Preparation of Anti-Le\textsuperscript{a} and Anti-Rh\textsubscript{0} (D) Sera by Immunization with Blood Group Substance Trapped in Autologous Red Cell Ghost

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YOKOI, T., SAGISAKA, K. and IWASA, M. Preparation of Anti-Le\textsuperscript{a} and Anti-Rh\textsubscript{0} (D) Sera by Immunization with Blood Group Substance Trapped in Autologous Red Cell Ghost. Tohoku J. exp. Med., 1984, 144 (3), 321-325 — Le\textsuperscript{a} substance or immune complex which was prepared with anti-D and D-active Band 3 was trapped in autologous red cell ghost. The trapped immunogens were administered intravenously to rabbits or guinea pigs. Rabbits immunized with Le\textsuperscript{a} substance loading ghost produced antisera of relatively low titers, but they contained specific incomplete anti-Le\textsuperscript{a} antibody (titer 1:128) after absorption procedure, which were higher than that of antisera prepared by the ordinary method. Guinea pig antiserum to the immune complex contained specific incomplete anti-D (titer 1:128). Immunoglobulin class analyses revealed that the anti-Le\textsuperscript{a} consisted mainly of IgM and the anti-D of IgG. It was considered that intravenous injection of the immunogen trapped in ghost is useful for preparing hemagglutinin of sufficient titers. — Le\textsuperscript{a} blood group; Rh-Hr blood group; antigen trapped in ghost; hemagglutinin

It is not extremely rare that anti-Le\textsuperscript{a} is found in human serum as natural cold agglutinin with a low titer. On the other hand, immune anti-Le\textsuperscript{a} was produced in animals such as rabbit, goat and chicken by immunizing with human saliva of Les group (Race and Sanger 1975). In these cases, a large quantity of Les saliva was injected intramuscularly or intravenously. We adopted the immunogen trapped in autologous ghost for preparing anti-Le\textsuperscript{a}.

As for anti-D, many efforts to prepare animal immune anti-D have ended in unsatisfactory results. It might be responsible to the animals which were less sensitive to Rh-Hr antigens. In this paper, the immunogen trapping method was introduced to prepare immune anti-D.
MATERIALS AND METHODS

Anti-Le. Pooled saliva of O. se. Les group was boiled at 100°C for 10 min and centrifuged at 13,000 rpm for 20 min. The supernatant was used for trapping into red cell ghost. An aliquot of the supernatant was mixed with the same volume of ethanol. The resulting precipitate was dissolved in saline at a protein concentration of 20 mg/ml and used for intramuscular injection. Trapping into red cell ghost was performed as described previously with slight modifications (Yokoi et al. 1983a). In brief, one volume of rabbit red cell ghost was mixed with 4 volumes of saliva which was dialyzed against following three buffers for 1 hr at 4°C each; K-PBS (137 mM KCl, 2.7 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄ and 4.4 mM MgCl₂, pH 7.2), 6-fold diluted K-PBS and PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄ and 4.4 mM MgCl₂, pH 7.2). In the third dialyzing step, the ruptured membrane was sealed and the immunogen was trapped into the ghosts. After washing adequately with PBS, the ghosts were used for immunization. When the ghosts containing the immunogen were mixed with anti-Le, no agglutination was observed under a phase contrast microscope. Randomly bred four rabbits (Group 1) were intravenously injected with 2 ml of the ghost suspension once a week for 10 weeks. Other rabbits (Group 2) were intramuscularly immunized with 1 ml of the immunogen prepared by the ethanol precipitation once a week for 6 weeks.

Anti-D. D-active Band 3 was prepared from red cell membrane solubilized with deoxycholic acid (DOC) as described previously (Yokoi et al. 1983b). Band 3 (protein concentration 4.0 mg/ml) was mixed with a half volume of human anti-D (Ortho Diagnostics, New Jersey, USA) and the resulting immune complex was isolated by gel filtrations (Yokoi et al. 1983c). Five animal groups (Groups 3, 4, 5, 6 and 7) were used for preparing anti-D. In Group 3, three rabbits were intravenously injected with 1 ml of Band 3, and intramuscular immunizations with 1 ml of Band 3 were carried out on three rabbits once a week for 5 weeks (Group 4). In Group 5, four guinea pigs were intramuscularly injected with 0.5 ml of Band 3 once a week for 6 weeks. Two animal groups, three rabbits (Group 6) and four guinea pigs (Group 7), were intravenously administered with 1.0 ml of the immune complex (protein concentration 3.0 mg/ml) trapped into autologous red cell ghost once a week for 7 weeks. In the intravenous immunizations (Groups 3, 6 and 7), 1 ml of Freund's complete adjuvant was intramuscularly injected at the initial two injections, separately. In the intramuscular immunizations (Groups 4 and 5), emulsion with 1 ml of Freund's complete adjuvant was injected at the initial two injections. Ten days after the last injection, all the animals were bled.

Absorption of antisera. Rabbit anti-Le was absorbed with one-tenth volume of non-treated or papainized red cell of Le (a-b+) group repeatedly. Crude anti-D sera were absorbed with non-treated or papainized red cells of D negative type.

Separation of immunoglobulin classes. Antisera were fractionated by DEAE-cellulose chromatography as described by Aberson and Rawson (1963).

Antisera. Anti-D of human origin and goat anti-Le and -Le were purchased from Ortho Diagnostics (New Jersey, USA). For anti-globulin test, anti-guinea pig IgG was raised in rabbits according to the method of Axelsen et al. (1977) with slight modifications.

RESULTS

Anti-Le

Rabbits produced antibody of various titers to human red cells according to the routes of immunization (Table 1). The most potent complete and incomplete antibodies (1: 256 and 1: 4,096) were produced in the rabbits of Group 1, but agglutinin activity as specific complete anti-Le was extremely low. However,
incomplete anti-Le\textsuperscript{a} (titer 1:32–128) was detected in all rabbits of Group 1. Of four rabbits of Group 2, one deceased during the immunization and only one of the remainder produced anti-Le\textsuperscript{a} of a sufficient titer. In general, absorptions of several times were necessary for preparing specific anti-Le\textsuperscript{a} in Group 2, whereas twice absorptions with both of non-treated and papainized red blood cells were enough for specification in Group 1. In the both Groups complete anti-Le\textsuperscript{a} was not induced by the present immunization methods.

\textit{Anti-D}

In Groups 3, 4 and 5, all rabbits produced anti-Band 3 precipitin antibody and the titer in Group 4 was very potent. However, absorption with D negative red cell indicated that no anti-D was detected in these antisera. On the other hand, only one of four guinea pigs in Group 7 produced anti-D antibody. As shown in Table 2, the agglutinin titers against normal and papainized D type red cells are listed.

\begin{table}[h]
\centering
\caption{Agglutinin activity of rabbit anti-Le\textsuperscript{a}}
\begin{tabular}{ccc}
\hline
\textbf{Rabbit group} & \textbf{Antiserum No.} & \textbf{Test cells} & \textbf{Before absorption} & \textbf{Agglutinin titer} & \textbf{After absorption} \\
\hline
1 & & Le (a+b−) & 1 : 8 (1 : 2,048) & — (1 : 32) & — (—) \\
 & & Le (a−b+) & 8 (2,048) & — (—) & — (—) \\
2 & & Le (a+b−) & 16 (2,048) & 1 : 2 (128) & — (—) \\
 & & Le (a−b+) & 8 (1,024) & — (—) & — (—) \\
3 & & Le (a+b−) & 256 (4,096) & — (64) & — (—) \\
 & & Le (a−b+) & 256 (2,048) & — (—) & — (—) \\
4 & & Le (a+b−) & 64 (512) & — (32) & — (—) \\
 & & Le (a−b+) & 32 (256) & — (—) & — (—) \\
\hline
\end{tabular}
\end{table}

Titer to papainized red cells are shown in parentheses.

\begin{table}[h]
\centering
\caption{Agglutinin activity of guinea pig anti-D}
\begin{tabular}{ccc}
\hline
\textbf{Test cells} & \textbf{Agglutinin titer} & \\
& \textbf{Before absorption} & \textbf{After absorption} \\
\hline
D (+) & 1 : 1,024 (1 : 8,192) & 1 : 2 (1 : 128) \\
D (—) & 512 (2,048) & — (—) \\
\hline
\end{tabular}
\end{table}

Titer to papainized red cells are shown in parentheses.
cell were 1:2 and 1:128, respectively. These titers were not enhanced by anti-globulin test using rabbit anti-guinea pig IgG.

**Immunoglobulin class composition**

Immunoglobulin class analyses revealed that most of the antibodies produced in Groups 1 and 2 were IgM. On the other hand, the guinea pig anti-D was composed mainly of IgG antibody.

**Discussion**

According to the review of Williams and Chase (1967) hapten-specific antibodies were prepared from many animals by various immunizing routes. However, the recommended method to prepare hemagglutinin of a high titer was not mentioned. To prepare specific and potent anti-M and -N, we reported an immunization method in which M- and N-active glycoproteins of red cell membrane were trapped into autologous red cell ghost (Yokoi et al. 1983a).

As for antibody in the Lewis blood type system, Iseki et al. (1957) first described that anti-Lea, -Leb and -Lei precipitin and agglutinin were produced in rabbits which were intramuscularly injected with alcohol precipitate from whole saliva. The activity of the antisera increased at low temperature. Levine and Celano (1960) used tanned red cells which were coated with saliva of Les type as an immunogen to prepare anti-Lea. The induced anti-Lea had low agglutinin activity so that blood typing should be performed at 5°C using papainized red cells. Goat was frequently employed as a donor of saline anti-Lea but a large quantity of immunogen was required for immunization. In the immunogen trapping method, a relatively small amount of immunogen (1 ml saliva) was enough for a single immunization of a rabbit.

On the antisera in the Rh-Hr blood type system, Prokop and Uhlenbruck (1969) reported that no anti-D had been produced in rabbits or guinea pigs with any route of immunization. Abraham and Bakerman (1975) isolated a D-active component from red cell membrane by ultrafiltration and electrofocusing methods. They indicated that immunization of guinea pigs with the component resulted in specific anti-D. Unfortunately, the results could not be reproduced in our laboratory. Our previous papers (Yokoi et al. 1983a, b) revealed that D-active Band 3 and anti-D formed immune complex which was adopted as an immunogen in this experiment. As a result, one guinea pig of four ones induced specific anti-D antibody (titer, 1:128) when tested with papainized red cells.

Immunoglobulin of anti-Lea in Group 1 was IgM and no IgG was detected, but anti-D in guinea pig was IgG. Anti-M and -N which were prepared by the similar immunizing method were composed mainly of IgM (Yokoi et al. 1983a). The difference of immunoglobulin classes between antibodies of MN, Lewis and Rh blood types might be responsible to animals immunized or the immunogens. The results obtained in this experiment indicated that the antigen trapped in
ghost was uptaken at a high concentration state in macrophages to stimulate antibody producing cells.

Recently many antibodies with strict specificity and high titers have been prepared by the monoclonal antibody technique (Brockhaus et al. 1981; Sonneborn and Uthemann 1983). It was considered, however, that the antigen trapping method was recommended for its simple procedure to prepare hemagglutinin with sufficient titers.

References