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Distribution of A and B antigens in organs of blood group AB individuals: observations disclosed by a double immunoenzymatic labeling method

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Summary. The localization of A and B antigens in the organs of blood group AB individuals has been studied using a double immunoenzymatic labeling method. Both A and B antigens were found in the various epithelial cells of these organs, but the epithelial cells could be classified into the following four types depending on the reaction pattern with anti-A and anti-B sera: type 1: cells that stained positive with both anti-A and anti-B sera; type 2: cells that stained positive with anti-A serum only; type 3: cells that stained positive with anti-B serum only; type 4: cells that were negative with both sera. The distribution of each of these epithelial cell types varied considerably, even in the same tissue and individual. Our results seem to suggest that a dissociation in the conversion to the A and B antigens occurs in the tissue of individuals belonging to blood group AB and that the degree of this dissociation varies from tissue to tissue and from cell to cell.

Key word: ABH blood group antigens, immunoenzymatic labeling method

Zusammenfassung. Die Lokalisation der A- und B-Antigene in den Organen von Personen der Blutgruppe AB wurde mit einer doppel-immunenzymatischen Methode studiert. In den verschiedenen Epithelzellen dieser Organe konnten beide Antigene nachgewiesen werden. Im Hinblick auf die A- und B-Antigene konnten 4 verschiedene Reaktionsmuster klassifiziert werden: Typ 1: Zellen, welche sich sowohl mit Anti-A als auch mit Anti-B-Seren anfärben ließen; Typ 2: Zellen, welche sich nur mit Anti-A-Serum anfärben ließen; Typ 3: Zellen, welche sich nur mit Anti-B-Serum anfärben ließen; Typ 4: Zellen, welche mit beiden Seren negativ reagierten. Die Verteilung jedes

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dieser epithelialen Zelltypen variierte beträchtlich, auch innerhalb desselben Gewebes und derselben Person. Unsere Ergebnisse legen die Vermutung nahe, daß bei Personen der Blutgruppe AB in der Phase der Konversion zu den A- und B-Antigenen eine Dissoziation stattfindet und daß das Ausmaß zu dieser Dissoziation von Gewebe zu Gewebe und von Zelle zu Zelle variiert.

Schlüsselwort: ABH-Blutgruppenantigene, Immunenzymatische Markierungsmethode

Introduction

The distribution of the ABH blood group antigens has been intensively investigated [1–6]. Ishiyama [7, 8] has studied the distribution patterns of the ABH blood group antigens in human tissue and has reported that the distribution of cells producing the ABH antigens varies considerably.

Recently, it has become widely acknowledged that ovarian cyst fluid [9], saliva [10–12], semen [10, 11, 13] and erythrocytes [14] from individuals belonging to blood group AB contain specific macromolecules, which in turn are believed to possess a similar amount of both the A and the B antigens, and that in cases where the blood group of a stain indicates AB the detection of these specific macromolecules is useful in determining whether the sample is genuinely AB or a mixture of blood groups A and B. Little, however, is known about how the A and B antigens are distributed in the tissue of individuals belonging to blood group AB, or whether both A and B antigens are always present in specific macromolecules in body fluids or on cell membranes.

In a previous study, we showed that in an individual with blood group AB the production of A and B antigens in mucous cells and duct cells of the salivary glands varies from cell to cell [15]. Ishiyama [7, 8] also arrived at a similar conclusion in some of the specimens that he studied. In this present study, we have further pursued our investigation of the distribution of the A and B antigens in various human organs identified as belonging to blood group AB by using a double immunoenzymatic labeling method.

Materials and methods

In this study, 14 autopsy cases (8 males and 6 females, aged 1–79 years) were used. ABO blood grouping was performed as a routine clinical examination in Gifu University Hospital using the hemagglutination test. Grouping into secretor and non-secretor, and identification of Lewis phenotypes and A_1B and A_2B were not carried out. Tissue samples investigated were taken from formalin-fixed lung, esophagus, stomach, duodenum, pancreas, kidney, prostata, testis and uterine cervix. It was impossible to obtain specimens of all organs in every case, however, and organs showing severe pathologic conditions or postmortem changes, such as autolysis or detachment of the epithelium, were excluded from our examination. Paraffin-embedded tissues were serially sectioned (3 μm) and the immunoperoxidase reaction was performed according to a double immunoenzymatic labeling method based on an indirect immunoperoxidase method (Table 1).

In order to obtain two different stains to identify the A and B antigens, the following substrates were employed: 3,3′-diaminobenzidine-4HCl (DAB) for brown and 4-chloro-1-naphthol for grayish-blue. Section 1 was prepared by first applying anti-A serum, followed by

Table 1. Procedure of the double immunoenzymatic labeling method. Modified from Nakane [16]

1.	Endogenous peroxidase activity inhibited by placing sections in 3% H_2O_2 solution for 10 min
2.	Two washings in PBS for 5 min each
3.	Suppression of non-specific background staining by the application of 4% calf albumin for 30 min
4.	Application of primary antisera for 15 min Section 1 – Anti-A serum Section 2 – Anti-B serum
5.	Three washings in PBS for 5 min each
6.	Application of secondary antisera for 15 min
7.	Three washings in PBS for 5 min each
8.	Immersion of sections in freshly prepared solution of DAB-4HCl with 0.01% H_2O_2 for 10 min
9.	Three washings in a glycine-HCl buffer (pH 2.0) for 40 min each
10.	Three washings in PBS for 5 min each
11.	Application of primary antisera for 15 min Section 1 – Anti-B serum Section 2 – Anti-A serum
12.	Three washings in PBS for 5 min each
13.	Application of secondary antisera for 15 min
14.	Three washings in PBS for 5 min each
15.	Immersion of sections in freshly prepared solution of 4-chloro-1-naphthol with 0.01% H_2O_2 for 10 min
16.	Counterstaining with neutral red and mounting in glycerin-gelatin

immersion in DAB solution. The same section was then treated with anti-B serum and then immersed in 4-chloro-1-naphthol solution. Thus, the cells that reacted with the anti-A serum in section 1 stained brown and those that reacted only with the anti-B serum stained grayish-blue. Using a reverse procedure, section 2 was prepared by applying first anti-B serum and then anti-A serum. Thus, the results obtained in section 2 were the exact opposite of those obtained in section 1. If the cells stained brown in both sections they were considered to express both A and B antigens.

The antisera and products used in this study were as follows: standard rabbit anti-A serum and goat anti-B serum (Tokyo Standard Serum, Tokyo, Japan); peroxidase-conjugated swine anti-rabbit immunoglobulin and rabbit anti-goat immunoglobulin (DAKO, Glostrup, Denmark); DAB (Nakarai Chemical, Kyoto, Japan); and 4-chloro-1-naphthol (Merck, Darmstadt, FRG).

Results

The staining reactions of the epithelial cells to anti-A and anti-B sera were used as the basis for division of them into the following four types: type 1: cells that stained positive with both anti-A and anti-B sera; type 2: cells that stained positive with only anti-A serum; type 3: cells that stained positive with only anti-B serum; and type 4: cells that were negative with both. The localization of both A and B antigens in the organs was as follows:

Lung

Sections from 14 autopsy cases were examined, including the bronchus, the bronchioles, and the bronchial glands. Both A and B antigens were observed in the epithelial cells from the bronchus and bronchioles and in the mucous cells and ductual cells of the bronchial glands. Most of the ciliated cells, goblet cells and basal cells of the bronchus and bronchioles were classified as type 1, but in two instances a few type 2 and type 3 cells also were observed. Most of the mucous cells of the bronchial glands also were classified as type 1. In eight cases, however, a few type 2 and type 3 cells were present, and in two of these cases a few type 4 cells were also observed. These results in the bronchial glands were similar to those obtained from our previous investigation of salivary glands from individuals belonging to the ABSe group [15].

Esophagus

Of 11 cases that were examined, the examination included the esophageal glands in 4. Both A and B antigens were observed in the mucosal epithelial cells, except for the basal layer, and in the mucous cells and ductal cells of the esophageal glands. In 10 cases, foci of type 2 and type 3 cells were present in the mucosal epithelium (Fig. 1). In 1 of 4 cases examined, a few type 2 and type 3 cells were observed in the esophageal glands.

Stomach

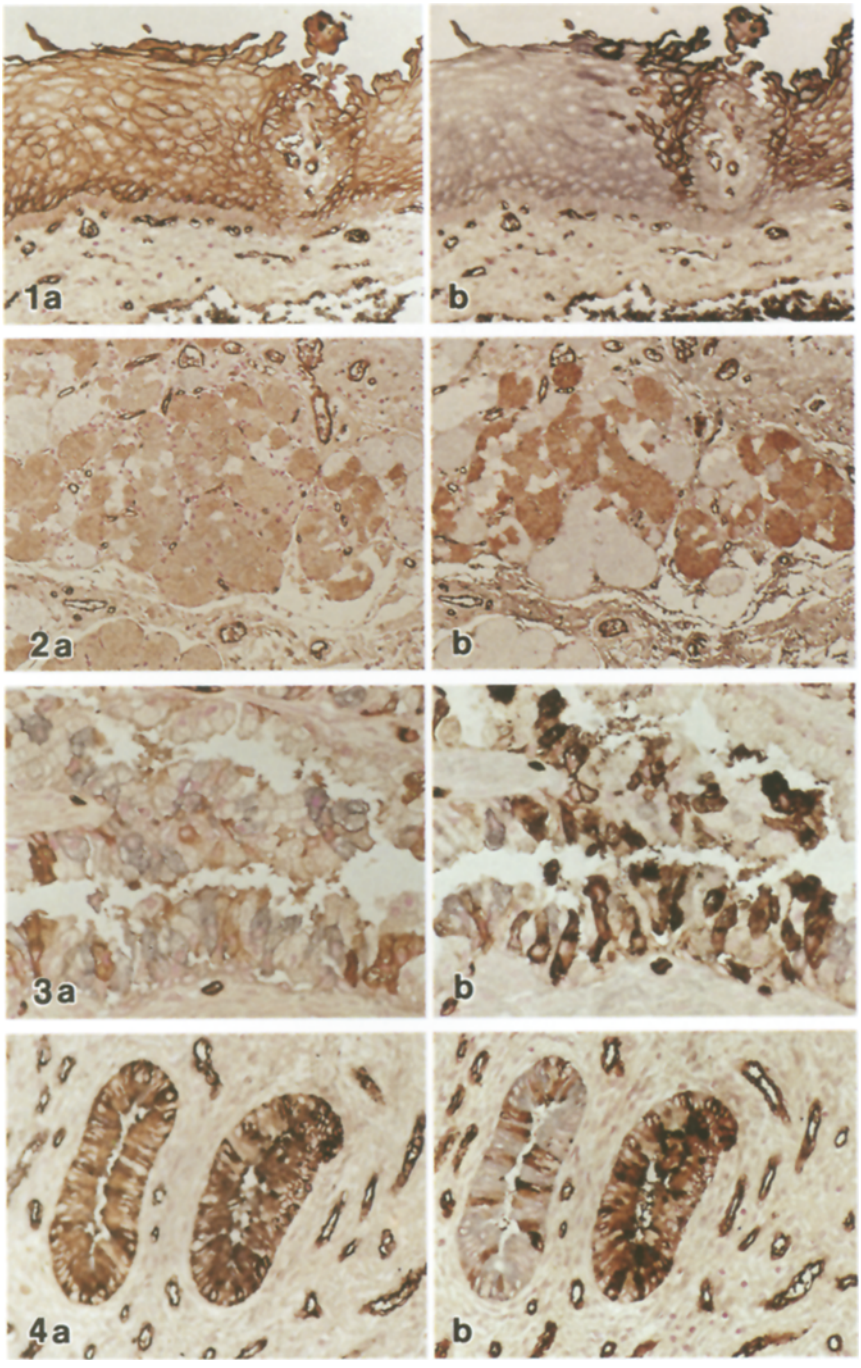
Nine cases were used to examine the localization of A and B antigens in the pyloric canal and four for their localization in the body of the stomach. A and B antigens were observed in the columnar mucous cells, the parietal cells and the mucous cells of the pyloric gland. Most of the columnar mucous cells and the parietal cells were classified as type 1. In two cases, however, a few of the columnar mucous cells were classified as type 2. Type 2 and type 3 cells were also observed in the stomach glands. The classification of the mucous cells of the pyloric gland ranged from type 1 to type 4, and the distribution of the four cellular types varied considerably from area to area and from case to case.

Fig. 1a, b. Esophageal mucosa from a 54-year-old man. A and B antigens are observed in the mucosal epithelium, except for basal layer. **a** Section 1; **b** section 2. In section 2, some cells stained positive with only the anti-A serum. × 230

Fig. 2a, b. Duodenal gland from a 74-year-old man. A and B antigens are observed in the mucous cells. These cells are shown to have a mixture of antigens from type 1 to type 4. **a** Section 1; **b** section 2. × 70

Fig. 3a, b. Prostatic gland from a 54-year-old man. A and B antigens are observed in the columnar cells. These cells are shown to have a mixture of antigens from type 1 to type 4. **a** Section 1; **b** section 2. × 230

Fig. 4a, b. Cervical gland from a 69-year-old woman. A and B antigens are observed in the mucous cells. **a** Section 1; **b** section 2. In section 2, some cells stain positive to anti-A serum only. × 110


1a
b
2a
b
3a
b
4a
b

Duodenum

Fourteen cases were used to examine the localization of A and B antigens in the duodenal gland. Severe postmortem changes made it impossible to localize these antigens in the mucosal epithelial cells. The mucous cells of the duodenal gland were seen to have a mixture of antigens from type 1 to type 4, and the distribution of all four cellular types varied considerably from case to case and from area to area (Fig. 2).

Pancreas

Nine cases were examined. Both A and B antigens were observed in the acinar cells and in the large interlobular ductal cells. In all cases the acinar cells ranged from type 1 to type 3 and in five cases, type 4 cells were also present. All large interlobular ductal cells were classified as type 1.

Kidney

In the 13 cases examined, both A and B antigens were present mainly in the epithelium of the collecting tubules and most of the epithelial cells were classified as being type 1. In 4 cases, however, type 2 and type 3 cells were present.

Bladder

In the five cases studied, both A and B antigens were present in the mucosal epithelium. Some of the superficial epithelial cells did not stain with either anti-A or anti-B and in one case, small foci of type 3 cells were observed.

Prostata

In the five cases studied, both A and B antigens were observed in the epithelial cells of the prostatic gland. Classification of the epithelial cells ranged from type 1 to type 4 (Fig. 3), and the distribution of these four cellular types varied considerably from area to area and from case to case.

Testis

In the four cases studied, comprising three adults and one infant, both A and B antigens were observed in the complex stratified epithelium of the seminiferous tubules. In two of the adults all cells were found to be type 1. In the remaining adult and infant, the epithelium of the seminiferous tubules consisted only of type 4 cells.

Uterine cervix

In the four cases studied, both A and B antigens were present in the columnar epithelial cells, in the mucous cells of the cervical gland and in the squamous cells of the portio. All cases exhibited varying numbers of type 2 and type 3 cells in the columnar epithelial cells and mucous cells of the cervical gland (Fig. 4), and in two cases a few type 4 cells were observed. All squamous cells of the portio were classified as type 1.

Discussion

In the present study, the grouping of A_1B and A_2B were not carried out. All cases might be considered A_1B, however, because the percentage of blood group A_2B among blood group AB individuals in the Japanese population is very low, about 2% [16].

Nakane [17] was the first to develop a method for detecting the simultaneous localization of multiple tissue antigens using an immunoenzymatic labeling method. Since then, several investigators have modified his method and have examined the localization of several antigens [18]. An advantage of this method is that both labels are simultaneously visible in a single histological section, but this method cannot detect trace amounts of other antigens, at sites where an antigen is highly concentrated. Heavy labeling with an enzyme or a substrate can potentially obscure weak labeling with another enzyme or substrate. Sternberger et al. [19, 20] have found that the DAB reaction masks the antigenic and catalytic sites of the first set of immunoreagents thus preventing an interaction with the second set. In the present study cells producing A and B antigens stained brown in both sections 1 and 2. This may have been due to the reason given by Sternberger et al. [19, 20].

Although the distribution of ABH blood group antigens in human tissue has been intensively studied, little is known about how A and B antigens are distributed in the tissue of individuals belonging to blood group AB. In our previous study of the salivary glands it was found that most of the mucous cells produced both A and B antigens and only a few, approximately 1%, were classified as type 2, 3, or 4. In contrast, there was no predominance of any particular type in ductal cells [15].

In the present study, the distribution of A and B antigens was found to vary among epithelial cells producing ABH blood group antigens. In all the organs studied, the duodenal gland, the pyloric gland and the prostatic gland showed the most severe dissociation of activity in the production of A and/or B antigens. In some cases, however, the acinar of the pancreas, the uterine cervical epithelium and the cervical gland also showed the same severe degree of dissociation. These results would seem to indicate that a dissociation in the conversion to A and B antigens occurs commonly among cells belonging to blood group AB and that the degree of this dissociation varies from tissue to tissue in the various organs of an individual.

In the present study, we could not clarify why the dissociation of conversion to the A and B antigens occurs among the epithelial cells of individuals with blood group AB. Nakajima et al. [3] have reported that although mature mucous granules of the human minor salivary glands react with group-specific lectin, immature mucous granules do not. These results may indicate that the conversion to A and B antigens in the secretory granules occurs in relation to the maturation process of the granules and that the dissociation of the conversion to A and B antigens found in the secreting cells of blood group AB individuals is attributable to the maturation process of the secretory granules. In the present study, however, this dissociation was also observed in non-secreting cells, such as squamous cells of the esophageal mucosa and transitional cells of the bladder. These results may suggest another possibility, i.e. that the dissociation of conversion to A and B antigens in the epithelial cells of individuals with blood group AB occurs under the control of A and B genes [7, 8].

Several investigators have reported that the existence of specific macromolecules containing both A and B antigens can be demonstrated in some body fluids, and have postulated that the detection of these specific macromolecules is useful in determining whether the sample is genuinely AB or a mixture of blood groups A and B [9–14]. They have not, however, clarified the exact nature of these body fluids, and the question as to whether the A and B antigens are present in only these macromolecules in these body fluids, or whether some cells of blood group AB individuals produce only the A or the B antigen remains unanswered.

The present study, however, has revealed that cells belonging to blood group AB individuals may not always produce the specific macromolecules and that the production of A and/or B antigens in these cells can vary from tissue to tissue and from cell to cell even in one individual. Therefore, body fluids produced by cells in individuals with blood group AB can be extremely complex.

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