DETECTION OF BLOOD GROUP H ANTIGENS OF RED CELLS, BLOOD AND SALIVA STAINS, AND HAIRS BY ANTI-H REAGENTS

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INTRODUCTION

Numerous anti-H active reagents have been found in normal and immunnized human and animal sera and the extracts of certain plant seeds. The reagents from different sources enabled us to undertake a study of the detection of the different nature of the human H substances. This papers reports the specificity differences among various anti-H reagents against red cells, blood and saliva stains and hairs.

MATERIALS AND METHODS

Human isoagglutinins, chicken immune serum, monoclonal antibodies, eel sera from Anguilla japonica and Ulex europaeus lectins were used in this examinations (Matsuki et al. 1990). Determination of agglutinating activities of red cells, agglutination inhibition experiments were identical to that previously reported (Matsuki et al. 1990). Absorption-elution experiments were done with O cells, using Bombay phenotype cells as a control by the heat elution method of Landsteiner and Miller.

RESULTS

The sera and lectins which preferentially react with group O red cells and differentiate group O secretor salivas from non-secretors were previously reported (Matsuki et al. 1990).

Table 1. Agglutinability of group O and A_1 red cells

		Agglutinability of red cells						
Red cell		Anti-H Bombay	Anti-H F.M.	Anti-H chicken	Anti-H mouse	Eel serum	Ulex I	Ulex II
Oh	Saline	0	0	4	0	0	0	0
O	Saline	32	32	4,096	64	1,024	32	32
A_1	Saline	8	4	2,048	0	32	8	8
Oh	Papain	2	1	16	0	2	0	0
O	Papain	128	128	16,384	128	8,192	1,024	1,024
A_1	Papain	32	32	8,192	0	1,024	256	256

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Agglutinability of saline and papain-treated group O and A1 red cells with anti-H active reagents were shown in Table 1. In the saline agglutination large differences of agglutinability between O and A_1 was demonstrated in mouse monoclonal anti-H and eel serum. After the red cells were treated with papain the agglutinability of the red cells were enhanced in all of the reagents, especially in Ulex europaeus lectins. The reactivity of red cells of group A genotypes, A_1O and A_1A_1 were tested with the anti-H reagents (Table 2). Most of the reagents except chicken anti-H were agglutinated A_1O cells stronger than A_1A_1 cells. Difference of the agglutinability between A_1O and A_1A_1 was remarkable in eel serum by saline agglutination. Reactivity of BO and BB red cells were the same strength as A_1O and A_1A_1. Therefore, in the E blood groups determined by eel type II serum (Furuhata 1974), type E red cells react with the eel serum as strongly as similar to O cells may be heterozygote A_1O and BO. Type e whose red cells agglutinate very weakly or not at all by the eel serum may be homozygote of A_1 and B.

Table 2. Agglutinability of A_1O and A_1A_1 red cells

Blood group genotype	Agglutinability of red cells						
	Anti-H Bombay	Anti-H F.M.*	Anti-H chicken	Anti-H mouse*	Eel	Ulex I	Ulex II
OO	32	128	1024	128	1024	64	64
A_1O	8	32	512	16	32	16	16
A_1A_1	4	0	512	1	0	4	4

* Papain treated cells were used

Table 3. Inhibition of agglutinations by L-fucose and fucose containing oligosaccharides

Sugar	Agglutination inhibition titer					
	Anti-H Bombay	Anti-H chicken	Anti-H mouse	Eel	Ulex I	Ulex II
L-Fuc (0.5M)	2	4	0	512	256	<4
2'-FL (0.1M)	128	512	32	256	2,048	2,048
LNF I (0.1M)	256	32	32	0	32	32
LNF II (0.1M)	0	0	0	0	0	0
LNDF I (0.1M)	0	32	0	32	0	0

L-Fuc: L-fucose, 2'-FL: 2'-fucosyllactose, LNF I: lacto-N-fucopentaose I, LNF II: lacto-N-fucopentaose II, LNDF I: lacto-N-difucohexaose I

The results of inhibition of anti-H reagents by L-fucose and fucose containing oligosaccharides were shown in Table 3. The human, chicken and mouse anti-H sera were inhibited by 2'-FL and LNF I which contains α-(1→2)-fucosyl residues in the structures. L-Fucose and 2'-FL were most strong inhibitor for eel serum and

Ulex I lectin. Ulex II lectin was hardly inhibited by L-fucose while 2'-FL is the highest inhibitor against the lectin. It is demonstrated that the Ulex II lectin is inhibited by di-N-acetyl-chitobiose which has unrelated structure of H active antigen determinants (Matsumoto and Oosawa 1969).
The results of the absorption test of anti-H reagents with blood and saliva stains indicated that chicken and mouse monoclonal antibodies, Ulex I and II lectins showed powerful affinity to the stains (Table 4).

Table 4. Absorption test and Absorption-elution test of anti-H sera and lectins with group O blood and saliva stains, and hairs

Anti-H active reagent	Absoption			Agglutination of eluate from					
	Before	After with		Test cells	Blood		Hair		Saliva
		Blood	Saliva		O	Oh	O	Oh	O
Anti-H F.M.	32	2	2	Saline	+	-	(+)	-	+
				Papain	+	-	(+)	-	+
Anti-H chicken	32	0	0	Saline	++	-	(+)	-	+
				Papain	++	-	(+)	-	++
Anti-H mouse	32	0	0	Saline	-	-	-	-	-
				Papain	-	-	-	-	-
Eel	32	2	16	Saline	+	-	-	-	-
				Papain	++	-	-	-	-
Ulex I	32	0	0	Saline	-	-	-	-	-
				Papain	+	-	(+)	-	+
Ulex II	32	0	0	Saline	-	-	-	-	-
				Papain	+	-	(+)	-	+

(+): not all hairs tested showed positive reaction

When the chicken serum was used for absorption and elution test of the stains and hairs strong agglutination of O red cells with the eluate was observed (Table 4). IgM anti-H mouse monoclonal antibodies and Ulex lectins were heardly eluted by the heating. Most strong agglutination of the eluates from hair was observed when Ulex I and II lectins were used and papain treated O red cells were used as test cells. Eluates from Ulex lectins were only reactive with papain treated O red cells.

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