

Early ovariectomy reveals the germline encoding of the “natural” mammalian anti-A-reactive IgM reflecting developmental malignancy.* Peter Arend

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Abstract. The germline encoding of a non-immune immunoglobulin M (IgM) molecule in mammals was experimentally documented for the first time as a result of the specifically timed ovariectomy of C57BL/10 mice. The target of this innate antibody involves a trans-species developmental antigen that signifies malignancy when expressed in any non-developmental tissue. Although ovariectomy and castration result in uncontrolled and/or enhanced humoral and cellular immunity involving increased weights of the spleen and thymus with pronounced B and T cell production, the development of mercaptoethanol-sensitive, complement-binding, non-immune anti-A reactivity in murine plasma was not enhanced after an ovariectomy that was performed in C57BL/10 mice prior to the onset of puberty. This non-immune murine anti-A, which is complementary to the trans-species, syngeneic ovarian GalNAc-glycan-bearing glycolipids and distinct from the cross-reactive adaptive anti-A antibody, was strongly down-regulated or did not appear in plasma of animals ovariectomized at the age of 20 days. Thus, contrary to our previous view, this reactivity is unlikely to originate from a somatic, primary immune or autoimmune response. All of the murine tissues expressed the species-specific Forssman reactivity, and further A-like structures were identified in the male and female reproductive and endodermal organs by reaction with innate human anti-A antibody, while the murine anti-A was exclusively inhibited by syngeneic ovarian glycolipids. Moreover, the early ovarian tissue, which represents a last evolutionary and/or developmental location, showed a developmental polymorphism characterized by reactivity to the lectin from *Dolichos biflorus* and the agglutinin from *Helix pomatia* indicating the functional involvement of the O-GalNAc-determined mucin-type A-like, Tn and T (Thomsen-Friedenreich) epitopes that signify malignancy when accumulated and expressed in non-developmental tissues.

Keywords: *Helix pomatia* reactivity, non-developmental tissue, O-GalNAc glycosylation, non-somatic, genital ridge, developmental location.

Introduction.

The genomes of human and mouse may lay the foundation of genome zoology¹, and although the murine-specific equivalent of the human AB-glycotransferase² and the difference in the carbohydrate-determined subspecificities between murine and human innate IgM³ make the mouse an unsuitable model of the ABO(H)-phenotype discordance in primates, the human innate anti-A reactive IgM or isoagglutinin and the murine anti-A demonstrate a common and serologically identical response to a trans-species A-like, ancestral antigen. However, the mercaptoethanol-sensitive and complement-binding anti-A reactivity in murine plasma is, in contrast to our first^{4, 5} and recently abandoned concept^{6, 7} unlikely to originate from a somatic, primary immune or autoimmune response. While ovariectomy and castrations result in uncontrolled and/or enhanced humoral and cellular immunity, involving increasing weights of spleen and thymus with pronounced B and T cell productions⁸⁻¹³ associated with autoimmune-related bone loss¹⁴, the development of this early murine IgM or IgM-like molecule was not enhanced after ovariectomy performed in C57BL/10 mice prior to the onset of puberty. On the contrary, the innate anti-A reactivity, which is complementary to syngeneic ovarian GalNAc glycan-bearing hydrophilic glycolipids¹⁵ and distinct from cross-reactive, adaptive anti-A¹⁶, appeared strongly retarded or did not appear at all in the plasmas of animals ovariectomized at the age of 20 days^{4, 5}, Fig. 1. While all murine tissues expressed the expected, species-specific Forssman reactivity and further A-like structures were identified in the male and female reproductive organs and endodermal tissues by innate human anti-A¹⁷, the murine anti-A was exclusively inhibited by syngeneic ovarian glycolipids, Fig. 2. These crude glycolipid preparations showed a developmental polymorphism that was revealed by reactions with the lectin from *Dolichos biflorus* (DBA) and the agglutinin from *Helix pomatia* (HPA)⁵, which indicated the functional involvement of mucin-type *O*-GalNAc-determined, serologically A-like, Tn and T (Thomsen-Friedenreich) epitopes. Indeed, such epitopes occur on both malignant and normal T cells¹⁸ and can involve NK cell populations^{19, 20}. They are not expressed by mature B cells and are largely restricted to B cell lymphomas and lymphocytic leukemias and signify malignancy when accumulating in non-developmental tissues of humans²¹⁻²⁴ and mammalian animals^{25, 26}.

Cross-specificities between mammalian anti-A/anti-Tn/T-reactive IgM and invertebrate defense proteins revealing the evolutionary/developmental position of the Tn/T epitope.

The “bulky” GalNAc-molecule³, possessing undefined biophysical properties, is assumed to be preferential in substrate competitions and hexosamine epimerizations²⁷ and to dominate the mammalian embryogenic stem cell (ESC)-germ cell (GC) transformation. While the role of specific carbohydrate in sperm-egg recognition remains a subject of discussion²⁸⁻³⁰, the non-somatic pro-

cess of germ cell maturation is initiated by transient *O*-GalNAc-glycosylations³¹⁻³². These especially occur on polypeptides³⁴ that express glycosidic sites on trans-species functional hydrophilic serine- and threonine-residues³⁵. Up to 20 distinct polypeptide *O*-GalNAc-transferases catalyze the first addition of GalNAc to proteins, which makes this step the most complex and differentially regulated step in protein glycosylation³⁶⁻³⁸. These developmental or early ontogenetic/non-somatic and genetically as-yet-undefined A-like *O*-GalNAc transferase functions dominate the early carbohydrate metabolism of all vertebrates. They are independent of the species or the phenotype and are characterized by extremely short half-lives, resulting in transient “immature” *O*-GalNAc expressions³¹ and potentially imply metabolic relationships with the mucin-type monosaccharide GalNAc α 1-*O*-Ser/Thr or Tn antigen, which signifies malignancy when accumulated in non-developmental tissues and is thus called “aberrant”. The historical naming of the Tn antigen or “T nouvelle” results from its discovery reported in 1957³⁹ and emphasizes the difference from the functionally similar T (Thomsen-Friedenreich) antigen reported in 1930⁴⁰, which is represented by the disaccharide Gal β 1-3GalNAc α 1-*O*-Ser/Thr. As shown by these structures, Tn is a less developed T; in cancerogenesis it predominates⁴¹ and while characterizing less developed cancer cells, it means a poorer prognosis than the more developed T. The complex chemistry and molecular biology and the impact of Tn and T antigens on cell differentiation and the potential metabolic pathways of the different types and stages of cancer and the perspectives in the constructions of vaccines are widely discussed and summarized in recent reviews^{42, 43}. In healthy organisms, these cryptic A-like or aberrant structures may be revealed by natural anti-Tn and T specificities that are part of the anti-glycan moiety occurring in the plasma of animals and humans⁴⁴. In humans their levels are highly dependent on the ABO(H) blood group phenotype and are primarily expressed by the cross-reactivities of isoagglutinins⁴⁵⁻⁴⁷. Blood group O(H) sera bind to both the Tn and T antigen, while the natural anti-Tn reactivity shows lower levels in blood group A individuals⁴⁸ but contributes to the higher anti-A levels in blood group O(H)⁴⁹. Moreover, the recent clinical investigations of Hofmann *et al.*⁵⁰ performed on patients with pancreatic cancer might demonstrate that blood group A sera and the anti-B of blood group O(H) sera bind to B and T glycoconjugates but not to A and Tn, while blood group B sera and the anti-A of blood group O(H) sera bind to A and Tn glycoconjugates and not to B and T. The authors could explain this phenomenon of a selective cross-reactivity by the positions of the phenotype-specific molecular terminals: indeed, the terminal N-acetylgalactosamine is shared by the antigens A and Tn, and the terminal α 1,3-galactose is shared by the antigens B and T. Nevertheless, while the presence of a potentially authentic anti-T antibody has already been reported by Friedenreich & Munck⁴⁰, monoclonal antibodies have been established after immunization of mice with membrane preparations of the human lung and react specifically with a majority of human adenocarcinomas, irrespective of the host's blood group ABO status, as well as with normal tissues and erythrocytes of blood group A individuals⁵¹. Furthermore, an IgG3 monoclonal

antibody, directed to the Tn glycoprotein antigen and not cross-reacting with blood group A antigen, has been demonstrated after immunization of mice with a purified Tn antigen⁵², and a similar Ig was alternatively generated through somatic cell hybridization after immunization of mice with a tumor cell line carrying a Tn-specific mucin⁵³.

Thus, the Tn- and T-bearing *O*-glycans appear to involve authentic antigenic potentials but most likely are synthesized by various *O*-GalNAc transferases. In the mammalian non-developmental tissues they occur as aberrant structures but are not restricted to higher organisms, and while produced in the differentiating ovarian tissue of the mouse, the T antigen appears to be normally expressed on the surface of the eggs and liver cells of *Schistosoma mansoni*. Sera from patients, who are infected with this worm, produce antibodies against cancerous tissue, while experimentally infected mice develop antibodies against the Tn and T epitopes⁵⁴. Furthermore, the Tn or aberrant A-like *O*-glycosidic structure is, when arising in tissues of vertebrates, in two ways specifically recognized by the immune system of invertebrates and may indicate common metabolic positions. The agglutinin from *Helix pomatia* (HPA) has meanwhile been established as tumor cell marker and prognostic indicator for different human tumor cell lineages⁵⁵, while the agglutinin-free hemocyanin (HPH) from the same snail demonstrates strong anti-proliferative effects in murine models of colon carcinoma⁵⁶, and the hemocyanin from *Concholepas* inhibits the growth of bladder tumors⁵⁷. In addition, GalNAc determinants of the hemocyanin from *Megathura crenulata* (Keyhole Limpet Hemocyanin, KLH) show cross-reactivity with T cells⁵⁸ and induce a potent Th1-dominant immune response⁵⁹. This hemocyanin is meanwhile used in adjuvant immunotherapy of several types of cancer⁶⁰.

Non-somatic, trans-species *O*-GalNAc glycosylations are distinct from somatic, species-intrinsic GalNAc glycosylations.

Again, the above developmental, non-somatic and genetically as-yet-undefined, A-like *O*-GalNAc transferases, which occur in any vertebrate ontogeny, are independent from the species or the phenotype. In fact, they have to be differentiated from the species-intrinsic and especially blood group A determining enzyme proteins or functions that are expressed only after formation of the zygote⁶¹. In humans, the somatic A-allelic, alpha 1-3-*N*-acetylgalactosaminyltransferase T2 and B-allelic, alpha 1,3 galactosyltransferase functions are encoded on chromosome 9, and in epistatic cooperation with the FUT1 and FUT2 transferases encoded on chromosome 19, they accomplish the mature human ABO(H) histo (blood) group epitope formations on red cells, mucoepithelial cells and plasma proteins. Furthermore, this epistatic cooperation, governed by two chromosomes,

guarantees the specificities of the ABO(H) isoagglutinins by glycosidic accommodation and exclusion of phenotype- or self-reactive antibody activities⁶¹.

In view of the historical experiments of Springer *et al.*⁶²⁻⁶⁴, the production of the human histo (blood) group ABO isoantibody populations involving the Tn and TF cross-specificities, are still thought to be exclusively induced by environmental, predominantly intestinal, well-documented microbial antigens, especially lipopolysaccharides from gram-negative bacteria⁶³. However, the administration of chemical immunosuppressants, which prior to ABO-incompatible transplantations are used to down-regulate the immunoglobulins of the recipient, does not completely eliminate the anti-A and B reactivities occurring in different immunoglobulin classes, because chemical immunosuppression does obviously not affect the mercaptoethanol-sensitive, between 20 and 24 degrees C reactive classic anti-A/B isoagglutinins⁶⁵, which always have to be removed by plasmapheresis or specific adsorption. In addition, early clinical studies in a limited number of patients with inflammatory bowel disease in comparison with healthy persons indicated that an adaptive enteral immunization as a source of the ABO isoantibody production might largely be restricted to blood group O(H) individuals, who apart from the “natural” anti-A isoagglutinin, produce anti-A/B cross-reactive immunoglobulins in connection with an anti-B-specific 7S (IgG) antibody, which was not developed in blood group A patients⁶⁶. Moreover, when the oral immunization of White Leghorn chickens fed with an extract of *Escherichia coli* O86:B7, has induced and/or stimulated^{62, 64} the production of an anti-B antibody^{62, 64}, the C57BL/10 mouse immunized over the intravenous route with the same antigen, developed strong anti-A/B cross-reactivities, which did not affect the preexisting levels of the “natural” anti-A that by specific adsorption could simply be separated from the adaptively produced cross-reactivities¹⁶. These findings might strongly argue for a non-immune or germline encoded origin of both the classic human anti-A isoagglutinin and the murine anti-A, which may represent complementary proteins to the transient or “immature” GalNAc α 1-O-Ser/Thr expressions and arise in conjunction with the first protein glycosylations.

The genomes of human and mouse may lay the foundation of genome zoology¹, and although the mouse might be an unsuitable model of the ABO(H)-phenotype discordance in primates^{2, 3}, the favorable experimental conditions that are present in the anatomy and physiology of the C57BL/10J inbred mouse contributed to reveal the germline encoded origin of an antibody, which independent from the species and the phenotype is directed against a common trans-species and human ontogenetic and/or developmental antigen. The result of the early ovariectomy might show that during germ cell maturation, non-somatic transferase activities have potentially been responsible for synthesizing the A-like, trans-species functional GalNAc-glycans that were identified on hydrophilic ovarian glycolipids and are volatily expressed by ESCs and/or pluripotent stem cells. In view of the early observations in the mouse related to the acquisitions of current stem cell physiology, it has

been hypothesized that the developmental “A-like” *O*-GalNAc-determined oligosaccharides and polypeptide precursors of the “natural” anti-A-“antibody” are conjunctively synthesized and combine ν -gene activation and *O*-GalNAc-glycosylation of the immunoglobulin heavy chain at its complementary regions⁶¹. After completion of germ cell maturation, these volatile “immature” transferase activities obviously undergo rapid depletion resulting in the loss of the glycan bonds between cell surfaces and complementary proteins, and the ancestral anti-A-reactive IgM, which has developed as a cell adhesion molecule (CAM), is released into the circulation, Fig. 1. However, an ovariectomy that was performed in limited experiments in mice beyond the age of 80 days, was not followed by the disappearance of anti-A reactivity from the plasma (data not shown). Thus, such ovariectomy executed prior to the onset of puberty might have revealed a developmental pluripotency, which in mature non-reproductive tissues most likely occurs in cell reprogramming and/or self-renewal processes and identically occurs in other mammalian and vertebrate species. In humans, this non-somatic process is accompanied by somatic, specific tissue maturation, in which depending on the blood group phenotype, ancestral, trans-species *O*-glycans on cell surfaces and plasma proteins are permanently “replaced” or followed by the formation of ABO-phenotypic, predominantly *N*-linked glycans⁶¹.

According to early reports on the effect of immunoglobulin glycosylation on the enhancement and/or inhibition of tumor growth⁶⁷ in connection with the recent discoveries of immunoglobulin fucosylation, in which the IgG molecule in initiating antibody-dependent cellular cytotoxicity can be increased 50-fold simply by removing the single fucose residue from the Fc glycan⁶⁸, the avidity of antibodies appears to be inversely proportional to the degree of glycosylation⁶¹. Strong antibody glycosylation might protect from autoimmunity, however, obviously favors tumor growth. Blood group A individuals are known to have an increased risk to develop gastric and pancreatic cancer, while a recent report showed a similar risk for lung cancer⁶⁹, and current studies indicate that the overall risk to develop cancer might be increased in any so-called none-O blood group^{70, 71}. Thus, aberrant *O*-GalNAc-glycosylations, which are recognizable by the immune system of invertebrates and potentially reflect common ancestral, evolutionary and/or developmental positions, most likely occur in volatile, not measurable expressions in any normal human growth but are, depending on the degree and quality of the phenotype-determined glycosylation of immunoglobulins⁶¹, revealed by the release of cross-reactive isoagglutinins. While statistically significant data on the difference between the anti-A levels of the blood groups O and B are not available, in the blood group O, in which transient aberrant *O*-GalNAc expressions are not followed by blood A/B phenotype formations and thus A/B-specific glycosylations of plasma proteins do not occur, the anti-A and B isoagglutinins are released after deglycosylation

from these transient expressions, and the anti-Tn antibody reactivity should be pronounced because the anti-A levels are always significantly higher than the levels of anti-B.

Evolutionary and developmental relationships between primordial germ cells and lymphomyeloid cell lineages.

The reproductive and immune systems have long been thought to share a common evolutionary origin. In the evolution and development of amniotes, these systems arise in topographic-anatomical connection and have their common extraembryonic origin in the yolk sac. In lower vertebrates, Ig secretion occurs in neonates and in adults along the genital ridge⁷², and even in skates and sharks, which are some of the oldest non-mammalian vertebrates, the development of the lymphomyeloid system and the ovarian and testicular tissues occurs in the epigonal organs^{73, 74}. In birds, the shutdown of somatic B cell functions requires the early removal of the avian bursa of *Fabricius* anlage⁷⁵, which develops near the cranial part of the genital ridge and is not anatomically formed in mammals. The mammalian ovary develops from the caudal end of the genital ridge and represents the last evolutionary and developmental location. Unknown numbers and qualities of lymphomyeloid cell populations and/or progenitors of the IgM-secreting B cells that occur in the peritoneal cavity of the mammalian female are likely to pass through the ovary and emerge through the fimbriae at the posterior tubal infundibulum, and thus the genetic relationships between B-lymphocytes and GC have been debated for decades. Although the adult mammalian ovary seems to be devoid of definitive germline stem cells and the specific “ovarian reserve” is limited⁷⁶, the ovary is considered to be its own type of organism⁶, that may generate the complete offspring genome not only in phylogenetically lower species but even in man, where parthenogenetic potential remains^{77, 78}. Moreover, the cellular traffic in oogenesis involves innumerable intercellular connections and is dominated by those between SC populations and oocytes. These cells have the potential to transform into each other^{79, 80} or to differentiate into any somatic tissue-intrinsic cell⁸¹. In males, an IL-4-inducible gene comes to be expressed on differentiating lymphocytes and on spermatozoa⁸², while classical V-(D) rearrangements have recently been located in the mouse epididymis⁸³. The B lymphocyte-induced maturation protein-1 (Blimp-1) is expressed in migrating PGCs as well⁸⁴, and membrane receptors that are complementary to each other induce cell divisions in the case of interactions⁸⁵. In addition, the lymphocyte antigen 75 (Ly75/CD205)⁸⁶, which is expressed on mitotic germ cells in rainbow trout and Atlantic salmon⁸⁷, contributes to speculations about the involvement of germ cell maturation, for example, in the production of the anti-A hemagglutinin in *Salmo trutta*⁸⁸. Nevertheless, due to different and complex migratory pathways, the developmental relationships involving PGCs, ESCs and LMC are not yet completely understood. The production of the IgM molecule, *per se*, is not restricted to B cells;

immunoglobulins also occur in chicken eggs⁸⁹, and an IgM-like glycoprotein was isolated from the oocytes of salmon⁹⁰. Furthermore, the sporadic production of functional IgM has been demonstrated in normal and malignant human epithelial cells⁹¹. Finally, 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,*”⁹² and there is speculation that the genetic information that governs the IgM polymerization process is primarily divided between germ cell precursors and LMC.

Conclusions.

Intriguingly, most ovarian and testicular tumors in humans appear to be B cell lymphomas^{93, 94} or develop as germ cell tumors together with non-Hodgkin lymphoma cells⁹⁵, while a primary ovarian tumor has been detected in a single lymph node⁹⁶. Moreover, the microenvironment of germ cell tumors harbors a prominent antigen-driven humoral response⁹⁷. Thus, it was speculated that the evolutionary and/or developmental mystery of the relationship between germ cells and B lymphocytes might become explainable sometime in the molecular biology of B cell tumors. Such an explanation may also reside in the topographically and molecularly connected synthesis of the trans-species, serologically A-like GalNAc epitope and its complementary, ancestral protein or IgM molecule arising in differentiating mammalian ovarian tissue. As outlined above, the mammalian ovary represents a last evolutionary and/or developmental location and exhibits developmental polymorphism on the basis of DBA and HPA reactivity⁵, suggesting the production of mucin-type O-GalNAc-determined, serologically A-like, Tn and T epitopes that signify malignancy when accumulated and expressed in non-developmental tissue. It is widely accepted that cancer is not an independent disease but predominantly results from the defeat of the defense systems. Thus, aberrant glycosylations, which are recognized by the defense proteins of invertebrates and potentially signify common ancestral, metabolic positions, most likely arise developmentally in transient, not measurable expressions in any normal human growth and cell renewal processes but are, depending on the degree and quality of the glycosylation of plasma proteins²¹, especially immunoglobulins⁶¹, revealed by the release of cross-reactive isoagglutinins. While statistically reliable data on the difference between the anti-A levels of the blood groups O and B are not available, in the blood group O, in which transient aberrant O-GalNAc expressions are not followed by blood A/B phenotype formations and thus A/ B-specific glycosylations of plasma proteins do not occur, the anti-A and B isoagglutinins are released after deglycosylation from these transient expressions, and the anti-Tn reactivity should be pronounced because of the dominance of anti-A.

Abbreviations. Ig = immunoglobulin; IgM = immunoglobulin M; GC = germ cells; SC = stem cells; ESC = embryonic stem cells; PGC = primordial germ cells; LMC = lymphomyloid cell lineages; DBA = *Dilochos biflorus* lectin; HPA = *Helix pomatia* agglutinin; NK = natural killer cells.

References.

1. Emes, R., Goodstadt, L., Winter, E. & Ponting, C. Comparison of the genomes of human and mouse lays the foundation of genome zoology. *Human Molecular Genetics* **12**, 701–709 (2003).
2. Larkin, J. & Porter, C. Mice are unsuitable for modelling ABO discordance despite strain-specific A cross-reactive natural IgM. *Br. J. Haematol.* **130**, 310–317 (2005).
3. Yamamoto, M., Lin, X., Kominato, Y., Hata, Y., Noda, R., Saitou, N. & Yamamoto, F. Murine Equivalent of the Human Histo-blood Group ABO Gene Is a cis-AB Gene and Encodes a Glycosyltransferase with Both A and B Transferase Activity. *J. Biol. Chem.* **276**, 13701–13708 (2001).
4. Arend, P. & Nijssen, J. Age-dependent appearance of A-specific ovarian glycolipids and syngeneic ‘natural’ anti-A hemolysin in mice. *Zeitschrift für Immunitätsforschung. Immunobiology* **153**, 74–84 (1977).
5. Arend, P. & Nissen, J. A-specific autoantigenic ovarian glycolipids inducing production of ‘natural’ anti-A antibody. *Nature* **269**, 255–257 (1977).
6. Arend, P. Ancestral gene and ‘complementary’ antibody dominate early ontogeny. *Immunobiology* **218**, 755–61. (2012).
7. Arend, P. 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 (2014).
8. Eidinger, D. & Garrett, T. Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. *J Exp Med.* **136**, 1098–116. (1972).
9. Erben, R., Raith, S., Eberle, J. & Stangassinger, M. Ovariectomy augments B lymphopoiesis and generation of monocyte-macrophage precursors in rat bone marrow. *Am J Physiol.* **274**, 476–83 (1998).
10. Rifkind, D., Frey, J. & Davis, J. Influence of gonadal factors on skin test reactivity of CFW mice to *Candida albicans*. *Infect Immun.* **7**, 322–8 (1973).
11. Cervantes-Rebolledo, C., Moreno-Mendoza, N., Morales-Montor, J., De La Torre, P., Laglette, J. P. & Carrero, J. C. Gonadectomy inhibits development of experimental amoebic liver abscess in hamsters through downregulation of the inflammatory immune response. *Parasite Immunol.* **31**, 447–456 (2009).
12. Frisancho-Kiss, S. *et al.* Gonadectomy of male BALB/c mice increases Tim-3+ alternatively activated M2 macrophages, Tim-3+ T cells, Th2 cells and Treg in the heart during acute coxsackievirus-induced myocarditis. *Brain. Behav. Immun.* **23**, 649–657 (2009).

13. Shang-xue Yan & Wei Wei. Castration reverses immunosenescence in aged mice. *Acta Pharmacol. Sin.* **32**, 1085–1086 (2011).
14. Malet, A., Bournaud, E., Lan, A., Mikogami, T., Tomé, D. & Blais, A. Bovine lactoferrin improves bone status of ovariectomized mice via immune function modulation. *Bone* **48**, 1028–1035 (2011).
15. Arend, P. & Nijssen, J. Significance of specific ovarian receptors for syngeneic naturally-occurring haemagglutinating anti-A antibodies. *Immunogenetics* **3**, 373–82 (1976).
16. Arend, P. Observations on different origins of ‘naturally-occurring’ antibodies. *Eur J Immunol* **1**, 398–402 (1971).
17. Arend, P. An auto-reactive A-like ovarian determinant distinct from xeno-reactive A-like structures. *Immunobiology* **156**, 410–7. (1980).
18. Axelsson, B., Kimura, A., Hammarström, S., Wigzell H., Nilsson, K. & Mellstedt, H. Helix pomatia A hemagglutinin: selectivity of binding to lymphocyte surface glycoproteins on T cells and certain B cells. *Eur J Immunol.* **8**, 757–64 (1978).
19. Haller, O., Gidlund, M., Hellström, U., Hammarström, S. & Wigzell, H. A new surface marker on mouse natural killer cells: receptors for Helix pomatia A hemagglutinin. *Eur J Immunol.* **8**, 765–71 (1978).
20. Poros, A., Ahrlund-Richter, L., Klein, E., Hammarström, S. & Koide, N. Expression of Helix pomatia (HP) haemagglutinin receptors on cytolytic lymphocytes activated in mixed cultures. *J. Immunol. Methods* **57**, 9–19 (1983).
21. Welinder, C., Jansson, B., Ferno, M., Olsson, H. & Baldetorp, B. Expression of Helix pomatia lectin binding glycoproteins in women with breast cancer in relationship to their blood group phenotypes. *J. Proteome Res.* **8**, 782–786 (2009).
22. Peiris, D., Ossondo, M., Fry, S., Loizidou, M., Smith-Ravin, J. & Dwek, M. Identification of O-linked glycoproteins binding to the lectin Helix pomatia agglutinin as markers of metastatic colorectal cancer. *PLoS One* **10**, (2015).
23. Laack, E., Nikbakht, H., Peters, A., Kugler, C., Jasiewicz, Y., Edler, L., Hossfeld, D. & Schumacher, U. Lectin histochemistry of resected adenocarcinoma of the lung: helix pomatia agglutinin binding is an independent prognostic factor. *Am. J. Pathol.* **160**, 1001–1008 (2002).
24. Thies, A., Moll, I., Berger, J. & Schumacher, U. Lectin binding to cutaneous malignant melanoma: HPA is associated with metastasis formation. *Br. J. Cancer* **84**, 819–23 (2001).
25. Hellström, U., Hammarström, S. & Klein, G. Enrichment of Helix pomatia (HP) lectin

- binding variant from the TA3St mouse ascites tumor by repeated column selection. *Eur. J. Cancer* **14**, 1665–1772 (1978).
26. Schreiber, S., Gocht, A., Wegwitz, F., Deppert, W. & Schumacher, U. Lectin Histochemistry of Murine WAP-T Mammary Cancer Reveals Similar Glycoconjugate Changes to Those in Human Breast Cancer. *Anticancer Res.* **34**, 7045–53 (2014).
 27. Cunneen, M., Liu, B., Wang, L. & Reeves, P. Biosynthesis of UDP-GlcNAc, UndPP-GlcNAc and UDP-GlcNAcA Involves Three Easily Distinguished 4-Epimerase Enzymes, Gne, Gnu and GnaB. *PLoS One* **8**, (2013).
 28. Clark, G. F. Molecular models for mouse sperm-oocyte binding. *Glycobiology* **21**, 3–5 (2011).
 29. Bianchi, E.; Doe, B.; Goulding, D.; & Wright, J. Juno is the egg Izumo receptor and is essential for mammalian fertilization. *Nat.* 483–487 **508**, 483–487 (2014).
 30. Aydin, H., Sultana, A., Li, S., Thavalingam, A. & Lee, J. Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex. *Nature* **16**, 562–5 (2016).
 31. Reisner, Y., Itzicovitch, L., Meshorer, A. & Sharon, N. Hemopoietic stem cell transplantation using mouse bone marrow and spleen cells fractionated by lectins. *Proc Natl Acad Sci U S A* **75**, 2933–6 (1978).
 32. Nash, R., Neves, L., Faast, R., Pierce, M. & Dalton, S. 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 (2007).
 33. Dodla, M., Young, A., Venable, A., Hasneen, K., Rao, R., Machacek, D. & Stice, S. Differing lectin binding profiles among human embryonic stem cells and derivatives aid in the isolation of neural progenitor cells. *PLoS One* **6**, e23266 (2011).
 34. Brockhausen, I., Schachter, H. & Stanley P in Varki, A. in (ed. Ajit Varki, Richard D Cummings, Jeffrey D Esko, Hudson H Freeze, Pamela Stanley, Carolyn R Bertozzi, Gerald W Hart, and M. E. E.) (Cold Spring Harbor Laboratory Press, 2009).
 35. Saini, S., Maiti, N. & Kaushik, A. Partial Characterization of Immunoglobulin C Gene of Water Buffalo (*Bubalus bubalis*) Predicts Distinct Structural Features of C1q-Binding Site in C 3 Domain. *ISRN Immunol. Vol. (2013)*, , **Article ID**, 6 pages (2013).
 36. Tenno, M., Ohtsubo, K., Hagen, F., Ditto, D., Zarbock, A., Schaerli, P., Andrian von, U., Ley, K., Le, D., Tabak, L. & Marth, J. Initiation of protein O glycosylation by the polypeptide GalNAcT-1 in vascular biology and humoral immunity. *Mol. Cell. Biol.* **27**, 8783–96 (2007).
 37. Bennett, E., Mandel, U., Clausen, H., Gerken, T., Fritz, T. & Tabak, L. Control of

- mucin-type O-glycosylation: A classification of the polypeptide GalNAc-transferase gene family. *Glycobiology* **22**, 736–56 (2012).
38. Schjoldager, K. & Clausen, H. Site-specific protein O-glycosylation modulates proprotein processing - Deciphering specific functions of the large polypeptide GalNAc-transferase gene family. *Biochimica et Biophysica Acta - General Subjects* **1820**, 2079–2094 (2012).
 39. Moreau R, Dausset J, Bernard J, M. J. Acquired hemolytic anemia with polyagglutinability of erythrocytes by a new factor present in normal blood (Article in French). *Bull Mem Soc Med Hop Paris.* **73**, 569–87 (1957).
 40. Friedenreich, V. & Munck, J. Intravital Effects of „Transformed” Blood Corpuscles in Guinea-Pigs. *Acta Pathol. Microbiol. Scand.* **7**, 134–145 (1930).
 41. Springer, G. F. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. *Journal of Molecular Medicine* **75**, 594–602 (1997).
 42. Ju, T., Otto, V. I. & Cummings, R. D. The Tn antigen-structural simplicity and biological complexity. *Angew. Chemie. Int. Ed. English* **50**, 1770–1791 (2011).
 43. Chia, J., Goh, G. & Bard, F. Short O-GalNAc glycans: regulation and role in tumor development and clinical perspectives. *Biochim. Biophys. Acta* (2016).
doi:10.1016/j.bbagen.2016.03.008
 44. Muthana, S. & Gildersleeve, J. Factors Affecting Anti-Glycan IgG and IgM Repertoires in Human Serum. *Sci Rep.* 619509 (2016). doi:10.1038/srep19509
 45. Dahr, W., Uhlenbruck, G., Gunson, H. & Van Der Hart, M. Molecular basis of Tn-polyagglutinability. *Vox Sang.* **29**, 36–50 (1975).
 46. Rögner, W., Renwantz, L. & Uhlenbruck, G. Comparison of a hemolymph lectin from *Octopus vulgaris* with hemocyanin. *Comp. Biochem. Physiol. -- Part B Biochem.* **85**, 119–123 (1986).
 47. Bird, G., Shinton, N. & Wingham, J. Persistent mixed-field polyagglutination. *Br J Haematol.* **21**, 443–53 (1971).
 48. Jaff, M. Higher frequency of secretor phenotype in O blood group – its benefits in prevention and/or treatment of some diseases. *Int J Nanomedicine* (2010).
doi:10.2147%2FIJN.S13980
 49. Smorodin, E., Kurtenkov, O., Sergeyev, B., Lilleorg, A. & Chuzmarov, V. Antibodies to tumor-associated epitopes in sera of cancer patients and blood donors. *Exp. Onkol.* **23**, 109–13 (2001).
 50. Hofmann, B. T. *et al.* ABO Blood Group IgM isoagglutinins interact with tumor-associated O-glycan structures in pancreatic cancer. *Clin. Cancer Res.* **20**, 6117–6126

- (2014).
51. Hirohashi, S., Clausen, H., Yamada, T., Shimosato, Y. & Hakomori, S. 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 (1985).
 52. Takahashi, H., Metoki, R. & Hakomori, S. 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 (1988).
 53. Welinder, C., Baldetorp, B., Borrebaeck, C., Fredlund, B. & Jansson, B. A new murine IgG1 anti-Tn monoclonal antibody with in vivo anti-tumor activity. *Glycobiology* **21**, 1097–1107 (2011).
 54. Thors, C., Jansson, B., Helin, H. & Linder, E. Thomsen-Friedenreich oncofetal antigen in *Schistosoma mansoni* : localization and immunogenicity in experimental mouse infection. *Parasitology* **132**, 73–81 (2006).
 55. Brooks, C., Schietinger, A., Borisova, S., Kufer, P., Okon, M., Hiramata, T., Mackenzie, C., Wang, L., Schreiber, H. & Evans, S. Antibody recognition of a unique tumor-specific glycopeptide antigen. *Proc Natl Acad Sci U S A* **107**, 10056–10066 (2010).
 56. Gesheva, V., Chausheva, S., Mihaylova, N., Manoylov, I., Doumanova, L., Idakieva, K. & Tchorbanov, A. Anti-cancer properties of gastropodan hemocyanins in murine model of colon carcinoma. *BMC Immunol.* **15**, 34 (2014).
 57. Moltedo, B., Faunes, F., Haussmann, D., De Ioannes, P., De Ioannes, A., Puente, J. & Becker, M. Immunotherapeutic Effect of Concholepas Hemocyanin in the Murine Bladder Cancer Model: Evidence for Conserved Antitumor Properties Among Hemocyanins. *J. Urol.* **176**, 2690–2695 (2006).
 58. Wirguin, I., Suturkova-Milosevic, L., Briani, C. & Latov, N. Keyhole limpet hemocyanin contains Gal(??1-3) GalNAc determinants that are cross-reactive with the T antigen. *Cancer Immunol. Immunother.* **40**, 307–310 (1995).
 59. Zhong, T., Arancibia, S., Born, R., Tampe, R., Villar, J., Del Campo, M., Manubens, A. & Becker, M. Hemocyanins Stimulate Innate Immunity by Inducing Different Temporal Patterns of Proinflammatory Cytokine Expression in Macrophages. *J Immunol.* **196**, 4650–62 (2016).
 60. Schumacher, K. Keyhole limpet hemocyanin (KLH) conjugate vaccines as novel therapeutic tools in malignant disorders. *Journal of Cancer Research and Clinical Oncology* **127 Suppl**, (2001).
 61. Arend, P. ABO (histo) blood group phenotype development and human reproduction as

- they relate to ancestral IgM formation: A hypothesis. *Immunobiology* **221**, 116–127 (2016).
62. Springer, G. Horton, H. & Forbes, M. [Origin of anti-human blood group B agglutinins in white Leghorn chicks]. *J Exp Med.* **110**, 221–244 (1959).
 63. Springer, G. F., Williamson, P. & Brandes, W. C. Blood Group Activity of Gram-Negative Bacteria. *J. Exp. Med.* **113**, 1077–93 (1961).
 64. Springer, F. & Horton, R. Blood group isoantibody stimulation in man by feeding blood group-active bacteria. *J Clin Invest.* **48**, 1280–91 (1969).
 65. Chuang, J., Hung, C., Chang, S., Chou, T. & Lee, P. Does Immunosuppressive Pharmacotherapy Affect Isoagglutinin Titers? *Transplant. Proc.* **40**, 2685–2687 (2008).
 66. Arend, P., & Fehlhauer, G. 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]. *Klin. Wochenschr.* **47**, 535–541 (1969).
 67. Kyogashima M., Mulshine, J., Linnoila, R.I., Jensen, S., Magnani, J.L., Nudelman, E., Hakomori, S. & Ginsburg, V. Antibody 624H12, which detects lung cancer at early stages, recognizes a sugar sequence in the glycosphingolipid difucosylneolactonorhexaosylcerami. *Arch Biochem Biophys.* **275**, 309–14. (1989).
 68. Shade, K. & Anthony, R. Antibody Glycosylation and Inflammation. *Antibodies* **2**, 392–414. (2013).
 69. Urun, Y., Utkan, G., Cangir, A., Oksuzoglu, O., Ozdemir, N., Oztuna, D., Kocaman, G., Coşkun, H., Kaplan, M., Yuksel, C., Demirkazik, A. & Icli, F. Association of ABO Blood Group and Risk of Lung Cancer in a Multicenter Study in Turkey. *Asian Pac. J. Cancer Prev.* **14**, 2801–3 (2013).
 70. Hsiao, L., Liu, N., You, S. & Hwang, L. ABO blood group and the risk of cancer among middle-aged people in Taiwan. *Asia Pac J Clin Oncol.* **11**, e31-6 (2015).
 71. Zhang, B., He, N., Huang, Y., Song, F. & Chen, K. ABO blood groups and risk of cancer: a systematic review and meta-analysis. *Asian Pac J Cancer Prev* **15**, 4643–4650 (2014).
 72. Rumfelt, L., Avila, D., Diaz, M., Bartl, S., McKinney, E. & Flajnik, M. . A shark antibody heavy chain encoded by a nonsomatically rearranged VDJ is preferentially expressed in early development and is convergent with mammalian IgG. *Proc Natl Acad Sci U S A.* **98**, 1775–80 (2001).
 73. Lutton, B. V. *Characterization of the Epigonal Organ-ovary Complex of the Little Skate, Leucoraja Erinacea: A Model for Reproductive-immune Interactions.* (Boston

- University, 2007).
74. McClusky, L. & Sulikowski, J. The epigonal organ and mature pole of the testis in the recreationally fished blue shark (*Prionace glauca*): histochemico-functional correlates. *J Anat.* **225**, 614–24 (2014).
 75. Davison, F. & Kaspers, B. & Schat, K. A. *Avian Immunology, Chemistry*. (Elsevier Ltd., 2008). doi:http://dx.doi.org/10.1016/B978-0-12-396965-1.00018-2
 76. Grive, K. & Freiman, R. The developmental origins of the mammalian ovarian reserve. *Development* **142**, 2554–2563 (2015).
 77. Polak de Fried, E. *et al.* Human parthenogenetic blastocysts derived from noninseminated cryopreserved human oocytes. *Fertil. Steril.* **89**, 943–947 (2008).
 78. Kim, K. *et al.* Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer. *Cell Stem Cell* **1**, 346–352 (2007).
 79. Virant-Klun, I., Skutella, T., Stimpfel, M. & Sinkovec, J. Ovarian surface epithelium in patients with severe ovarian infertility: A potential source of cells expressing markers of pluripotent/multipotent stem cells. *J. Biomed. Biotechnol.* **2011**, (2011).
 80. White, Y., Woods, D., Takai, Y., Ishihara, O., Seki, H. & Tilly, J. . Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nat. Med.* **18**, 413–421 (2012).
 81. Pacchiarotti, J. *et al.* Differentiation potential of germ line stem cells derived from the postnatal mouse ovary. *Differentiation* **79**, 159–170 (2010).
 82. Nabavi, N., Grusby, M., Finn, P., Wolgemuth, D. & Glimcher, L. Identification of an IL-4-Inducible Gene Expressed in Differentiating Lymphocytes and Male Germ Cells. *Dev. Immunol.* **1**, 19–30 (1990).
 83. Huang, J., Zhang, L., Ma, T.; Zhang, P. & Xiaoyan, Q. Expression of immunoglobulin gene with classical V-(D)-J rearrangement in mouse testis and epididymis. *J Histochem Cytochem.* **57**, 339–49 (2009).
 84. Chang, D., Cattoretti, G. & Calame, K. The dynamic expression pattern of B lymphocyte induced maturation protein-1 (Blimp-1) during mouse embryonic development. *Mech Dev.* **117**, 205–9 (2002).
 85. Metz, C. *Biology Of Fertilization. Model Systems And Oogenesis (2012)*. (Elsevier Ltd., 2012).
 86. Nagasawa, K., Shikina, S., Takeuchi, Y. & Yoshizaki, G. Lymphocyte antigen 75 (Ly75/CD205) is a surface marker on mitotic germ cells in rainbow trout. *Biol Reprod.* **83**, 597–606 (2010).
 87. Nagasawa, J., Fernandes, M., Yoshizaki, G., Miwa, M. & Babiak, I. Identification and

- Migration of Primordial Germ Cells in Atlantic Salmon, *Salmo salar*: Characterization of Vasa, Dead End, and Lymphocyte Antigen 75 Genes. *Mol Reprod Dev.* **80**, 118–31 (2013).
88. Holt, P. & Anstee, D. A natural anti-A agglutinin in the serum of the brown trout (*Salmo trutta*). *Vox Sang.* ; **29**, 286–91. (1975).
89. Yamamoto, H., Watanabe, H., Sato, G. & Mikami, T. Identification of immunoglobulins in chicken eggs and their antibody activity. *Jpn. J. Vet. Res.* **23**, 131–140 (1975).
90. Yousif, A. N., Albright, L. J. & Evelyn, T. P. Immunological evidence for the presence of an IgM-like immunoglobulin in the eggs of coho salmon *Oncorhynchus kisutch*. *Dis. Aquat. Organ.* **23**, 109–114 (1995).
91. Hu, F. *et al.* Spontaneous Production of Immunoglobulin M in Human Epithelial Cancer Cells. *PLoS One* **7**, (2012).
92. Jerne, N. K. The somatic generation of immune recognition. *Eur. J. Immunol.* **1**, 1–9 (1971).
93. Sun, J., Zhang, J., Ling, Q., Luo, Y., Wu, S., Liang, Z., Zhong, D. & Zeng, X. Primary diffuse large B-cell lymphoma of the ovary is of a germinal centre B-cell-like phenotype. *Virchows Arch.* **466**, 93–100 (2015).
94. Valli, R., Froio, E., Alvarez de Celis, M., Mandato, V. & Piana, S. 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 (2014).
95. Valdez, R., McKeever, P., Finn, W., Gebarski, S. & Schnitzer, B. Composite germ cell tumor and B-cell non-Hodgkin's lymphoma arising in the sella turcica. *Hum Pathol.* **33**, 1044–7. (2002).
96. Carrabin, N., Treilleux, I., Meeus, P., Tredan, O. & Ray-Coquard, I. Primary ovarian borderline tumor in the inguinal lymph node. *Int. J. Gynecol. Pathol.* **32**, 167–70 (2013).
97. Willis, S., Mallozzi, S., Rodig, S., Cronk, K., McArdel, S., Caron, T., Pinkus, G., Lovato, L., Champain, K., Anderson, D., Anderson, R., Bruce, J. & O'Connor, K. The microenvironment of germ cell tumors harbors a prominent antigen-driven humoral response. *J. Immunol.* **182**, 3310–3317 (2009).

Early ovariectomy unmasking the non-somatic
origin of murine anti-A-reactive IgM

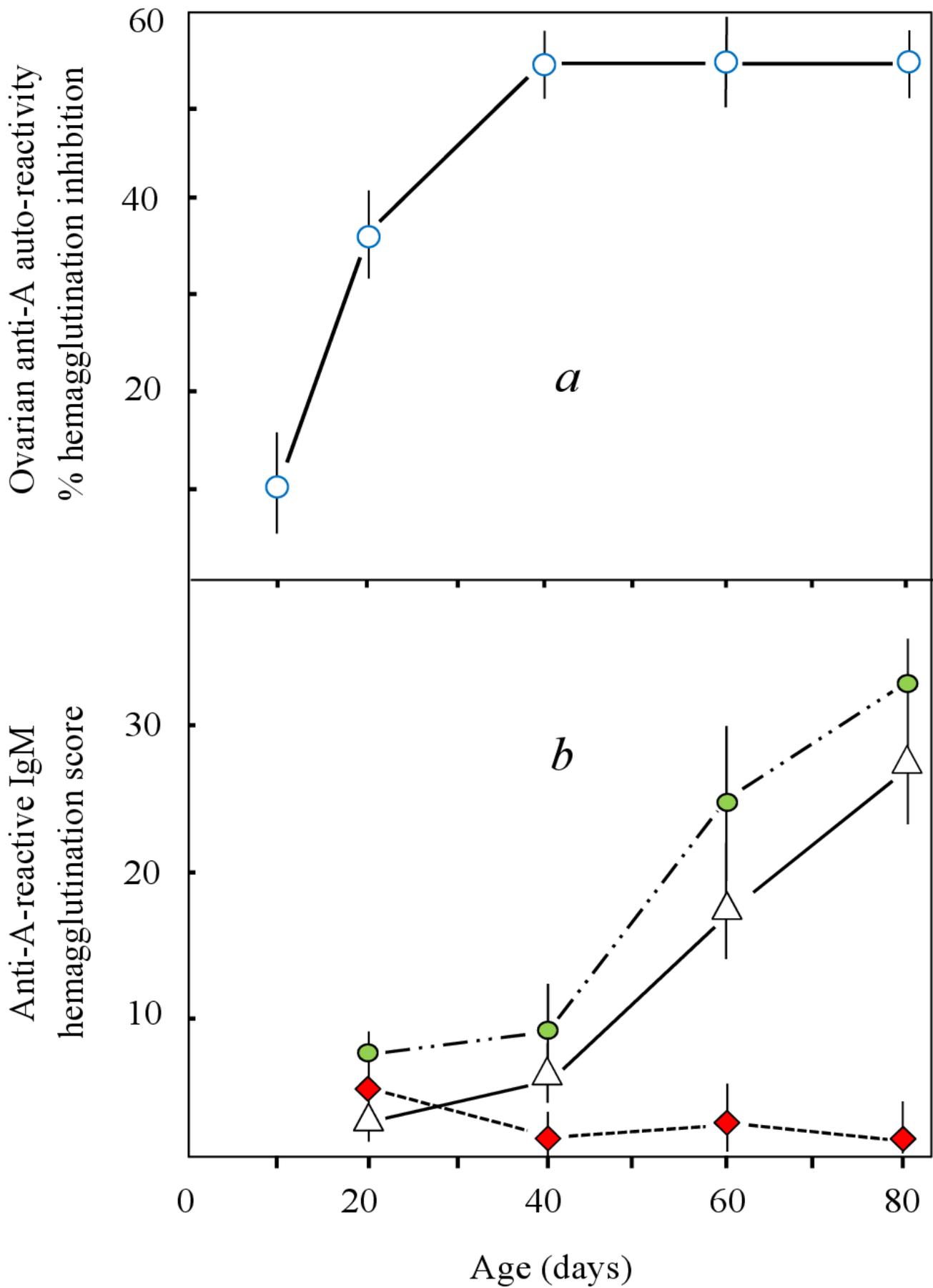


Fig. 1.

Fig. 1.

a) Growth-related appearance of auto-reactive, non-somatic GalNAc glycan-bearing hydrophilic glycolipids in the differentiating ovarian tissue with peak levels occurring at puberty: 

b) Subsequently arising, complementary, innate anti-A-like IgM reactivity:

 serum of untreated (non-operated) animals;

 sham-operated;

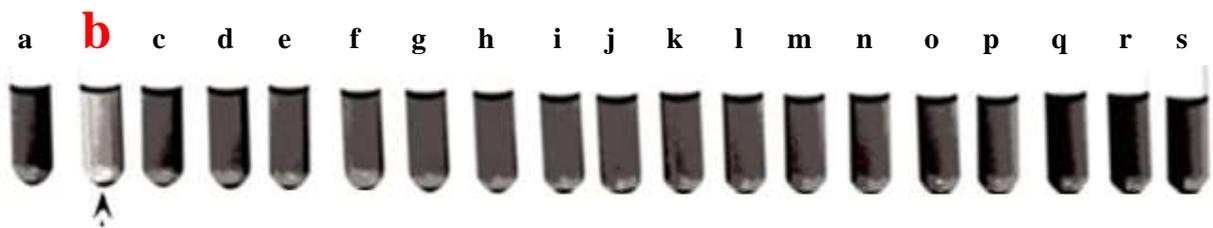
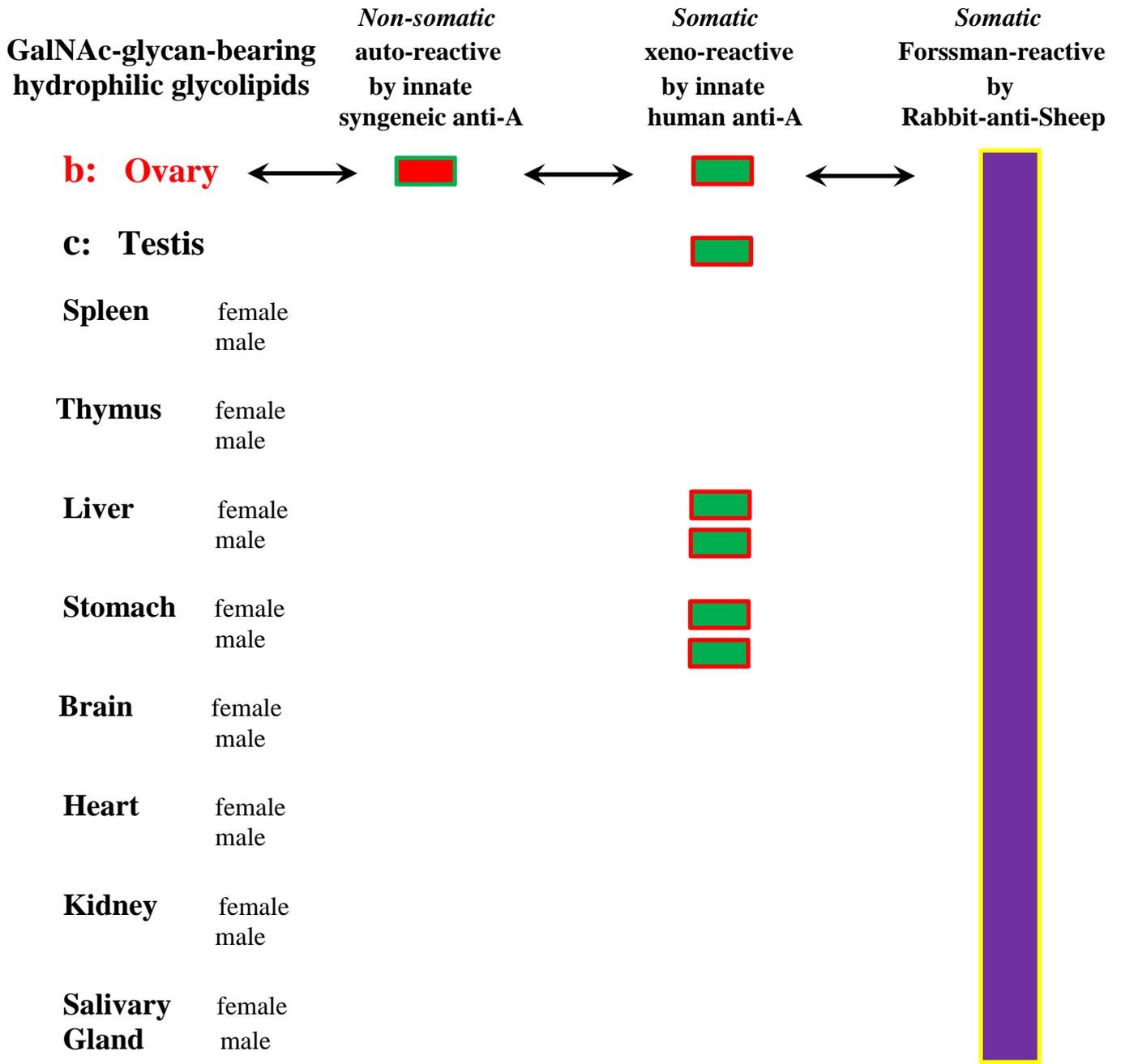
 ovariectomized.

Figure reconstructed from⁴ Arend, P. and Nijssen, J., 1977 Nature, 269, 255–257, cited in⁵⁶

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Non-somatic, auto-reactive A-like ovarian determinant distinct from somatic, xeno-reactive A-like structures



Ovary

a) = phosphate-buffered saline

Fig. 2

Fig. 2

Distribution of auto-reactive/non-somatic and xeno-reactive/somatic GalNAc glycan-bearing, hydrophilic glycolipids in C57BL/10 murine ovarian and non-reproductive tissues. While all the murine tissues express the species-typical Forssman reactivity and other xeno-reactive A-like structures are, by innate human anti-A, identified in male and female reproductive and endodermal organs, the murine anti-A is exclusively inhibited by syngeneic ovarian glycolipids. The photo was taken during the hemolysis-inhibition experiments as described in¹⁶ Arend, P. 1980, *Immunobiology* 156, 410–417; cited in⁵⁶ Arend, 2014, *Immunobiology* 219; 285–29; doi:10.1016/j.imbio.2013.10.01. PMID:26433867