**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...
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 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 ovariecotmy 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.

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), ovariecotomized (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].
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. 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].

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| GalNAC-glycan-bearing hydrophilic glycolipids | **Nonsomatic auto-reactive** | **Somatic xeno-reactive** | **Somatic Forssman-reactive** |
|-----------------------------------------------|-----------------------------|--------------------------|-------------------------------|
|                                               | by innate syngeneic anti-A  | by innate human anti-A    | by rabbit-anti-sheep          |
| **b:** Ovary                                  | ![Green](#)                  | ![Green](#)               |                               |
| **c:** Testis                                 | ![Green](#)                  | ![Green](#)               |                               |
| Spleen                                        | ![Green](#)                  | ![Green](#)               |                               |
| Thymus                                        | ![Green](#)                  | ![Green](#)               |                               |
| Liver                                         | ![Green](#)                  | ![Green](#)               |                               |
| Stomach                                       | ![Green](#)                  | ![Green](#)               |                               |
| Brain                                         | ![Green](#)                  | ![Green](#)               |                               |
| Heart                                         | ![Green](#)                  | ![Green](#)               |                               |
| Kidney                                        | ![Green](#)                  | ![Green](#)               |                               |
| Salivary gland                                | ![Green](#)                  | ![Green](#)               |                               |

**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].
Germline Encoded Anti-A/Tn cross-reactive IgM

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 Galβ1-3Galα1-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 antiglycan 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
Germline Encoded Anti-A/Tn cross-reactive IgM

1605

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Germline Encoded Anti-A/Tn cross-reactive IgM

P. Arend

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 suggested the presence of a potentially authentic anti-T antibody 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
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 glycosylation from the coat proteins of fertilized eggs. They most likely reflect the snail-intrinsic, reversible O-GalNAc 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 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 Forssman 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-acetylgalactosaminy 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, 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
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
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 v-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 fleeting 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 GalNAcα1-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
Germline Encoded Anti-A/Tn cross-reactive IgM

P. Arend

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.

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Conflict of Interest

None declared.

References

1. Zhou, R., S. P. O’Hara, and X. M. Chen. 2011. MicroRNA regulation of innate immune responses in epithelial cells. Cell. Mol. Immunol. 8:371–379.

2. Shao, W., F. Hu, J. Ma, C. Zhang, Q. Liao, Z. Zhu, et al. 2016. Epithelial cells are a source of natural IgM that contribute to innate immune responses. Int. J. Biochem. Cell Biol. 73:19–29.

3. Hu, F., L. Zhang, J. Zheng, et al. 2012. Spontaneous production of immunoglobulin M in human epithelial cancer cells. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0051423.

4. Muthana, S., and J. Gildersleeve. 2016. Factors affecting Anti-Glycan IgG and IgM repertoires in human serum. Sci. Rep. 6:19509.

5. Springer, G., H. Horton, and M. Forbes. 1959. Origin of anti-human blood group B agglutinins in white Leghorn chicks. J. Exp. Med. 110:221–244.

6. Springer, G. F., P. Williamson, and W. C. Brandes. 1961. Blood group activity of gram-negative bacteria. J. Exp. Med. 113:1077–1093. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2137423&tool=pmcentrez&rendertype=abstract.

7. Springer, F., and R. Horton. 1969. Blood group isoantibody stimulation in man by feeding blood group-active bacteria. J. Clin. Invest. 48:1280–1291.

8. Arend, P., and G. Fehlhaber. 1969. Varying influence of increased enteral antigen absorption on the behavior of “natural” antibodies in O and A blood group subjects. Comparative blood group serological studies on patients with ulcerative colitis and healthy persons [Article in German]. J. Mol. Med. (Klinische Wochenschrift) 47:535–541.

9. O’Keefe, D. S., and A. Dobrovic. 1996. A rapid and reliable PCR method for genotyping the ABO blood group. II: A2 and O2 alleles. Hum Mutat. 8:358–361. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8956041.

10. Yazer, M. H., B. Hosseini-Maaf, and M. L. Olsson. 2008. Blood grouping discrepancies between ABO genotype and phenotype caused by O alleles. Curr Opin Hematol. 15:618–624.

11. Arend, P. 2017. Central immunological position of the human histo (blood) group O(H). https://doi.org/10.6084/m9.figshare.4714618.v1.

12. Chuang, J., C. Hung, S. Chang, T. Chou, and P. Lee. 2008. Does Immunosuppressive Pharmacotherapy Affect Isoagglutinin Titer?. Transplant. Proc. 40:2685–2687.

13. Bennett, E., U. Mandel, H. Clausen, T. Gerken, T. Fritz, and L. Tabak. 2012. Control of mucin-type O-glycosylation: A classification of the polypeptide GalNac-transferase gene family. Glycobiology 22:736–756.

14. Arend, P., and J. Nijssen. 1976. Significance of specific ovarian receptors for syngeneic naturally-occurring haemagglutinating anti-A antibodies. Immunogenetics 3:373–382.

15. Arend, P., and J. Nijssen. 1977. Age-dependent appearance of A-specific ovarian glycolipids and
syngeneic “natural” anti-A hemolysin in mice. Z. Immunitatforsch. Immunobiolog. 153:74–84.

16. Arend, P., and J. Nissen. 1977. A-specific autoantigenic ovarian glycolipids inducing production of “natural” anti-A antibody. Nature 269:255–257.

17. Arend, P. 1971. Observations on different origins of “naturally-occurring” antibodies. Eur. J. Immunol. 1:398–402.

18. Arend, P. 1980. An auto-reactive A-like ovarian determinant distinct from xeno-reactive A-like structures. Immunobiology 156:410–417.

19. Axelsson, B., A. Kimura, S. Hammarström, H. Wigzell, K. Nilsson, and H. Mellstedt. 1978. Helix pomatia A hemagglutinin: selectivity of binding to lymphocyte surface glycoproteins on T cells and certain B cells. Eur. J. Immunol. 8:757–764.

20. Moreau, R., J. Dausset, J. Bernard, and J. Moullé. 1957. Acquired hemolytic anemia with polyaagglutinability of erythrocytes by a new factor present in normal blood (Article in French). Bull. Mem. Soc. Med. Hop. Paris. 73:569–587.

21. Haller, O., M. Gidlund, U. Hellström, S. Hammarström, and H. Wigzell. 1978. A new surface marker on mouse natural killer cells: receptors for Helix pomatia A hemagglutinin. Eur. J. Immunol. 8:765–771.

22. Poros, A., L. Ahrlund-Richter, E. Klein, S. Hammarström, and N. Koide. 1983. Expression of Helix pomatia (HP) haemagglutinin receptors on cytolytic lymphocytes activated in mixed cultures. J. Immunol. Methods 57:9–19.

23. van Vliet, S. J., I. M. Vuist, K. Lenos, B. Tefsen, H. Kalay, J. J. García-Vallejo, et al. 2013. Human T cell activation results in extracellular signal-regulated kinase (ERK)-calcineurin-dependent exposure of Tn antigen on the cell surface and binding of the macrophage galactose-type lectin (MGL). J. Biol. Chem. 288:27519–27532.

24. Steentoft, C., S. Y. Vakhrushev, H. J. Joshi, et al. 2013. Precision mapping of the human O-GalNAc glycoproteome through simplecell technology. EMBO J. 32:1478–1488.

25. Brockhausen, I., H. Schachter, and P. Stanley. 2009. O-GalNAc Glycans. Pp. 1–16 in A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler, eds. Source Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press. Chapter 9.

26. Staudacher, E. 2015. Mucin-Type O-Glycosylation in Invertebrates. Molecules 20:10622–10640.

27. Peiris, D., M. Ossondo, S. Fry, M. Loizidou, J. Smith-Ravin, and M. Dwek. 2015. Identification of O-linked glycoproteins binding to the lectin Helix pomatia agglutinin as markers of metastatic colorectal cancer. PLoS ONE 10. https://doi.org/10.1371/journal.pone.0138345.

28. Welinder, C., B. Jansson, M. Ferno, H. Olsson, and B. Baldetorp. 2009. Expression of Helix pomatia lectin binding glycoproteins in women with breast cancer in relationship to their blood group phenotypes. J. Proteome Res. 8:782–787.

29. Laack, E., H. Nikbakht, A. Peters, C. Kugler, Y. Jasiewicz, L. Edler, et al. 2002. Lectin histochemistry of resected adenocarcinoma of the lung: helix pomatia agglutinin binding is an independent prognostic factor. Am. J. Pathol. 160:1001–1008.

30. Thies, A., I. Moll, J. Berger, and U. Schumacher. 2001. Lectin binding to cutaneous malignant melanoma: HPA is associated with metastasis formation. Br. J. Cancer 84:819–823.

31. Hellström, U., S. Hammarström, and G. Klein. 1978. Enrichment of Helix pomatia (HP) lectin binding variant from the TA3St mouse ascites tumor by repeated column selection. Eur. J. Cancer 14:1665–1772.

32. Schreiber, S., A. Gocht, F. Wegwitz, W. Deppert, and U. Schumacher. 2014. Lectin histochemistry of murine WAP-T mammary cancer reveals similar glycoconjugate changes to those in human breast cancer. Anticancer Res. 34:7045–7053.

33. O’Sullivan, J. M., P. V. Jenkins, O. Rawley, et al. 2016. Galectin-1 and galectin-3 constitute novel-binding partners for Factor VIII. Arterioscler. Thromb. Vasc. Biol. 36:855–863.

34. O’Donnell, J. S., T. A. J. McKinnon, J. T. B. Crawley, D. A. Lane, and M. A. Laffan. 2005. Bombay phenotype is associated with reduced plasma-VWF levels and an increased susceptibility to ADAMTS13 proteolysis. Blood 106:1988–1991.

35. Matsui, T., J. Hamako, Y. Ozeki, and K. Titani. 2001. Galectin-1 and -3 expression as markers of metastatic colorectal cancer. PLoS ONE 10. https://doi.org/10.1371/journal.pone.0067646.
39. Clark, G. F. 2011. Molecular models for mouse sperm-oocyte binding. Glycobiology 21:3–5.
40. Bianchi, E., B. Doe, D. Goulding, and G. J. Wright and Sanger Mouse Genetics Project 2. 2014. Juno is the egg Izumo receptor and is essential for mammalian fertilisation. Nature 508:483–487.
41. Aydin, H., A. Sultana, S. Li, A. Thavalingam, and J. Lee. 2016. Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex. Nature 16:562–565.
42. Reisner, Y., L. Itzcovitch, A. Meshorer, and N. Sharon. 1978. Hemoipoietic stem cell transplantation using mouse bone marrow and spleen cells fractionated by lectins. Proc. Natl Acad. Sci. USA 75:2933–2936.
43. Nash, R., L. Neves, R. Faast, M. Pierce, and S. Dalton. 2007. The Lectin dolichos biflorus agglutinin recognizes glycan epitopes on the surface of murine embryonic stem cells: a new tool for characterizing pluripotent cells and early differentiation. Stem Cells 25:974–982.
44. Saini, S., N. Maiti, and A. Kaushik. 2013. Partial characterization of immunoglobulin C gene of water buffalo (Bubalus bubalis) predicts distinct structural features of C1q-binding site in C 3 domain. Int J Microbiol Adv Immunol. 1:19–23. doi: dx.doi.org/10.19070/2329-9967-130004.
45. Tenno, M., K. Ohtsubo, F. Hagen, D. Ditto, A. Zarbock, P. Schaerli, et al. 2007. Initiation of protein O glycosylation by the polypeptide GalNACT-1 in vascular biology and humoral immunity. Mol. Cell. Biol. 27:8783–8796.
46. Schjoldager, K., and H. Clausen. 2012. Site-specific protein O-glycosylation modulates proprotein processing - Deciphering specific functions of the large polypeptide GalNAc-transferase gene family. Biochim Biophys Acta - Gen Subj. 1820:2079–2094.
47. Arend, P. 2014. Complementary innate (anti-A-specific) IgM emerging from ontogenic O-GalNAc-transferase depletion (Innate IgM complementarity residing in ancestral antigen completeness). Immunobiology 219:285–295. www.elsevier.com/locate/imibio.
48. Friedenreich, V., and J. Munck. 1930. Intravital Effects of „Transformed” Blood Corpuscels in Guinea-Pigs. Acta Pathol, Microbiol, Scand. 7:134–145.
49. Springer, G. F. 1997. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J. Mol. Med. 75:594–602.
50. Ju, T., V. I. Otto, and R. D. Cummings. 2011. The Tn antigen-structural simplicity and biological complexity. Angew Chemie Int Ed English. 50:1770–1791.
51. Chia, J., G. Goh, and F. Bard. 2016. Short O-GalNAc glycans: regulation and role in tumor development and clinical perspectives. Biochim. Biophys. Acta. 1860:1623–1639. https://doi.org/10.1016/j.bbagen.2016.03.008.
52. Dahr, W., G. Uhlenbruck, H. Gunson, and M. Van Der Hart. 1975. Molecular basis of Tn-polyagglutinability. Vox Sang. 29:36–50.
53. Rögner, W., L. Renwrantz, and G. Uhlenbruck. 1986. Comparison of a hemolymph lectin from Octopus vulgaris with hemocyanin. Comp. Biochem. Physiol. – Part B Biochem. 85:119–123.
54. Bird, G., N. Shinton, and J. Wingham. 1971. Persistent mixed-field polyagglutination. Br. J. Haematol. 21:443–453.
55. Jaff, M. 2010. Higher frequency of secretor phenotype in O blood group – its benefits in prevention and/or treatment of some diseases. Int. J. Nanomedicine. 5:901–905. https://doi.org/10.2147%2FINJ.S13980.
56. Smorodin, E., O. Kurtenkov, B. Sergeyev, A. Lilleorg, and V. Chuzmarov. 2001. Antibodies to tumor-associated epitopes in sera of cancer patients and blood donors. Exp. Onkol. 23:109–113.
57. Hofmann, B. T., A. Stehr, T. Dohrmann, C. Gungör, L. Herich, J. Hiller, et al. 2014. ABO Blood Group IgM isoagglutinins interact with tumor-associated O-glycan structures in pancreatic cancer. Clin. Cancer Res. 20:6117–6126.
58. Hirohashi, S., H. Clausen, T. Yamada, Y. Shimosato, and S. Hakomori. 1985. Blood Group A cross-reacting epitope defined by monoclonal antibodies NCC-LU–35 and -81 expressed in cancer of blood group O or B individuals: its identification as Tn antigen. Proc. Natl Acad. Sci. 82:7039.
59. Takahashi, H., R. Metoki, and S. Hakomori. 1988. Immunoglobulin G3 monoclonal antibody directed to Tn antigen (tumor-associated α-N-acetylgalactosaminyl) epitope that does not cross-react with blood group A antigen. Cancer Res. 48:4361–4367.
60. Welinder, C., B. Baldetorp, C. Borrebaeck, B. Fredlund, and B. Jansson. 2011. A new murine IgG1 anti-Tn monoclonal antibody with in vivo anti-tumor activity. Glycobiology 21:1097–1107.
61. Blixt, K., O. Lavrova, D. Mazurov, E. Cló, K. Stjepan, N. Bovin, et al. 2012. Analysis of Tn antigenicity with a panel of new IgM and IgG1 monoclonal antibodies raised against leukemic cells. Glycobiology 21:452–459.
62. Thors, C., B. Jansson, H. Helin, and E. Lindner. 2006. Thomsen-friedenreich oncofetal antigen in schistosoma mansoni localization and immunogenicity in experimental mouse infection. Parasitology 132(Pt 1):73–81.
63. Brooks, C., A. Schietinger, S. Borisova, P. Kufer, M. Okon, T. H irama, et al. 2010. Antibody recognition of a unique tumor-specific glycopeptide antigen. Proc. Natl Acad. Sci. USA 107:10056–11006.
64. Sanchez, J. F., J. Lescar, V. Chazalet, A. Audfray, J. Gagnon, R. Alvarez, et al. 2006. Biochemical and
structural analysis of Helix pomatia agglutinin: a hexameric lectin with a novel fold. J. Biol. Chem. 281:20171–20180.

65. Stepán, H., M. Pabst, F. Altmann, H. Geyer, R. Geyer, and E. Staudacher. 2012. O-Glycosylation of snails. Glycoconj. J. 29:189–198.

66. Gesheva, V., S. Chausheva, N. Mihaylova, I. Manoylov, L. Doumanova, K. Idakieva, et al. 2014. Anti-cancer properties of gastropodan hemocyanins in murine model of colon carcinoma. BMC Immunol. 15:34.

67. Molledo, B., F. Faunes, D. Haussmann, P. De Ioannes, A. De Ioannes, J. Puente, et al. 2006. Immunotherapeutic effect of concholepas hemocyanin in the murine bladder cancer model: evidence for conserved antitumor properties among hemocyanins. J. Urol. 176:2690–2695.

68. Wirguin, I., L. Suturkova-Milosevic, C. Briani, and N. Latov. 1995. Keyhole limpet hemocyanin contains Gal(beta 1–3)GalNAc determinants that are cross-reactive with the T antigen. Cancer Immunol. Immunother. 40:307–310.

69. Zhong, T., S. Arancibia, R. Born, R. Tampe, J. Villar, M. Del Campo, et al. 2002. Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. Blood. 99:1517–1526.

70. Hakomori, S., S. M. Wang, and W. W. Young. 1977. Isoantigenic expression of Forssman glycolipid in human gastric and colonic mucosa: its possible identity with “A-like antigen” in human cancer. Proc. Natl Acad. Sci. USA 74:3023–3027.

71. Kijimoto-Ochiai, S., W. Takahashi, and A. Makita. 1981. Anti-Forssman antibody in human sera: properties and cross-reactive natural IgM. Br. J. Haematol. 45:1501–1513.

72. Gill, D. J., H. Clausen, and F. Bard. 2011. Location, location, location: new insights into O-GalNAc protein glycosylation. Trends Cell Biol. 21:149–158.

73. Emes, R., L. Goodstadt, E. Winter, and C. Ponting. 2008. The acquisition of narrow binding specificity by polyspecific natural IgM antibodies in a semi-physiological environment. Mol. Immunol. 45:1501–1513.

74. Williams, R., B. S. Briney, S. L. DeLuca, J. E. Jr. Crowe, and J. Meiler. 2013. Human Germline Antibody Gene Segments Encode Polyspecific Antibodies. PLoS Comput Biol 9:e1003045. https://doi.org/10.1371/journal.pcbi.1003045.

75. Agarwal, K., R. Kaul, M. Garg, A. Shajahan, S. K. Jha, and S. G. Sampathkumar. 2013. Inhibition of Mucin-type O-glycosylation through metabolic processing and incorporation of N-thioglycolyl-D-galactosamine peracetate (Ac 5GalNTGc). J. Am. Chem. Soc. 135:14189–14197.

76. Bhende, Y. M., C. K. Deshpande, H. M. Bhatia, R. Sanger, R. R. Race, W. T. Morgan, et al. 2008. A "new" blood-group character related to the ABO system. 1952. Natl Med. J. India 21:3 p.

77. Nagai, M., V. Davè, B. Kaplan, and A. Yoshida. 1978. Human blood group glycosyltransferases. I. Purification of n-acetylgalactosaminyltransferase. J. Biol. Chem. 253:377–379.

78. Stevenson, L., E. Laursen, G. J. Cowan, B. Bandoth, L. Barfod, D. R. Cavanagh, et al. 2015. α2-Macroglobulin can crosslink multiple plasmadium falciparum erythrocyte membrane protein 1 (PfEMP1) molecules and may facilitate adhesion of parasitized erythrocytes. PLoS Pathog. 11:e1005022.

79. Wang, H., J. E. Coligan, and H. C. Morse. 2016. Emerging functions of natural IgM and its Fc receptor FCMR in immune homeostasis. Front Immunol. 7:99. https://doi.org/10.3389/fimmu.2016.00099PMID: 27014278.

80. Arend, P. 2016. ABO (histo) blood group phenotype development and human reproduction as they relate to ancestral IgM formation: a hypothesis. Immunobiology 221:116–127.

81. Chu, Q., J. J. Ludtke, V. M. Subbotin, A. Blockhin, and A. V. Sokoloff. 2008. The acquisition of narrow binding specificity by polyspecific natural IgM antibodies in a semi-physiological environment. Mol. Immunol. 45:1501–1513.
Molecular Biology Education, 29:126–128. Freeman & Co., New York, NY, 2000, 1084 pp. https://doi.org/10.1016/s1470-8175(01)00023-6.

89. Angeletti, R. H. 2012. Proteins: Analysis and Design. Pp. 363. in R. H. Angeletti, ed. Proteins: Analysis and Design. Academic Press, San Diego, London, New York, Sydney, Boston, Tokyo, Toronto, ISBN-10: 0123885418 - ISBN-13: 978-0123885418.

90. Hsiao, L., N. Liu, S. You, and L. Hwang. 2015. ABO blood group and the risk of cancer among middle-aged people in Taiwan. Asia. Pac. J. Clin. Oncol. 11:e31–e36.

91. Zhang, B., N. He, Y. Huang, F. Song, and K. Chen. 2014. ABO blood groups and risk of cancer: a systematic review and meta-analysis. Asian Pac. J. Cancer Prev. 15:4643–4650.

92. Hoek, A., J. Schoemaker, and H. A. Drexhage. 1997. Premature Ovarian Failure and Ovarian Autoimmunity. 1. Endocr. Rev. 18:107–134.

93. Jerne, N. K. 1971. The somatic generation of immune recognition. Eur. J. Immunol. 1:1–9.

94. Sun, J., J. Zhang, Q. Ling, Y. Luo, S. Wu, Z. Liang, et al. 2015. Primary diffuse large B-cell lymphoma of the ovary is of a germinal centre B-cell-like phenotype. Virchows Arch. 466:93–100.

95. Valli, R., E. Froio, deAlvarez Celis M., V. Mandato, and S. Piana. 2014. Diffuse large B-cell lymphoma occurring in an ovarian cystic teratoma: expanding the spectrum of large B-cell lymphoma associated with chronic inflammation. Hum. Pathol. 45:2507–2511.

96. Valdez, R., P. McKeever, W. Finn, S. Gebarski, and B. Schnitzer. 2002. Composite germ cell tumor and B-cell non-Hodgkin’s lymphoma arising in the sella turcica. Hum. Pathol. 33:1044–1047.

97. Carrabin, N., I. Treilleux, P. Meeus, O. Tredan, and I. Ray-Coquard. 2013. Primary ovarian borderline tumor in the inguinal lymph node. Int. J. Gynecol. Pathol. 32:167–170.

98. Willis, S., S. Mallozzi, S. Rodig, K. Cronk, S. McArdel, T. Caron, et al. 2009. The microenvironment of germ cell tumors harbors a prominent antigen-driven humoral response. J. Immunol. 182:3310–3317.

99. Kim, K., K. Ng, P. Rugg-Gunn, J. Shieh, O. Kirak, R. Jaenisch, et al. 2007. Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer. Cell Stem Cell 1:346–352.

100. Polak de Fried, E., P. Ross, G. Zang, A. Divita, K. Cunniff, F. Denaday. et al. 2008. Human parthenogenetic blastocysts derived from noninseminated cryopreserved human oocytes. Fertil. Steril. 89:943–947.

101. Mullainadhan, P., and L. Renwrantz. 1989. Comparative analysis of agglutinins from hemolymph and albumin gland of Helix pomatia. J. Comp. Physiol. B. 159:443–452.

102. Ishiyama, I., W. Dietz, and G. Uhlenbruck. 1973. Comparative studies of anti-a agglutinins from various snails of the genus helix (Helix pomatia and Helix aspersa). Comp. Biochem. Physiol. – Part B Biochem. 44:529–547. https://doi.org/10.1016/0305-0491(73)90027-8.