interaction that likely occurs via the N-glycans of MUC16, allows the cancer cells to attach to themselves to form spheroids and also for the cancer cells to metastasize within the peritoneal cavity (78-81). Heparan sulfate proteoglycans expressed by tumors regulate angiogenesis by serving as co-receptors for VEGF and FGF (82-85). Thus; glycoconjugates play important roles during the progression of ovarian and other cancers. In this review we will discuss the immunomodulatory properties of glycoproteins (especially of the glycoproteins expressed by ovarian cancer cells) that allow the tumor cells to circumvent immune responses throughout the life cycle of the cancer.

While considering the immunomodulatory effects of glycoproteins in ovarian and other tumors, it is important to consider the biology occurring at the surface of tumor cells as well as in the extracellular milieu around the tumor cells and the immune cells. This is because immunomodulation can arise through the glycoproteins that are expressed on the surface as well as those secreted by the tumor cells.

Glycoproteins can modulate immune activity via at least four prominent mechanisms- (i) providing a glycoprotein shield around tumor cells that prevents the immune cells from forming immune synapses; (ii) interaction with immune cell receptors; (iii) serve as scavengers of cytokines or chemokines; and (iv) generation of autoantibodies against aberrantly glycosylated self-glycoproteins. We will now discuss specific examples to illustrate these four operational mechanisms by which glycoproteins are known to modulate immune responses against ovarian tumors.

It is important to mention that another mechanism used by tumor cells to induce apoptosis of T cells is not directly related to expression of glycoproteins but instead via the expression of galectins that can induce T cell death (86). Expression of galectin-1 is associated with aggressiveness of tumor cells (87-89). Galectin-1 and galectin-3 are expressed by ovarian tumors (90-92). Significant data is now available indicating that galectin-1 binding to N-linked and O-linked glycans of CD45 and other molecules expressed on T cells induces immune cell apoptosis (93). Galectin-3 also regulates T cell apoptosis by controlling the aggregation of CD3 and CD8 on T cells (94). The expression of galectin-1 and galectin-3 by ovarian tumors may allow direct modulation of the function and viability of T cells and other types of immune cells thereby allowing the cancer cells to attenuate anti-tumor immune response.

7.1. Glycoprotein shield

Both the T cells and the NK cells require close cellular interactions with the cancer cells (Figure 1 and 2). These interactions are characterized by the polarization of cell adhesion molecules (LFA-1, ICAM-1), immune activating receptors, and cell signaling molecules at the contact sites between the tumor cells and the immune cells (53, 95-98). Such contact sites are referred to as the immune synapse. There are two types of immune synapses- inhibitory and activating. The inhibitory synapses occur through the engagement of inhibitory immune cell receptors with their corresponding ligands on the target cells. Similarly, activating immune synapses are a result of binding of immune activating receptors with their ligands. These inhibitory synapses result in the delivery of perforin and granzyme to the target cell resulting in cytotoxicity (Figure 2).

Since activating immune synapse formation is essential for the lysis of targets, prevention of such interactions leads to immune protection of the cancer cells. There is one specific strategy that ovarian tumors use to reduce the formation of activating immune synapses with NK cells. This strategy involves the expression of high molecular weight mucins. Epithelial ovarian tumors over-express the mucins MUC1, MUC4 and MUC16 (99-106). Mucins are large molecular weight glycoproteins that are heavily glycosylated with both O-linked as well as N-linked oligosaccharides that add to the bulk of the molecule (100, 107). Structural studies have shown that the mucins display a linear extended structure. A glycopeptide consisting of 28 amino acids is estimated to extend up to 7 nm in length (108). Based on this estimate, MUC16 which is composed of ~22,000 amino acids is expected to extend between 1-5 micrometer on the surface of the tumor cells (109).

Because of this extended structure, the normal expression of mucins is known to provide a shield on the surface of epithelial cells. For example, the shielding effect of MUC16 prevents adhesion of *Staphylococcus aureus* to corneal epithelial cells (110, 111). Similarly, expression of MUC1 and MUC4 on endometrial epithelial cells prevents the implantation of the embryo (112-117). Shedding of mucins, an event regulated by estradiol, TGF-beta and other mediators, leads to development of sites where the embryo can attach to the endometrial wall (116). In all of these studies it appears that because of their extended structure, mucins have the ability to introduce steric hindrance by exhibiting anti-adhesive effects that prevent cell-cell interactions.

A similar effect is also observed on the interaction between immune cells and cancer cells. Studies by Carraway and colleagues showed that the presence of MUC4 protected target cells from cytolysis by Lymphokine Activated Killer (LAK) cells (118). Recombinant fragments of MUC4 containing between 1-8 tandem repeats were tested in these experiments. It was demonstrated that the inhibitory effect of MUC4 was directly proportional to the number of tandem repeats within MUC4, as the constructs with 8 tandem repeats provided maximum protection (118). Although such a protective effect for MUC4 on ovarian cancer cells has not been demonstrated, recent data obtained in our laboratory on MUC16 gives credence to the importance of the shielding effect in protecting ovarian tumor cells from NK cell attack (109).

In co-cultures of the MUC16 expressing ovarian tumor cell line OVCAR-3 and human NK cells, it was invariably observed that the immune cells preferably formed conjugates with OVCAR-3 cells that expressed lower levels of the mucin (109). This initial observation led us to investigate the potential shielding effect of MUC16. Indeed, MUC16-knockdown OVCAR-3 cells formed increased numbers of immune synapses with the NK cells as compared to MUC16^{POS} OVCAR-3 subclones. This increase in synapse formation was correlated with increased lysis of the MUC16-knockdown OVCAR-3 cells. The selective lysis of the MUC16-knockdown was not a result of increased expression of activating receptor ligands or conversely due to decreased expression of the ligands of inhibitory receptors on the MUC16-knockdown target cells. Similarly, no major difference was observed in the expression level of ICAM-1 between the MUC16 expressing and the MUC16-knockdown cells. All of these experiments indicated that the expression of MUC16 provided a steric barrier around the ovarian cancer cells resulting in the protection of the cancer cells from NK cell-mediated attack.

7.2. Immunomodulation via interaction of immune receptors with carbohydrate ligands

7.2.1. Ovarian tumor glycome—Glycoproteomic analysis of ovarian cancer cells and serum from ovarian cancer patients has resulted in the identification of aberrant glycosylation patterns of glycoproteins (119-121). Such aberrant glycosylation patterns may serve as diagnostic markers for ovarian malignancy (119-122). While a glycome-based diagnostic test may prove useful in early detection or monitoring of ovarian cancer, it will also be of great importance if the presence of a specific aberrant glycosylation pattern could predict poor survival of patients. To the best of our knowledge, such an analysis has not been conducted, although it would likely suggest that the biology elicited by specific glycans may contribute to the malignant potential of ovarian tumors. Initial analysis has not detected any changes in the expression patterns of Lewis^y and Sialyl Lewis^x and the tumor associated Sialyl Tn and Tn antigen levels on tumors from long term or short term survivors (123). However, in this study a trend of higher expression of Lewis^y on tumors from short term survivors was observed (123). Several studies have indicated the expression of specific oligosaccharide sequences on ovarian tumor cells that have been previously shown to alter anti-tumor immune responses. We will review prominent oligosaccharide ligands expressed on the cell surface as well as secreted glycoproteins expressed by ovarian tumor cells that have been shown to possess the potential to attenuate immune responses.

7.2.2. Tn and Sialyl Tn antigens—The expression of Tn and sialyl Tn antigens have been observed on ovarian tumors and studies have also attempted to determine if monitoring the levels of these carbohydrate antigens in coordination with serum CA125 levels could be used to detect and monitor ovarian cancer progression (123, 124). Tn and sialyl Tn antigens have been detected on the O-linked glycans expressed on the ovarian tumor cell surface and these glycans have also been detected on MUC16 and other specific glycoproteins isolated from ovarian tumor cells (125).

The abundance of the Tn antigen in tumor associated mucins is linked with mutations in the molecular chaperone Cosmc (126-129). The Tn antigen (GalNAc alpha-Ser/Thr) is predominantly modified by the core-beta1-3Galactosyltransferase (T synthase) to the T antigen in normal cells (130). Mutations in Cosmc result in a deficit in the proper functioning of the T synthase, leading to increased expression of the Tn antigen in cancer cells (127, 128). The Tn antigen is further modified by sialylation in tumor cells to the sialyl Tn antigen (NeuAc alpha6-GalNAc alpha-Ser/Thr). Although mutations in Cosmc have been detected in cervical tumors, a correlation has not been made in ovarian cancer (127). From an immunologic standpoint, the expression of Tn and sialyl Tn antigen is an important factor as these antigens possess the ability to inhibit the cytolytic responses of NK cells (131, 132).

The exact mechanism by which the Tn or sialyl Tn antigens inhibit NK cell responses are not clear. However, specific receptors for these antigens on immune cells exist. As we will discuss later, the Tn antigen may serve as a ligand for I-type lectins such as the Siglecs that are known to attenuate immune responses.

On the other hand, the Tn antigen is a ligand of the Macrophage galactose-type lectin (MGL, CD301), a C-type lectin expressed on immature dendritic cells (133, 134). This receptor was first identified on tumoricidal macrophages and exists as one single isoform in humans but as two separate isoforms, MGL-1 and MGL2, in mice (135-137). MGL binds to terminal GalNAc residues such as those presented by the Tn antigen (134). Binding of MGL to its ligands leads to internalization of the ligand-receptor complex (138). The internalized complex is transported to the endosomal compartments that contain MHC class II molecules. Hence, it has been postulated that MGL may play a role in cross presentation of the glycoprotein/glycopeptides ligands to the MHC class II molecules, thereby facilitating downstream adaptive response through the T cells.

MUC1 glycopeptides containing up to 3 tandem repeats of the mucin and engineered to express 9-15 mol of Tn antigen are internalized by dendritic cells through the binding with MGL (139). These glycopeptides are delivered to endosomes containing both MHC class I and Class II molecules. However, these same studies have demonstrated that Tn antigen expressing MUC1 glycoprotein, although internalized by the dendritic cells through MGL was presented to endosomal compartments containing only the MHC class II and not the MHC class I antigens.

The presentation of MUC1 glycopeptides to endosomal compartments containing MHC class I molecules is an important finding from the perspective of developing anti-tumor

vaccine strategies. Dendritic cells loaded with MUC1 peptides or glycopeptides are being investigated as a route to induce anti-tumor immunity (140, 141). Thus the interaction of the glycopeptides with MGL and their subsequent cross-presentation to the MHC class I molecules may indeed serve as an important route to develop novel anti-cancer dendritic vaccine strategies.

While internalization of MGL-ligand complex has been demonstrated in several studies, only recent studies have provided direct evidence for the development of T cell responses against the internalized antigens. GalNAc or MUC1 peptides expressing GalNAc when conjugated with streptavidin were internalized by dendritic cells (142). Such internalization was inhibited in MGL2^{-/-} mice indicating that MGL2 was the primary receptor for these glycoconjugates. Incubation of the GalNAc-Streptavidin or the GalNAc-expressing MUC1-streptavidin complexes with dendritic cells resulted in high proliferation of CD4 T cells and increased ability of these T cells to produce IFN- γ and IL-17 by streptavidin primed CD4 T cells (142). Thus, MGL binding to its glycan ligands can result in presentation of peptide epitopes of glycoproteins on MHC class I molecules.

Presentation of the Tn antigen to MGL expressing dermal dendritic cells also results in internalization of the complex and delivery of the glycans to MHC class II (143). The net result is development of T and B cell responses against the Tn antigen, further suggesting that the use of Tn based vaccination strategies may play an important role in controlling tumor growth (143).

7.2.3. Thomsen-Friedenrich (T or Tf) antigen—The Tn antigen was first described by Dausset et al. in a patient with hemolytic anemia (144). Prior to this observation, another antigen described as the T antigen was also identified by Thomsen and Friedenreich (145). The T antigen (Gal β 1-3GalNAc), also known as the Thomsen-Friedenreich or Tf antigen, is exposed on red blood cells infected with viruses and bacteria or after digestion with neuraminidase (146). Pioneering research conducted by George Springer led to the understanding that the majority of solid tumors express high levels of T antigen on their surface (147, 148). Springer and colleagues further demonstrated that the majority of adults have circulating IgM autoantibodies against T antigen (149). A quotient developed by this group (Q_{Me} ; calculated as the square of the anti-T antigen IgM concentration divided by the concentration of the total IgM times 100) led to the observation of significantly lower levels in patients with breast, colorectal, gastric and many other carcinomas (148, 150). Interestingly, the Q_{Me} provided high specificity to not only differentiate between healthy individuals and cancer patients but also between benign conditions and malignancies (148, 150). The passing of Dr. Springer halted the development of the Q_{Me} quotient as a pan-cancer diagnostic tool for early detection of ovarian and other tumors and a clinical trial involving the repeated administration of a T/Tn antigen vaccine to breast cancer patients. Data available from this trial indicated that 94% (17/18) and 100% (18/18) of the patients with stage III and IV breast cancer, respectively, survived for five years after initiation of treatment (151, 152). This impressive survival advantage led others to develop T and Tn antigen-based vaccination strategies that involved the activation of the immune response by the Keyhole Limpet Hemocyanin and other adjuvants or by using synthetic constructs of T and Tn antigens (153, 154). Clinical trials are currently underway to determine the efficacy

of these T and Tn antigen based vaccination approaches. The inability of carbohydrate antigens to produce T cell responses is likely the cause of the lack of a robust clinical response in these T/Tn antigen based vaccination approaches (155).

7.2.4. LacDiNAc glycans—Glycomic analysis of the ovarian cancer cell line SKOV-3 has indicated the expression of LacdiNAc (GalNAc β 1-4GlcNAc-) sequence on surface glycoproteins (156). Recombinant erythropoietin expressed by SKOV-3 cells also expresses the LacdiNAc sequence. The very first reports of LacdiNAc expression was on the surface of helminthic parasites (157, 158). Glycomic profiling of the pregnancy associated human glycoprotein, glycodelin-A, showed for the first time the presence of LacdiNAc terminated N-linked oligosaccharides on this glycoprotein (159). Early studies had shown that glycodelin-A (also referred to previously as PP14 and PAEP (159-161)) was a potent inhibitor of human NK cell mediated cytotoxic responses against the erythroleukemia cell line K562 (162). Although the exact mechanism by which glycodelin inhibits NK cell responses has not been elucidated, the LacdiNAc sequences are known to be ligands of galectin-3, a galactose binding lectin that is expressed on regulatory T cells and is known to induce apoptosis of T cells, and regulate T cell receptor-mediated activation of T cells (163-167).

7.2.5. Bisecting-type N-linked glycans—The enzyme N-acetylglucosaminyltransferase III, encoded by the Mgat3 gene, is responsible for the addition of the bisecting β 1-4-linked GlcNAc residues to complex type N-glycans (168). Deletion of the Mgat3 gene is associated with reduced development of mammary, liver, and other tumors (169, 170). Mass spectrometric analysis of MUC16 indicated abundant expression of the bisecting glycans on this mucin (125). Two crucial studies had previously implicated the bisecting type N-linked oligosaccharides in protecting tumor cells from NK cell-mediated lysis (171, 172). These initial reports originally inspired us to investigate the immunosuppressive activities of MUC16 (173). However, while the bisecting glycans are expressed not only on cancer associated glycoproteins but also on glycoproteins expressed by HIV (174, 175) and other pathogens, a clear immune receptor through which these oligosaccharide chains may develop an immunosuppressive response has not emerged.

7.2.6. Sialylated N-linked or O-linked glycans—Sialylation of glycolipids and glycoproteins affects the recognition of tumor cells by immune cells (176-181). It is generally accepted that a decrease in sialylation of cell surface glycans results in increased recognition of the cancer cells by immune cells. The data is particularly striking for NK cell recognition of tumor cells. The ovarian tumor mucin MUC16 is a potent inhibitor of NK cell mediated cytolytic response (173). We have recently demonstrated that MUC16 is a high affinity ligand of the NK cell receptor Siglec-9 (182). An I-type lectin, Siglec-9 specifically recognizes α 2-3-linked sialic acid residues (183, 184) that are abundant on MUC16 (125). Siglec-9 is primarily expressed on the surface of innate immune cells (182). High levels of Siglec-9 are expressed on neutrophils and monocytes whereas intermediate levels of this receptor are present on approximately 30-40% of CD16^{pos}/CD56^{dim} NK cells and approximately 5-10% of CD19^{pos} B cells (182, 185). Low levels of Siglec-9 are present on 1-5% of CD3^{pos} T cells (186).

In our studies on understanding the mechanism by which MUC16 inhibits NK cell cytolytic responses, we observed that NK cells and monocytes from the peripheral blood and peritoneal fluid of ovarian cancer patients were positive for this mucin (42, 182). RT-PCR indicated that the immune cells from the patients were not expressing endogenous MUC16. Instead, it was demonstrated that immune cells were binding to MUC16 released by ovarian tumor cells (42). Additional experiments led us to conclude that MUC16 was binding via its terminal sialic acid residues to Siglec-9 on the immune cells (182).

Siglec-9 is an inhibitory immune cell receptor. Engagement of Siglec-9 results in attenuation of T cell and NK cell cytolytic responses and data originating from monocytic cell lines suggests that signaling through this I-type lectin may induce monocytes to express the immunosuppressive cytokine IL-10 (186-188). The interaction between MUC16 and Siglec-9 likely leads to a reduced ability of NK cells to react against ovarian tumor targets. Based on the shielding effect of MUC16 and the interactions of the mucin with Siglec-9^{pos} NK cells, we are proposing a model by which ovarian tumor cells are protected from immune attack (Figure 4) (109, 182). According to this model, MUC16 shed from the surface of the ovarian tumor cells binds to NK cells via Siglec-9. This receptor-ligand interaction occurs locally in the tumor microenvironment as well as systemically, as evidenced by the binding of the mucin in the peritoneal environment as well as in systemic circulation (42). The interaction between Siglec-9 and MUC16 provides an opportunity for the mucin to mediate suppression of NK cell cytolytic activity even before the NK cells engage the cancer cells. Siglec-9 engagement causes phosphorylation of tyrosine residue in the distal ITIM domain of this receptor followed by recruitment and activation of the phosphatases, SHP-1 and SHP-2 (187). As a result, the phosphorylation events (phosphorylation of Vav-1, WASp, and other proteins) necessary for the formation of an NK cell activating synapse are inhibited and hence the NK cells are unable to lyse tumor targets (96, 98, 189). Therefore, the selective expression of Siglec-9 on the CD16^{pos}/CD56^{dim} NK cells likely leads to protection of the ovarian cancer cells from the cytolytic responses of a major innate immune cell subset (Figure 4).

Our data indicates that the ovarian tumor cells also utilize the molecular properties of MUC16 to protect themselves from the cytolytic responses of Siglec-9^{neg} CD16^{pos}/CD56^{dim} cytolytic NK cells. This is achieved via the shielding effect of MUC16 that hinders the ability of the Siglec-9^{neg} NK cells to form activating immune synapses with the ovarian cancer cells.

There are two additional prominent examples of the immunoprotective effect afforded by sialylated glycans. The first example we will consider is glycodefin since this glycoprotein is also expressed by ovarian tumors (190). Glycodefin expression has been observed in immunohistological specimens of ovarian tumors as well as in the peritoneal fluid of cancer patients. In addition to being expressed by ovarian tumors, the major research on this molecule has been focused on its role in protecting the human embryo/fetus from the maternal immune responses. In this context, several reports have demonstrated the ability of glycodefin in regulating the immunologic function of dendritic cells, NK cells, and CD4^{pos} and CD8^{pos} T cells (162, 191-196). As mentioned previously, glycomic analysis of glycodefin from amniotic fluid revealed the presence of LacdiNAc glycans (159). However,

the isoform of glycodelin expressed in human seminal fluid showed predominant expression of high mannose type and fucosylated N-glycans and a total absence of the LacdiNAc type oligosaccharides (197). Subsequent analysis of glycodelin isolated from other tissues and fluids revealed differences in the glycan expression profile of the different isoforms and no reported changes in the primary amino acid sequence of this glycoprotein (198). Therefore, the glycodelin isoforms have provided an excellent opportunity to reveal the biologic activities of oligosaccharides from the different tissue-specific isoforms of the molecule. It is now confirmed that glycodelin expressed in the amniotic fluid (glycodelin-A, Gd-A) and follicular fluid (Gd-F) modulate immune responses whereas glycodeilins present in the seminal fluid (Gd-S) and the cumulus matrix (Gd-C) that surrounds the human oocyte are not immunomodulatory (198). These differences in the activities of the glycodeilin isoforms likely arise from their different glycosylation patterns. Glycodelin isolated from the peritoneal fluid of ovarian cancer patients decreases dendritic cell maturation and induces increased expression of the immunosuppressive cytokine IL-10 (191). These results suggest that the ovarian cancer associated glycodeilin likely possesses the appropriate glycosylation pattern that facilitates the immunomodulatory properties of this glycodeilin.

Glycomic analysis of the Gd-A, Gd-F, Gd-S, and Gd-C isoforms indicated major differences in the glycosylation patterns of the glycodeilins (198). Immunologic assays showed that only the Gd-A and Gd-F isoforms and not Gd-C or Gd-S inhibited Jurkat T cell proliferation, induced apoptosis in the cells, and impaired their ability to respond to IL-2 stimulation. Enzymatic desialylation of Gd-A reversed its suppressive effects on the Jurkat T cells indicating that the sialylation of glycoprotein may serve as an important factor in modulating immune responses against ovarian tumors (198).

Another line of evidence indicating the importance of sialylation in tumor immunology is a recent study in the methylcholanthrene induced fibrosarcoma model (199, 200). It was observed that methylcholanthrene induced fibrosarcomas grown in immunocompetent mice expressed high levels of sialic acid on their cell surface. However, the same type of tumors grown in IL-1 α ^{-/-} and IFN- γ ^{-/-} immunocompromised mice expressed lower levels of sialylation (199). Extensive analysis in this model resulted in the understanding that the immune cells were selectively lysing fibrosarcoma cells that were expressing low levels of sialic acids while those expressing high levels of sialic acid were being selectively excluded from immune cell attack. Selective elimination of the tumors expressing low levels of sialic acid is mediated by NK cells and is associated with increase immune synapse formation and increased unmasking of the ligands for the NK cell activating receptor NKG2D. Thus degree of sialylation of the tumor cells was serving as an important factor that contributed to immunoediting in this model (199).

In this context it is also important to mention that in our experiments we have found that ovarian tumor cells surviving an initial NK cell challenge in in vitro experiments express higher levels of MUC16 (109). These examples suggest that a consequence of the immunomodulatory properties of glycoconjugates may be their direct contribution to the development of highly immune resistant tumors via the process of immunoediting.

7.3. Cytokine binding to glycoprotein

Cytokines are important mediators of the immune response. Their activities can be either stimulatory or inhibitory and are mediated via specific receptors expressed on defined subsets of immune cells. Typically, cytokines attach to their receptor via specific peptide epitopes. However, it has been proposed that in addition to their peptide binding epitopes, cytokines also possess novel carbohydrate recognition domains that allow them to recognize a variety of oligosaccharide ligands (201, 202). IL-1alpha IL-1beta, IL-2, IL-3, IL-4, IL-6, IL-7, and other cytokines have been shown to possess carbohydrate recognition domains that allow them to bind to specific oligosaccharides (201, 203-210). Cytokines bind to specific glycan sequences expressed on glycoproteins such as ribonuclease B, Tamm Horsfall glycoprotein and uromodulin (211-215). To the best of our knowledge, specific instances of ovarian cancer associated glycoproteins interacting with cytokines via their glycan chains have not been reported. However, mucins such as MUC1 and MUC16 have been postulated to modulate immune responses by scavenging cytokines and thereby hindering their ability to interact with the cytokine receptors on the immune cells (216). It is worth noting that IL-2 was shown to bind to high mannose type oligosaccharide ligands with binding constants in the micromolar range (212). However, a recent exhaustive study of IL-2 that used ELISA, chromatography, equilibrium dialysis, and NMR spectroscopic methods did not show any significant binding interaction between IL-2 and the high mannose chains (217). Thus the subject of cytokine-glycan interactions should be approached with caution.

7.4. Aberrant glycosylation and the autoantibody response

Cancer associated mucins are aberrantly glycosylated, as evidenced by the abundant expression of Tn and sialyl Tn antigens. In addition, mucins expressed by cancer cells are also not fully glycosylated leading to existence of peptide epitopes that are devoid of glycans (100, 103, 216, 218). The expression of such aberrant glycans and non-glycosylated peptide epitopes results in their detection by the immune system and the subsequent generation of an autoantibody response against these antigens. In the case of ovarian cancer, autoantibodies have been detected against MUC1 and MUC16 (219-226). The generation of humoral responses against the cancer associated antigens may imply another mechanism of active immune surveillance. A careful characterization of the aberrantly glycosylated epitopes of glycoproteins that generate a more robust autoantibody response will result in development of novel immunologic strategies to combat ovarian and other tumors. The use of dendritic cells loaded with MUC1 glycopeptides is another approach that may likely lead to better anti-tumor vaccine strategies (139, 140, 226, 227).

In the case of cancer patients, aberrant glycosylation is detected not only in the glycoproteins that are expressed by the tumors but also by proteins expressed by healthy tissues. A classic example is the development of antibodies in cancer patients with glycosylation profiles that are diverse from those found in antibodies developing in non-cancer patients. IgG fractions of ovarian cancer patients carry a higher percentage of the agalactosylated and core fucosylated N-linked oligosaccharide chains (228). Another group has demonstrated an approximately fourfold increase in the binding of the total IgG fraction to immobilized ConA as compared to IgG derived from the serum of healthy controls (38% versus 9%) (229). Greater than 87% of the IgGs that were reactive against ovarian cancer

antigens bound to immobilized Con A. Such changes in glycosylation are observed not only on the antibodies but also on other serum glycoproteins including haptoglobin, alpha1-acid glycoprotein, and alpha1-antichymotrypsin (228, 230). It has been proposed that identification of the glycosylation alterations in ovarian cancer may be used to develop novel diagnostic tests for the detection and monitoring of this disease (230).

8. CONCLUSIONS

Glycoconjugates display redundant mechanisms for immunomodulation. The plasticity of the glycosylation process leads to development of glycoproteins and glycolipids that express a diverse array of glycans in healthy individuals versus cancer patients. The tumors likely utilize this plasticity to develop mechanisms to modulate immune responses that allow them to survive during the different stages of the development of cancer. Thus, the glycoproteins may allow them to withstand immune surveillance, contribute towards development of immune resistant tumors (as predicted by the immunoediting hypothesis), and in the advanced stages of tumorigenesis, may alter immune cell function and phenotype thus hijacking an important tumor control mechanism. Several different mechanisms of immunomodulation are displayed by ovarian tumors, indicating a very diverse and redundant strategy. The silver lining is that while such strategies may allow the cancer cells to overcome native immune responses, these mechanisms may not always be able to overpower immune responses induced via the application of immunologic anti-cancer agents. Thus, the administration of antibodies, cytokines and other strategies lead to development of strong immune responses against tumor cells and in some cases have also been associated with efficient cancer control. Indeed, in our own in vitro experiments we have demonstrated that the immune shielding provided by MUC16 to ovarian cancer cells can be surpassed with administration of immunotherapeutic agents that lead to antibody mediated tumor cell death (231).

9. PERSPECTIVE

Several of the examples of glycoproteins discussed in this review are expressed not only by ovarian tumors but are also temporally expressed in the human endometrium and/or the human decidua where protection of the embryo/fetus from maternal immune responses is paramount. The role of glycoconjugates in suppressing the maternal immune responses has previously been elaborated (175, 232-234). We propose that ovarian, and quite possibly other tumors, are co-opting these already existing glycoprotein based strategies that are central to human reproduction, to effectively neutralize the immune system. It is important that we recognize the glycobiological connections between pregnancy and cancer to fully understand the immune system, a concept that was introduced several decades ago with the identification of onco-fetal antigens.

ACKNOWLEDGEMENTS

Support for this study was provided by the National Institutes of Health (1R21CA143616-01 and T32 fellowship to JAAG), the Wisconsin Alumni Research Foundation, UW School of Medicine and Public Health Wisconsin Partnership Program, Research and Development funds from the Department of Obstetrics and Gynecology and a charitable donation from Jean McKenzie. Support provided to JAAG through the NIH Grant Number 2 P20

RR016479 from the INBRE Program of the National Center for Research Resources is also acknowledged. We thank Drs. Arvinder Kapur and Mian Shahzad for critical reading of this manuscript.

REFERENCES

- Gubbels JA, Claussen N, Kapur AK, Connor JP, Patankar MS. The detection, treatment, and biology of epithelial ovarian cancer. *J Ovarian Res.* 2010; 3:8. [PubMed: 20350313]
- Permuth-Wey J, Sellers TA. Epidemiology of ovarian cancer. *Methods Mol Biol.* 2009; 472:413–37. [PubMed: 19107446]
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin.* 2009; 59(4):225–49. [PubMed: 19474385]
- Randall L, Berman M. Management of Adnexal Masses. ACOG Practice Bulletin- Clinical Management Guidelines for Obstetrician-Gynecologists. 2007
- Zagouri F, Dimopoulos MA, Bournakis E, Papadimitriou CA. Molecular markers in epithelial ovarian cancer: their role in prognosis and therapy. *Eur J Gynaecol Oncol.* 31(3):268–77. [PubMed: 21077467]
- Hoskins, W.; Perez, CA. *Epithelial Ovarian Cancer.* Lippincott Williams & Wilkins; Philadelphia: 2005.
- Bast RC Jr. Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest.* 1981; 68(5):1331–7. [PubMed: 7028788]
- Bast RC Jr. Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, Berkowitz RS, Leavitt T, Griffiths CT, Parker L, Zurawski VR Jr. Knapp RC. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med.* 1983; 309(15):883–7. [PubMed: 6310399]
- Bast RC Jr. Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. *Int J Biol Markers.* 1998; 13(4):179–87. [PubMed: 10228898]
- Rustin GJ. Use of CA-125 to assess response to new agents in ovarian cancer trials. *J Clin Oncol.* 2003; 21(10 Suppl):187s–193s. [PubMed: 12743133]
- Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev.* 2001; 22(2):255–88. [PubMed: 11294827]
- Ahmed N, Thompson EW, Quinn MA. Epithelialmesenchymal interconversions in normal ovarian surface epithelium and ovarian carcinomas: an exception to the norm. *J Cell Physiol.* 2007; 213(3): 581–8. [PubMed: 17708542]
- Dubeau L. The cell of origin of ovarian epithelial tumors and the ovarian surface epithelium dogma: does the emperor have no clothes? *Gynecol Oncol.* 1999; 72(3):437–42. [PubMed: 10053122]
- Dubeau L. The cell of origin of ovarian epithelial tumours. *Lancet Oncol.* 2008; 9(12):1191–7. [PubMed: 19038766]
- Jarboe E, Folkins A, Nucci MR, Kindelberger D, Drapkin R, Miron A, Lee Y, Crum CP. Serous carcinogenesis in the fallopian tube: a descriptive classification. *Int J Gynecol Pathol.* 2008; 27(1): 1–9. [PubMed: 18156967]
- Callahan MJ, Crum CP, Medeiros F, Kindelberger DW, Elvin JA, Garber JE, Feltmate CM, Berkowitz RS, Muto MG. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. *J Clin Oncol.* 2007; 25(25):3985–90. [PubMed: 17761984]
- Crum CP, Drapkin R, Miron A, Ince TA, Muto M, Kindelberger DW, Lee Y. The distal fallopian tube: a new model for pelvic serous carcinogenesis. *Curr Opin Obstet Gynecol.* 2007; 19(1):3–9. [PubMed: 17218844]
- Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol.* 2007; 211(1):26–35. [PubMed: 17117391]
- Garcia-Lora A, Algarra I, Garrido F. MHC class I antigens, immune surveillance, and tumor immune escape. *J Cell Physiol.* 2003; 195(3):346–55. [PubMed: 12704644]

20. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*. 1999; 285(5428):727–9. [PubMed: 10426993]
21. Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, Kimura R, Miyagi T, Mochizuki K, Sasaki Y, Hayashi N. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. *Int J Cancer*. 2003; 104(3):354–61. [PubMed: 12569559]
22. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature*. 2002; 419(6908):734–8. [PubMed: 12384702]
23. Li K, Mandai M, Hamanishi J, Matsumura N, Suzuki A, Yagi H, Yamaguchi K, Baba T, Fujii S, Konishi I. Clinical significance of the NKG2D ligands, MICA/B and ULBP2 in ovarian cancer: high expression of ULBP2 is an indicator of poor prognosis. *Cancer Immunol Immunother*. 2009; 58(5):641–52. [PubMed: 18791713]
24. Giuntoli RL 2nd, Webb TJ, Zoso A, Rogers O, Diaz-Montes TP, Bristow RE, Oelke M. Ovarian cancer-associated ascites demonstrates altered immune environment: implications for antitumor immunity. *Anticancer Res*. 2009; 29(8):2875–84. [PubMed: 19661290]
25. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ, Odunsi K. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A*. 2005; 102(51):18538–43. [PubMed: 16344461]
26. Barnett B, Kryczek I, Cheng P, Zou W, Curiel TJ. Regulatory T cells in ovarian cancer: biology and therapeutic potential. *Am J Reprod Immunol*. 2005; 54(6):369–77. [PubMed: 16305662]
27. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*. 2004; 10(9):942–9. [PubMed: 15322536]
28. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res*. 1970; 13:1–27. [PubMed: 4921480]
29. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol*. 2002; 3(11):991–8. [PubMed: 12407406]
30. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol*. 2006; 90:1–50. [PubMed: 16730260]
31. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, Diamond MS, Koebel CM, Arthur C, White JM, Schreiber RD. A critical function for type I interferons in cancer immunoediting. *Nat Immunol*. 2005; 6(7):722–9. [PubMed: 15951814]
32. Dunn GP, Ikeda H, Bruce AT, Koebel C, Uppaluri R, Bui J, Chan R, Diamond M, White JM, Sheehan KC, Schreiber RD. Interferon-gamma and cancer immunoediting. *Immunol Res*. 2005; 32(1-3):231–45. [PubMed: 16106075]
33. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol*. 2004; 22:329–60. [PubMed: 15032581]
34. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 2004; 21(2):137–48. [PubMed: 15308095]
35. Teng MW, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: an equilibrium with cancer. *J Leukoc Biol*. 2008; 84(4):988–93. [PubMed: 18515327]
36. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, Smyth MJ, Schreiber RD. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature*. 2007; 450(7171):903–7. [PubMed: 18026089]
37. Feldman GB, Knapp RC, Order SE, Hellman S. The role of lymphatic obstruction in the formation of ascites in a murine ovarian carcinoma. *Cancer Res*. 1972; 32(8):1663–6. [PubMed: 5044130]
38. Shen-Gunther J, Mannel RS. Ascites as a predictor of ovarian malignancy. *Gynecol Oncol*. 2002; 87(1):77–83. [PubMed: 12468346]

39. Mantovani A, Allavena P, Sessa C, Bolis G, Mangioni C. Natural killer activity of lymphoid cells isolated from human ascitic ovarian tumors. *Int J Cancer*. 1980; 25(5):573–82. [PubMed: 6154662]
40. Haskill S, Koren H, Becker S, Fowler W, Walton L. Mononuclear-cell infiltration in ovarian cancer. II. Immune function of tumour and ascites-derived inflammatory cells. *Br J Cancer*. 1982; 45(5):737–46. [PubMed: 6177328]
41. Melichar B, Freedman RS. Immunology of the peritoneal cavity: relevance for host-tumor relation. *Int J Gynecol Cancer*. 2002; 12(1):3–17. [PubMed: 11860531]
42. Belisle JA, Gubbels JA, Raphael CA, Migneault M, Rancourt C, Connor JP, Patankar MS. Peritoneal natural killer cells from epithelial ovarian cancer patients show an altered phenotype and bind to the tumour marker MUC16 (CA125). *Immunology*. 2007; 122(3):418–429. [PubMed: 17617155]
43. Lai P, Rabinowich H, Crowley-Nowick PA, Bell MC, Mantovani G, Whiteside TL. Alterations in expression and function of signal-transducing proteins in tumor-associated T and natural killer cells in patients with ovarian carcinoma. *Clin Cancer Res*. 1996; 2(1):161–73. [PubMed: 9816103]
44. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med*. 2003; 348(3):203–13. [PubMed: 12529460]
45. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, Rubin SC, Kaiser LR, June CH. Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res*. 2001; 61(12):4766–72. [PubMed: 11406550]
46. Santin AD, Hermonat PL, Ravaggi A, Bellone S, Roman JJ, Smith CV, Pecorelli S, Radominska-Pandya A, Cannon MJ, Parham GP. Phenotypic and functional analysis of tumor-infiltrating lymphocytes compared with tumor-associated lymphocytes from ascitic fluid and peripheral blood lymphocytes in patients with advanced ovarian cancer. *Gynecol Obstet Invest*. 2001; 51(4):254–61. [PubMed: 11408737]
47. Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. *J Immunol*. 1989; 143(10):3183–91. [PubMed: 2530273]
48. Carson WE, Fehniger TA, Caligiuri MA. CD56bright natural killer cell subsets: characterization of distinct functional responses to interleukin-2 and the c-kit ligand. *Eur J Immunol*. 1997; 27(2):354–60. [PubMed: 9045904]
49. Freud AG, Caligiuri MA. Human natural killer cell development. *Immunol Rev*. 2006; 214:56–72. [PubMed: 17100876]
50. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol*. 2008; 9(5):495–502. [PubMed: 18425106]
51. Lanier LL. NK cell recognition. *Annu Rev Immunol*. 2005; 23:225–74. [PubMed: 15771571]
52. Wu J, Lanier LL. Natural killer cells and cancer. *Adv Cancer Res*. 2003; 90:127–56. [PubMed: 14710949]
53. Orange JS, Harris KE, Andzelm MM, Valter MM, Geha RS, Strominger JL. The mature activating natural killer cell immunologic synapse is formed in distinct stages. *Proc Natl Acad Sci U S A*. 2003; 100(24):14151–6. [PubMed: 14612578]
54. Olcese L, Lang P, Vely F, Cambiaggi A, Marguet D, Blery M, Hippen KL, Biassoni R, Moretta A, Moretta L, Cambier JC, Vivier E. Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases. *J Immunol*. 1996; 156(12):4531–4. [PubMed: 8648092]
55. Fry AM, Lanier LL, Weiss A. Phosphotyrosines in the killer cell inhibitory receptor motif of NKB1 are required for negative signaling and for association with protein tyrosine phosphatase 1C. *J Exp Med*. 1996; 184(1):295–300. [PubMed: 8691146]
56. Valiante NM, Phillips JH, Lanier LL, Parham P. Killer cell inhibitory receptor recognition of human leukocyte antigen (HLA) class I blocks formation of a pp36/PLC-gamma signaling complex in human natural killer (NK) cells. *J Exp Med*. 1996; 184(6):2243–50. [PubMed: 8976179]
57. Valiante NM, Lienert K, Shilling HG, Smits BJ, Parham P. Killer cell receptors: keeping pace with MHC class I evolution. *Immunol Rev*. 1997; 155:155–64. [PubMed: 9059891]

58. Lanier LL, Corliss B, Phillips JH. Arousal and inhibition of human NK cells. *Immunol Rev.* 1997; 155:145–54. [PubMed: 9059890]
59. Sivori S, Vitale M, Sanseverino L, Parolini S, Barbaresi M, Bottino C, Moretta A. Inhibitory CD94 molecules identified by the Z199 monoclonal antibody recognize different HLA-class I molecules. *Transplant Proc.* 1996; 28(6):3199–203. [PubMed: 8962240]
60. Braud VN, Allan DS, O'Callaghan CA, Soderstrom K, Andrea AD, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ. HLA-E binds to natural killer cell receptors CD94/NKG2A, B, and C. *Nature.* 1998; 391:795–799. [PubMed: 9486650]
61. Shiroishi M, Kuroki K, Ose T, Rasubala L, Shiratori I, Arase H, Tsumoto K, Kumagai I, Kohda D, Maenaka K. Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. *J Biol Chem.* 2006; 281(15):10439–47. [PubMed: 16455647]
62. Gonen-Gross T, Achdout H, Gazit R, Hanna J, Mizrahi S, Markel G, Goldman-Wohl D, Yagel S, Horejsi V, Levy O, Baniyash M, Mandelboim O. Complexes of HLA-G protein on the cell surface are important for leukocyte Ig-like receptor-1 function. *J Immunol.* 2003; 171(3):1343–51. [PubMed: 12874224]
63. Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, Kelm S, Le Douarin N, Powell L, Roder J, Schnaar RL, Sgroi DC, Stamenkovic K, Schauer R, Schachner M, van den Berg TK, van der Merwe PA, Watt SM, Varki A. Siglecs: a family of sialic-acid binding lectins [letter]. *Glycobiology.* 1998; 8(2):v. [PubMed: 9498912]
64. Crocker PR, Varki A. Siglecs in the immune system. *Immunology.* 2001; 103(2):137–45. [PubMed: 11412300]
65. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol.* 2007; 7(4):255–66. [PubMed: 17380156]
66. Varki A, Angata T. Siglecs—the major subfamily of I-type lectins. *Glycobiology.* 2006; 16(1):1R–27R. [PubMed: 16118287]
67. Binstadt BA, Brumbaugh KM, Dick CJ, Scharenberg AM, Williams BL, Colonna M, Lanier LL, Kinet JP, Abraham RT, Leibson PJ. Sequential involvement of Lck and SHP-1 with MHC-recognizing receptors on NK cells inhibits FcR-initiated tyrosine kinase activation. *Immunity.* 1996; 5(6):629–38. [PubMed: 8986721]
68. Yusa S, Campbell KS. Src Homology Region 2-Containing Protein Tyrosine Phosphatase-2 (SHP-2) Can Play a Direct Role in the Inhibitory Function of Killer Cell Ig-Like Receptors in Human NK Cells. *J Immunol.* 2003; 170(9):4539–47. [PubMed: 12707331]
69. Wu C, Sun M, Liu L, Zhou GW. The function of the protein tyrosine phosphatase SHP-1 in cancer. *Gene.* 2003; 306:1–12. [PubMed: 12657462]
70. Vyas YM, Maniar H, Lyddane CE, Sadelain M, Dupont B. Ligand binding to inhibitory killer cell Ig-like receptors induce colocalization with Src homology domain 2-containing protein tyrosine phosphatase 1 and interruption of ongoing activation signals. *J Immunol.* 2004; 173(3):1571–8. [PubMed: 15265884]
71. Conejo-Garcia JR, Benencia F, Courreges MC, Gimotty PA, Khang E, Buckanovich RJ, Frauwirth KA, Zhang L, Katsaros D, Thompson CB, Levine B, Coukos G. Ovarian carcinoma expresses the NKG2D ligand *Letal* and promotes the survival and expansion of CD28-antitumor T cells. *Cancer Res.* 2004; 64(6):2175–82. [PubMed: 15026360]
72. Conejo-Garcia JR, Benencia F, Courreges MC, Khang E, Zhang L, Mohamed-Hadley A, Vinocur JM, Buckanovich RJ, Thompson CB, Levine B, Coukos G. *Letal*, A tumor-associated NKG2D immunoreceptor ligand, induces activation and expansion of effector immune cells. *Cancer Biol Ther.* 2003; 2(4):446–51. [PubMed: 14508119]
73. Carlsten M, Bjorkstrom NK, Norell H, Bryceson Y, van Hall T, Baumann BC, Hanson M, Schedvins K, Kiessling R, Ljunggren HG, Malmberg KJ. DNAX accessory molecule-1 mediated recognition of freshly isolated ovarian carcinoma by resting natural killer cells. *Cancer Res.* 2007; 67(3):1317–25. [PubMed: 17283169]
74. Barber A, Zhang T, Sentman CL. Immunotherapy with chimeric NKG2D receptors leads to long-term tumor-free survival and development of host antitumor immunity in murine ovarian cancer. *J Immunol.* 2008; 180(1):72–8. [PubMed: 18097006]

75. Barber A, Zhang T, DeMars LR, Conejo-Garcia J, Roby KF, Sentman CL. Chimeric NKG2D receptor-bearing T cells as immunotherapy for ovarian cancer. *Cancer Res.* 2007; 67(10):5003–8. [PubMed: 17510432]
76. Carlsten M, Malmberg KJ, Ljunggren HG. Natural killer cell-mediated lysis of freshly isolated human tumor cells. *Int J Cancer.* 2009; 124(4):757–62. [PubMed: 19035446]
77. Lanier LL. Turning on natural killer cells. *J Exp Med.* 2000; 191(8):1259–62. [PubMed: 10770793]
78. Bergan L, Gross JA, Nevin B, Urban N, Scholler N. Development and in vitro validation of anti-mesothelin biobodies that prevent CA125/Mesothelin-dependent cell attachment. *Cancer Lett.* 2007; 255(2):263–74. [PubMed: 17560019]
79. Gubbels JA, Belisle J, Onda M, Rancourt C, Migneault M, Ho M, Bera TK, Connor J, Sathyanarayana BK, Lee B, Pastan I, Patankar MS. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. *Mol Cancer.* 2006; 5(1):50. [PubMed: 17067392]
80. Scholler N, Garvik B, Hayden-Ledbetter M, Kline T, Urban N. Development of a CA125-mesothelin cell adhesion assay as a screening tool for biologics discovery. *Cancer Lett.* 2006; 247(1):130–136. [PubMed: 16677756]
81. Rump A, Morikawa Y, Tanaka M, Minami S, Umesaki N, Takeuchi M, Miyajima A. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J Biol Chem.* 2004; 279(10):9190–8. [PubMed: 14676194]
82. Raman K, Ninomiya M, Nguyen TK, Tsuzuki Y, Koketsu M, Kuberan B. Novel glycosaminoglycan biosynthetic inhibitors affect tumor-associated angiogenesis. *Biochem Biophys Res Commun.* 404(1):86–9. [PubMed: 21094131]
83. Raman K, Kuberan B. Chemical Tumor Biology of Heparan Sulfate Proteoglycans. *Curr Chem Biol.* 4(1):20–31. [PubMed: 20596243]
84. Folkman J, Klagsbrun M, Sasse J, Wadzinski M, Ingber D, Vlodavsky I. A heparin-binding angiogenic protein--basic fibroblast growth factor--is stored within basement membrane. *Am J Pathol.* 1988; 130(2):393–400. [PubMed: 3277442]
85. Klagsbrun M. Mediators of angiogenesis: the biological significance of basic fibroblast growth factor (bFGF)-heparin and heparan sulfate interactions. *Semin Cancer Biol.* 1992; 3(2):81–7. [PubMed: 1378313]
86. Kovacs-Solyom F, Blasko A, Fajka-Boja R, Katona RL, Vegh L, Novak J, Szebeni GJ, Krenacs L, Uher F, Tubak V, Kiss R, Monostori E. Mechanism of tumor cell-induced T-cell apoptosis mediated by galectin-1. *Immunol Lett.* 127(2):108–18. [PubMed: 19874850]
87. Danguy A, Camby I, Kiss R. Galectins and cancer. *Biochim Biophys Acta.* 2002; 1572(2-3):285–93. [PubMed: 12223276]
88. Camby I, Decaestecker C, Lefranc F, Kaltner H, Gabius HJ, Kiss R. Galectin-1 knocking down in human U87 glioblastoma cells alters their gene expression pattern. *Biochem Biophys Res Commun.* 2005; 335(1):27–35. [PubMed: 16051185]
89. Le QT, Shi G, Cao H, Nelson DW, Wang Y, Chen EY, Zhao S, Kong C, Richardson D, O'Byrne KJ, Giaccia AJ, Koong AC. Galectin-1: a link between tumor hypoxia and tumor immune privilege. *J Clin Oncol.* 2005; 23(35):8932–41. [PubMed: 16219933]
90. van den Brule F, Califice S, Garnier F, Fernandez PL, Berchuck A, Castronovo V. Galectin-1 accumulation in the ovary carcinoma peritumoral stroma is induced by ovary carcinoma cells and affects both cancer cell proliferation and adhesion to laminin-1 and fibronectin. *Lab Invest.* 2003; 83(3):377–86. [PubMed: 12649338]
91. Chow SN, Chen RJ, Chen CH, Chang TC, Chen LC, Lee WJ, Shen J, Chow LP. Analysis of protein profiles in human epithelial ovarian cancer tissues by proteomic technology. *Eur J Gynaecol Oncol.* 31(1):55–62. [PubMed: 20349782]
92. Iurisci I, Tinari N, Natoli C, Angelucci D, Cianchetti E, Iacobelli S. Concentrations of galectin-3 in the sera of normal controls and cancer patients. *Clin Cancer Res.* 2000; 6(4):1389–93. [PubMed: 10778968]
93. Earl LA, Bi S, Baum LG. N- and O-glycans modulate galectin-1 binding, CD45 signaling, and T cell death. *J Biol Chem.* 285(4):2232–44. [PubMed: 19920154]

94. Peng W, Wang HY, Miyahara Y, Peng G, Wang RF. Tumor-associated galectin-3 modulates the function of tumor-reactive T cells. *Cancer Res.* 2008; 68(17):7228–36. [PubMed: 18757439]
95. Andzel MM, Chen X, Krzewski K, Orange JS, Strominger JL. Myosin IIA is required for cytolytic granule exocytosis in human NK cells. *J Exp Med.* 2007; 204(10):2285–91. [PubMed: 17875677]
96. Krzewski K, Chen X, Orange JS, Strominger JL. Formation of a WIP-, WASp-, actin-, and myosin IIA-containing multiprotein complex in activated NK cells and its alteration by KIR inhibitory signaling. *J Cell Biol.* 2006; 173(1):121–32. [PubMed: 16606694]
97. Orange JS. The lytic NK cell immunological synapse and sequential steps in its formation. *Adv Exp Med Biol.* 2007; 601:225–33. [PubMed: 17713009]
98. Orange JS, Ramesh N, Remold-O'Donnell E, Sasahara Y, Koopman L, Byrne M, Bonilla FA, Rosen FS, Geha RS, Strominger JL. Wiskott-Aldrich syndrome protein is required for NK cell cytotoxicity and colocalizes with actin to NK cell-activating immunologic synapses. *Proc Natl Acad Sci U S A.* 2002; 99(17):11351–6. [PubMed: 12177428]
99. Vlad AM, Diaconu I, Gantt KR. MUC1 in endometriosis and ovarian cancer. *Immunol Res.* 2006; 36(1-3):229–36. [PubMed: 17337783]
100. Bafna S, Kaur S, Batra SK. Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells. *Oncogene.* 2010; 29(20):2893–904. [PubMed: 20348949]
101. Ponnusamy MP, Singh AP, Jain M, Chakraborty S, Moniaux N, Batra SK. MUC4 activates HER2 signalling and enhances the motility of human ovarian cancer cells. *Br J Cancer.* 2008; 99(3):520–6. [PubMed: 18665193]
102. Davidson B, Baekelandt M, Shih Ie M. MUC4 is upregulated in ovarian carcinoma effusions and differentiates carcinoma cells from mesothelial cells. *Diagn Cytopathol.* 2007; 35(12):756–60. [PubMed: 18008338]
103. Chauhan SC, Singh AP, Ruiz F, Johansson SL, Jain M, Smith LM, Moniaux N, Batra SK. Aberrant expression of MUC4 in ovarian carcinoma: diagnostic significance alone and in combination with MUC1 and MUC16 (CA125). *Mod Pathol.* 2006; 19(10):1386–94. [PubMed: 16880776]
104. Giuntoli RL 2nd, Rodriguez GC, Whitaker RS, Dodge R, Voynow JA. Mucin gene expression in ovarian cancers. *Cancer Res.* 1998; 58(23):5546–50. [PubMed: 9850092]
105. Yin BW, Lloyd KO. Molecular cloning of the ca125 ovarian cancer antigen. identification as a new mucin, muc16. *J Biol Chem.* 2001; 276(29):27371–5. [PubMed: 11369781]
106. Yin BW, Dnistrian A, Lloyd KO. Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. *Int J Cancer.* 2002; 98(5):737–40. [PubMed: 11920644]
107. Hattrop CL, Gendler SJ. Structure and Function of the Cell Surface (Tethered) Mucins. *Annu Rev Physiol.* 2007; 70:431–457. [PubMed: 17850209]
108. Jentoft N. Why are proteins O-glycosylated? *Trends Biochem Sci.* 1990; 15(8):291–4. [PubMed: 2204153]
109. Gubbels JA, Felder M, Horibata S, Belisle JA, Kapur A, Holden H, Petrie S, Migneault M, Rancourt C, Connor JP, Patankar MS. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. *Mol Cancer.* 2010; 9:11. [PubMed: 20089172]
110. Argueso P, Tisdale A, Spurr-Michaud S, Sumiyoshi M, Gipson IK. Mucin characteristics of human corneal-limbal epithelial cells that exclude the rose bengal anionic dye. *Invest Ophthalmol Vis Sci.* 2006; 47(1):113–9. [PubMed: 16384952]
111. Blalock TD, Spurr-Michaud SJ, Tisdale AS, Heimer SR, Gilmore MS, Ramesh V, Gipson IK. Functions of MUC16 in corneal epithelial cells. *Invest Ophthalmol Vis Sci.* 2007; 48(10):4509–18. [PubMed: 17898272]
112. Brayman MJ, Julian J, Mulac-Jericevic B, Conneely OM, Edwards DP, Carson DD. Progesterone receptor isoforms A and B differentially regulate MUC1 expression in uterine epithelial cells. *Mol Endocrinol.* 2006; 20(10):2278–91. [PubMed: 16740655]
113. DeSouza MM, Surveyor GA, Price RE, Julian J, Kardon R, Zhou X, Gendler S, Hilken J, Carson DD. MUC1/episialin: a critical barrier in the female reproductive tract. *J Reprod Immunol.* 1999; 45(2):127–58. [PubMed: 10674981]

114. DeSouza MM, Mani SK, Julian J, Carson DD. Reduction of mucin-1 expression during the receptive phase in the rat uterus. *Biol Reprod.* 1998; 58(6):1503–7. [PubMed: 9623612]
115. Hoffman LH, Olson GE, Carson DD, Chilton BS. Progesterone and implanting blastocysts regulate Muc1 expression in rabbit uterine epithelium. *Endocrinology.* 1998; 139(1):266–71. [PubMed: 9421424]
116. Idris N, Carraway KL. Regulation of sialomucin complex/Muc4 expression in rat uterine luminal epithelial cells by transforming growth factor-beta: implications for blastocyst implantation. *J Cell Physiol.* 2000; 185(2):310–6. [PubMed: 11025453]
117. Idris N, Carraway KL. Sialomucin complex (Muc4) expression in the rat female reproductive tract. *Biol Reprod.* 1999; 61(6):1431–8. [PubMed: 10569986]
118. Komatsu M, Yee L, Carraway KL. Overexpression of sialomucin complex, a rat homologue of MUC4, inhibits tumor killing by lymphokine-activated killer cells. *Cancer Res.* 1999; 59(9):2229–36. [PubMed: 10232613]
119. An HJ, Miyamoto S, Lancaster KS, Kirmiz C, Li B, Lam KS, Leiserowitz GS, Lebrilla CB. Profiling of glycans in serum for the discovery of potential biomarkers for ovarian cancer. *J Proteome Res.* 2006; 5(7):1626–35. [PubMed: 16823970]
120. Li B, An HJ, Kirmiz C, Lebrilla CB, Lam KS, Miyamoto S. Glycoproteomic analyses of ovarian cancer cell lines and sera from ovarian cancer patients show distinct glycosylation changes in individual proteins. *J Proteome Res.* 2008; 7(9):3776–88. [PubMed: 18642944]
121. Leiserowitz GS, Lebrilla C, Miyamoto S, An HJ, Duong H, Kirmiz C, Li B, Liu H, Lam KS. Glycomics analysis of serum: a potential new biomarker for ovarian cancer? *Int J Gynecol Cancer.* 2008; 18(3):470–5. [PubMed: 17655680]
122. Abbott KL, Pierce JM. Lectin-based glycoproteomic techniques for the enrichment and identification of potential biomarkers. *Methods Enzymol.* 480:461–76. [PubMed: 20816222]
123. Davidson B, Gotlieb WH, Ben-Baruch G, Kopolovic J, Goldberg I, Nesland JM, Berner A, Bjamer A, Bryne M. Expression of carbohydrate antigens in advanced-stage ovarian carcinomas and their metastases-A clinicopathologic study. *Gynecol Oncol.* 2000; 77(1):35–43. [PubMed: 10739688]
124. Toba S, Tenno M, Kurosaka A. An O-acetylated sialyl-Tn is involved in ovarian cancer-associated antigenicity. *Biochem Biophys Res Commun.* 2000; 271(2):281–6. [PubMed: 10799288]
125. Kui Wong N, Easton RL, Panico M, Sutton-Smith M, Morrison JC, Lattanzio FA, Morris HR, Clark GF, Dell A, Patankar MS. Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *J Biol Chem.* 2003; 278(31):28619–34. [PubMed: 12734200]
126. Ju T, Aryal RP, Stowell CJ, Cummings RD. Regulation of protein O-glycosylation by the endoplasmic reticulum-localized molecular chaperone Cosmc. *J Cell Biol.* 2008; 182(3):531–42. [PubMed: 18695044]
127. Ju T, Lanneau GS, Gautam T, Wang Y, Xia B, Stowell SR, Willard MT, Wang W, Xia JY, Zuna RE, Laszik Z, Benbrook DM, Hanigan MH, Cummings RD. Human tumor antigens Tn and sialyl Tn arise from mutations in Cosmc. *Cancer Res.* 2008; 68(6):1636–46. [PubMed: 18339842]
128. Ju T, Cummings RD. Protein glycosylation: chaperone mutation in Tn syndrome. *Nature.* 2005; 437(7063):1252. [PubMed: 16251947]
129. Ju T, Cummings RD. A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. *Proc Natl Acad Sci U S A.* 2002; 99(26):16613–8. [PubMed: 12464682]
130. Ju T, Brewer K, D'Souza A, Cummings RD, Canfield WM. Cloning and expression of human core 1 beta1,3-galactosyltransferase. *J Biol Chem.* 2002; 277(1):178–86. [PubMed: 11677243]
131. Ogata S, Maimonis PJ, Itzkowitz SH. Mucins bearing the cancer-associated sialosyl-Tn antigen mediate inhibition of natural killer cell cytotoxicity. *Cancer Res.* 1992; 52(17):4741–6. [PubMed: 1511439]
132. Blottiere HM, Burg C, Zennadi R, Perrin P, Blanchardie P, Bara J, Meflah K, Le Pendu J. Involvement of histo-blood-group antigens in the susceptibility of colon carcinoma cells to natural killer-mediated cytotoxicity. *Int J Cancer.* 1992; 52(4):609–18. [PubMed: 1399144]

133. Saeland E, van Vliet SJ, Backstrom M, van den Berg VC, Geijtenbeek TB, Meijer GA, van Kooyk Y. The C-type lectin MGL expressed by dendritic cells detects glycan changes on MUC1 in colon carcinoma. *Cancer Immunol Immunother.* 2007; 56(8):1225–36. [PubMed: 17195076]
134. Singh SK, Streng-Ouwehand I, Litjens M, Weelij DR, Garcia-Vallejo JJ, van Vliet SJ, Saeland E, van Kooyk Y. Characterization of murine MGL1 and MGL2 C-type lectins: distinct glycan specificities and tumor binding properties. *Mol Immunol.* 2009; 46(6):1240–9. [PubMed: 19162326]
135. Higashi N, Fujioka K, Denda-Nagai K, Hashimoto Si S, Nagai S, Sato T, Fujita Y, Morikawa A, Tsuiji M, Miyata-Takeuchi M, Sano Y, Suzuki N, Yamamoto K, Matsushima K, Irimura T. The macrophage C-type lectin specific for galactose/N-acetylgalactosamine is an endocytic receptor expressed on monocyte- derived immature dendritic cells. *J Biol Chem.* 2002; 27:27.
136. Higashi N, Morikawa A, Fujioka K, Fujita Y, Sano Y, Miyata-Takeuchi M, Suzuki N, Irimura T. Human macrophage lectin specific for galactose/N-acetylgalactosamine is a marker for cells at an intermediate stage in their differentiation from monocytes into macrophages. *Int Immunol.* 2002; 14(6):545–554. [PubMed: 12039906]
137. Tsuiji M, Fujimori M, Ohashi Y, Higashi N, Onami TM, Hedrick SM, Irimura T. Molecular cloning and characterization of a novel mouse macrophage C-type lectin, mMGL2, which has a distinct carbohydrate specificity from mMGL1. *J Biol Chem.* 2002; 16:16.
138. van Vliet SJ, Aarnoudse CA, Broks-van den Berg VC, Boks M, Geijtenbeek TB, van Kooyk Y. MGL-mediated internalization and antigen presentation by dendritic cells: a role for tyrosine-5. *Eur J Immunol.* 2007; 37(8):2075–81. [PubMed: 17616966]
139. Napoletano C, Rughetti A, Agervig Tarp MP, Coleman J, Bennett EP, Picco G, Sale P, Denda-Nagai K, Irimura T, Mandel U, Clausen H, Frati L, Taylor-Papadimitriou J, Burchell J, Nuti M. Tumor-associated Tn-MUC1 glycoform is internalized through the macrophage galactose-type C-type lectin and delivered to the HLA class I and II compartments in dendritic cells. *Cancer Res.* 2007; 67(17):8358–67. [PubMed: 17804752]
140. Loveland BE, Zhao A, White S, Gan H, Hamilton K, Xing PX, Pietersz GA, Apostolopoulos V, Vaughan H, Karanikas V, Kyriakou P, McKenzie IF, Mitchell PL. Mannan-MUC1-pulsed dendritic cell immunotherapy: a phase I trial in patients with adenocarcinoma. *Clin Cancer Res.* 2006; 12(3 Pt 1):869–77. [PubMed: 16467101]
141. Apostolopoulos V, Loveland BE, Pietersz GA, McKenzie IF. CTL in mice immunized with human mucin 1 are MHC-restricted. *J Immunol.* 1995; 155(11):5089–94. [PubMed: 7594517]
142. Denda-Nagai K, Aida S, Saba K, Suzuki K, Moriyama S, Oo-Puthinan S, Tsuiji M, Morikawa A, Kumamoto Y, Sugiura D, Kudo A, Akimoto Y, Kawakami H, Bovin NV, Irimura T. Distribution and function of macrophage galactose-type C-type lectin 2 (MGL2/CD301b): efficient uptake and presentation of glycosylated antigens by dendritic cells. *J Biol Chem.* 2010; 285(25):19193–204. [PubMed: 20304916]
143. Freire T, Zhang X, Deriaud E, Ganneau C, Vichier-Guerre S, Azria E, Launay O, Lo-Man R, Bay S, Leclerc C. Glycosidic Tn-based vaccines targeting dermal dendritic cells favor germinal center B-cell development and potent antibody response in the absence of adjuvant. *Blood.* 2010; 116(18):3526–36. [PubMed: 20720186]
144. Dausset J, Moullec J, Bernard J. Acquired hemolytic anemia with polyagglutinability of red blood cells due to a new factor present in normal human serum (Anti-Tn). *Blood.* 1959; 14:1079–93. [PubMed: 13814231]
145. Friedenreich V. Investigations into the Thomsen hemagglutination phenomena. *Acta Pathol. Microbiol. Scand.* 1928; 5:59.
146. Springer GF, Tegtmeier H. Origin of anti-Thomsen-Friedenreich (T) and Tn agglutinins in man and in White Leghorn chicks. *Br J Haematol.* 1981; 47(3):453–60. [PubMed: 7006674]
147. Springer GF, Tegtmeier H. Tn, a universal carcinoma (CA) marker, frequently strongly expressed in anaplastic, aggressive CA. *Naturwissenschaften.* 1983; 70(12):621–2. [PubMed: 6674816]
148. Springer GF. T and Tn, general carcinoma autoantigens. *Science.* 1984; 224(4654):1198–206. [PubMed: 6729450]

149. Springer GF, Taylor CR, Howard DR, Tegtmeier H, Desai PR, Murthy SM, Felder B, Scanlon EF. Tn, a carcinoma-associated antigen, reacts with anti-Tn of normal human sera. *Cancer*. 1985; 55(3):561–9. [PubMed: 2981150]
150. Desai PR, Ujjainwala LH, Carlstedt SC, Springer GF. Anti-Thomsen-Friedenreich (T) antibody-based ELISA and its application to human breast carcinoma detection. *J Immunol Methods*. 1995; 188(2):175–85. [PubMed: 8551046]
151. Springer GF. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. *J Mol Med*. 1997; 75(8):594–602. [PubMed: 9297627]
152. Desai PR. Immunoreactive T and Tn antigens in malignancy: role in carcinoma diagnosis, prognosis, and immunotherapy. *Transfus Med Rev*. 2000; 14(4):312–25. [PubMed: 11055076]
153. Gilewski TA, Ragupathi G, Dickler M, Powell S, Bhuta S, Panageas K, Koganty RR, Chin-Eng J, Hudis C, Norton L, Houghton AN, Livingston PO. Immunization of high-risk breast cancer patients with clustered sTn-KLH conjugate plus the immunologic adjuvant QS-21. *Clin Cancer Res*. 2007; 13(10):2977–85. [PubMed: 17504999]
154. Keding SJ, Danishefsky SJ. Prospects for total synthesis: a vision for a totally synthetic vaccine targeting epithelial tumors. *Proc Natl Acad Sci U S A*. 2004; 101(33):11937–42. [PubMed: 15280546]
155. Guo Z, Wang Q. Recent development in carbohydrate-based cancer vaccines. *Curr Opin Chem Biol*. 2009; 13(5-6):608–17. [PubMed: 19766052]
156. Machado E, Kandzia S, Carilho R, Altevogt P, Conradt HS, Costa J. N-Glycosylation of total cellular glycoproteins from the human ovarian carcinoma SKOV3 cell line and of recombinantly expressed human erythropoietin. *Glycobiology*. 2010
157. Neeleman AP, van der Knaap WP, van den Eijnden DH. Identification and characterization of a UDPGalNAc:GlcNAc beta-R beta 1-->4-N-acetylgalactosaminyltransferase from cercariae of the schistosome *Trichobilharzia ocellata*. Catalysis of a key step in the synthesis of N,N'-diacetyllactosedi amino (lacdiNAc)-type glycans. *Glycobiology*. 1994; 4(5):641–51. [PubMed: 7881179]
158. Van den Eijnden DH, Neeleman AP, Van der Knaap WP, Bakker H, Agterberg M, Van Die I. Novel glycosylation routes for glycoproteins: the lacdiNAc pathway. *Biochem Soc Trans*. 1995; 23(1):175–9. [PubMed: 7758724]
159. Dell A, Morris HR, Easton RL, Panico M, Patankar M, Oehninger S, Koistinen R, Koistinen H, Seppala M, Clark GF. Structural analysis of the oligosaccharides derived from glycodeclin, a human glycoprotein with potent immunosuppressive and contraceptive activities. *J Biol Chem*. 1995; 270(41):24116–26. [PubMed: 7592613]
160. Seppala M, Bohn H, Tatarinov Y. Glycodeclins. *Tumour Biol*. 1998; 19(3):213–20. [PubMed: 9591048]
161. Seppala M, Koistinen H, Koistinen R, Mandelin E, Oehninger S, Clark GF, Dell A, Morris HR. Glycodeclins: role in regulation of reproduction, potential for contraceptive development and diagnosis of male infertility. *Hum Reprod*. 1998; 13(Suppl 3):262–91. [PubMed: 9755428]
162. Okamoto N, Uchida A, Takakura K, Kariya Y, Kanzaki H, Riittinen L, Koistinen R, Seppala M, Mori T. Suppression by human placental protein 14 of natural killer cell activity. *Am J Reprod Immunol*. 1991; 26(4):137–42. [PubMed: 1840727]
163. van den Berg TK, Honing H, Franke N, van Remoortere A, Schiphorst WE, Liu FT, Deelder AM, Cummings RD, Hokke CH, van Die I. LacdiNAcglycans constitute a parasite pattern for galectin-3-mediated immune recognition. *J Immunol*. 2004; 173(3):1902–7. [PubMed: 15265923]
164. Stillman BN, Hsu DK, Pang M, Brewer CF, Johnson P, Liu FT, Baum LG. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J Immunol*. 2006; 176(2):778–89. [PubMed: 16393961]
165. Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mga5 N-glycosylation. *Nature*. 2001; 409(6821):733–9. [PubMed: 11217864]
166. Chen HY, Fermin A, Vardhana S, Weng IC, Lo KF, Chang EY, Maverakis E, Yang RY, Hsu DK, Dustin ML, Liu FT. Galectin-3 negatively regulates TCR-mediated CD4+ T-cell activation at the

- immunological synapse. *Proc Natl Acad Sci U S A*. 2009; 106(34):14496–501. [PubMed: 19706535]
167. Hsu DK, Chen HY, Liu FT. Galectin-3 regulates T-cell functions. *Immunol Rev*. 2009; 230(1): 114–27. [PubMed: 19594632]
 168. Bhaumik M, Seldin MF, Stanley P. Cloning and chromosomal mapping of the mouse Mgat3 gene encoding N-acetylglucosaminyltransferase III. *Gene*. 1995; 164(2):295–300. [PubMed: 7590346]
 169. Yang X, Tang J, Rogler CE, Stanley P. Reduced hepatocyte proliferation is the basis of retarded liver tumor progression and liver regeneration in mice lacking N-acetylglucosaminyltransferase III. *Cancer Res*. 2003; 63(22):7753–9. [PubMed: 14633700]
 170. Song Y, Aglipay JA, Bernstein JD, Goswami S, Stanley P. The bisecting GlcNAc on N-glycans inhibits growth factor signaling and retards mammary tumor progression. *Cancer Res*. 70(8): 3361–71. [PubMed: 20395209]
 171. el Ouagari K, Teissie J, Benoist H. Glycophorin A protects K562 cells from natural killer cell attack. Role of oligosaccharides. *J Biol Chem*. 1995; 270(45):26970–5. [PubMed: 7592944]
 172. Yoshimura M, Ihara Y, Ohnishi A, Ijuhin N, Nishiura T, Kanakura Y, Matsuzawa Y, Taniguchi N. Bisecting N-acetylglucosamine on K562 cells suppresses natural killer cytotoxicity and promotes spleen colonization. *Cancer Res*. 1996; 56(2):412–8. [PubMed: 8542600]
 173. Patankar MS, Yu J, Morrison JC, Belisle JA, Lattanzio FA, Deng Y, Wong NK, Morris HR, Dell A, Clark GF. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecol Oncol*. 2005; 99(3):704–713. [PubMed: 16126266]
 174. Mizuochi T, Matthews TJ, Kato M, Hamako J, Titani K, Solomon J, Feizi T. Diversity of oligosaccharide structures on the envelope glycoprotein gp 120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. Presence of complex-type oligosaccharides with bisecting N- acetylglucosamine residues. *J Biol Chem*. 1990; 265(15):8519–24. [PubMed: 2341393]
 175. Clark GF, Dell A, Morris HR, Patankar M, Oehninger S, Seppala M. Viewing AIDS from a glycobiological perspective: potential linkages to the human foetoembryonic defence system hypothesis. *Mol Hum Reprod*. 1997; 3(1):5–13. [PubMed: 9239703]
 176. Wolfl M, Batten WY, Posovszky C, Bernhard H, Berthold F. Gangliosides inhibit the development from monocytes to dendritic cells. *Clin Exp Immunol*. 2002; 130(3):441–8. [PubMed: 12452834]
 177. Miyazaki K, Ohmori K, Izawa M, Koike T, Kumamoto K, Furukawa K, Ando T, Kiso M, Yamaji T, Hashimoto Y, Suzuki A, Yoshida A, Takeuchi M, Kannagi R. Loss of disialyl Lewis(a), the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) associated with increased sialyl Lewis(a) expression on human colon cancers. *Cancer Res*. 2004; 64(13):4498–505. [PubMed: 15231659]
 178. von Gunten S, Simon HU. Sialic acid binding immunoglobulin-like lectins may regulate innate immune responses by modulating the life span of granulocytes. *Faseb J*. 2006; 20(6):601–5. [PubMed: 16581967]
 179. Aarnoudse CA, Garcia Vallejo JJ, Saeland E, van Kooyk Y. Recognition of tumor glycans by antigen-presenting cells. *Curr Opin Immunol*. 2006; 18(1):105–11. [PubMed: 16303292]
 180. Kopitz J, von Reitzenstein C, Burchert M, Cantz M, Gabius HJ. Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. *J Biol Chem*. 1998; 273(18):11205–11. [PubMed: 9556610]
 181. van Kooyk Y, Rabinovich GA. Protein-glycan interactions in the control of innate and adaptive immune responses. *Nat Immunol*. 2008; 9(6):593–601. [PubMed: 18490910]
 182. Belisle JA, Horibata S, Gubbels JA, Petrie S, Kapur A, Andre S, Gabius HJ, Rancourt C, Connor J, Paulson JC, Patankar MS. Identification of Siglec-9 as the receptor for MUC16 on human NK cells, B cells, and monocytes. *Mol Cancer*. 2010; 9(1):118. [PubMed: 20497550]
 183. Alpey MS, Attrill H, Crocker PR, Van Aalten DM. High resolution crystal structures of Siglec-7- insights into ligand specificity in the Siglec family. *J Biol Chem*. 2002

184. Angata T, Varki A. Cloning, characterization and phylogenetic analysis of Siglec-9, a new member of the CD33-related group of Siglecs. Evidence for co-evolution with sialic acid synthesis pathways. *J Biol Chem.* 2000; 275(29):22127–35. [PubMed: 10801860]
185. von Gunten S, Yousefi S, Seitz M, Jakob SM, Schaffner T, Seger R, Takala J, Villiger PM, Simon HU. Siglec-9 transduces apoptotic and nonapoptotic death signals into neutrophils depending on the proinflammatory cytokine environment. *Blood.* 2005; 106(4):1423–31. [PubMed: 15827126]
186. Ikehara Y, Ikehara SK, Paulson JC. Negative regulation of T cell receptor signaling by Siglec-7 (p70/AIRM) and Siglec-9. *J Biol Chem.* 2004; 279(41):43117–25. [PubMed: 15292262]
187. Avril T, Floyd H, Lopez F, Vivier E, Crocker PR. The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by Siglecs-7 and -9, CD33-related Siglecs expressed on human monocytes and NK cells. *J Immunol.* 2004; 173(11):6841–9. [PubMed: 15557178]
188. Ando M, Tu W, Nishijima K, Iijima S. Siglec-9 enhances IL-10 production in macrophages via tyrosine-based motifs. *Biochem Biophys Res Commun.* 2008; 369(3):878–83. [PubMed: 18325328]
189. Krzewski K, Chen X, Strominger JL. WIP is essential for lytic granule polarization and NK cell cytotoxicity. *Proc Natl Acad Sci U S A.* 2008; 105(7):2568–73. [PubMed: 18258743]
190. Tsviliana A, Mayr D, Kuhn C, Kunze S, Mylonas I, Jeschke U, Friese K. Determination of glycodelin-A expression correlated to grading and staging in ovarian carcinoma tissue. *Anticancer Res.* 2010; 30(5):1637–40. [PubMed: 20592354]
191. Scholz C, Rampf E, Toth B, Brunnhuber R, Weissenbacher T, Friese K, Jeschke U. Ovarian cancer-derived glycodelin impairs in vitro dendritic cell maturation. *J Immunother.* 2009; 32(5):492–7. [PubMed: 19609241]
192. Scholz C, Toth B, Brunnhuber R, Rampf E, Weissenbacher T, Santoso L, Friese K, Jeschke U. Glycodelin A induces a tolerogenic phenotype in monocyte-derived dendritic cells in vitro. *Am J Reprod Immunol.* 2008; 60(6):501–12. [PubMed: 19032611]
193. Rachmilewitz J, Riely GJ, Tykocinski ML. Placental protein 14 functions as a direct T-cell inhibitor. *Cell Immunol.* 1999; 191(1):26–33. [PubMed: 9918684]
194. Soni C, Karande AA. Glycodelin A suppresses the cytolytic activity of CD8+ T lymphocytes. *Mol Immunol.* 47(15):2458–66. [PubMed: 20638131]
195. SundarRaj S, Soni C, Karande AA. Glycodelin A triggers T cell apoptosis through a novel calcium-independent galactose-binding lectin activity. *Mol Immunol.* 2009; 46(16):3411–9. [PubMed: 19683346]
196. Seppala M, Jiang S, Strick N, Lin K, Li YY, Koistinen H, Koistinen R, Neurath AR. Glycodelins GdA and GdS modified by 3-hydroxyphthalic anhydride inhibit gp120-CD4 binding and HIV-1 infection in vitro. *Lab Invest.* 1997; 77(2):127–30. [PubMed: 9274854]
197. Morris HR, Dell A, Easton RL, Panico M, Koistinen H, Koistinen R, Oehninger S, Patankar MS, Seppala M, Clark GF. Gender-specific glycosylation of human glycodelin affects its contraceptive activity. *J Biol Chem.* 1996; 271(50):32159–67. [PubMed: 8943270]
198. Lee CL, Pang PC, Yeung WS, Tissot B, Panico M, Lao TT, Chu IK, Lee KF, Chung MK, Lam KK, Koistinen R, Koistinen H, Seppala M, Morris HR, Dell A, Chiu PC. Effects of differential glycosylation of glycodelins on lymphocyte survival. *J Biol Chem.* 2009; 284(22):15084–96. [PubMed: 19240032]
199. Cohen M, Elkabets M, Perlmutter M, Porgador A, Voronov E, Apte RN, Lichtenstein RG. Sialylation of 3-methylcholanthrene-induced fibrosarcoma determines antitumor immune responses during immunoediting. *J Immunol.* 185(10):5869–78. [PubMed: 20956342]
200. Avidan A, Perlmutter M, Tal S, Oraki O, Kapp T, Krelm Y, Elkabets M, Dotan S, Apte RN, Lichtenstein RG. Differences in the sialylation patterns of membrane stress proteins in chemical carcinogen-induced tumors developed in BALB/c and IL-1alpha deficient mice. *Glycoconj J.* 2009; 26(9):1181–95. [PubMed: 19430902]
201. Cebo C, Dambrouck T, Maes E, Laden C, Strecker G, Michalski JC, Zanetta JP. Recombinant human interleukins IL-1alpha, IL-1beta, IL-4, IL-6, and IL-7 show different and specific

- calcium-independent carbohydrate-binding properties. *J Biol Chem.* 2001; 276(8):5685–91. [PubMed: 11050099]
202. Cebo C, Vergoten G, Zanetta J. Lectin activities of cytokines: functions and putative carbohydrate-recognition domains. *Biochim Biophys Acta.* 2002; 1572(2-3):422. [PubMed: 12223284]
 203. Fukushima K, Yamashita K. IL-2 carbohydrate recognition modulates CTLL-2 cell proliferation. *J Biol Chem.* 2000
 204. Fukushima K, Hara-Kuge S, Ideo H, Yamashita K. Carbohydrate recognition site of interleukin-2 in relation to cell proliferation. *J Biol Chem.* 2001; 276(33):31202–8. [PubMed: 11390392]
 205. Fukushima K, Yamashita K. Interleukin-2 carbohydrate recognition modulates CTLL-2 cell proliferation. *J Biol Chem.* 2001; 276(10):7351–6. [PubMed: 11076950]
 206. Fukushima K, Yamashita K. Carbohydrate recognition of interleukin-2 in cell proliferation. *Methods Enzymol.* 2003; 363:518–25. [PubMed: 14579600]
 207. Fukushima K, Hara-Kuge S, Yamashita K. [Carbohydrate recognition modulates cell proliferation]. *Tanpakushitsu Kakusan Koso.* 2003; 48(8 Suppl):1120–5. [PubMed: 12807018]
 208. Yamashita K, Fukushima K. The carbohydrate recognition by cytokines modulates their physiological activities. *Glycoconj J.* 2004; 21(1-2):31–4. [PubMed: 15467395]
 209. Fukushima K, Ishiyama C, Yamashita K. Recognition by TNF-alpha of the GPI-anchor glycan induces apoptosis of U937 cells. *Arch Biochem Biophys.* 2004; 426(2):298–305. [PubMed: 15158680]
 210. Yamashita K, Fukushima K. [Functional roles of glycans for interaction between cytokines and receptors]. *Tanpakushitsu Kakusan Koso.* 2008; 53(12 Suppl):1513–9. [PubMed: 21089358]
 211. Sherblom AP, Sathyamoorthy N, Decker JM, Muchmore AV. IL-2, a lectin with specificity for high mannose glycopeptides. *J Immunol.* 1989; 143(3):939–44. [PubMed: 2787353]
 212. Zanetta JP, Alonso C, Michalski JC. Interleukin 2 is a lectin that associates its receptor with the T-cell receptor complex. *Biochem J.* 1996; 318(Pt 1):49–53. [PubMed: 8761451]
 213. Sherblom AP, Decker JM, Muchmore AV. The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. *J. Biol. Chem.* 1988; 263:5418. [PubMed: 3356692]
 214. Muchmore AV, Sathyamoorthy N, Decker J, Sherblom AP. Evidence that specific high-mannose oligosaccharides can directly inhibit antigen-driven T-cell responses. *J Leukoc Biol.* 1990; 48(5): 457–64. [PubMed: 2146352]
 215. Hession C, Decker JM, Sherblom AP, Kumar S, Yue CC, Mattaliano RJ, Tizard R, Kawashima E, Schmeissner U, Heletky S, Chow EP, Burne CA, Shaw A, Muchmore AV. Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. *Science.* 1987; 237(4821):1479–84. [PubMed: 3498215]
 216. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer.* 2004; 4(1):45–60. [PubMed: 14681689]
 217. Papalia GA, Rini JM. Re-examining the proposed lectin properties of IL-2. *Mol Immunol.* 2008; 45(5):1241–7. [PubMed: 17983654]
 218. Moniaux N, Andrianifahanana M, Brand RE, Batra SK. Multiple roles of mucins in pancreatic cancer, a lethal and challenging malignancy. *Br J Cancer.* 2004; 91(9):1633–8. [PubMed: 15494719]
 219. Cramer DW, O'Rourke DJ, Vitonis AF, Matulonis UA, Dijohnson DA, Sluss PM, Crum CP, Liu BC. CA125 immune complexes in ovarian cancer patients with low CA125 concentrations. *Clin Chem.* 56(12):1889–92. [PubMed: 20943848]
 220. Piura B, Piura E. Autoantibodies to tumor-associated antigens in epithelial ovarian carcinoma. *J Oncol.* 2009; 2009:581939. [PubMed: 20145720]
 221. Wandall HH, Blixt O, Tarp MA, Pedersen JW, Bennett EP, Mandel U, Ragupathi G, Livingston PO, Hollingsworth MA, Taylor-Papadimitriou J, Burchell J, Clausen H. Cancer biomarkers defined by autoantibody signatures to aberrant O-glycopeptide epitopes. *Cancer Res.* 70(4): 1306–13. [PubMed: 20124478]
 222. Taylor DD, Gercel-Taylor C, Parker LP. Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer. *Gynecol Oncol.* 2009; 115(1):112–20. [PubMed: 19647308]

223. Pinheiro SP, Hankinson SE, Tworoger SS, Rosner BA, McKolanis JR, Finn OJ, Cramer DW. Anti-MUC1 antibodies and ovarian cancer risk: prospective data from the Nurses' Health Studies. *Cancer Epidemiol Biomarkers Prev.* 19(6):1595–601. [PubMed: 20501761]
224. Terry KL, Titus-Ernstoff L, McKolanis JR, Welch WR, Finn OJ, Cramer DW. Incessant ovulation, mucin 1 immunity, and risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2007; 16(1):30–5. [PubMed: 17220329]
225. Cramer DW, Titus-Ernstoff L, McKolanis JR, Welch WR, Vitonis AF, Berkowitz RS, Finn OJ. Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2005; 14(5):1125–31. [PubMed: 15894662]
226. Hiltbold EM, Ciborowski P, Finn OJ. Naturally processed class II epitope from the tumor antigen MUC1 primes human CD4+ T cells. *Cancer Res.* 1998; 58(22):5066–70. [PubMed: 9823312]
227. Soares MM, Mehta V, Finn OJ. Three different vaccines based on the 140-amino acid MUC1 peptide with seven tandemly repeated tumor-specific epitopes elicit distinct immune effector mechanisms in wild-type versus MUC1-transgenic mice with different potential for tumor rejection. *J Immunol.* 2001; 166(11):6555–63. [PubMed: 11359807]
228. Saldova R, Royle L, Radcliffe CM, Abd Hamid UM, Evans R, Arnold JN, Banks RE, Hutson R, Harvey DJ, Antrobus R, Petrescu SM, Dwek RA, Rudd PM. Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG. *Glycobiology.* 2007; 17(12):1344–56. [PubMed: 17884841]
229. Gercel-Taylor C, Bazzett LB, Taylor DD. Presence of aberrant tumor-reactive immunoglobulins in the circulation of patients with ovarian cancer. *Gynecol Oncol.* 2001; 81(1):71–6. [PubMed: 11277653]
230. Saldova R, Wormald MR, Dwek RA, Rudd PM. Glycosylation changes on serum glycoproteins in ovarian cancer may contribute to disease pathogenesis. *Dis Markers.* 2008; 25(4-5):219–32. [PubMed: 19126966]
231. Connor JP, Felder M, Hank J, Harter J, Gan J, Gillies SD, Sondel P. Ex vivo evaluation of anti-EpCAM immunocytokine huKS-IL2 in ovarian cancer. *J Immunother.* 2004; 27(3):211–9. [PubMed: 15076138]
232. Patankar MS, Oehninger S, Barnett T, Williams RL, Clark GF. A revised structure for fucoidan may explain some of its biological activities. *J Biol Chem.* 1993; 268(29):21770–6. [PubMed: 8408031]
233. Clark GF, Oehninger S, Patankar MS, Koistinen R, Dell A, Morris HR, Koistinen H, Seppala M. A role for glycoconjugates in human development: the human feto-embryonic defence system hypothesis. *Hum Reprod.* 1996; 11(3):467–73. [PubMed: 8671249]
234. Clark GF, Patankar MS. Opinion: Hu-FEDS: in search of 'universal self'. *Mol Hum Reprod.* 1997; 3(11):985–7. [PubMed: 9433925]

Figure 1.

NK immune synapse. Localization of actin (red) at the contact site between NKL (an NK leukemia cell line) and K562 (target) cells is shown in the left panel. Nucleus stained with DAPI (blue) and perforin (green) is in transition towards the synapse. Recruitment of LFA (green) to the contact site is shown in the right panel.

Figure 2.

Recruitment of perforin granules to the NK immune synapse. Synapse between K562 cells (labeled with phalloidin to stain actin, red) and NKL (NK cell leukemia cell line) is shown. Perforin granules (green) are recruited to the synapse. Nucleus is stained with DAPI (blue).

Figure 3.

Reduced susceptibility of ovarian tumor cells to NK cell mediated lysis. Ability of NK cells derived from healthy donor peripheral blood to lyse the ovarian tumor cell line OVCAR-3 in comparison to the NK cell susceptible cell line, K562, is shown. Cytotoxicity was determined in a 4 h chromium release assay.

Figure 4.

Model for MUC16 induced NK cell suppression. MUC16 binding to Siglec-9 leads to phosphorylation of proximal ITIM (yellow box) of the receptor and subsequently phosphorylation and activation of SHP-1 and/or SHP-2. SHP-1 and SHP-2 are phosphatases that dephosphorylate Vav-1 and other signaling molecules. As a result, polymerization of actin, polarization of actin associated proteins (WASp, WIP, Myosin II), LFA-1, and perforin granules is not achieved. Lack of activating synapse formation leads to protection of the tumor cell. csMUC16-Siglec-9 binding triggers an inhibitory immune synapse that protects the tumor cells from NK cell attack. MUC16 binding to Siglec-9 upregulates IL-10 and other Th2-type cytokines that inhibit the anti-tumor immune responses.