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*Short Communication*

# Production of a new anti-A monoclonal reagent

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**Monoclonal antibodies are essential tools in molecular and cellular immunology research. They have essentially replaced the polyclonal antibodies in identifying blood groups and detecting cell markers and pathogenic agents. The aim of the present study is to produce monoclonal antibody identifying the ABO blood groups using the murine hybridoma technology. An anti-A monoclonal antibody A907 was selected and estimated for its use in the manufacture of a reagent anti-A. The selected antibody specifically reacts with $A_1$, $A_2$, $A_1B$ and $A_2B$ erythrocytes. It does not recognize B, O, $A_3$ and $A_x$ erythrocytes. The A907 monoclonal antibody can be used in blood grouping in association with a reagent recognizing the A weak phenotypes.**

**Key words:** Anti-A monoclonal antibodies, murine myeloma cells, haemagglutination.

## INTRODUCTION

Monoclonal antibodies are essential tools in research, therapy and diagnostic techniques (Pelegrin et al., 2004; Borrebaeck, 2000). Certain markers detected by monoclonal antibodies can provide information on prognosis in patients with cancer (Laack et al., 2002). For example, monoclonal antibodies can help to establish the tumor nature. But, their large success is especially their use in immuno-hematology. Thus, monoclonal reagents replaced polyclonal reagents because of their fine specificity, availability in great quantities and reduced production cost (Nelson et al., 2000). Our purpose is to produce anti-A monoclonal antibodies in order to manufacture blood grouping reagents.

## MATERIALS AND METHODS

### Immunization and fusion

A female mouse Balb/c aged of 6 weeks was immunized intraperitoneally 3 times with 20 x $10^6$ A human erythrocytes suspended in physiological serum. The injections were spaced by 15 days. Ten days before the fusion, the P3X63Ag8 murine myeloma cells at 0.2 to 0.5 x $10^6$ cells/ml were cultured in 75 $cm^2$ flask (Costar, United-States) containing RPMI medium 1640 supplemented with 10% of fetal bovine serum (EuroBio, France) and incubated at 37°C in humidified atmosphere with 5% $CO_2$.

Four days after the last injection, the mouse was sacrificed and collected splenocytes were fused with P3X63Ag8 murine myeloma cells according to the modified protocol of Galfré et al. as described previously (Habti et al., 2003) by using polyethylene glycol 4000 (BDH laboratories, Britain) at 45% in RPMI medium, pH 7.2 and containing 5% of dimethylsulfoxyde (BDH laboratory, Britain). The hybrid cells were selected by culture in RPMI medium, containing 15% fetal bovine serum (FBS), 1.3 µg/ml hypoxanthine, 1.7 µg/ml azaserine, 100 UI/ml penicillin and 100 µg/ml streptomycin. Then, they were distributed in six 96-well plates (Costar, United-States) at 15 x $10^6$ splenocytes/plate and incubated at 37°C in humidified atmosphere with 5% $CO_2$.

### Screening

The identification of the secreting hybridomas was realized in microplate with A erythrocytes treated with bromelin. The specificity of the antibodies was determined by using panels of O erythrocytes presenting majority of blood groups systems antigens (Sanofi Diagnostic Pasteur, France) and panels of $A_1$, $A_2$, B and AB erythrocytes. The secreting clones were cryopreserved in the liquid nitrogen, after their clonings by limiting dilution.

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**Table 1.** Stability (titer and score) of A907 antibody at +37°C with time.

| ER | D0 (T/S) | D15 (T/S) | D30 (T/S) | D45 (T/S) | D60 (T/S) |
|---|---|---|---|---|---|
| $A_1$, AB | 256/70 | 256/70 | 256/68 | 128/60 | 128/60 |
| $A_2$ | 32/38 | 32/38 | 32/36 | 16/31 | 16/28 |

ER, erythrocytes; T/S, titer/score; D, day.

**Table 2.** Reactivity of A907 antibody with various groups.

| ER/N | Plat of opaline | Tube | Microplate | Avidity (s) | Titer | Score |
|---|---|---|---|---|---|---|
| $A_2$/20 | ++ | +++ | +++ | < 5 | 32 | 38 |
| $A_2$B/7 | ++ | +++ | +++ | < 5 | 32 | 40 |
| $A_3$B/1 | negative | negative | negative | - | - | - |
| $A_x$/3 | negative | negative | negative | - | - | - |
| $A_x$B/2 | negative | negative | negative | - | - | - |

ER/N, erythrocytes/number; s, second.

**Evaluation of an anti-A clone**

After several clonings, the production of the anti-A monoclonal antibody was carried out in culture supernatant. The precipitation in ammonium sulfate and HPLC chromatography analysis were both performed in order to deduce the monoclonal antibody's isotype. The elution profile of monoclonal antibody was compared at those of the IgG and IgM antibodies of reference products, respectively, by anti-D clones (Rh1) P3 x 35 and HM10 (Diagast, France).

The stability of the clone was verified monthly in continuous culture by titration of culture supernatant products for 1 year. The stability of the antibody molecule was controlled weekly, by titration of culture supernatant stored at +37°C for 2 months and at +4°C for 1 year. The scores were assigned according to the notation recommended by the international code. After 1 year of culture, the precloned hybridomas was cloned again to control its stability and to determine the percentage of secreting clones.

The O red cells of 10 panels of anti-erythrocytes antibodies identification and a panel constituted of 30 $A_1$, 20 $A_2$, 50 B and 50 AB erythrocytes were used to study the antibody specificity by saline and enzymatic techniques. Further, the $A_2$B, $A_3$B, $A_x$B and $A_x$ erythrocytes were used for antibody haemagglutination tests on opaline plate, in tube and in microplate.

A private or rare epitope can exist within the normal population. It can also be expressed in individuals with certain pathologies. To verify if such was present, the selected antibody reactivity was compared to those of the anti-A and anti-AB commercial reagents (Sanofi Diagnostics Pasteur and Diagast, France) against erythrocytes from 20000 blood donors and 1000 patients with an automaton PK7200 (Olympus, Japan).

## RESULTS AND DISCUSSION

Only one clone (A907) was selected and evaluated. The elution profile of the A907 antibody in chromatography is similar to IgM antibodies profile. The A907 hybridoma clone gave 100% secreting clones. The titer of culture supernatant is stable; after 1 year at +4°C, it is 256 with a score of 70 with $A_1$ and AB red cells, and it is 32 with a score of 38 with the $A_2$ red cells. On the other hand, the reactivity of the antibody decreases after 45 days of storage at +37°C (Table 1).

The A907 antibody agglutinates the $A_1$, $A_2$ and AB erythrocytes exclusively. The study of the A907 antibody reactivity with erythrocytes panels confirms its anti-A specificity. The A907 antibody does not recognize the A weak groups such as $A_3$ and $A_x$ (Table 2). The comparative study among blood donors and patients did not show any discordance.

In this work, a murine monoclonal antibody was obtained. In order to characterize the antibody, it is necessary to clone the hybridoma several times by limiting dilution to assure the clone stability and therefore its monoclonality (Nelson et al., 2000). The study of the antibody reactivity with different erythrocytes confirmed its anti-A specificity. The A907 antibody is stable at +4°C for more than one year. Clonal and antibody stability would make it possible to produce the A907 antibody continually as culture supernatant for one year thereby reducing the reagent production cost. However, culture supernatant concentration is necessary for obtaining good reactivity with $A_2$ erythrocytes, and good titer superior to the international standards.

The A907 antibody does not recognize the weak groups as $A_3$ and $A_x$. However, its use in association with another antibody recognizing the $A_3$ and $A_x$ groups will provide information on ABO system polymorphisms. It can also be helpful in studying of their expression in cancer. The specificity of the A907 antibody will be determined better by studying its reactivity with leukocytes and normal and tumorous tissues (Nakagoe et al., 2001; Marionneau et al., 2001; Oberhuber et al., 1997).

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