Lectin-Binding Patterns in the Microenvironment of the Mouse Developing T-Cells

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ABSTRACT

Glycoconjugates and their programmed changes during the course of development in the cell-surface as well as in the extracellular matrix, are known to affect cell differentiation, cellular interaction and other developmental phenomena during embryogenesis. The purpose of this study was to localize N-acetylgalactosamin as well as fucose-containing glycoconjugates *in situ* during thymus development. Staged embryos or thoracic segments were fixed and processed for lectin histochemistry studies. Five microns paraffin-embedded sections were incubated with horseradish peroxidase conjugated lectins from *Dolichos biflorus* and *Aleuria aurantia* specific for N-acetylgalactosamine and fucose, respectively. Our results revealed unique reaction of T-cells with *Dolichos biflorus* and the presence of a fucosylated glycoconjugate in microenvironments of the developing thymus including extracellular matrix and developing Hassall bodies. The time and distribution of staining with these two lectins suggest that fucosylated glycoconjugates and N-acetylgalactosamin terminal sugars may play significant role in intrathymic microenvironment that might cause differentiation of T-lymphocytes. *Iran. Biomed. J. 7 (1): 19 22 2003*

Keywords: Lectin histochemistry, Thymus, T-lymphocytes, Extracellular matrix

INTRODUCTION

It is well known that carbohydrate-containing macromolecules of the cell surface and extracellular matrix, play a significant role in many developmental phenomena, including cell-cell interactions and differentiation [1-4]. As in the other developing systems, the developing thymus undergoes critical events during the course of its morphogenesis including molecular and structural modification [5-9]. A unique surface glyco-conjugates with terminal alpha-N-acetylgalactos-amine (alpha-GalNac), has been shown to occur on the surface of the early embryonic mouse T-cells exclusively only during their maturation [10]. The microenvironment of these developing cells is believed to drive from neural crest cells [9, 1]. Virtually, every developing cell including embryonic T-cells encounters some form of extracellular matrix (ECM) and there are good reasons to believe that their interactions are of importance for the development of the mature T-cells [8, 12 13].

Little attention has been paid to the distribution and structural changes in glycoconjugates of the thymic microenvironment including on the surface of the developing T-cells and within the ECM during the course of morphogenesis. Previous studies suggested that fucosylated glycoconjugates are present in the surface of the developing neural crest cells and may play an important role in mediating cell-cell and cell-extracellular matrix interactions necessary to the process of normal thymus development [3, 14]. The purpose of the present study was to localize and characterize fucose as well as N-acetylgalactosamine containing glycoconjugates *in situ* during thymus development.

MATERIALS AND METHODS

Female BALB/C mice were mated with male of the same strain overnight and the following morning was designated day 0 of gestation. At gestational ages of 1 days through 18 days, pregnant mice were sacrificed by cervical dislocation and the embryos were dissected from the uterus and extraembryonic membranes. The

embryos were fixed in a solution of 6% mercuric chloride, 1.0% sodium acetate and 0.1% glutar-aldelyde overnight at room temperature [3]. The tissues were dehydrated through graded alcohols and xylene and embedded in paraffin blocks in orientations providing sagittal, and transverse sections.

Paraffin-embedded sections were serially cut at the thickness of 5 µm. The sections were treated with lugol solution to remove mercuric salts prior to histochemical staining. HRP-conjugated *Dolichos biflorus* (DBA) (for N-acetylgalactosamine), UEA-1, LTA (for -fucose) [14, 20] were purchased from Sigma Chemical Co. (USA). Orange peel fungus (OFA) [19] was kindly supplied by Dr. N. Kochibe, (Department of biology, faculty of education, Ganna University, Ganna, Japan). The histochemical procedure was similar to that of described previously [3]. Briefly, the sections were flooded with a solution containing 10-20 µg/ml of lectin-HRP conjugate in 0.1 M PBS, pH 7.2, for 2 hours. After extensive rinsing in PBS, the sections were incubated in diaminobenzidine-hydrogen peroxidase substrate medium (pH 7.0) for 15 minutes at room temperature. All sections were counterstained with a 1% solution of Alcian blue at pH 2.5 for 5 minutes.

RESULTS

DBA. This lectin binds strongly to the surface of the T-lymphocyte population from day 11 to day 18 of gestation. The Golgi zones also show strong reaction (Figs. 1a & b). Extracellular matrix and number of other cells within the ECM had no reaction with DBA (stars in Fig. 1b) and stained only with the Alcian blue at pH 2.5.

Fucose-binding lectins. Among the three fucose-binding lectins, only OFA lectin (*Aleuria aurantia*) reacted with the extracellular matrix of the developing thymus and to certain clusters of cells (Figs. 2a and b). Both the Golgi zones and the surface of these cells reacted strongly with OFA.

DISCUSSION

Intrathymic T-cell differentiation and cell-mesenchyme interaction is not completely understood. The developing thymus contains an extensive ECM. Components of the ECM are essentially produced by sustain thymic micro-environmental mesenchymal cells, which are mostly originated from the neural crest cells [8-11]. These mesenchymal derivatives of the neural crest, through participation in the early development of the endodermal-derived thymic primordium from the third pharyngeal pouches, play an important role in thymic and immune system development [6]. It has been shown that omitting of the involved neural crest cells results in thymic craniofacial and cardiovascular defects similar to human DiGeorge syndrome [9, 15].

Fig. 1. Section of Thymus (T) on day 13 of gestation incubated with DBA . **(a),** Only developing lymphocytes reacted with this lectin (all cells in brown). Other part stained with Alcian blue. Developing vessels appear at this time of development (V); **(b),** High magnification of the area pointed by an arrow in Figure 1. Golgi zones and all surface of the developing lymphocytes reacted intensively with DBA (arrows). Stars represent non-reacted areas in the developing thymus.

Fig. 2. (a and b), Section of thymus on day 18 of gestation treated with OFA . Extracellular matrix (small arrows in part b) and clusters of cells and ECM (big arrows) reacted very strong and intense with OFA. Part (b) is high magnification of rectangular area in part (a).

Glycoconjugates, in particular their constituent carbohydrate moieties are major components of the ECM. These glycoconjugates and those in the cell-surface play an important role in many critical events during morphogenesis [16]. Sugars such as fucose and N-acetylgalactosamine are of particular interest in developmental studies. The terminal position of these sugars in the side chain of many structurally defined glycoconjugates makes them potential candidates as ligands for developmentally regulated recognition molecules such as endogenous lectins, glycosyl transferases or glycosidase on the surface of similar or different embryonic cell type [17, 18].

Previous studies with DBA have demonstrated the temporal expression of the terminal (beta 1-3)-GalNac during intrathymic T-lymphocyte develop-ment [unpublished data]. In the present study, in addition to DBA, microenvironment of the developing thymus has been investigated with three fucose-specific lectins in an

effort to establish a relationship between morphogenesis of non-lymphoid parts and stages and specific changes in focusyl residues during development. Out of these fucose-binding lectins, only lectin from *Aleuria aurantia* (OFA) recognized glycoconjugate in the ECM and related cells of the thymic micro-environment during fetal period [18, 19]. Although, each of the fucose-binding lectins has a nominal binding specificity for alpha fucose, their preferences as to linkage and underlying sugar chain structure are known to be different.

OFA binds to fucose residues of carbohydrate side chains, but does not require a particular linkage to the penultimate moiety [19]. UEA-1 and LTA, on the other hand, are thought to show the highest affinity for fucose linked (alpha 1-2) to galactose or other more complex difucosyl structures [14, 20]. It has been shown that migrating neural crest cells might show reactivity with OFA [3]. It is tempting to speculate that the pattern of OFA binding sites in the developing thymus correlates with neural crest derived mesenchyme during early thymic morpho-genesis including ECM and Hassall bodies and perhaps other type of reticuloepithelial cells. Our results also indicate that only the ECM and cluster of cells within the ECM reacted with OFA during thymus development. Developing T-lymphocytes reacted only with DBA, which match with our previous unpublished pilot lectin histochemical studies.

Data from immuno-histochemical studies of T-lymphocytes in which Thy-1 receptors could be detected during development showed the same pattern of reaction as our results with OFA study [22, 23]. All of these cells might be originated from the neural crest cells [9, 21]. Our results also provide evidence that specific fucosylated glycoconjugate are involved in normal development of the thymic microenvironments that interacts continuously with lymphoid cells to develop mature T-lymphocytes.

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