Age-Related Changes of Lectin Bindings on the Cell Surface of Unfertilized Mouse Ova

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Abstract: *Lectin bindings on the surface of unfertilized mouse ova immediately after ovulation were histochemically examined, and were compared among 30-day-old, 60- to 90-day-old, 180- to 210-day-old and 270-day-old mice. The bindings of PNA, GS-I, DBA, SBA, BPA, MPA, GS-II, WGA and Con A were observed from a weak to an intense degree on the cell surface of ova in mice from different age groups, but those of UEA-I and LPA were not. Although the strength in bindings of PNA, DBA, SBA, GS-II, BPA, WGA and Con A did not differ among the age groups, the strength of GS-I and MPA bindings intensified in 270-day-old mice. From the results, it may be said that the glycoconjugates on the cell surface of mouse ova immediately after ovulation contain galactose, N-acetylgalactosamine, N-acetylglucosamine and mannose, and that the amount of galactose and N-acetylgalactosamine increases as the animals get older.*

Key words: *Mouse ovum, Cell surface, Glycoconjugate, Lectin, Histochemistry.*

It is generally accepted that glycoconjugates on the cell surface of mammals play an important role not only in the protection of cells and in the ingestion of external substances into cells, but also in cell-to-cell recognition and the formation of tissues. It has also been clarified by lectin-histochemical techniques that glycoconjugates on the cell surface change during the course of differentiation, aging and the malignancy of cells [1–4].

Lectins are proteins that have plural regions, where they each bind with peculiar oligosaccharides of glycoconjugates [5]. Using the lectins labeled by fluorescent pigments, ferritins or by enzymes, it is possible to examine the status of glycoconjugates on the cell surface [1–4].

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In the mammalian ova and early embryos, the changes in glycoconjugates on their cell-surfaces have been studied by lectin-histochemical techniques, using hamsters [6] and mice [7–24]; and it has been deduced from these investigations that the glycoconjugates on the cell surface play important roles in fertilization, compaction and implantation. Lectin bindings on the cell surface of unfertilized ova immediately after ovulation also have been examined using hamsters [6] and mice [7, 9, 13, 20, 21, 24], and it has been confirmed that lectins such as *Ricinus communis* agglutinin I (RCA-I), *Ricinus communis* agglutinin II (RCA-II), *Dolichos biflorus* agglutinin (DBA), *Glycine max* agglutinin (SBA), *Sophora japonica* agglutinin (SJA), *Bauhinia purpurea* agglutinin (BPA), *Wistaria floribunda* agglutinin (WFA), *Visea villosa* agglutinin (VVA), *Helix pomatia* agglutinin (HPA), *Canavalia ensiformis* agglutinin (Con A), *Lens culinaris* agglutinin (LCA), *Helix aspersa* agglutinin (HAA), *Solanum tuberosum* agglutinin (STA), *Triticum vulgaris* agglutinin (WGA), *Griffonia simplicifolia* agglutinin I (GS-I), *Griffonia simplicifolia* agglutinin II (GS-II), *Maclura pomifera* agglutinin (MPA), *Phaseolus vulgaris* agglutinin (PHA), *Arachis hypogaea* agglutinin (PNA), succinylated *Canavalia ensiformis* agglutinin (s-Con A), *Pisum sativum* agglutinin (PSA), *Lotus tetragonolobus* agglutinin (FBP) bind with glycoconjugates on the cell surface from a weak to an intense degree, whereas lectins such as *Ulex europeus* agglutinin I (UEA-I), *Ulex europeus* agglutinin II (UEA-II), *Limulus polyphemus* agglutinin (LPA) and *Phaseolus limensis* agglutinin (LBA) do not. Age-related changes and the ability of lectin binding in mammalian ova, however, remained unknown.

The present investigation, therefore, deals with age-related changes in the lectin binding ability of mouse ova immediately after ovulation, using PNA, GS-I, DBA, SBA, BPA, MPA, GS-II, WGA, Con A, UEA-I and LPA as lectins.

Materials and Methods

Fifty-two female mice of the ICR strain were divided into four age groups; 30-day-old, 60- to 90-day-old, 180- to 210-day-old and 270-day-old mice. They were kept and fed normally in a room at 24°C and lit 14 hrs a day, 4 a.m. through 6 p.m., and were peritoneally injected with 5 i.u. of PMSG (Serotropin, Teikoku Hormone Manufacturing Co., Ltd., Tokyo, Japan), and with 5 i.u. of hCG (Gonatropin, Teikoku Hormone Manufacturing Co., Ltd.) 48 hrs later in order to induce superovulation.

In order to observe lectin bindings on the cell surface of mouse ova, ova-containing oviducts were taken 13 hrs after an hCG injection, fixed in Bouin solution, embedded in paraffin, serially cut at a thickness of 6 μm, and then only ova-containing sections were selected. The direct method was applied to deparaffinized sections, using eleven kinds of lectins labeled by peroxidase (E-Y Laboratories, San Mateo, U.S.A.): namely, PNA, GS-I, DBA, SBA, BPA, MPA, GS-II, WGA, Con A, UEA-I and LPA. The deparaffinized sections were immersed in a phosphate buffer saline [25] (PBS, pH 7.4) containing 0.1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, U.S.A.) (BSA-PBS), at room temperature for 30 min. Rinsed in PBS, they were immersed in 1 ml of BSA-PBS containing 100 μg of lectin at room temperature for 30 min. The sections thus treated were rinsed in PBS, and then immersed in a PBS containing 0.02% diaminobenzidine (DAB, Nakarai Chemicals Ltd., Kyoto, Japan) (DAB-PBS) at room temperature for 10 min, and then immersed in 100 ml of DAB-PBS containing 100 μl of 5% H_2O_2 at room temperature for another 10 min. The same procedures for the demonstration of lectin bindings were applied 3 times to 20 to 25 ova from each age group of mice. As for the control groups, some sections were pre-treated in a 0.2 M inhibiting monosaccharide-containing solution, and then were immersed either in a plain BSA-PBS, in place of a lectin-containing BSA-PBS, or in a BSA-PBS which contained a 0.2 M inhibiting monosaccharide in addition to the lectin. As inhibiting monosaccharides, D-galactose (Fluka Biochemika, Buchs, Switzerland) was used for PNA and GS-I, N-acetyl-D-galactosamine (Wako Pure Chemical Industries Ltd., Osaka, Japan) for DBA, SBA, BPA and MPA, N-acetyl-D(+)-glucosamine (Wako Pure Chemical Industries Ltd.) for GS-II and WGA, α-methyl-D-mannoside (Fluka Biochemika) for Con A, L-fucose (Fluka Biochemika) for UEA-I, and N-acetylneuraminic acid (Nakarai Tesque, Kyoto, Japan) for LPA, respectively. The sections thus treated were dehydrated, and mounted in Balsam (Wako Pure Chemical Industries Ltd.).

Results

When the sections that contained ova were treated by the lectin-histochemical method as described above, brown reaction products of benzidine which show the presence of lectin bindings were found on the cell sur-

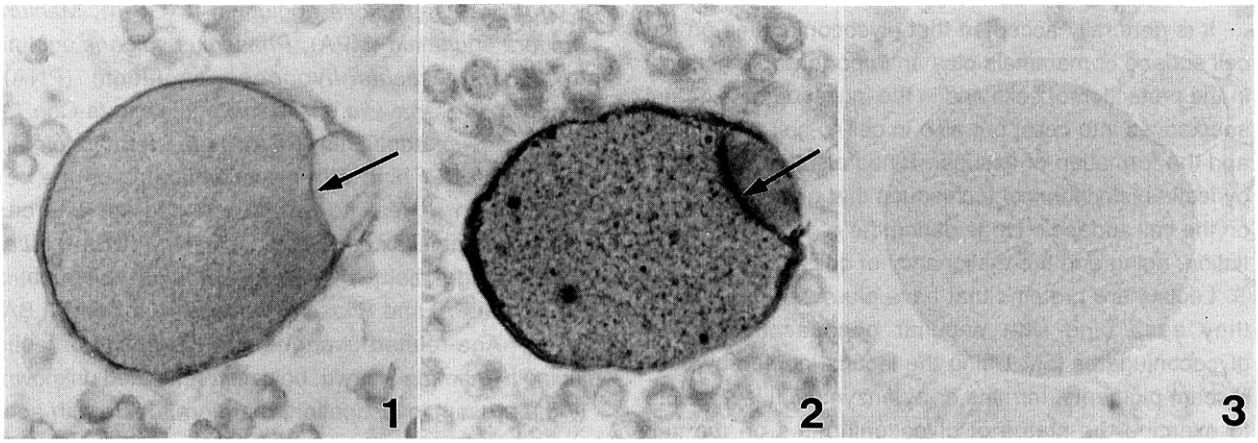

Fig. 1. An unfertilized mouse ovum from a 60-day-old mouse, treated with Con A. A small amount of reaction products showing the binding of Con A (an arrow) are seen on the cell surface. × 450.

Fig. 2. An unfertilized mouse ovum from a 270-day-old mouse, treated with GS-I. A large amount of reaction products showing the binding of GS-I (an arrow) are seen on the cell surface. × 450.

Fig. 3. A control unfertilized mouse ovum from a 30-day-old mouse, treated with PNA and galactose. No reaction products are seen on the cell surface. × 450.

face of ova (Figs. 1 and 2). No reaction products were observed when the sections were treated with a lectin-free BSA-PBS, or treated with a solution including an inhibiting monosaccharide (Fig. 3). From these results, it was confirmed that lectin binding was specific, and that the amount of products indicated the intensity of lectin binding activity.

The results with respect to the intensity of lectin binding on the cell surface of ova from different age groups of mice are given in Table 1. As shown in Table 1, PNA, DBA, SBA, BPA, GS-II, WGA and Con A were bound with glycoconjugates from a weak to an intense degree on the cell surface of ova from mice in every age group (Fig. 1), showing no age-related changes. On the other hand, GS-I and MPA were bound with glycoconjugates from a weak to a moderate degree on the cell surface of ova from 30- to 210-day-old mice. The binding of GS-I and MPA was intensified to a moderate or to a high degree on the cell surface of ova in 270-day-old mice (Fig. 2).

Discussion

Past lectin-histochemical studies have shown that in unfertilized mouse ova, lectins such as RCA-I, RCA-II, DBA, SBA, SJA, BPA, WFA, VVA, HPA, Con A, WGA, LCA, PNA, HAA, PHA, PSA, FBP, MPA, s-Con A, GS-I, GS-II and STA are bound with glycoconjugates on their cell-surfaces, but UEA-I, UEA-II, LBA and LPA are not [7, 9, 13, 20, 21, 24]. In this present investigation on unfertilized mouse ova also, the bindings of PNA, DBA, SBA, BPA, WGA, GS-I, GS-II, Con A and MPA were observed, while UEA-I and LPA were not, thus confirming past reports. From the results described above, it may be said that glycoconjugates which contain galactose, N-acetylgalactosamine, N-acetylglucosamine and mannose do exist on the cell surface of mouse ova immediately after ovulation, while those containing fucose do not.

Table 1. Intensity of lectin bindings on the cell surface of unfertilized ova from different aged mice

Lectins	Age (days)			
	30	60–90	180–210	270
PNA	+++	+++	+++	+++
DBA	+++	+++	+++	+++
SBA	+++	+++	+++	+++
BPA	++	++	++	++
WGA	++	++	++	++
GS-II	+	+	+	+
Con A	+	+	+	+
GS-I	++	++	++	+++
MPA	+	+	+	++
UEA-I	–	–	–	–
LPA	–	–	–	–

– Negative, + weak, ++ moderate, +++ strong.

As for age-related changes in glycoconjugates on the cell surface of somatic cells, a few investigators have studied several kinds of cells using different lectins, and have confirmed that glycoconjugates containing galactose in the surface of human liver cells decrease with age [26, 27]. The same occurs in those containing fucose, mannose, galactose, glucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid in cell-surfaces of human erythrocytes [28–30]. The amount of these glycoconjugates on cell-surfaces of such cells was reported to have decreased as the cell culturing period was extended [26, 27, 29]. Further, the number of Con A-binding cells increased with aging in rat hepatic cells [31] and in fibroblasts of chick embryos [32] as well as humans [33]. These experiments *in vitro* proved (1) the increase of glycoconjugates that contain mannose with the advance in age, and (2) a vast diversity in age-related changes of glycoconjugates on the cell surface. In the present investigation, the binding of GS-I and MPA on the cell surface of ova from 270-day-old mice increased. This seems to prove that there is an increase in glycoconjugates which contain galactose and N-acetylgalactosamine as the animals age.

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マウスの加齢に伴う未受精卵子表面のレクチン結合の変化

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30日齢，60ないし90日齢，180ないし210日齢および270日齢のマウスの未受精卵子について，表面のレクチン結合が動物の加齢に伴ってどのように変化するかを組織化学的に調べた．UEA-IとLPAの結合はすべての動物の卵子でみられなかったが，PNA，DBA，SBA，BPA，GS-II，WGAおよびCon Aの結合は，いずれの日齢の動物の卵子においても常に弱度ないし強度に認められ，各日齢の動物の間で結合の強さに変化はみられなかった．一方，GS-IおよびMPAの結合は，30ないし210日齢の動物の卵子では中等度および弱度であったが，270日齢の動物の卵子では強まり，強度および中等度になった．以上の結果から，排卵直後のマウス未受精卵子表面の複合糖質はガラクトース，N-アセチルガラクトサミン，N-アセチルグルコサミンおよびマンノースを含んでおり，このうちのガラクトースとN-アセチルガラクトサミンの量は加齢動物の卵子で増加することが推察された．

キーワード：マウス卵子，細胞表面，複合糖質，レクチン，組織化学．