On the New Secretor Status Recognized by Saliva Specific ABH Antigens

KAORU SAGISAKA, MINEO IWASA* and TSUYOSHI YOKOI

*Department of Forensic Medicine, Tohoku University School
of Medicine, Sendai 980 and *Department of Legal
Medicine Gifu University School of Medicine, Gifu 500*

SAGISAKA, K., IWASA, M. and YOKOI, T. *On the New Secretor Status Recognized by Saliva Specific ABH Antigens.* Tohoku J. exp. Med., 1984, **143** (3), 255-259 — The present authors have reported that each of ABH antigens of saliva consisted of saliva specific antigen and common antigen between saliva and red cells. Using saliva specific anti-A and -B, 77 samples of A, B and AB groups could be determined as strong secretor (Se) or weak secretor (se). Frequency of Se was 62% for A group saliva, 45% for B, and 76% for A antigen and 75% for B antigen of AB group saliva, respectively. The secretor status of twenty-four O salivas could not be determined by saliva specific anti-H. Compared with the ordinary secretor system, some subjects belonging to Se in the ordinary system were typed as se in the new system, whereas all se saliva (three cases) in the ordinary system were typed as se in the new system. ——— saliva; ABH blood group; secretor system

Physicochemical and serological differences between ABH antigens of red cell and saliva were pointed out (Morgan and Watkins 1948); the former consisted of glycolipid insoluble in water, whereas the latter was glycoprotein soluble in water. In the preceding papers (Sagisaka et al. 1983, 1984a, b), we reported that ABH antigens of red cell and saliva could be identified according to their serological differences. Quantity of ABH antigens in saliva was genetically controlled, and the secretor system of saliva was established (Schiff and Sasaki 1932). Our reports demonstrated that ABH antigens of saliva were composed of saliva specific antigen and common antigen between saliva and red cell (Sagisaka et al. 1983, 1984a, b). The secretor status of saliva was determined by the amount of whole blood group antigen which was measured by agglutination inhibition test. In this paper, we investigated the amount of ABH antigens specific for only saliva on healthy 101 subjects and discussed the relation with the ordinary secretor status.

MATERIALS AND METHODS

Preparation of antisera. Rabbit antisera specific for saliva A (As), B (Bs) and H (Hs)

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were the same as described in preceding papers (Sagisaka et al. 1983, 1984a, b, c). In brief, rabbits were immunized with O, A or B saliva and the resulting antisera were absorbed with red cells of corresponding group.

Determination of secretor status. Saliva collected was boiled for 10 min and stored at -20°C until use. Before the test, mucin of saliva was removed by centrifugation. Secretor in the ordinary system was determined as follows. Saliva was serially diluted ($0.025\ \mu\text{l}$ each) in microtitre plate (U type) to which was added anti-A, -B (human origin) or -H (*Ulex europaeus*) of titer 1:8. After stirring for 10 min, the same volume of 1% red cell suspension was added. After standing for 2 hr at room temperature, agglutination was read. By the method, saliva with an inhibition titer less than 1:2 was determined as non-secretor (se). To examine the new secretor status appropriate titers of anti-Hs, -As and -Bs was beforehand established using latex coated with O, B, or A group saliva. Anti-As and -Bs of titers 1:32 and 1:128 gave satisfactory results, but the difference in an amount of Hs was not distinguished by anti-Hs of any titers.

Coating latex with O, A, or B saliva. Latex particles ($0.6\ \mu\text{m}$ in diameter, specific gravity 1.16) were kindly provided by Matsuzawa, T., Professor of Legal Medicine, Juntendo University. Latex was coated with saliva antigen as described previously (Sagisaka et al.

TABLE 1. *Relationship of secretor status determined by total amount of blood group antigen and saliva specific antigen*

Blood group	Secretor status			
	I*	Number	II†	Number
O	Se	15		
	se	8	‡	
A	Se	33	Se	21
			se	12
	se	1	Se	0
			se	1
B	Se	21	Se	10
			se	10 (1)
	se	2	Se	0
			se	2
AB	Se	21	Se	16
			se	5
	A		Se	0
	se	0	se	0
	Se	20	Se	15
			se	4 (1)
	B		Se	0
	se	1	se	1

* I is the ordinary secretor system determined by usual agglutination inhibition test.

† II is the new secretor status determined by anti-Hs, -As, and -Bs.

‡ The new secretor status of O group saliva cannot be determined by anti-Hs.

The cases indicated in parentheses could not be clearly determined.

1983). To prepare desirable latex particles, suitable saliva should be chosen. If not so, non-specific agglutination occurred presumably because of mucin in the saliva.

Elution test. Some A or B group saliva which was determined as new Se or se was dropped on gauze threads. After being fixed by heating at 100°C for 10 min, the threads were sensitized with corresponding anti -As or -Bs. After being washed with cold saline one drop of 0.05 M glycine-NaOH pH 8.2 buffer was added. Elution was performed by heating at 55°C for 10 min. Eluate was applied on a well of microtitre plate (U type) to which 0.05% latex coated with A or B saliva was added.

Lewis type. Lewis type of saliva was examined with agglutination inhibition test.

RESULTS

Examinations of secretor status on 101 samples are summarized in Table 1. Of all samples 89 subjects were grouped as Se and 11 as se in the ordinary secretor system. One case of AB group was typed as Se for A antigen but se for B one.

By analyses with anti-As, 67% of A and AB group subjects were typed as Se and 33% as se. As for Bs antigen, 60% and 40% were typed as Se and se, respectively (Fig. 1). On the O group samples, the new secretor system could not be determined by anti-Hs of any titers. Some of A, B and AB subjects belonging to Se in the ordinary system were typed as se in the new system. However, all three se type subjects of which groups were A, B and AB were typed as se in the new system. For example, an AB group subject had inhibition titers of 1:64 to anti-A and 1:1 to anti-B, and the new secretor types of the subject were determined as se for both As and Bs. No relationship between the new secretor system and Le^a type was noted. Saliva stains from Se and se types in the new system were examined by the elution test using anti-As and -Bs, resulting in that the saliva stains of both types give positive reaction. The saliva of both types was diluted serially and each the diluent was dropped on gauze. The elution test of the diluted saliva samples showed that se saliva diminished at 1:4 dilution whereas Se kept yet the activity.

Fig. 1. Agglutination inhibition test of 34 subjects of B group saliva with anti-Bs. Twelve subjects were determined as Se and the remainder as se. Subjects of 2D, 3D and 3J were typical cases of Se and 1A, 4B and 4C were se. In control experiments, both 4E (A group saliva) and 4F (saline) give negative results.

DISCUSSION

Amounts of saliva specific antigens were investigated using anti-Hs, -As, and -Bs. As for H antigen, Morgan and Watkins (1948) pointed out that there were serological differences between red cells and saliva. In the preceding paper (Sagisaka et al. 1983), the difference of H antigen in red cells and saliva was identified with rabbit anti-Hs and anti-O red cell. We reported also that rabbits and guinea pigs could produce specific antibody to saliva A and B antigens when the animals were immunized (Sagisaka et al. 1984a, b). It meant that these animals recognized the minute differences in A and B antigenicities between red cell and saliva. Analyses of amounts of As and Bs showed that individual difference equivalent to the ordinary secretor status was detected by agglutination inhibition test. Moreover, frequency of the new secretor system did not correspond with that of the ordinary system. Of the 101 samples tested, some subjects of Se in the ordinary system were proved to be se by the test with anti-As or -Bs. On the contrary, no case belonging to se in the ordinary system was typed as Se in the new system. Therefore, it was suggested that the new secretor system was determined by factors other than the ordinary Se-se genes.

Few individual difference in the amount of Hs antigen was detected. It agreed that deviation in an amount of H antigen was considerably small as compared with those of A and B in the ordinary system (Kanemitsu et al. 1981).

Saliva stains of Se and se in the new system were similarly detected by the elution test. Accordingly se in the new system did not denote to secrete nothing but to secrete relatively few amount of the antigen.

Genetic study and biochemical analyses of As and Bs antigens are under consideration.

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