

Early ovariectomy unmasking the non-somatic origin of murine anti-A-reactive IgM reflecting developmental malignancy.*

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Abstract. The germline encoding of a non-immune IgM molecule in mammals was experimentally documented for the first time by specifically timed ovariectomy of C57BL/10 mice, and the target of this innate antibody is a trans-species developmental antigen, which when expressed in any non-developmental tissue signifies malignancy. Although ovariectomy and castrations have been described to result in uncontrolled and/or enhanced humoral and cellular immunity, involving increasing weights of spleen and thymus with pronounced B and T cell productions, the development of the mercaptoethanol-sensitive and complement-binding, non-immune anti-A reactivity in murine plasma was not enhanced after ovariectomy performed in C57BL/10 mice before the onset of puberty. This non-immune murine anti-A, which is complementary to trans-species, syngeneic ovarian GalNAc-glycan-bearing glycolipids and distinct from cross-reactive adaptive anti-A antibody, was strongly retarded or did not appear at all in plasmas 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 murine tissues expressed the species-typical *Forssman* reactivity and further A-like structures were, by reaction with innate human anti-A antibody, identified in male and female reproductive and endodermal organs, 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 DBL and HPA reactivity indicating the functional involvement of *O*-GalNAc-determined mucin-type A-like TN or TF epitopes that, when expressed by non-developmental tissues, signify malignancy.

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

Introduction.

The mercaptoethanol-sensitive and complement-binding innate anti-A reactivity in murine plasma is, contrary to our first (1; 2) and recently abandoned concept (3; 4) unlikely to originate from a somatic, primary immune or autoimmune response. Although ovariectomy and castrations are described to result in uncontrolled and/or enhanced humoral and cellular immunity, involving increasing weights of spleen and thymus with pronounced B and T cell productions (5; 10) associated with autoimmune-related bone loss (11), the development of this early murine IgM or IgM-like molecule was not enhanced after ovariectomy performed in the C57BL/10 mouse before the onset of puberty. On the contrary, the innate anti-A reactivity, which is complementary to syngeneic ovarian GalNAc glycan-bearing hydrophilic glycolipids (12) and distinct from cross-reactive, adaptive anti-A (13), appeared strongly retarded or did not appear at all in plasmas of animals ovariectomized at the age of 20 days (1; 2), Fig.1. While all murine tissues expressed the expected, species-typical *Forssman* reactivity and further A-like structures were, by innate human anti-A identified in male and female reproductive organs and endodermal tissues (14), the murine anti-A was exclusively inhibited by syngeneic ovarian glycolipids, Fig. 2. Moreover, these crude glycolipid preparations showed a developmental polymorphism (3), revealed by reactions with the lectin from *Dolichos biflorus* (DBL) and the agglutinin from *Helix pomatia* (HPA), which indicated the functional involvement of mucin-type *O*-GalNAc-determined, serologically A-like, Tn or TF (Thomsen-Friedenreich) epitopes. They are established to occur on both malignant and normal T cells (15), involving NK cell populations (16, 17). On the other hand, they are not expressed by mature B cells and are basically restricted to B cell lymphomas and lymphocytic leukemias, and when displayed by human (18 - 21) and animal (22; 23) non-developmental tissues, signify malignancy.

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

The “bulky” GalNAc-molecule (24), possessing undefined biophysical properties, is assumed to be preferential in substrate competitions and hexosamine epimerizations (25) and to dominate the mammalian embryogenic stem cell (ESC)-germ cell (GC) transformation (26 - 28). While the role of specific carbohydrate in sperm-egg recognition is still subject to discussion (29) (30) (31), the non-somatic process of germ cell maturation is initiated by transient *O*-GalNAc-glycosylations,. They especially occur on polypeptides (32) which are expressing glycosidic sites by trans-species functional hydrophilic serine- and threonine-residues (33). 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 glycosylation (34 - 36). These develop-

mental or early ontogenic/non-somatic and genetically as-yet-undefined A-like *O*-GalNAc transferase functions dominate the early carbohydrate metabolism of all vertebrates, independent from the species or the phenotype and are characterized by extremely short half-lives, resulting in transient “immature” *O*-GalNAc expressions (26). They potentially imply functional relationships with the structurally related mucin-type TN or TF (Thomsen-Friedenreich) antigen (Gal β 1–3GalNAc α -1-*O*-Ser/Thr), which reacts more or less pronounced with any mammalian innate anti-A-reactive immunoglobulin and thus may still cause diagnostic confusion. However, this aberrant *O*-glycosidic structure, which when arising in tissues of vertebrates, is recognized in two different ways by the immune system of invertebrates and thus may suggest common ancestral functions. Indeed, the agglutinin from *Helix pomatia* (HPA) has meanwhile been established as tumor cell marker and prognostic indicator for different human tumor cell lineages (38), while the agglutinin-free hemocyanin (HPH) from the same snail demonstrates strong anti-proliferative effects in murine models of colon carcinoma (39), and the hemocyanin from *Concholepas* inhibits the growth of bladder tumors (40). Furthermore, GalNAc determinants of the hemocyanin from *Megathura crenulata* (Keyhole Limpet Hemocyanin) show cross-reactivity with T cells and induce a potent Th1-dominant immune response (37). This hemocyanin is meanwhile used in adjuvant immunotherapy of several types of cancer (42).

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 become expressed only after formation of the zygote (43). These somatic functions are encoded on chromosome 9, and in epistatic cooperation with the FUT1 and FUT2 transferases encoded on chromosome 19, 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 self-reactive antibody activities (43).

The specifically timed ovariectomy of the C57BL/10 mouse has experimentally established the existence and the origin of a germline-encoded anti-A-reactive IgM in mammals: over the course of germ cell maturation, non-somatic, *O*-glycotransferase activities have potentially synthesized the serologically A-like, trans-species functional *O*-GalNAc-glycans, which were identified on hydrophilic ovarian glycolipids and are volatily expressed by ESC and/or pluripotent hematopoietic stem cells. After completion of germ cell maturation, these volatile “immature” transferase activities obviously undergo rapid depletion, resulting in the loss of 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. This non-somatic process occurring in reproductive tissues and/or in cell renewal events is accompanied by somatic tissue maturation, in which ancestral, trans-species *O*-glycans on cell surfaces and plasma proteins are permanently “replaced” by phenotypical *N*-linked glycans (33). According to early observations on the effect of immunoglobulin glycosylation on the enhancement and/or inhibition of tumor growth (44) in connection with the recent discoveries of immunoglobulin fucosylation (45), the avidity of antibodies appears to be inversely proportional to the degree of glycosylation (43): strong antibody glycosylation might protect from autoimmunity, however, obviously favors tumor growth. In fact, it is known for decades that blood group A individuals have an increased risk to develop gastric and pancreatic cancer, while a recent report showed a similar risk for lung cancer (46). Furthermore, current studies indicate that the overall risk to develop cancer might be increased in any none-O blood group (47; 48). Thus, aberrant *O*-GalNAc-glycosylations, which are even recognizable by the immune system of invertebrates and thus potentially reflect common ancestral, evolutionary and/or developmental functions, most likely occur in not measurable expressions in any normal human growth but become, depending on the degree of phenotype-determined immunoglobulin glycosylation, unmasked by the release of anti-A reactivity. Consequently, such release appears most pronounced in blood group O individuals.

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, IgM secretion occurs in neonates and in adults along the genital ridge (49), 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 are associated in the epigonal organs (50; 51). In birds, the shutdown of somatic B cell functions requires the early removal of the avian bursa of *Fabricius* Anlage (52), 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 likely have passed the ovary and emerged through the fimbriae at the posterior tubal infundibulum, and debates about the genetic relationships between B-lymphocytes and GC have been underway for decades. Although the adult mammalian ovary seems to be devoid of definitive

germline stem cells and the specific “ovarian reserve” is limited (53), the ovary has to be considered as kind of an organism on its own (3), which may generate a complete offspring genome not only in phylogenetically lower species but even in man, where parthenogenetic potentials are still alive (54; 55). The cellular traffic in oogenesis involving uncountable intercellular correlations is dominated by those between SC populations and oocytes, first of all by their potentials to be transformable in each other (56; 57), or to differentiate into all somatic tissue intrinsic cells (58). In males, an IL-4-inducible gene comes to be expressed on differentiating lymphocytes and on spermatozoa (59), while classical V-(D) rearrangements have recently been located in the mouse epididymis (60). The B lymphocyte-induced maturation protein-1 (Blimp-1) is expressed in migrating PGCs as well (61), and membrane receptors that are complementary to each other induce cell divisions in the case of interactions (62). In addition, the lymphocyte antigen 75 (Ly75/CD205) (63) is expressed on mitotic germ cells in rainbow trout and Atlantic salmon (64), which might raise speculation about the involvement of germ cell maturation, for example, in the production of the anti-A hemagglutinin in *Salmo trutta* (65). 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* appears to not be restricted to B cells; immunoglobulins also occur in chicken eggs (66), and an IgM-like glycoprotein has been isolated from the oocytes of salmon (67). Furthermore, sporadic production of functional IgM has been demonstrated in normal and malignant human epithelial cells (68). Finally, “*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,*” Jerne (69) and may give rise to speculations that the genetic information, which governs the IgM polymerization process is primarily divided between germ cell precursors and LMC.

Conclusions.

Most ovarian and testicular tumors in humans appear to be B cell lymphomas (70 - 72) or develop as germ cell tumors together with Non-Hodgkin lymphoma cells (73), while a primary ovarian tumor has been detected in a single lymph node (74). Moreover, the microenvironment of germ cell tumors obviously harbors a prominent antigen-driven humoral response (75). Thus, it has been 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. However, such explanation may also reside in the topographically connected synthesis of the trans-species, serologically A-like GalNAc epitope and its complementary, ancestral protein or IgM molecule arising in a mammalian differentiating ovarian tissue. As outlined

above, the mammalian ovary represents a last evolutionary and/or developmental location, which on the basis of DBL and HPA reactivity (2) showed a developmental polymorphism suggesting the production of mucin-type *O*-GalNAc-determined, serologically A-like, Tn or TF epitopes, which when occurring in non-developmental tissue signify malignancy. Nevertheless, these aberrant *O*-GalNAc-glycosylations, which are recognizable by the defense proteins of invertebrates and thus potentially reflect common ancestral, evolutionary and/or developmental functions, most likely occur in not measurable expressions even in any normal human growth but become, depending on the degree of phenotype-determined immunoglobulin glycosylation (43), unmasked by the release of anti-A reactivity. Consequently, such release appears most pronounced in the plasma of blood group O individuals.

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

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

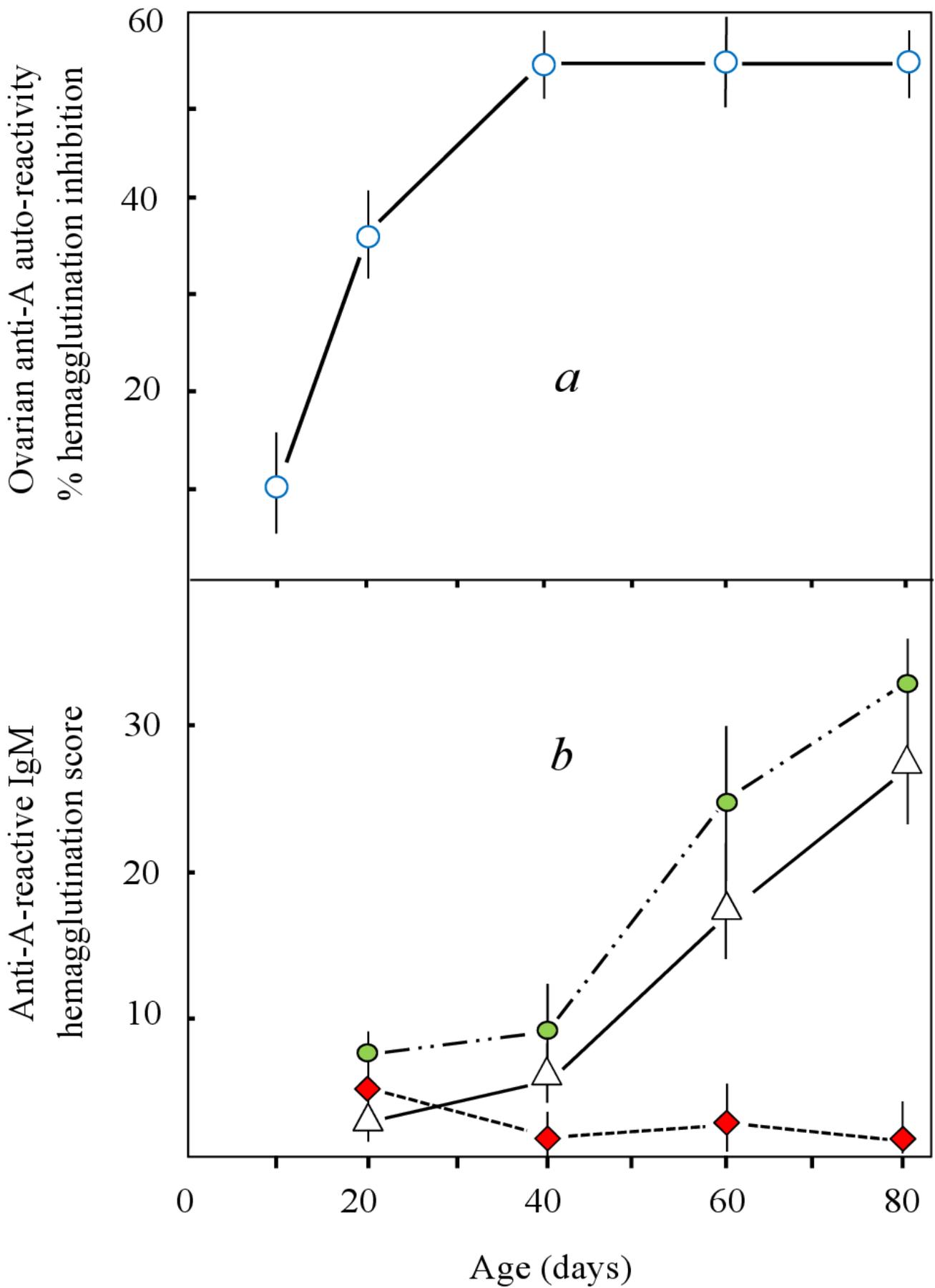


Fig. 1.

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 from (2), Arend and Nijssen 1977 *Nature*, 269, 255–257, cited in

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PMID:26433867

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

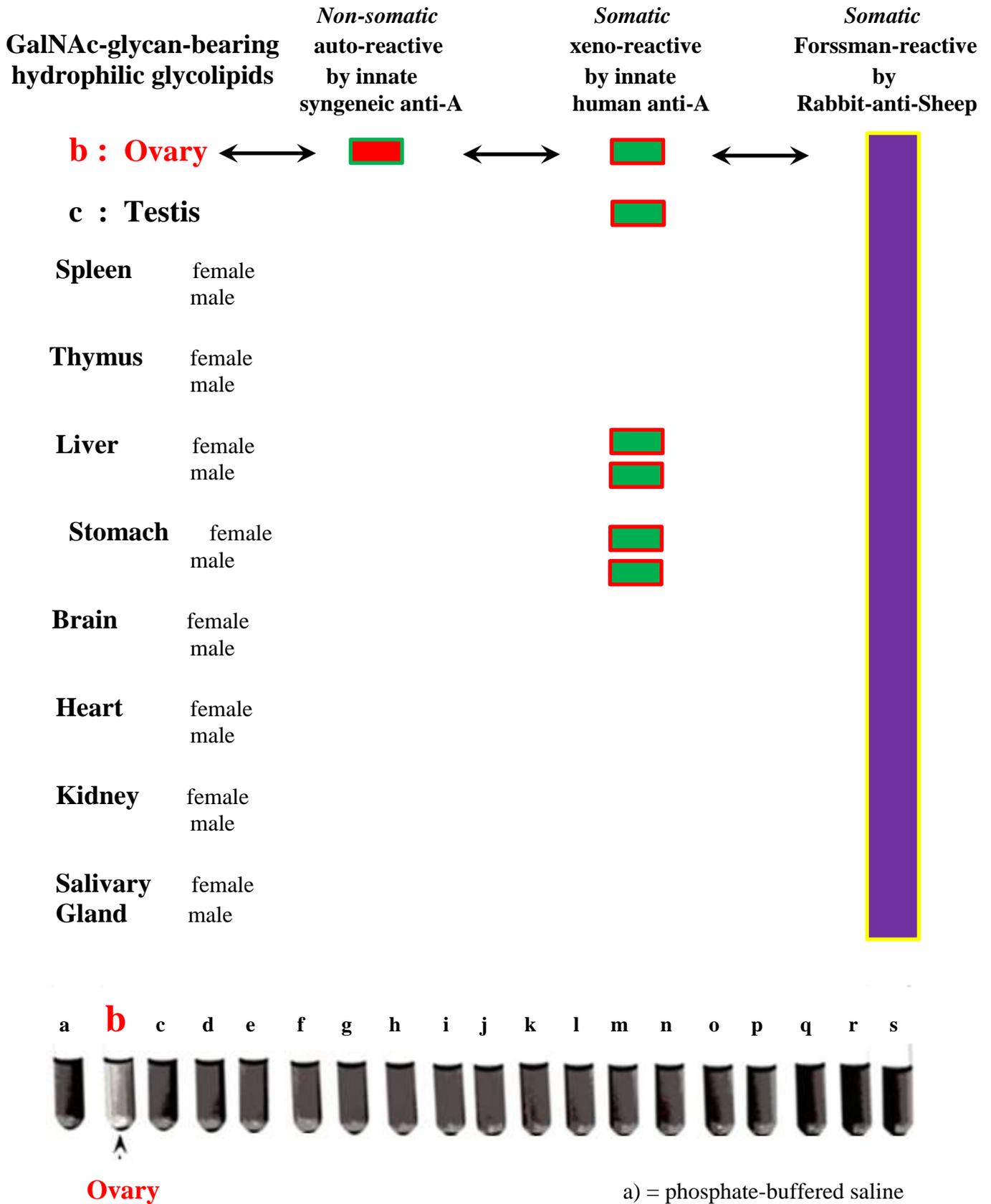


Fig. 2

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 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 in Arend, P. 1980, *Immunobiology* 156, 410–417; cited in Arend, 2014, *Immunobiology* 219; 285–29; doi:10.1016/j.imbio.2013.10.01. PMID:26433867