COMPARATIVE LECTIN AND IMMUNO-HISTOCHEMISTRY ON ANTIGEN EXPRESSION IN BLOOD GROUP A1 AND A2 INDIVIDUALS

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Blood group A has been divided into the major subgroups, A1 and A2, since it was first described by von Dungern and Hirszfeld in 1911[1].

Although the serology and chemical structures, and cDNA encoding transferases of A1 and A2 have been well established[2], the different of antigen expressionin tissue sites between these subgroups has remained an unresolved problem.

The aim of the present work was to elucidate possible differences in the antigen expression and the reactivity with lectins within several organs from A1 and A2 secretor individuals using lectin- and immunohistochemical methods.

Materials and Methods

Specimens of the sublingual gland, trachea, and tongue were obtained at autop-sies. Blood goup A subtypes and Lewis phenotypes of the donors were determined by the routine hemagglutination test. Twenty A1 secretor and 8 A2 secretor individuals were used in this study. Specimens of the thymus from 5 A1 and 3 A2 donors were also examined. The specimens were fixed in 10% formalin, embedded in paraffin and serially sectioned at 5μm. The experiments were performedas previously described using monoclonal anti-A or -H antibodies, and blood groupA or H specific lectins conjugated with horseradish peroxidase(HRP)[3,4]. Monoclonal anti-A and -A1(type 3 and 4 specific) antibodies were purchased from Biotest, Germany. Anti-H(type 1 and 2 chain specific) antibody was obtained from Chembiomed, Canada. HRP-labelled Helix pomatia agglutinin(HPA), Dolichos biflorus agglutinin(DBA) and Ulex europaeus agglutinin I(UEA) were purchased from E.Y.Laboratories, USA. Following incubation with antibodies and lectins, tissue sites reactive with the reagents were visualized using a Strept-Avidin Biotin immunostaining kit(Nichirei,Japan) and/or diaminobenzidine-H_2O_2 medium.

Results

Hassall's corpuscles

The Hassall's corpuscles from A1 secretor individuals showed good reactivity with anti-A, HPA and DBA, and relatively weak with anti-A1 and UEA. Weak or no staining was observed with Anti-H. The Hassall's corpuscles from A2 secretors were clearly stained with anti-A and -H antibodies. They also reacted with HPA and UEA but not with anti A1 and DBA.

Sublingual gland

Nearly all the mucous cells from A1 individuals were stained with anti-A, HPA,DBA and UEA. Anti-H antibody exhibited good reactivity with a small number of the mucous cells and no reactivity with the remaining cells showing a mosaic distribution pattern of H antigen. Although the mucous cells from A2 individuals were clearly stained with anti-A,-H, HPA and UEA, DBA exhibited feeble or no reactivity with these cells. Anti A1 antibody showed no reactivity with the mucous cells in the sublingual gland. No positive reactions were recognized with these reagents in the serous cells of the glands.

Tracheal gland

In the mucous cells of the tracheal glands, the reactivity with monoclonal antibodies and lectins was essentially the same as that observed in the sublingual glands except that DBA showed good reactivity with the mucous cells from both A1 and A2 groups. The serous cells of the tracheal glands exhibited

no reactivity with the reagents used in this study.

Lingual gland

The mucous cells in the posterior lingual glands from A1 individuals showed homogeneous staining with anti-A, HPA, DBA and UEA. Anti-H antibody exhibited feeble or no staining with these cells. The serous cells in von Ebner's glands showed strong but mosaic reactivity with these reagents including anti-H antibody. The mucous cells from A2 individuals exhibited a mosaic reactivity with anti-A, HPA and DBA and homogeneous reactivity with anti H and UEA. The serous cells in von Ebner's glands from A2 individuals exhibited essentially the same reactivity with these reagents except that DBA showed feeble reactivity.

Endothelial cells of blood vessels

Endothelial cells from A1 individuals were strongly stained with anti-A and HPA, feebly with UEA and not with anti-H, whereas cells from A2 individuals were clearly stained with anti-H and UEA as well as anti-A and HPA. DBA exhibited no reactivity with these cells from both A1 and A2 individuals.

The results obtained in this study are summarized in Table 1.

Discussion

The differences in the chemical nature of A1 and A2 erythrocytes has been defined. In the A1 phenotype, the determinant structure of A antigen is found on type 1, 2, 3 and 4 core structures. However, the corresponding structure in the A2 erythrocytes is found only on type 1 and 2, and is less abundant[5].

The results obtained in this study indicate that the pattern of expression of A and H antigen varies between the organs and is complicated. Since the A type3 or 4 determinants recognized by anti-A1 have been found on glycolipids[5], these antigens were presumed to be lost during the embedding process. In fact, the anti-A1 antibody used in this study exhibited no reactivity with the secretory cells, endothelial cells and erythrocytes but reacted with Hassall's corpuscles from A1 donors. The anti-H used in this study, which recognizes type 1 and/or 2 H antigen, exhibited little or weak reactivity with the mucous cells of tracheal glands, sublingual glands, and posterior lingual glands from A1 donors, and showed good reactivity with those from A2 donors. These results indicate that nearly all the H antigens were converted into A antigens in the mucous cells of A1 phenotypes while substantial amounts of H antigen remained unchanged in A2 phenotypes. The serous cells in von Ebner's glands from both A1 and A2 individuals were stained with anti-A and -H, showing a mosaic-like pattern. This result may indicates the presence of a mechanism for inhibiting the conversion of H antigen into A antigen in von Ebner's glands as observed in the serous cells of the submandibular glands. Here only the H antigen was expressed irrespective of the ABO group but dependent on the secretor status of the individual[4].

The reactivity with HPA or DBA varied among the tissues and organs, as seen in Hassall's corpuscles, secretory mucous cells, von Ebner's glands, endothelial cells. DBA did not react with endothelial cells from both A1 and A2 donors, and showed good reactivity with secretory cells from A1 donors, weak to moderate reactivity with secretory mucous cells from A2 donors and feeble reactivity with the serous cells of von Ebner's glands and Hassall's corpuscles from A2 donors. On the other hand, HPA and anti-A showed intense reactivity with these cells from both A1 and A2 individuals. These differences in staining intensity may be explained by differences in binding specificity of these lectins and structurural differences of the A antigens at each tissue site. Torres et al.[6] and Baker et al.[7] reported that HPA has six carbohydrate binding sites and exhibits much broader specificity for terminal GalNac than DBA which has only two carbohydrate binding sites. Since it seems that nearly all the H antigens are converted into A antigens in the secretory cells from A1 donors but remained unchanged in A2 donors, more A antigens are present in tissues from A1 donors than those from A2 individuals. Thus, the present results may be alternatively explained simply by a difference in the amount of A antigens at each tissue sites.

The results of the present study also showed that nearly all the H antigens in the endothelial cells from A1 donors may be converted into A antigen while substantial amount of H antigens remained unchanged in A2 individuals.

Although anti-H did not stain the mucous cells in the posterior lingual glands and tracheal glands from A1 donors, UEA showed moderate to weak reactivity in these cells. This result indicates that UEA reacts with at least two different types of terminal fucose residue in the mucous cells; one is the UEA specific residue which is unreactive with anti-H, and the other is the terminal residue of H antigen recognized by the anti-H used in this study.

The results obtained in this study showed that the expression of A and H anti-gens in selected tissues is different between A1 and A2 individuals.

Table 1. Staining with monoclonal antibodies and lectins in various human tissues from blood group A1 and A2 secretor donors.

	Hassalls corpuscles		Sublingual gl.		Tracheal gl.		Posterior lingual		Ebner's gl.		Endothelial cells	
Blood group	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Reagents												
MoAB-A	++	++	++	++	++	++	++	++	++	++	++	++
MoAB-A1	−	−	−	−	−	−	−	−	−	−	−	−
MoAB-H	W	++	M	++	−	+	−	M	M	+	−	+
HPA	++	++	++	++	++	++	++	M	++	++	++	++
DBA	+	−	+	M	+	+	+	M	M	W	−	−
UEA	W	++	+	++	W	+	+	+	+	+	W	+

MoAB;monoclonal antibody, ++;intense reactivity, +;positive reactivity, -;negative reactivity, W;weak reactivity, M;mosaic pattern,

Summary

The expression of blood group antigens in selected tissues from blood group A1and A2 donors was examined using monoclonal anti-A,-H antibodies and blood group A or H specific lectins. Monoclonal anti-A antibody and HPA showed good reactivity with secretory cells of salivary glands and tracheal glands from both A1 and A2 donors. Endothelial cells and Hassall's corpuscles were also stained by these reagents. DBA showed good reactivity with these cells from A1donors and with mucous cells of tracheal and posterior lingual glands from A2 donors. However, it reacted feebly with the serous cells of von Ebner's glandsand exhibited no reactivity with Hassall's corpuscles from A2 donors. Endothlial cells from both A1 and A2 donors did not stain with DBA. Monoclonal anti-H antibody showed better reactivity with endothelial cells from A2 donors than those from A1.

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