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Short Communication

CHARACTERIZATION OF ANTI-Le^b ANTIBODY PURIFIED FROM SERUM OF IMMUNIZED RABBITS

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SUMMARY: An anti-Le^b antibody was produced in sera of rabbits by immunization with human saliva from blood group O Le(a-b+) secretor and purified by sequential use of silica beads immobilized with H type 1, Le^a and Le^b. The purified antibody agglutinated only Le(a-b+) red cells irrespective of their ABO blood type. Hemagglutination reaction with the antibody of blood group O Le(a-b+) red cells was inhibited not only by saliva samples from blood group Le(a-b+) secretors and Synsorb beads immobilized with Le^b hapten, but also weakly by Synsorb immobilized with Y and H type 2 haptens.

Anti-Lewis antibodies naturally occurring in human sera are commonly in a low concentration and react best at low temperature. Some anti-Le^a and anti-Le^x and less frequently anti-Le^b antibodies have been reported to cause hemolysis even at the first transfusion and to affect the survival of transplanted kidney in recipients (1).

For blood typing, anti-Le^b as well as anti-Le^a antibodies were produced in goats or rabbits by injecting Le^b- and Le^a-active substances, respectively (2-4). The anti-Le^b antibody derived from rabbits was purified by affinity chromato-

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graphy on Lewis blood group-active immunoadsorbents (4). Its precise specificity for Lewis-related antigens, however, has not yet been identified.

In the present paper, we describe the characterization of the rabbit anti-Le^b antibody purified with Synsorb beads immobilized with various kinds of haptens, and compare its specificity with that of a mouse monoclonal antibody.

Synsorb H type 1 (Fuc α 1-2Gal β 1-3GlcNAc β -R), H type 2 (Fuc α 1-2Gal β 1-4GlcNAc β -R), Le^a (Gal β 1-3[Fuc α 1-4]GlcNAc β -R), Le^b (Fuc α 1-2Gal β 1-3[Fuc α 1-4]GlcNAc β -R), X (Gal β 1-4[Fuc α 1-3]GlcNAc β -R), Y (Fuc α 1-2Gal β 1-4[Fuc α 1-3]GlcNAc β -R), Le^c (Gal β 1-3GlcNAc β -R) and H disaccharide (Fuc α 1-2Gal β -R) beads are obtained from Chembiomed (Edmonton, Canada). These Synsorb beads are prepared by coupling each synthetic oligosaccharide to a solid matrix, silica beads (0.41-0.88 μ mol hapten/g). Human red blood cells and saliva samples, whose ABO and Lewis blood types and secretor status had been determined, were supplied from healthy volunteers.

Preparation and purification of rabbit anti-Le^b antibody: Human saliva of blood group O Le(a-b+) secretors was boiled in a water bath for 30 min. After centrifugation, the supernatant (63 ml) was dialyzed against distilled water, and the non-dialyzed portion was lyophilized. The resulting powder was dissolved in 6.3 ml of saline and used for immunization. Two Japanese white rabbits were injected every seven days (three times in total) with 1.5 ml of the solution of saliva in Freund's complete adjuvant and bled in approximately one month after the last injection.

The purification of the antibody from rabbit sera was performed by the method of Lemieux et al. (4) with some modifications. Each serum (2.0 ml) was dialyzed against 10 mM phosphate buffered saline, pH 7.0 (PBS) and applied to Synsorb H type 1, Le^a and Le^b bead columns (0.5 $\times$ 5 cm), successively. The materials which passed through Synsorb H type 1 and Le^a bead columns was applied on a Synsorb Le^b bead column. After washing the column with PBS, the bound materials were eluted with 1% NH₄OH-PBS (Fig. 1). The fractions showing hemagglutinability with papain-treated blood group O Le(a-b+) red cells were pooled, neutralized with saturated KH₂PO₄, dialyzed against PBS, and concentrated up to 1 ml by pressure dialysis through PM-10 membrane (Amicon Corp., Denver, CO).

In place of Synsorb Le^b beads, the antibody was also applied on Synsorb H type 2 and Y bead columns (0.5 $\times$ 3 cm). The antibody was completely bound to each column and eluted by the same manner as described above.

Fig. 1. Affinity chromatography of the rabbit (No. 1) anti-Le^b antibody on a Le^b Synsorb column.

The arrow shows the position where 1% NH₄OH-PBS was added. The hemagglutination titer (○) against papain treated blood group O Le(a-b+) red cells was determined as described in "Hemagglutination tests".

The antibody solution prepared with Synsorb H type 1, Le^a and Le^b columns was used for the following studies. All the tests were carried out at least in duplicate.

Hemagglutination tests: Hemagglutination tests were carried out as described previously (5) with papain-treated red cells. The hemagglutination titer was expressed as the reciprocal of the maximum dilution where hemagglutination was observed. Anti-Le^b antibodies were successfully purified from rabbits (Nos. 1 and 2), and their hemagglutination titers were 32- to 64-fold against blood group Le(a-b+) red cells of A, B, and O blood types (n=12). The purified antibody agglutinated only blood group Le(a-b+) red cells but not Le(a+b-) ones, irrespective of their ABO blood type (data not shown).

Hemagglutination inhibition tests: Hemagglutination inhibition tests were performed with human saliva samples fromh different blood groups (n=8). Serially diluted samples (25 μl) were mixed with an equal volume of the aforementioned purified antibody after adjusting the hemagglutination titer against

blood group O Le(a-b+) red cells to four-fold, and the mixture was incubated at 4 C for 2 hr. The remaining activity of the antibody was measured with blood group O Le(a-b+) red cells. The hemagglutination inhibition titer was expressed as the reciprocal of the maximum dilution where the hemagglutination reaction was inhibited. The hemagglutination reaction was inhibited more strongly by salivas from blood group Le(a-b+) secretors than those from blood group Le(a+b-) non-secretors. Thus, the inhibition titers were from 64- to 256-fold and from twofold to 16-fold, respectively. The hemagglutination inhibition tests with the same saliva samples were conducted also with mouse monoclonal anti-Le^b antibody (Chembiomed), and showed that the monoclonal antibody was also inhibited weakly by saliva samples from blood group Le(a+b-) non-secretors (inhibition titers were 4- to 16-fold).

Absorption and hemagglutination inhibition tests: Absorption tests of the purified antibody was tested with various kinds of Synsorb beads (H type 1, H type 2, Le^a, Le^b, X, Y, Le^c and H disaccharide). Twenty milligrams of each kind of beads was mixed in a plastic tube with 50 μ l of the antibody (No. 1 rabbit) whose titer against blood group O Le(a-b+) red cells was adjusted to 32-fold, and the mixture was gently rotated for 2 hr at 4 C.

The hemagglutination titer of the supernatant was measured with blood group O Le(a-b+) red cells. Absorption of the antibody was observed with Le^b, Y and H type 2, and the remaining hemagglutination titers were 4-, 16-, and 16-folds, respectively. The same results were obtained with the purified antibody from No. 2 rabbit.

The specificity of the purified antibody for Lewis-related oligosaccharides was determined also by hemagglutination inhibition tests with Synsorb Le^b, Y and H type 2 beads. Each suspension (20 μ l) of beads which containing 2.5 nmol haptens (2.9 - 6.0 mg beads/tube) was serially diluted, and an equal volume of the purified antibody (titer, twofold) was added to each tube. After standing at 4 C for 2 hr, the hemagglutination inhibition titer of the solution was determined with blood group O Le(a-b+) red cells as described above. Synsorb Le^b was the best inhibitor, and Y and H type 2 were weak inhibitors (Table I). It has been reported previously that human and goat polyclonal and mouse monoclonal anti-Le^b antibodies reacted not only with Le^b, but also with Y, H type 1 and/or H type 2 structures (6), and that the lectin recognizes both Le^b and Y antigens (7). Not a few similarities between Le^b and Y haptens have theoretically been predicted from the analysis of three-dimensional structures of these haptens (8).

Table I. Hemagglutination inhibition titers of the purified anti-Le^b antibody Lewis-related Synsorb beads

Synsorb	Structure	Minimum amount of hapten required for complete inhibition
Le ^b	$ \begin{array}{cc} \text{Gal}\beta 1 \rightarrow 3 \text{GlcNAc}\beta \text{R} & \\ \begin{array}{c} 2 \\ \uparrow \\ \alpha 1 \\ \text{Fuc} \end{array} & \begin{array}{c} 4 \\ \uparrow \\ \alpha 1 \\ \text{Fuc} \end{array} \end{array} $	15.5*
Y	$ \begin{array}{cc} \text{Gal}\beta 1 \rightarrow 4 \text{GlcNAc}\beta \text{R} & \\ \begin{array}{c} 2 \\ \uparrow \\ \alpha 1 \\ \text{Fuc} \end{array} & \begin{array}{c} 3 \\ \uparrow \\ \alpha 1 \\ \text{Fuc} \end{array} \end{array} $	62.5
H type 2	$ \begin{array}{c} \text{Gal}\beta 1 \rightarrow 4 \text{GlcNAc}\beta \text{R} \\ \begin{array}{c} 2 \\ \uparrow \\ \alpha 1 \\ \text{Fuc} \end{array} \end{array} $	125.0

*Values indicated are in nmol giving complete inhibition of hemagglutination with 1.0 ml of rabbit (No. 1) anti-Le^b antibody.

On the other hand, the conformational analysis indicated that the *N*-acetyl groups of GlcNAc residue are close to the fucosyl residue on H type 1 structure but are on the opposite side on H type 2 structure (9). The difference in the position of the *N*-acetyl group might influence the reactivity of the anti-Le^b antibody with H type 1 and 2 structures. Thus, some antibodies might react with Y as well as Le^b hapten. It remains to be classified whether the purified antibody obtained in this study can recognize the different conformational structures between types 1 and 2.

In our previous papers (5,10), we stated that anti-H and anti-B antibodies from egg yolks of immunized hens were purified and characterized by use of Synsorb beads immobilized with blood group haptens. In the present study, we showed not only that the anti-Le^b antibody was produced in sera of rabbits by immunization with human saliva from blood group O Le(a-b+) secretors but also that it was purified easily with Synsorb H type 1, Le^a and Le^b beads. The higher

reactivity with Leb-active structure suggests that the purified antibody is suitable for use as a Lewis blood grouping reagent.

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