ABO Blood Grouping and Species Identification of Bloodstains by Sandwich ELISA Using Monoclonal Antibody Specific for Human Erythrocyte Band 3

A. Kimura, T. Uda, S. Nakashima, M. Osawa, H. Ikeda, S. Yasuda and T. Tsuji

Department of Legal Medicine, Wakayama Medical College, 27 Kyuban-cho, 640 Wakayam, Japan

INTRODUCTION

Species identification and ABO blood grouping of bloodstains constitute the main subjects in medicolegal practices. The methods employed for species identification and ABO blood grouping, e.g. immunodiffusion with anti-human hemoglobin antibody and absorption -elution test using anti-ABO blood group sera, are well established and highly reliable. However, methods of distinguish between species, especially between humans and other primates, and methods of ABO blood grouping of bloodstains contaminated by other body fluids are not yet satisfactory. In our study of species -specific epitopes on human erythrocyte membrane, we produced monoclonal antibodies (mAbs) to species specific-epitopes on human erythrocyte membrane band 3. One (P3-9H) of these mAbs could even discriminate erythrocytes of human from those of chimpanzee (Kimura 1990). Since erythrocyte membrane band 3 carries ABO blood group active carbohydrate chains, the use of P3-9H may be applicable not only to human blood identification but also to ABO blood grouping.

In this paper, we describe the applications of P3-9H for human blood identification and for ABO blood grouping from bloodstains contaminated by other body fluids.

MATERIALS AND METHODS

Production of mAbs: Anti-human erythrocyte membrane band 3 mAb (P3-9H) was produced by using human erythrocyte membrane as an immunogen as described previously(Kimura 1990).
SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting: SDS-PAGE was performed by method of Laemmli and immunoblotting was performed as described by Towbin.
Sandwich ELISA: A microwell plate was coated with P3-9H (10μg/ml PBS) and blocked with 0.3% gelatin (10mM TBS, pH7.4). Erythrocyte membrane proteins were extracted from bloodstains with PBS containing 1% Triton X-100 and 1mM EDTA and the extracts were incubated on the P3-9H coated plate. ABO blood group epitopes on band 3 captured by P3-9H were detected with mAbs to ABO blood group and with peroxidase conjugated goat anti-mouse IgM.

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RESULTS AND DISCUSSION

As shown in Fig. 1, anti-human band 3 mAb P3-9H (IgG1) bound to whole band 3, and to its 60 and 42 kDa amino-terminal fragments (N-60 and N-42) in the human erythrocyte membrane but not to that of the chimpanzee on the blot, indicating that the epitope defined by P3-9H existed on N-42 and was specific for human. P3-9H did not cross-react with the erythrocytes of other primates (spider monkey, capuchin monkey, rhesus monkey, Japanese monkey or orang-utan) or other mammals (dog, cat,cow, pig and rabbit).

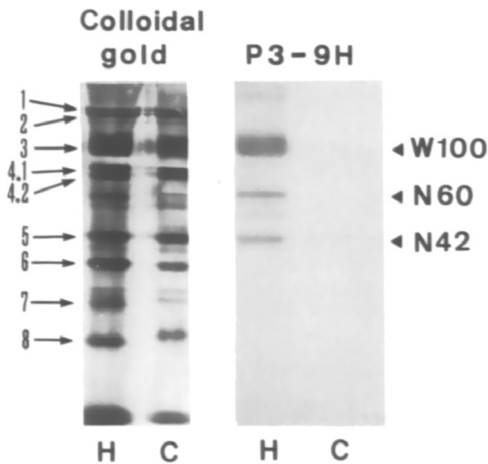

Fig. 1. Identification of the antigen defined by P3-9H by immunoblotting. Proteins in the erythrocyte membranes of human and chimpanzee were separated by SDS-PAGE (10% acrylamide gel) and transferred onto PVDF membranes. The blots were stained with colloidal gold or P3-9H. H, human; C, chimpanzee: W100, whole band 3; N60 and N42, 60kDa and 42kDa amino-terminal fragments respectively, of band 3. Nomenclature for human erythrocyte membrane proteins is according to Steck (1974).

Band 3 is an integral membrane protein in erythrocytes; it acts as an anion channel in erythrocyte membrane and also carries the ABO blood group active sugar chain (Tsuji 1981). About 25% of the ABO blood group determinants in the erythrocyte membrane are distributed on the sugar chain which links to an asparagine residue on band 3 (Finne 1980). Since the human-specific epitope and the ABO blood group epitopes are located apart from each other on the band 3 molecule, the binding of each antibody to each epitope is not disturbed by the binding of other antibody. Therefore, it appeared to be appropriate to apply P3-9H to a capture antibody in sandwich ELISA for the ABO blood grouping of bloodstains. ABO blood group epitopes were detected in extracts from minute bloodstains by sandwich ELISA using P3-9H. Even when bloodstains were contaminated by other body fluids (e.g., semen, saliva and sweat),

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only the ABO blood group epitopes on band 3 captured by P3-9H were detected by the ABO blood group antibodies without interference from other body fluids. Sakata et al. reported the same sandwich ELISA using a mAb which recognized the intramembrane domain of band 3 and cross-reacted with only some primates, for the ABO blood grouping of bloodstains (1988). In a blind trial, all A, B and O bloodstains (a 1cm long thread) were precisely typed, but some AB bloodstains were typed as blood group A (Table 1). In general, B epitopes are detected less than A epitopes by the present method. However, when increased amounts (a 1.5cm long thread) of AB bloodstain specimens were used the ABO blood group was determined precisely. Furthermore, since P3-9H is specific for human erythrocyte band 3, it is evident that if ABO blood group epitopes are detected on the specimens by the present method, they are those of human blood.

Table 1. Blind trials of ABO blood grouping of bloodstains by sandwich ELISA using P3-9H

bloodstains	**number**	**correct**
A	**16**	**16**
B	**21**	**21**
O	**19**	**19**
AB	**13**	**11**[a]

a) Two specimens were typed as blood group A.

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