Cancer Medicine

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REVIEW

Early ovariectomy reveals the germline encoding of natural anti-A- and Tn-cross-reactive immunoglobulin M (IgM) arising from developmental *O*-GalNAc glycosylations. (Germline-encoded natural anti-A/Tn cross-reactive IgM)

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Keywords
Developmental location, glycosidic accommodation, *Helix pomatia* reactivity, nonimmune IgM, invertebrate defense proteins, non-developmental tissue, *O*-GalNAc glycosylation(s)

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Funding Information
This work was supported by the Paul Martini Stiftung Frankfurt/Main, Germany, National Institute of Health, Bethesda MD, USA (Grant Number: 'AM11242 and AM 02534 ') and Deutsche Forschungsgemeinschaft, Bonn, Germany.

Received: 1 December 2016; Revised: 26 February 2017; Accepted: 24 March 2017

***Cancer Medicine* 2017; 6(7):1601–1613**

doi: 10.1002/cam4.1079
*Where the experiments of the author are performed.

Abstract

While native blood group A-like glycans have not been demonstrated in prokaryotic microorganisms as a source of human "natural" anti-A isoagglutinin production, and metazoan eukaryotic *N*-acetylgalactosamine *O*-glycosylation of serine or threonine residues (*O*-GalNAc-Ser/Thr-R) does not occur in bacteria, the *O*-GalNAc glycan-bearing ovarian glycolipids, discovered in C57BL/10 mice, are complementary to the syngeneic anti-A-reactive immunoglobulin M (IgM), which is not present in animals that have undergone ovariectomy prior to the onset of puberty. These mammalian ovarian glycolipids are complementary also to the anti-A/Tn cross-reactive *Helix pomatia* agglutinin (HPA), a molluscan defense protein, emerging from the coat proteins of fertilized eggs and reflecting the snail-intrinsic, reversible *O*-GalNAc glycosylations. The hexameric structure of this primitive invertebrate defense protein gives rise to speculation regarding an evolutionary relationship to the mammalian nonimmune, anti-A-reactive immunoglobulin M (IgM) molecule. Hypothetically, this molecule obtains its complementarity from the first step of protein glycosylations, initiated by GalNAc via reversible *O*-linkages to peptides displaying Ser/Thr motifs, whereas the subsequent transferase depletion completes germ cell maturation and cell renewal, associated with loss of glycosidic bonds and release of *O*-glycan-depleted proteins, such as complementary IgM revealing the structure of the volatilely expressed "lost" glycan carrier through germline Ser residues. Consequently, the evolutionary/developmental first glycosylations of proteins appear metabolically related or identical to that of the mucin-type, potentially "aberrant" monosaccharide GalNAcα1-*O*-Ser/Thr-R, also referred to as the Tn (T "nouvelle") antigen, and explain the anti-Tn cross-reactivity of human innate or "natural" anti-A-specific isoagglutinin and the pronounced occurrence of cross-reactive anti-Tn antibody in plasma from humans with histo-blood group O. In fact, A-allelic, phenotype-specific GalNAc glycosylation of plasma proteins does not occur in human blood group O, affecting anti-Tn antibody levels, which may function as a growth regulator that contributes to a potential survival advantage of this group in the overall risk of developing cancer when compared with non-O blood groups.

Introduction

While the naturally occurring immunoglobulin M (IgM) is permanently engaged in recognition and elimination of aberrant growth and cancerous tissue, the secretion of IgM molecules is not restricted to B cells but spontaneously occurs in murine [1, 2] and human [3] normal and malignant epithelial cells as well. Moreover, although

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many anti-glycan antibodies do not adhere to the paradigm of an adaptive immune response and are often referred to as "natural antibodies" [4], to date, based on the historical experiments of Springer et al. [5–7], the production of human histo-blood group ABO(H) isoantibodies or isoagglutinins with Tn and T antigen cross-specificity is believed to be exclusively induced by environmental, predominantly intestinal, well-documented microbial antigens, particularly lipopolysaccharides from gram-negative bacteria. However, prokaryotic "blood group A/B-like" antigenic structures basically induce cross-reactive anti-A/B immunoglobulins, which due to clonal selection neither arise in blood group A nor in B individuals. While bacterial endotoxins nonspecifically stimulate the formation of all immunoglobulins, most likely involving the anti-A/B isoagglutinins, a definitive adaptive, enteral immunization with ABO(H)-reactive, environmental antigens is a source of antibody production that in humans might largely be restricted to blood group O(H) individuals. When adaptive production of anti-blood group B-reactive immunoglobulins occurring in White Leghorn chickens fed a diet containing E.coli O86:B7 lipopolysaccharide [5], was demonstrated for the first time to occur spontaneously in humans [8], this way of isoagglutinin production could exclusively be documented for the histo (blood) group O(H). Although this blood group can no longer be considered a genetic entity, which in particular is contaminated by OA hybrid or weak A alleles [9, 10], even a small number of blood group O(H) patients, suffering from ulcerative colitis associated with increased enteral absorption, showed a statistically significant adaptive immune response, measured by an anti-B-reactive 7S (IgG)- and 19S (IgM) immunoglobulin, involving asymetrically cross-reactive, less pronounced anti-A-reactive IgG, whereas the anti-B-reactive IgG and IgM antibody levels in plasma from blood group A patients remained within normal range [11].

Chemical immunosuppressants, which are used prior to major ABO-incompatible transplantations to downregulate immunoglobulin synthesis by the recipient, do not completely eliminate the anti-A and anti-B reactivity of different immunoglobulin classes [12]. In fact, chemical immunosuppression does not affect the formation of the mercaptoethanol-sensitive, complement-binding anti-A/B "classic" isoagglutinins that preferentially induce hemagglutination at 22–24°C. These hemagglutinins must be removed via plasmapheresis or specific adsorption.

Neither *N*- nor *O*-linked native blood group A-like glycans have been demonstrated in prokaryotic microorganisms; in particular, mucin-type GalNAc-*O*-Ser/Thr glycosylation does not occur in bacteria [13]. The synthesis of *O*-linked GalNAc glycan-bearing ovarian glycolipids, discovered in C57BL/10 mice, is associated with the

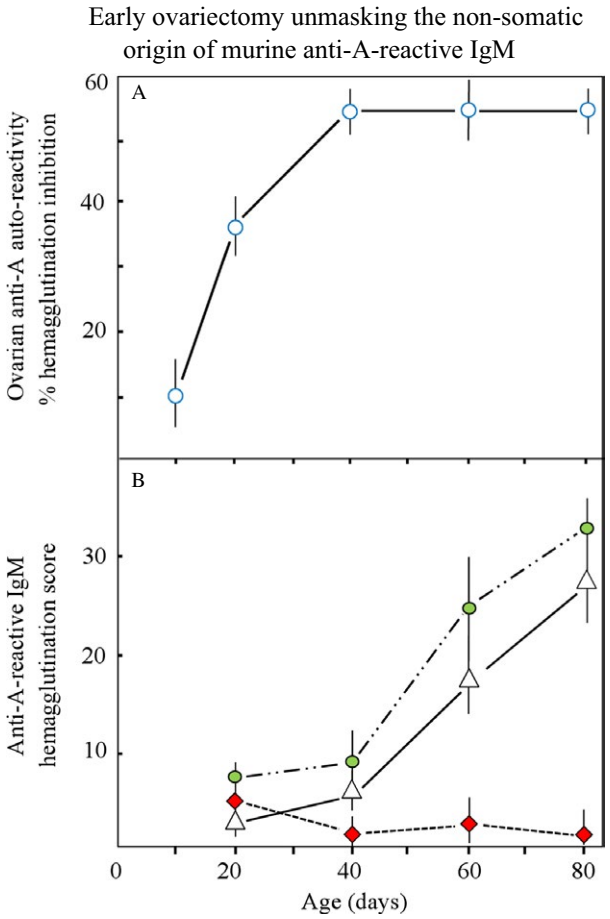

Figure 1. (A) Growth-related appearance of autoreactive, nonsomatic GalNAc glycan–bearing hydrophilic glycolipids in differentiating ovarian tissue, with peak levels appearing at puberty. (B) Subsequently arising complementary, innate anti-A reactive IgM: Serum of untreated animals (white triangles), sham-operated (green ovals), ovariectomized (red squares). This development of innate anti-A reactivity does not reflect (auto) immune response but signifies the completion of cell differentiations and shows IgM release during deglycosylations. Figure reconstructed from Arend and Nijssen (1977, *Nature*, 269, 255–257) [16], cited in Arend (2016, ABO (histo) blood group phenotype development and human reproduction as they relate to ancestral IgM formation: A hypothesis. *Immunobiology*, 221(1), 116–127, PMID: 26433867) [80].

formation of a syngeneic, complementary anti A-reactive IgM [14, 15], which demonstrates identical serological reaction patterns to human innate anti-A isoagglutinin but is not present in animals that have undergone ovariectomy prior to the onset of puberty [15, 16] (Fig. 1). Furthermore, the anti-A/B cross-reactive antibody produced when White Leghorn chickens were fed a diet containing *Escherichia coli* O86:B7 lipopolysaccharide [5], appeared identically in C57BL/10 mice when immunized with the same antigen; however, this immunization did not affect pre-existing levels of the syngeneic "natural" anti-A

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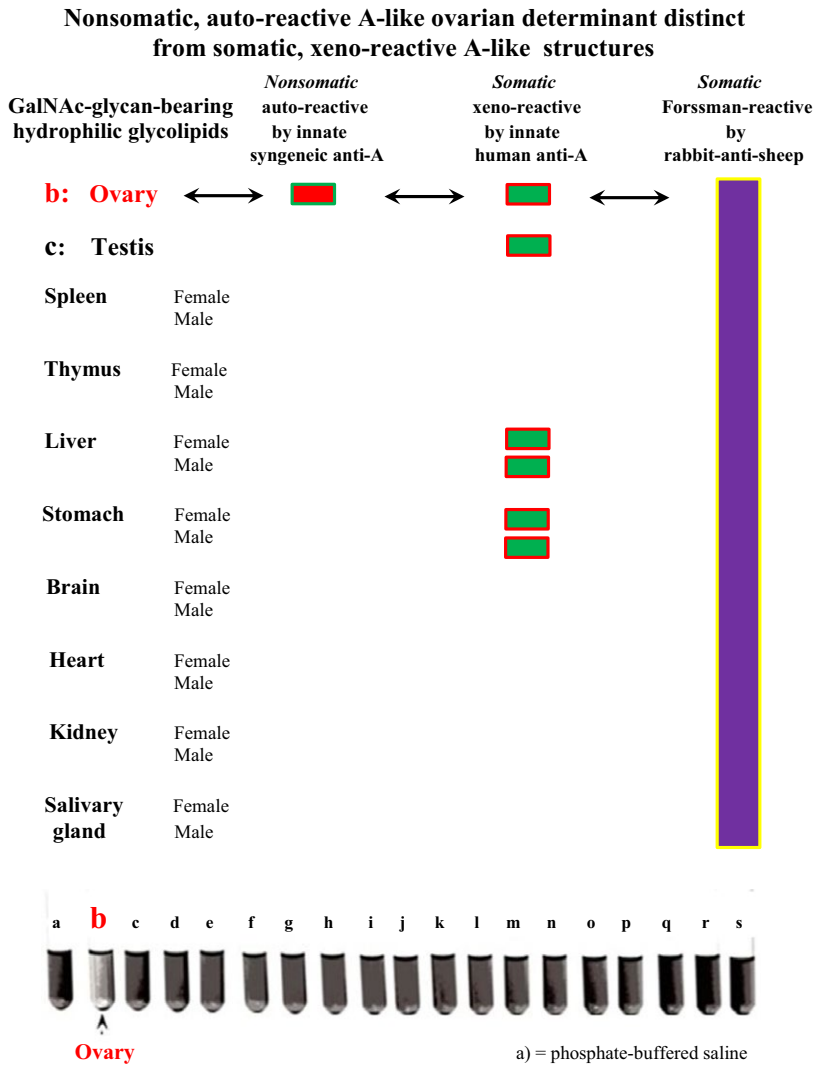

Figure 2. Distribution of autoreactive/nonsomatic and xenoreactive/somatic GalNAc glycan-bearing hydrophilic glycolipids in C57BL/10 murine ovarian and nonreproductive tissues. While all the murine tissues exhibit characteristic species-intrinsic Forssman reactivity and other xenoreactive A-like structures in male and female reproductive, and endodermal organs are detected using innate human anti-A antibodies, the murine anti-A antibody was exclusively inhibited by syngeneic ovarian glycolipids. The image was captured during the hemolysis inhibition experiments described in Arend (1980, *Immunobiology*, 156, 410–417) [18], cited in Arend (2016, *Immunobiology*, 219, 285–29, PMID: 26433867) [80].

antibody, which was simply separated from the adaptive, cross-reactive antibody via specific adsorption [17].

All murine tissues expressed the expected species-intrinsic Forssman-type structure, and additional A-like structures in the male and female reproductive organs and endodermal tissues were detected using human anti-A antibody, whereas the murine anti-A molecule was exclusively inhibited by syngeneic ovarian glycolipids [18] (Fig. 2). These crude glycolipid preparations showed developmental polymorphism, which was identified through reactions with *Dolichos biflorus* lectin and *Helix pomatia* agglutinin (HPA) [16], revealing the involvement of mucin-type *O*-GalNAc-determined serologically "A-like" glycans in complex protein glycosylation processes, such as characterizing T-cell development. A major cell surface glycoprotein (apparent mol. wt. = 150,000) on human lymphocytes has been reported to provide HPA-binding or the presence of HPA receptor activity on normal and malignant thymus-derived (T) lymphocytes [19]. Such binding was not found on various B cells at different steps of differentiation, whereas two of four B cell lymphoma lines and a myeloma line had another HPA-binding surface glycoprotein (mol. wt. = 200,000) instead of the 150,000–mol. wt. protein. The serologically "A-like" HPA receptor, or mucin-type GalNAcα1-*O*-Ser/

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Thr-R glycan, also referred to as the Tn antigen [20], has been reported as a surface marker on natural killer cells (NK) in normal mouse spleen after neuraminidase treatment [21, 22], while its expression appears to be dependent on the level of Ser/Thr-specific protein kinase C [23]. This enzyme obviously activates to the family of those glycotransferases, providing the first step of protein glycosylation that in metazoan eukaryotes is initiated by GalNAc via *O*-linkages [24, 25], and is essential in T cell activation and downregulation, performed through macrophage galactose lectin (MGL), also termed "Tn lectin".

Not spontaneously occurring in plant species [13], the Tn antigen is a common metazoan eukaryotic structure, which arises from *O*-GalNAc glycosylations, used already by mollusks and insects, like the fruit fly *Drosophila melanogaster* [26] but when arrested in nondevelopmental tissues of higher metazoans, such as mammals, signifies malignancy, while the degree of HPA binding correlates with the stages of various kinds of metastatic cancer, irrespectively of the organ [27–30]. Furthermore, when animal tumors are associated with Tn antigen expression [31], it is important to mention that the HPA binding sites are identical to that in human tumors and appear to change similarly with tumor stage. The histochemistry of murine WAP-T mammary cancer has revealed glycoconjugate changes similar to that in human breast cancer [32]. In plasma, the major HPA-binding proteins are blood group ABO(H)-reactive glycoproteins, such as clotting factor VIII (FVIII)[33] and von Willebrand factor (vWF) [34], carried by α2-macroglobulin (A2M) [35]. This is an abundant polyfunctional protein occurring in plasma of mammals and considered an evolutionarily conserved arm of the innate immune system [36], while in the human is expressing the ABO(H) phenotype in plasma, strictly in accordance with the expression on red cell surfaces [35]. Thus, when using HPA for identifying cancer biomarkers in sera and plasma [28], determining ABO(H) phenotype is of utmost importance.

Cross-specificity between mammalian anti-A/anti-Tn/T-reactive IgM and invertebrate defense proteins reveals the evolutionary/developmental position of Tn/T epitopes

The "bulky" GalNAc molecule [37] is a preferred substrate and target of hexosamine epimerization in microorganisms [38] due to undefined biophysical properties, which also dominate the carbohydrate metabolism in mammalian embryonic stem cell-germ cell (ESC-GC) transformation. While the role of specific carbohydrates in sperm-egg recognition remains the subject of discussion [39–41], the nonsomatic process of GC maturation is initiated by transient *O*-GalNAc glycosylation [42, 43], which occurs in particular on polypeptides [25] that express trans-species functional hydrophilic Ser and Thr residues [44]. As the most complex and differentially regulated step in protein glycosylation, up to 20 distinct polypeptide *O*-GalNAc transferases catalyze the first addition of GalNAc to a protein [13, 45, 46]. resulting in transient "immature"[42] *O*-GalNAc expressions, which are characterized by extremely short half-lives and identical with Tn antigen formation (Fig. 3). Contrary to a previous report [47], these ancestral, early ontogenetic and genetically undefined functions of "A-like" *O*-GalNAc transferases, while used by all metazoan eukaryotes, must be differentiated from species-intrinsic and human A-allelic enzyme functions, which are expressed only after formation of the zygote and involve both *N*- and *O*-glycosylations determining phenotype formation based on human-specific fucosylations (Fig. 4).

Historically, the Tn antigen, or "T nouvelle", was named upon its discovery in 1957[20] to emphasize its distinction from the functionally similar T (Thomsen-Friedenreich) antigen reported in 1930 [48], which refers to the disaccharide Galβ1-3GalNAcα-1-*O*-Ser/Thr (Fig. 3). Thus, the Tn antigen appears structurally to be less developed than the T antigen; the former predominates in carcinogenesis [49] and is associated with poorer prognosis compared with the latter. Recent reviews have widely discussed and summarized the complex biochemistry of these "A-like" glycans, their impact on cell differentiation, and their roles in metabolic pathways related to different cancer types and stages, as well as the development of vaccines targeting "A-like" glycans [50, 51].

In healthy organisms, these cryptic and potentially "aberrant" structures may be specifically reflected by natural anti-Tn and anti-T antibodies that are among the anti-glycan moieties present in the plasma of all mammals [4]. In humans, anti-Tn and anti-T antibody levels are highly dependent on the ABO(H) blood group and are primarily expressed through their cross-reactivity with anti-A/B isoagglutinins [52–54]. Blood group O(H) sera bind to both the Tn and T antigens, whereas blood group A individuals exhibit poor natural anti-Tn reactivity [55] that in blood group O(H) individuals [56] contributes to elevated anti-A reactivity. Recent clinical investigations of patients with pancreatic cancer by Hofmann et al. [57] demonstrated that the anti-A isoagglutinin levels in blood group O(H) and blood group B sera are associated with strong anti-Tn antibody, which does not react with B or T glycoconjugates. In contrast, the anti-B antibodies of blood group A sera and O(H) sera bind to B and T glycoconjugates but not to A or Tn glycoconjugates. The authors suggested that this selective cross-reactivity of isoagglutinins with Tn and T structures is due to their phenotype-specific terminal moieties; indeed, the terminal

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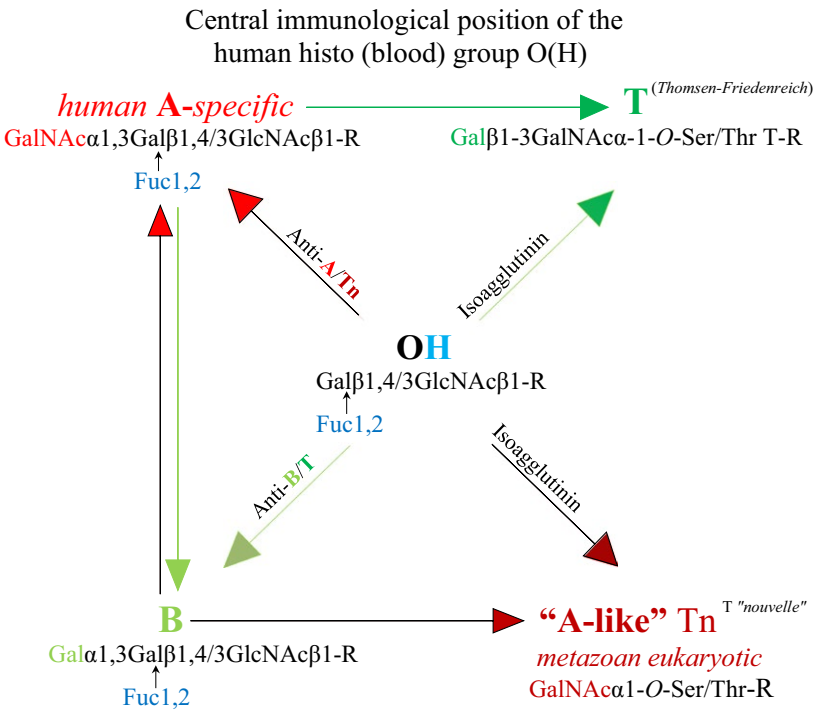

Figure 3. The central immunological position of blood group O(H) is evident in its comprehensive presentation of adaptive and innate "natural" antibodies against all mature A and B glycans and their cross-reactive developmental structures Tn and T. The human A-specific (A-allelic) glycosylation and trans-species "A-like" Tn formation are developmentally connected via the formation of cross-reactive anti-A/Tn isoagglutinin. According to Hofmann et al. [57], blood O(H) sera bind to both Tn and T antigens, and the anti-A isoagglutinin levels in blood group O(H) and blood group B sera are associated with anti-Tn antibody, which does not react with blood group B red cells or T glycoconjugates. In contrast, the anti-B antibodies of blood group A sera and blood group O(H) sera bind to B and T glycoconjugates but not to A or Tn glycoconjugates. The authors explain this selective cross-reactivity of isoagglutinins with Tn and T antigens via phenotype-specific terminal moieties; the terminal *N*-acetylgalactosamine is shared by A and Tn antigens, and the terminal galactose is, although with different configuration, shared by B and T antigens.

N-acetylgalactosamine is shared by A and Tn antigens, and the terminal galactose is, although with different configuration, shared by B and T antigens (Fig. 3). Friedenreich and Munck had suggested the presence of a potentially authentic anti-T antibody [48] but to date this antibody has not been confirmed. Thus, in view of the most likely common molecular origin of anti-A and anti-Tn reactivity, it is tempting to speculate that the natural anti-Tn-reactive IgM and natural human anti-A isoagglutinin represent a single antibody quality. However, monoclonal anti-Tn-specific antibodies have been produced; mice immunized with membrane preparations of human lung samples reacted specifically with the majority of human adenocarcinoma specimens, irrespective of the ABO status of the host, as well as with normal tissues and red cells of blood group A individuals [58]. Furthermore, a monoclonal anti-IgG3 antibody directed against the Tn antigen and not cross-reactive with the A antigen was generated after mice were immunized with purified Tn antigen [59]. A similar immunoglobulin was generated through somatic cell hybridization after mice were immunized with a tumor cell line carrying a Tn-specific mucin [60]. Thus, although the Tn- and T-bearing *O*-glycans may only represent the metabolic accumulation of short *O*-glycans, which develop in various cancers for innumerable reasons, these molecules and/or their derivatives clearly show authentic antigenic potential but are potentially synthesized by different *O*-GalNAc transferase qualities. In view of the more recent experiments by Blixt et al. [61]., the chemical simplicity of the Tn antigen does not necessarily stand for an antigenic unity. The authors generated different anti-Tn monoclonal antibodies of IgM and IgG classes and showed that monoclonal IgM binds to the terminal GalNAc residue of the Tn antigen irrespective of the peptide context and with low selectivity to the glycoproteins, while monoclonal IgG recognizes the Tn antigen in the context of a specific peptide motif. Thus, the Tn antigen-antibody binding capacity appeared to be determined by the peptide context of the Tn antigen, moreover, antigenic specificity of the antibody and class of the immunoglobulins. Nevertheless, the broad specificity of the "naturally occurring" anti-A/Tn cross-reactive IgM molecule most likely covers the

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major spectrum of antigenic sites and lets distinct anti-A and Tn reactivities look like a single antibody quality.

Tn- and T- glycosylation is not restricted to higher metazoan organisms. These *O*-glycosylations are already used by mollusks [26], and the T antigen appears to be normally expressed on the surface of eggs and liver cells of *Schistosoma mansoni*. Sera from patients infected with this worm produce antibodies against cancerous tissue, whereas experimentally infected mice generate antibodies against Tn and T antigenic epitopes [62]. Furthermore, upon their accumulation in vertebrate tissue, invertebrate immune systems recognize Tn and T antigenic epitopes or aberrant "A-like" structures specifically via two pathways. The egg-protecting hemagglutinating protein from *H. pomatia* has been established as a tumor cell marker and a prognostic indicator of different human tumor cell lineages [63], and its hexameric structure [64] may give rise to speculation regarding an evolutionary relationship to the mammalian nonimmune or ancestral immunoglobulin M. These molluscan agglutinins are produced in the albumen gland (connected to the oviduct), and emerging from the coat proteins of fertilized eggs. They most likely reflect the snail-intrinsic, reversible *O*-GalNAc glycosylations [64, 65], initiating protein glycosylations even in mollusks [26]. While the agglutinins are engaged in self-defense, the agglutinin-free hemocyanin from *H. pomatia* (HPH) exerted strong anti-proliferative effects in murine models of colon carcinoma [66]. In addition, *Concholepas* hemocyanin inhibits the growth of bladder tumors [67], and the Gal(β1-3)GalNAc-bearing hemocyanin of *Megathura crenulata* (keyhole limpet hemocyanin, KLH) shows cross-reactivity with T antigen [68], inducing a potent Th1-dominant immune response [69] and was used as an effective immunogenic carrier in dendritic cell vaccination developed for immunotherapy of human B cell lymphoma [70].

The metabolic relationship of the Tn and T antigens to other developmental antigens, such as the heterogenetic Forssman antigen, with the structure GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-R, remains unknown. While Tn and T are common trans-species, metazoan structures that occur even in mollusks [26], one must differentiate between Forssman-positive (F+) metazoans, such as mice, and the Forrsman negative (F-) human. Hakomori et al. described chemically and immunologically detectable levels of the Forssman glycolipid as a normal component of the human gastrointestinal mucosa [71], while they discovered Forssman glycolipids in the tumors of F- individuals but did not find them in F+ individuals. Although such F+ tumors arise independently of the ABO(H) blood group, they exert strong cross-reactivity with blood group A determinants, whereas the Forssman antibody also occurs independently of the ABO(H) blood group [72].

Nonsomatic trans-species, A-like *O*-GalNAc glycosylations are distinct from somatic species-intrinsic and blood group A phenotype-determining GalNAc glycosylations

The above-described developmental, nonsomatic, and genetically undefined A-like *O*-GalNAc transferases are present in any developing metazoan independent of species and phenotype. In fact, these ancestral transferases must be differentiated from strain- and species-intrinsic enzymes, and contrary to a previous report [47], they must be differentiated especially from the human blood group A phenotype-determining enzyme proteins or functions, as illustrated in Figures 3 and 4.

After generation of the zygote, the complex construction of human ABO(H) phenotypes is accomplished in the Golgi apparatus trans-cisternae and vesicles through the membrane-bound, human-specific, A-allelic α1-3-*N*-acetylgalactosaminyl transferase T2 and B-allelic α1,3-galactosyl-transferase, encoded on chromosome 9. This occurs in human-specific, epistatic cooperation with the fucosyltransferase 1 (FUT1) and 2 (FUT2), encoded by the H and Se genes on chromosome 19. The membrane-located *N*-linked glycosylations are associated with soluble enzyme versions, which independently of the secretor status, are involved in identically specific *N*- and *O*-linked glycosylations on (muco) epithelial cells and plasma proteins [73, 74], such as clotting factor VIII [33] and vWF [34], carried by A2M [35, 75]. It is important to mention, that the dynamic, functional connection between the A2M structure and FVIII/vWF activity is based on both *N*- and *O*-glycosylations [33], while the levels of A2M-bound ABO(H) blood group reactivity correlate strictly with the phenotype expression on red cell surfaces [34]. Consequently, O'Donnell et al. [34] could show that the ABO(H) blood group reactivity associated with A2M carrying vWF, is markedly reduced in plasma from the *Bombay* blood type that lacks ABO(H) epitope synthesis [76]. Although blood group ABO(H)-specific plasma glycoproteins are primarily cellular products, the functionality of soluble plasma glycotransferase is evident in the experiments by Nagai et al. 1978 [77], who transferred UDP-GalNAc to a blood group O red cell surface by means of an enzyme purified from blood group A_1 plasma, and converted blood O into blood group A in vitro. Furthermore, when A2M is considered an evolutionarily conserved arm of the innate immune system [36], its functional synergism with the structurally related IgM molecule [78] providing Ser/Thr residues [79], might be essential in relation to ontogenetic immunoglobulin modulation that was termed glycosidic exclusion [80] and/or accommodation, and suggests the functions of soluble plasma or serum transferases. According to this concept, which was inspired

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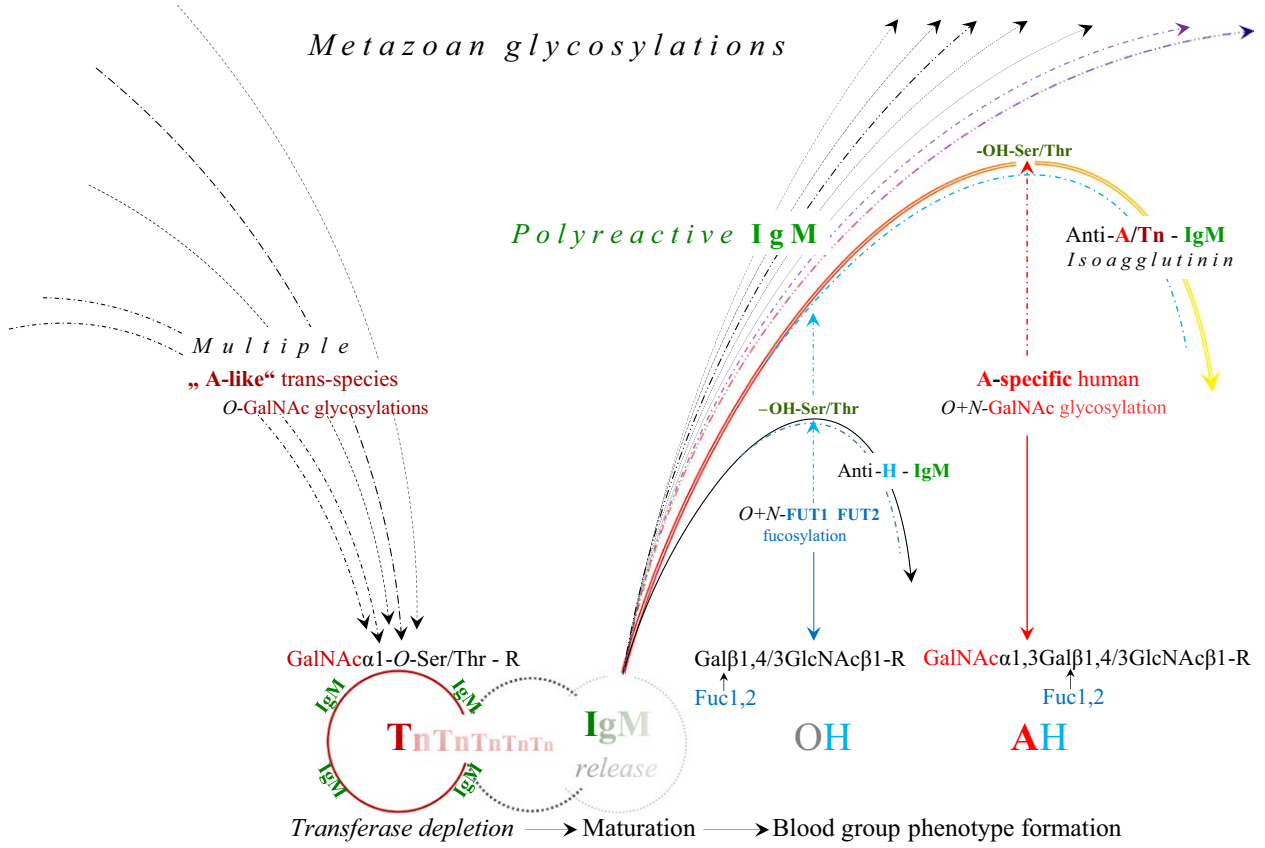

Figure 4. Hypothetical germline encoding of nonimmune, natural anti-A/cross-reactive anti-Tn IgM and phenotype-specific, glyosidic accommodation of plasma proteins. The *metazoan* trans-species "A-like" *O*-GalNAc glycosylations of proteins, involving the formation of the mucin-type *O*-glycan, GalNAcα1-*O*-Ser/Thr-R, also referred to as the Tn antigen, are distinct from the species-specific *N*+*O*-linked GalNAc glycosylation or blood group A phenotype formation. The naturally occurring anti-A isoagglutinin and the anti-Tn reactivity in human plasma cannot be separated from each other; they are expressed together by a secretory, primarily polyreactive IgM molecule, which arises in connection with the transient expression of the Tn antigen during the course of ESC-GC transformation and cell renewal processes.The in normal conditions extremely short half-live of this expression concerns the binding of the nonimmune IgM molecule, which has developed as a cell adhesion molecule and is released after Tn depletion. This secretory IgM molecule retains the Ser/Thr-OH functional groups that in the normal human undergo the ABOH) phenotype formation, occurring on both cell surfaces and plasma proteins, and involves *N*- and *O*-glycosylations. This process is based on human-specific FUT1 and FUT2 fucosylations, which exclude the formation of significant anti-H antibody levels, restricted to the rare *Bombay* type (Oh) individual [76]. The plasma of the blood group O(H) individual exerts strong anti-A/Tn reactive IgM, or anti-A isoagglutinin activity. In blood group A, the appearance of this ancestral anti A activity is, independently of classic clonal selection, reduced or excluded by human-specific, A-allelic GaNAc glycosylation, termed glycosidic exclusion [80] or accommodation, which hypothetically provides the conversion of synthesized glycoconjugates into phenotype-specific plasma (glyco) proteins and/or molecular complexes that become subject to internalization [82].

by a report that natural IgM loses its polyspecificity in undiluted sera [81], the formation of natural anti-self-reactive anti-A/B reactivity is, aside from classic clonal selection of adaptive immunoglobulin production, reduced or excluded by phenotype-specific glycosylation or accommodation of plasma proteins (Fig. 4). The resulting glycoconjugates may be subject to complex internalization [82], whereas in blood group O(H) individuals, the unaffected anti-A and Tn-cross-reactive IgM remains involved in the internal and external immune defense processes. Finally, the binding of this nonimmune IgM to an antigen might, like a primary immune response, initiate a secondary response and induce the production of anti-A/Tn-reactive IgG [49, 56] associated with T and NK cell activation [19, 21, 22], which in the non-O blood groups hypothetically is affected by glycosidic competition between phenotype and HPA receptor formation occurring on the T and NK cell surfaces.

The human and mouse genomes are described as laying the foundation of genome zoology [83], and although the mouse might be an unsuitable model for the discordance in the ABO(H) phenotype observed in primates [37,84], the favorable experimental conditions resulting from the anatomy and physiology of the C57BL/10J inbred mouse strain has contributed to the identification of the

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germline-encoded origin of an antibody molecule. This antibody is directed against a common trans-species and human ontogenetic and/or developmental antigen. As a consequence of early ovariectomy, nonsomatic transferase activities during GC maturation might be responsible for synthesizing A-like trans-species functional GalNAc-modified glycans that have been identified on hydrophilic ovarian glycolipids and are transiently expressed by ESCs and/or pluripotent stem cells (SCs). Together with recent advances in SC physiology, these early observations in mice have led to the hypothesis that the developmental "A-like" *O*-GalNAc-determined oligosaccharides and polypeptide precursors of the natural anti-A "antibody" are conjunctively synthesized and combine υ-gene activation and *O*-GalNAc-glycosylation of the immunoglobulin heavy chain at its complementary regions [80]. After cell differentiation and/or maturation are completed, these transient "immature" transferase activities are rapidly depleted [42, 43], resulting in downregulation of the developmentally synthesized GalNAcα1-*O*-Ser/Thr-R glycan or Tn antigen, and causing the loss of the glycosidic bonds between cell surfaces and complementary proteins. Consequently, the ancestral anti-A-reactive IgM, which has developed as an *O*-linked cell surface molecule, is released into the circulation (Figs. 1 and 4) and displays the respective breaking points, as there are the hydroxy (-OH) groups of the germline-specific Ser and/or Thr residues (Fig. 4).

The polyreactivity of the secretory "natural" IgM molecule is assumed to be provided primarily by hydrophilic amino acids. Ser residues, in particular, located on the V regions [79] and assumed to guarantee energy-rich polyspecificity [85], are appropriate targets for *O*-GalNAc glycosylation, while the characteristic lack of *O*-glycans and the presence of Ser/Thr residues on the secretory IgM strongly argue for a "broken linkage" to the developmental "lost" GalNAcα1-*O*-Ser/Thr-R glycan or Tn antigen. For example, the presence of *O*-GalNAc glycan-bearing glycolipids in differentiating murine ovarian tissue and appearance of the complementary IgM in plasma [14–16], could represent such "broken linkage". Enzymes catalyze forward and backward reactions, and in view of the dynamics of *O*-GalNAc glycosylation [24, 25, 86, 87], the binding of some short *O*-glycans on cell surfaces and antibody molecules might occur only fleetingly in reversible *O*-glycosylations [88]. Moreover, apart from *N*-glycosylations, dominating the complex ABO(H) phenotype construction, the hydroxy groups (-OH) of Ser and Thr residues may serve as predetermined breaking points, on which trans-species glycans hypothetically are replaced by species-specific ones in a fast deglycosylation/glycosylation process that may be termed "single cycle event"[89]. In the human blood group O(H), such predetermined breaking points are suggested in the anti-A-complementary domain of the IgM molecule and the vis-à-vis ABO(H)-convertible red cell surface [77], on which "lost" ancestral glycans are not replaced by phenotype-specific ones. While in the phenotype A(H) such replacement has been accomplished and excluded the formation of anti-self-reactive IgM, this hypothesis explains the pronounced occurrence of anti-A and cross-reactive anti-Tn in blood group O(H). Clearly, the central immunological position of the human histo (blood) group O(H) [11] is evident in its comprehensive production of both nonimmune and adaptive, environmentally acquired antibodies against all mature A and B glycans involving their cross-specific developmental glycans, Tn and T, as illustrated in Figure 3. While IgM polyreactivity in the phenotype A(H) individual thus is impaired, the anti-A/Tn cross-reactivity in the phenotype O(H) individual potentially contributes to a currently discussed survival advantage [90, 91] in the overall risk of developing cancer when compared with non-O(H) blood group individuals.

Conclusions

IgM molecule production *per se* is not restricted to B cells and lymphoid tissues; functional IgM secretion has been demonstrated in normal [1, 2] and malignant human epithelial cells [3], while the formation of immunoglobulins that arise *de novo* from ovarian tissue appears to be established [92]. According to Jerne, "*Germ cells of an animal carry a set of v-genes determining the combining sites of antibodies directed against a complete set of a certain class of histocompatibility antigens of the species to which this animal belongs*" [93]. Intriguingly, most ovarian and testicular tumors in humans appear to be B-cell lymphomas [94, 95] or develop as GC tumors together with non-Hodgkin lymphoma cells [96], while a primary ovarian tumor has been detected in a single lymph node [97]. Moreover, the microenvironment of GC tumors harbors a prominent antigen-driven humoral response;[98] thus, these authors speculated that the evolutionary and/or developmental mystery of the relationship between GCs and B lymphocytes might be explained through the molecular biology of B-cell tumors. However, because the ovary represents the last evolutionary/developmental location in mammals [80], where parthenogenetic potential remains, even in humans [99, 100], such an explanation may also reside in the topographically and molecularly connected synthesis of the trans-species evolutionary/developmental GalNAc1α-*O*-Ser/Thr-R Tn epitope and its authentic complementary protein or nonimmune ancestral IgM molecule occurring in mammalian ovarian tissue. This dynamic connection might be documented by the early experiments of the author, in particular, a timed ovariectomy performed on C57BL/10 mice [14–16], and in view of the molecular biological data accumulated over the decades in the

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literature, the 40-year-old prediction that the majority of the human isoantibody populations basically reflects growth processes [16] may be substantiated. In fact, the ancestral, innate anti-A/Tn cross-reactive IgM dominates these antibody populations and may give rise to speculation of an evolutionary relationship to the hexameric structure [64] of the *O*-glycan–reactive HPA. This hemagglutinin emerges from the coat proteins of fertilized eggs and most likely reflects the snail–intrinsic, reversible *O*-GalNAc glycosylations [26, 65], synthesizing the hemocyanins, while all GalNAc expression in *Helix pomatia* and other snails appears to be normal and does not signify malignancy. In these lower metazoans, the fundamental evolutionary missions of reproduction and defense occur topographically and molecularly connected with the function of the albumen gland [101, 102], which produces the multifunctional egg coat proteins that protect the egg against fungal or bacterial attacks. It is intriguing how the female C57BL/10 mouse mimics this developmental connection of reproduction and primitive immunological defense, in which similarly to HPA release from fertilized eggs, the anti-A/Tn cross-reactive, nonimmune protein or ancestral IgM is released after completion of CG maturation (Figs. 1 and 2). In humans, these functions are strongly divided topographically and molecularly. Beyond that, in the non-O blood groups, the physiological anti-A and cross-reactive anti-Tn complementarity of the ancestral IgM molecule undergoes a complex phenotype-specific enzymatic accommodation [80]. It is, aside from clonal selection, primarily this human phenotype-specific, glycosidic accommodation of plasma proteins that clearly affects the natural IgM polyreactivity, and the reduction of physiological anti-self-reactivity potentially increases the risk of developing "aberrant" structures and/or cancerous tissue, which might be the price of species specializing and phenotype diversity.

Acknowledgments

I thank Prof. James A. Clifton, MD (1923 – 2014), Gastroenterology Research Laboratory, College of Medicine, University of Iowa, Iowa City, IA, USA, who provided the conditions for the early experiments on the C57BL/10J strain of mice that became the basis for this research [17].

Conflict of Interest

None declared.

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