Heterologous Immunization, a Way out of the Problem of Monoclonal Antibody Production against Carcinoma Antigen 125

Sorour Shojaeian[1], Amir Hassan Zarnani[2,3], Mahmood Jeddi-Tehrani[4], Forouzandeh Fereidooni[5], Ebrahim Torkabadi[6], Mahammad Mehdi Akhondi[6], Akram Sadat Tabatabaei-Panah[7], Abdolamir Allameh[1]*

[1]Department of Biochemistry, Tarbiat Modares University, [2]Nanobiotechnology, [4]Monoclonal Antibody and [6]Reproductive Biotechnology Research Centers, Avicenna Research Institute, ACECR, [3]Immunology Research Center, Iran University of Medical Science, [5]Cancer Institute, Imam Khomeini Medical Center, [7]Science and Research Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

Background: Monoclonal antibodies (mAbs) are essential tools for many molecular immunology investigations, epitope mapping and molecular modelling, clinical laboratory diagnostic tests and immunotherapy. Humoral immune response of immunized animals largely depends on the nature of antigen and the immunization technique. Polysaccharides and heavily-glycosylated proteins are very elusive targets incapable of mounting long-lasting, high affinity antibody responses. Carcinoma antigen 125 (CA 125), a well known tumor marker of ovarian cancer, is a mucin type antigen consisting of repetitive units of heavily glycosylated moieties which render production of mAbs very difficult. **Objective:** To evaluate the efficacy of heterologous antigen preparations as a way of mouse immunization in the production of anti-CA 125 mAb. **Methods:** Two different protocols of immunization were used for priming of NMRI mice. In the first method, mice conventionally immunized by three intraperitoneal injections of purified CA 125 and boosted by the antigen three days before fusion. In the second approach, mice were primed by three intraperitoneal injections of living CA 125 positive cells of OVCAR-3 cell line, and boosted by intravenous injection of the purified extracellular domain of CA 125. Production of mAb was performed by standard hybridoma technology and mAbs were characterized by different immunoassays. **Results:** The first method failed to produce stable clones despite six time fusion. A total of ten stable clones, however, were produced in the second approach. Some of the clones were characterized and found to have excellent immunoreactivity when tested by ELISA assay, western blotting, intracellular and surface immunofluorescent staining of OVCAR-3 cell line and immunohistochemical staining of ovarian cancer tissues. **Conclusion:** Altogether the results of the present study clearly showed that heterologous antigen preparation is the method of choice for immunization when production of monoclonal antibody against highly glycosylated poorly immunogenic antigens is concerned.

Keywords: Heterologuos Immunization, Monoclonal Antibody, Glycosylated Protein, CA 125

*Corresponding author: Dr Abdolamir Allameh, Department of Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Tel: (+) 98 21 82883585, Fax: (+) 98 21 82883590, e-mail: allameha@modares.ac.ir

INTRODUCTION

After introduction by Kohler and Milstein in the mid-1970s (1), tremendous efforts have been made in the development, optimization of production and characterization of monoclonal antibodies. These types of antibodies have found many applications in different aspects of medicine ranging from diagnosis and research to therapies of such diseases as cancer and autoimmunity (2,3).

Although some species have been employed successfully in production of monoclonal antibodies, more than 95% of researches on mAbs are still represented by mouse mAbs (4). The first prerequisite for successful manufacturing of mAbs is, of course, effective immunization of the animals. The ability to generate high titers of circulating antibodies varies with the chemical nature of the antigen, the animal used, and the immunization protocol. Employment of a purified antigen for immunization raises the probability of obtaining hybridomas producing specific antibodies. Whole cells, membrane-enriched fractions, purified receptors, protein antigens produced by expression in prokaryotic or eukaryotic cells, and synthetic peptides have been successfully used as immunogens (5).

Various immunization techniques can be used for the successful production of mAbs. The choice of method used depends on the nature of the antigen and the type of antibody needed by the researcher. Despite effectiveness of conventional methods of immunization in mounting of humoral immunity against highly immunogenic proteins or peptide-carrier molecules, they fail to induce long-lasting and vigorous antibody responses directed to highly glycosylated, poorly immunogenic antigens, which is a prerequisite for production of monoclonal antibodies. When highly glycosylated antigens are used for immunization, it is highly unlikely that the animal will ever produce a classic full immune response. Usually these antigens do not produce memory B cell response after the rest period and each immunization is seen as the primary challenge. Antibodies produced against such antigens are usually IgM (6).

Epithelial mucins are heavily O-glycosylated proteins found in the mucus layer or at the cell surface of many epitheliums (7). The biophysical properties of mucins depend on their extensive O-linked glycosylation rather than on their polypeptide sequences (8). Several immunologically based clinical therapy trials target mucins that are expressed by adenocarcinomas, including monoclonal antibody-based therapies and tumor vaccines. CA 125 (MUC16), a mucin which is highly expressed by epithelial cells in ovarian carcinoma, is routinely tested to confirm the diagnosis of ovarian carcinoma and to monitor stabilization, progression or regression of the disease during therapy (9).

CA 125 is poorly immunogenic and the rarity of CA 125 antigenic domains may be due to its unusual structure which consists of more than 60 repeat units of 156 amino acids (10,11), hyperglycosylated state and to its immunosuppressive effects (12) that may prevent immunized mice from developing a diversified population of anti-CA 125 antibodies.

Here we describe the production of anti-CA 125 monoclonal antibodies using heterologous antigen preparations and conclude that this approach may overcome difficulties facing the production of mAbs against highly glycosylated proteins.

MATERIALS AND METHODS

Production and Purification of Anti-CA 125 Monoclonal Antibodies. Two

approaches were used for immunization. In the first method, NMRI mice (Pasteur Institute, Tehran, Iran) received, intra-peritoneally, 100 µg of purified CA 125 (Biodesign, USA) emulsified in complete Freund adjuvant (Sigma, USA). Immunization was repeated two times in conjunction with incomplete Freund adjuvant (Sigma, USA) with 3 weeks intervals. Mice received a final booster of antigen given intravenously three days before fusion. In the second approach, mice were immunized three times by intraperitoneal injection of 5×10^6 living $CA125^+$ OVCAR-3 cell line. After completion of immunization schedule, enzyme-linked immunosorbent (ELISA) assay was performed as described later and two mice with the highest titers were selected for fusion. Booster immunization was performed by intravenous injection of 66 µg purified CA 125 four and three days before fusion. Hybridomas were produced and culture supernatants of hybridoma clones were screened by ELISA against purified CA 125 and the positive clones were subcloned by limiting dilution. Subcloning was performed four times to ensure monoclonality and stability of the clones. All works with animals were conducted according to the guidelines of Avicenna Research Institute Ethics Committee.

mAbs were isotyped by isostrip (Roche, Germany). For large scale production, clones were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS) (Invitrogen, USA) and culture supernatant was collected.

A total of ten clones were produced, three of which had IgG isotype and the rest IgM. Characteristics of 10A6, 7A3 and 9F7 clones have been reported elsewhere (13). Here, we report the features of the fourth clone, 11B8. Given the IgM isotype of this clone, a rabbit anti-mouse IgM affinity chromatography column was set up for antibody purification. Purified antibody was quantified by spectrophotometry at 280 nm. Purity of antibody was tested by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The antibody was further characterized by ELISA, western blot, immunofluorescent staining of living and fixed OVCAR-3 cells and immunofluorescent labeling of ovarian cancer tissue.

ELISA. Ninety-six well plates (Nunc, Denmark) were coated with 3 µg/ml of CA 125 overnight at 4 °C. After washing with phosphate buffered saline containing 0.05% Tween-20 (PBS-T), blocking was performed with 2.5% BSA for 1 h at 37 °C. Two fold serial dilutions (from 1:500) of the mouse serum, hybridoma culture supernatant or two fold dilution series (from 20 µg/ml) of the purified antibody were added at the next step and the plates were incubated for 1.5 h at 37 °C. The plates were then washed with PBS-T and 1:1000 dilution of HRP-conjugated rabbit anti-mouse Ig (Avicenna Research Institute, Iran) was added and incubation was continued for another hour at 37 °C. Finally, color was developed after adding 3,3',5,5' tetra methylbenzidine (TMB) (US Biologicals, USA) for 15 min. The reaction was stopped by 20% H_2SO_4 and the absorbances were measured at 450 nm. The negative controls included omission of the first layer (antigen), the second layer (anti-CA 125 antibodies) or combination of both aforementioned controls. The results of such tests were always negative showing that there is no non-specific binding of our anti-CA 125 antibody.

Indirect Immunlofluorescent Staining. 5×10^5 living OVCAR-3 cells (National Cell Bank of Iran, Pasture Institute, Iran) were incubated with 100 µl of 5 µg/ml purified antibody for 60 min on ice, washed twice with PBS and stained with FITC-conjugated sheep anti-mouse Ig (dilution 1:50) (Avicenna Research Institute, Iran) for 30 min on ice. After two washes with PBS, cells were examined under fluorescent microscope (Olympus, BX 51, Japan). As negative cell control, human fibroblast cell line, HFFF-PI6,

was stained in the same manner. For negative reagent control, isotype matched irrelevant antibody (5 µg/ml) was used instead of primary antibody. Immunostaining of fixed OVCAR-3 cells was performed as above except that the cells were cytospinned and fixed by ice-cold acetone for 2 min prior to staining. The cell nuclei were stained by DAPI stain.

Flow cytometric analysis. In order to determine whether 11B8 is able to detect extracellular expression of CA 125, three $CA125^+$ cell lines, namely, OVCAR-3, CAOV-4 and SKOV-3 and one negative cell control, HFFF-PI6, (all from National Cell Bank of Iran, Pasture Institute, Iran) were assayed by flow cytometry. Briefly, 5×10^5 living cells were washed three times with PBS containing 2% fetal calf serum (PBS-FCS) and centrifuged at 500 g for 5 min at 4°C. All incubations were performed on ice. Cells were first incubated with 5 µg/ml of primary anti-CA 125 antibody (clone 11B8) diluted in PBS-FCS for 30 min followed by three washes with PBS-FCS. In negative reagent control tubes, isotype matched irrelevant antibody was used instead of the primary antibody. Cells were then incubated with FITC-conjugated sheep anti-mouse Ig (Avicenna Research Institute, Tehran, Iran) at 1:50 dilution in PBS-FCS for 20 min. After washing as above, signals were analyzed by Partec FloMax flow cytometer (Partec, Germany).

Western Blotting. OVCAR-3 cell lysate was prepared in NP-40 (Nonidet P-40) buffer. Briefly, OVCAR-3 cells were incubated in NP-40 buffer (150 mM NaCl, 1% Triton X-100, 0.1% SDS, 50 mM Tris-HCl pH 7.5) in the presence of 1% protease inhibitor cocktail (Sigma, USA) at 4 °C for 30 min. Concentration of protein in cell lysate was determined by BCA Protein Assay Kit (Pierce, USA). Lysate was boiled in sample buffer (10% SDS, 0.5% Bromophenol Blue, 50% (v/v) Glycerol, Tris-HCl pH 6.8), and loaded (20 µg/well) onto a 7% SDS-PAGE. After electrophoretic separation, proteins were transferred onto PVDF (polyvinylidine difluoride) membranes (Roche, Germany) and blocked overnight with TBS-TT (50 mM NaCl, 0.5% Tween-20, 2% Triton X-100, 20 mM Tris-HCl pH 7.5) supplemented with 5% nonfat dry milk**.** Thereafter, the membranes were incubated for 1 h at room temperature with the 2.5 µg/ml purified 11B8 anti-CA 125 antibody. The membranes were washed three times with TBS-TT and incubated for 1 h at room temperature with HRP-conjugated rabbit anti-mouse Ig (dilution: 1:1000). Blots were developed using an ECL (GE-Healthcare, Sweden) system according to manufacturer's instruction.

Immunofluorescent Labeling of Tissue Section. For further characterization of our monoclonal antibody, immunofluorescent labeling was performed on formalin-fixed paraffin-embeded (FFPE) ovarian cancer tissue samples and normal ovarian tissue as negative control according to a previously published protocol (14). Tissues were obtained from the Cancer Instituite of Imam Khomeini Medical Center. All tissues had been fixed in 4% buffered formalin and embedded in paraffin by conventional techniques. Tissue sections were cut in 3 mm slices and transferred onto poly L-lysine-coated slides. Sections were dewaxed upon treating with xylene (2×5 min), hydrated in graduated alcohol (100%, 96%, 80% and 70%, 3 min each) and rinsed for 5 min in distilled water. Antigen unmasking was performed in a water bath for 25 min at 95°C in 0.1 M citrate buffer (pH 6.0). Following two washes with Tris-buffered saline, pH 7.4, containing 0.1% bovine serum albumin (TBS-BSA), endogenous biotin was blocked with biotin blocking system (Dako, Sweden) according to the manufacturer's instruction. Pretreated slides were then blocked for 15 min at room temperature with 5% normal sheep serum and then incubated at 4°C overnight with 2.5 µg/ml of 11B8 or 1.8 µg/ml of OC125 (Invitrogen, USA) as positive control primary antibody. After overnight

incubation, slides were washed in TBS-TT and incubated with biotin-labeled rabbit anti-mouse antibody (Avicenna Research Institute, Iran) at a concentration of 2.5 µg/ml for 45 min, followed by washing three times with TBS-BSA and incubation in the dark for 30 min with 1:100 dilution of streptavidin conjugated to FITC (Biosource, USA). They were again washed in TBS, mounted with glycerol-PBS (50% v/v) and stored at 4°C in the dark if not viewed immediately. Sections were analyzed on an Olympus BX51 microscope with epifluorescence. Images were acquired using a 12.5 megapixel cooled CCD camera (Olympus, Japan). In negative reagent control slides, primary antibody was substituted by isotype matched irrelevant antibody.

RESULTS

Monoclonal Antibody Production. Three mice were immunized with each protocol. Production of specific antibody was traced in each mouse by ELISA. As depicted in figure 1, substantial levels of anti-CA 125 antibodies were produced in all the animals after completion of immunization schedule.

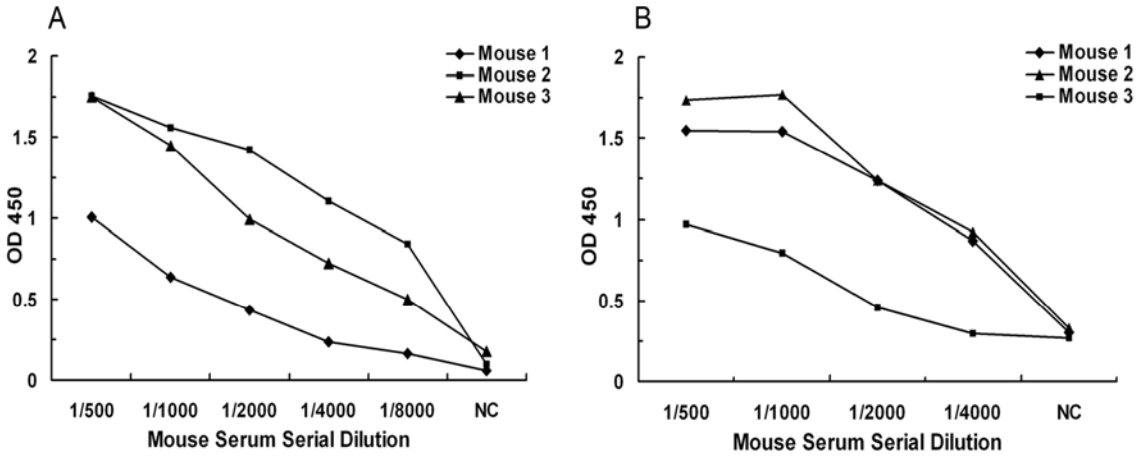

Figure 1. Serum anti-CA 125 titer of mice immunized with different protocols. In the first protocol (A), mice were immunized with purified CA 125 and boosted with the same preparation. In the second protocol (B), mice were primed with living OVCAR-3 cells and boosted with purified CA 125. NC: negative control.

Purification of mAb. Based on the results of ELISA and immunofluorescent staining of OVCAR-3 cells by hybridoma culture supernatant, 11B8 mAb was selected for purification and further analysis. Antibody purification was performed by a house-made Rabbit anti-mouse IgM affinity chromatography column. The purity of antibody was confirmed by SDS-PAGE and silver staining which showed a single band at interface of stacking and separating gels under non-reducing conditions (Figure 2). Purification yield was calculated and found to be around 11.2 mg/l.

ELISA. Clone 11B8 showed excellent reactivity when tested by indirect ELISA. Titration with two fold serial dilutions from 20 µg/ml to 0.04 µg/ml showed that this clone gives OD>1 even at the concentration of 0.2 µg/ml (Figure 3). OC 125 served as a positive control for anti-CA 125 primary antibody.

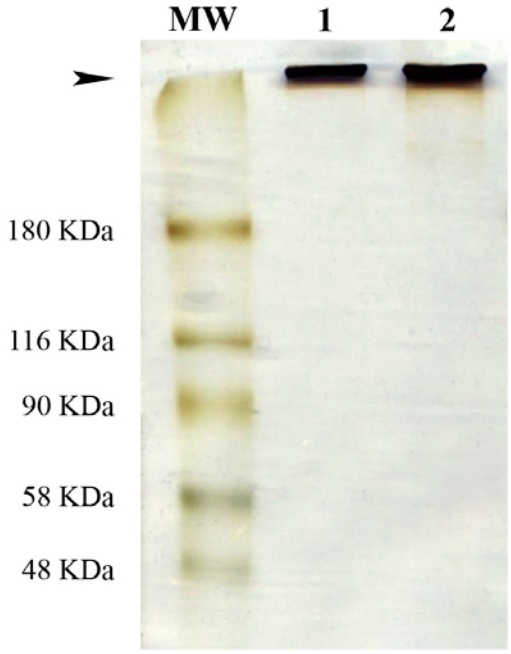

Figure 2. SDS-PAGE electrophoresis of purified anti-CA 125 monoclonal antibody (11B8). 11B8 mAb was purified over anti-mouse IgM affinity column, separated by 7% SDS-PAGE under non-reducing condition followed by silver staining. Lane 1: Normal mouse IgM, Lane 2: Purified monoclonal antibodies of 11B8 (5 µg/well), MW: Molecular Weight marker. Arrow indicates the interface between stacking and separating gel.

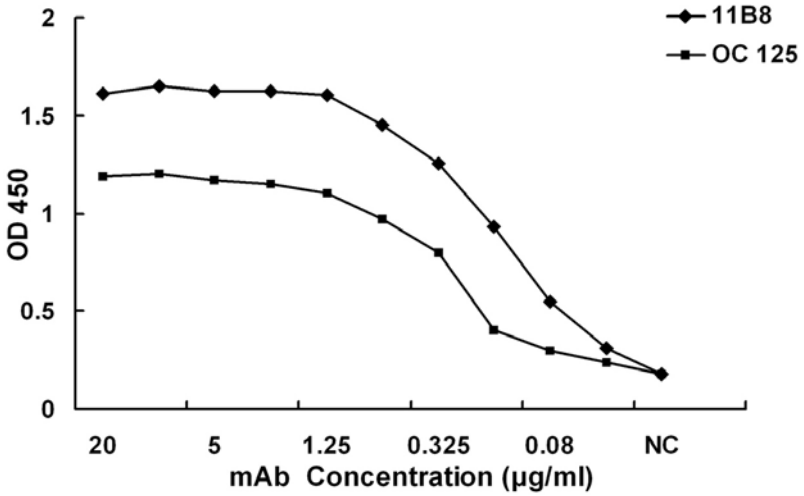

Figure 3. Titration of purified anti-CA 125 monoclonal antibody (11B8) by ELISA. CA 125 antigen was coated in ELISA plate. The purified 11B8 mAb was titrated in two fold dilution series from 20 µg/ml to 0.04 µg/ml. OC 125 served as a positive control for anti-CA 125 primary antibody. NC: negative control.

Immunofluorescent Staining of OVCAR-3 Cell Line. Immunofluorescent staining of living OVCAR-3 cells was performed to confirm reactivity of purified 11B8 with native form of extracellular domain of CA 125. 11B8 showed reactivity with living OVCAR-3 cells as judged by OC 125 antibody as positive control (Figure 4), but did not show any reactivity with CA 125 negative HFFF-PI6 cell line (data not shown). The mAb reacted

with the rim of living OVCAR-3 cells which is characteristic of surface staining pattern. When tested on fixed OVCAR-3 cells, the same pattern was observed indicating suitability of this antibody for immunofluorescent and immunocytochemistry applications (Figure 5).

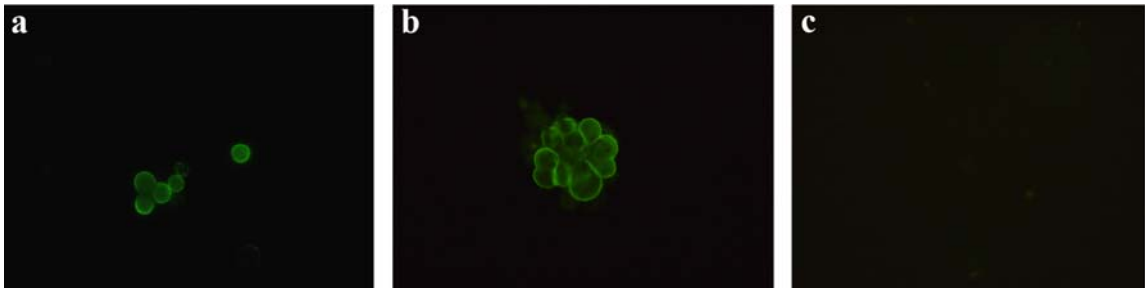

Figure 4. Immunofluorescent staining of living OVCAR-3 cells with purified anti-CA 125 monoclonal antibody (11B8). Immunostaining of extracellular domain of CA 125 positive cells, OVCAR-3, was performed by incubation of living cells with appropriate dilution of 11B8 mAb (a), OC 125 (b) or isotype matched irrelevant antibody (c), followed by FITC-conjugated anti-mouse antibody. Magnification: 400×.

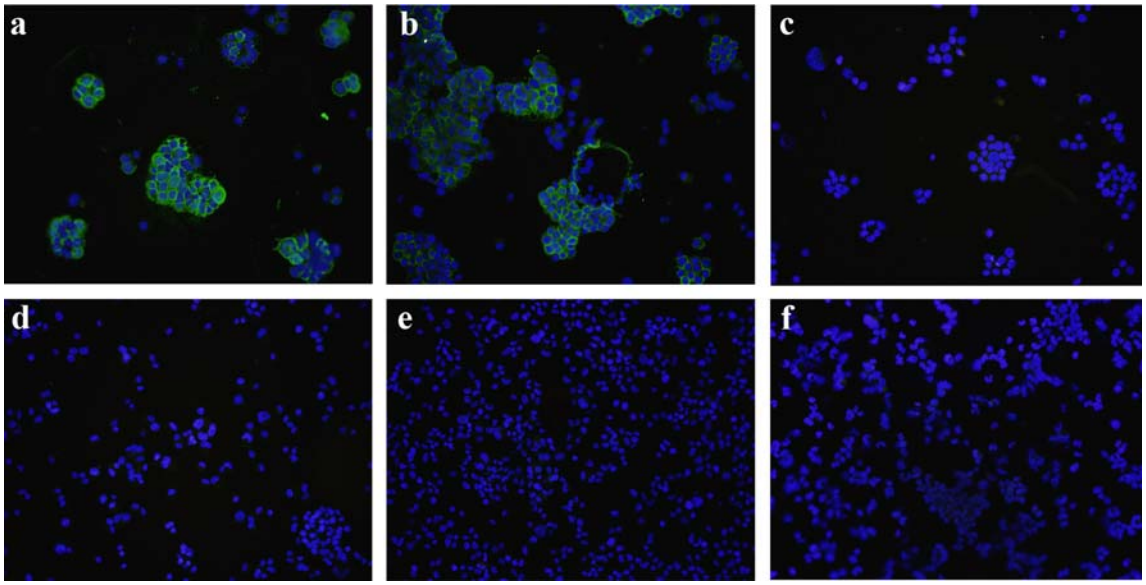

Figure 5. Immunofluorescent staining of fixed OVCAR-3 cells with purified anti-CA 125 monoclonal antibody (11B8). Acetone-fixed OVCAR-3 CA 125 positive cells (a-c) and HFFF-PI6 (negative cell control) (d-f), were incubated with 11B8 mAb (a,d), OC 125 (b,e) or isotype matched irrelevant antibody (c,f) and specific signal was traced by FITC-conjugated anti-mouse antibody. Nuclei were counter stained with DAPI. Magnification: 200×.

Flow Cytometric Analysis. Extracellular CA 125 expression was assayed in three CA 125^{+} cell lines by flow cytometry. HFFF-PI6 was used as the negative cell control. 11B8 was able to detect specifically extracellular expression of CA 125 in all positive cell lines, while no signal was achieved when fibroblast cell line, HFFF-PI6, was tested (Figure 6).

Western Blot Analysis. CA 125 exists in various molecular weight forms (>1000 to <200 KDa) (15). Consequently, the major part of the antigen usually migrates in the stacking gel and does not enter the separating gel (16). We also showed that this is the case for our mAb where western blot analysis of OVCAR-3 cell lysate by 11B8 re-

vealed a variety of high molecular weight species of CA 125 that mostly migrated in the 3% stacking gel and this pattern was also observed when OC 125 was used as primary antibody. In addition, we observed a band at the interface of stacking and separating gels. A band just beneath the interface was detected by 11B8 mAb and OC 125. A band of about 200 KDa could be traced by 11B8 as well as OC 125 which might represent smaller version of CA 125 (Figure 7).

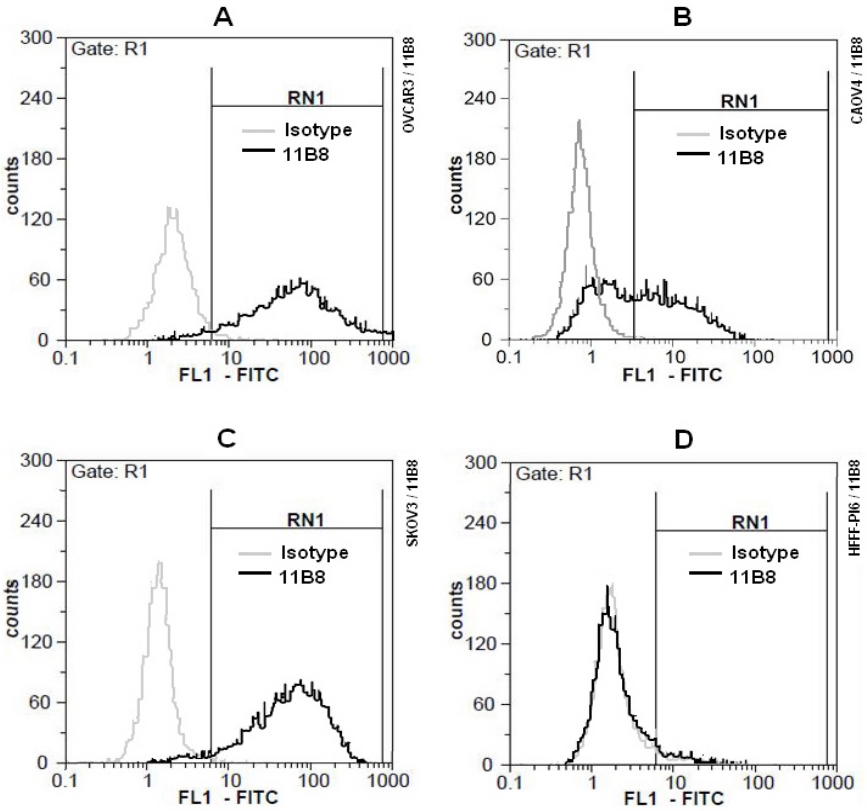

Figure 6. Flow cytometric analysis of purified anti-CA 125 monoclonal antibody (11B8). Immunoreactivity of 11B8 mAb was assessed by flow cytometry in three CA 125^{+} cell lines, OVCAR-3, CAOV-4 and SKOV-3. HFFF-PI6 was used as the negative cell control. Gray and black lines are showing isotope control and 11B8 reactivity, respectively.

Immunofluorescent Labeling of Tissue Section. To confirm the results of immunofluorescent staining of OVCAR-3 cells and to address capability of our anti-CA 125 mAb to detect in situ expression of CA 125, immunofluorescent labeling was done on formalin fixed paraffin embeded ovarian cancer tissues. The results of such experiment clearly showed that 11B8 has strong immunoreactivity with CA 125 expressing epithelial cells (Figure. 8a). Immunoreactivity was predominantly observed in cell membrane and to a lesser extent in cytoplasm of positive cells. In the negative control slide, primary antibody was substituted by isotype matched irrelevant antibody. Slide was always shown to be negative (Figure. 8c). 11B8 did not show any reactivity when tested on normal ovarian tissues (data not shown) pointing to the specificity of our mAb.

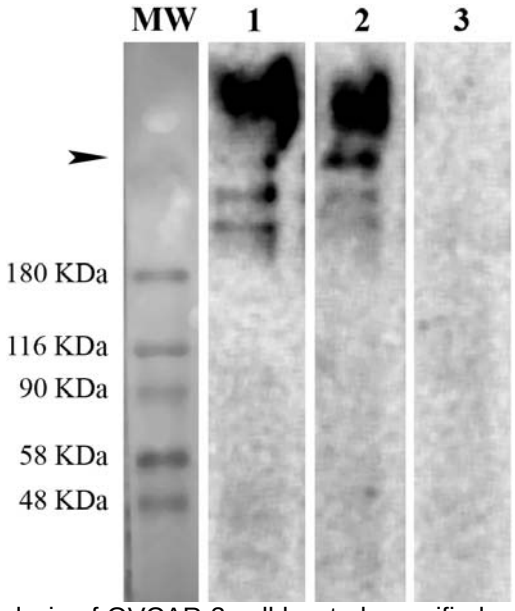

Figure 7. Western blot analysis of OVCAR-3 cell lysate by purified anti-CA 125 monoclonal antibody (11B8). OVCAR-3 cell lysate was separated by SDS-PAGE under reducing conditions and was analyzed using purified monoclonal antibody (2.5 µg/ml) after blotting on PVDF membrane. Lane 1: 11B8, Lane 2: OC 125 (positive control), Lane 3: normal mouse Ig (negative control), MW: Molecular Weight marker. Arrow indicates the interface between stacking and separating gel.

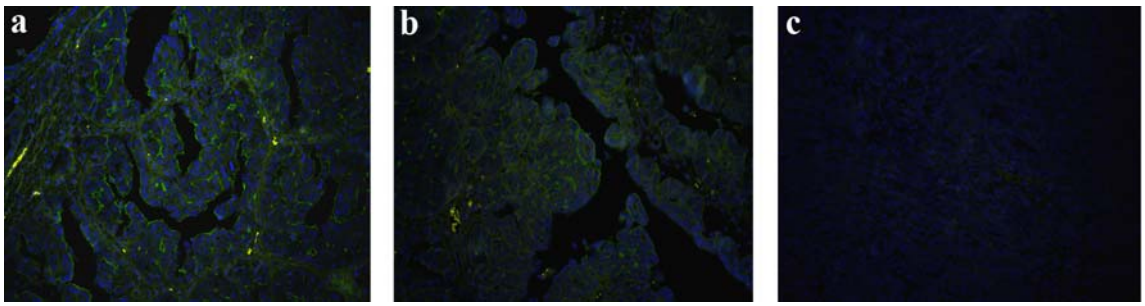

Figure 8. Immunofluorescent staining of ovarian cancer tissue by purified anti-CA 125 monoclonal antibody (11B8). Expression of CA 125 was assessed in FFPE sections of ovarian cancer tissue by 11B8 mAb using labeled-biotin-streptavidin procedure (a). OC 125 (b) and isotype matched irrelevant antibody (c) were used as positive and negative reagent controls, respectively. Nuclei were counter stained with DAPI. Magnification: 200×.

DISCUSSION

Monoclonal antibodies target a single epitope and usually have very high specificity. They are popular for many research applications and diagnostic assays because of their very high specificity for the antigen of interest. Production of monoclonal antibodies is a highly sophisticated procedure and is considerably affected by several factors including host species, structure and immunization route of antigen, immunization intervals and the type of antibody and its application, to list a few (17,18).

While protein antigens are highly immunogenic and elicit vigorous humoral immune responses, other types of antigens induce immunity to a lesser extent and such determinants as route of immunization, immunization schedule and antigen preparation are

among the factors that should be optimized. Carbohydrates or highly glycosylated glycoproteins are prototype of such entity, as they are very elusive targets for immune system and do not mount long lasting high affinity antibody responses (9,12,19). In order to overcome such obstacles, conjugation of T cell dependent antigens to polysaccharides has been introduced since 1990 as an effective vaccination strategy against haemophilus influenzae B in which immunity is considerably dependent on antibody against capsular polysaccharides. In fact, the protein component in such preparations can be processed and presented on the surface of B cells associated with MHC class II molecules and in this way can activate T cells which are principal for affinity maturation and class switching (19). Interestingly, helper effect of protein components in vaccine preparation does not restrict to polysaccharides and they are also able to promote immunity against protein moieties.

In a recently reported work by our group (20), it was shown that in immunization of mice with dendritic cells co-pulsed with two irrelevant protein antigens, each antigen serves to promote immune response against the other one, a condition that is termed "mutual helper effect". Based on the subjects mentioned above, in the present study, we used heterologous immunization strategy for monoclonal antibody production against CA 125 which is a highly glycosylated protein.

Our results showed that immunization with purified CA 125 does not elicit effective humoral immunity and all six fusions failed to produce stable clones. Vaccination with CA 125 expressing ovarian cancer cell line and boosting with purified CA 125, however, was found to be a very triumphant approach as this led to ten stable clones in the first round of fusion. We think that protein components present in the cell membrane may serve as helper partner to stimulate high affinity antibody production of CA 125-responding B cell clones.

On the other hand, efficiency of fusion is highly dependent on the procedure of the final booster immediately preceding fusion. Thus, B cell activation should be synchronous and vigorous in our protocol where we used purified CA 125 antigen for final intravenous boosting. Final intravenous boosting leads to the full activation of responding B cell clones which preferentially fuse to the myeloma partners. Given the fact that in the priming period, mice were immunized with whole cells, one may expect that several clones against different antigens including CA 125 are raised. In such a situation, final boosting with purified antigen of interest would preferentially activate clones with cognate specificity.

It is well accepted that long term immunization leads to IgG formation, while highly efficient IgM formation needs a short term immunization. However, it does not always necessarily follow that long term immunization method with 4-weeks intervals that produce IgG isotype (17) and such factors as antigen structure should be taken into consideration. In our study, seven of ten anti-CA 125 clones were IgM and the rest were IgG. Predominancy of IgM isotype may stem from the inherent highly glycosylated structure of CA 125. Different protocols were applied to produce anti-CA 125 murine monoclonal antibodies (16, 21, 22).

In this paper, we presented the characteristics of anti-CA 125 clone, 11B8. Our results clearly showed that 11B8 had excellent immunoreactivity in different immunoassays including ELISA, western blotting, flow cytometry, immunofluorescent staining of fixed and living CA 125 positive cell lines and immunohistochemical staining of ovar-

ian cancer tissues. With no doubt, this is ideal for those working in the research and diagnostic field of ovarian cancer.

Anti-CA 125 monoclonal antibodies are now widely used in pathology and clinical diagnosis. The application of 22 anti-CA 125 monoclonal antibodies in immunohistochemistry was reported in the second report from TD-1 workshop (23). Such antibodies are also invaluable tools for the detection of serum levels of CA 125 as a diagnostic marker of ovarian cancer (24).

In conclusion, it seems that prime-boost strategy is the method of choice for immunization as far as production of stable and multi-functional monoclonal antibodies against heavily glycosylated proteins is concerned. Such antibodies may provide invaluable information about the structure, composition and immunodominant epitopes of such complex molecules as CA 125 by epitope mapping.

ACKNOWLEDGEMENTS

The authors wish to thank A.A. Bayat, G.E. Kazemi Sefat, F. Davoudi, S.N. Madani, R. Rabiee, S. Zarei and M. Salavati for technical assistance. This work was supported by a grant from Iranian Nanotechnology Initiative.

REFERENCES

1 Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975; 256:495-7.
2 Hendriksen CF, de Leeuw W. Production of monoclonal antibodies by the ascites method in laboratory animals. Res Immunol. 1998; 149:535-42.
3 Chatenoud L. Monoclonal Antibody-Based Strategies in Autoimmunity and Transplantation. Methods Mol Med. 2005; 109:297-328.
4 Mechetner E. Development and Characterization of Mouse Hybridomas. Methods Mol Biol. 2007; 375:1-13.
5 Johnson DR. Murine Monoclonal Antibody Development. Methods Mol Biol. 1995; 51:123-37.
6 Burns R. Immunization Strategies for Antibody Production. Methods Mol Biol. 2005; 295:1-12.
7 Desseyn JL, Tetaert D, Gouyer V. Architecture of the large membrane-bound mucins. Gene. 2008; 410:215-22.
8 Dekker J, Rossen JW, Büller HA, Einerhand AW. The MUC family: an obituary. Trends Biochem Sci. 2002; 27:126-31.
9 Hollingsworth MA, Swanson BJ. Mucins in cancer: Protection and control of the cell surface. Nat Rev cancer. 2004; 4:45-60.
10 O'Brien TJ, Beard JB, Underwood LJ, Dennis RA, Santin AD, York L. The CA125 gene: an extracellular superstructure dominated by repeat sequences. Tumour Biol. 2001; 22:348-66.
11 Yin BW, Dnistrian A, Lloyd KO. Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. Int J Cancer. 2002; 98:737-40.
12 Patankar MS, Jing Y, Morrison JC, Belisle JA, Lattanzio FA, Deng Y, et al. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. Gynecol Oncol. 2005; 99:704-13.
13 Shojaeian S, Allameh A, Zarnani AH, Chamankhah M, Ghods R, Bayat AA, Jeddi-Tehrani M. Immunological Investigations, Accepted, 2009.
14 Jeddi-Tehrani M, Abbasi N, Dokouhaki P, Ghasemi J, Rezania S, Ostadkarampour M et al. Indoleamine 2,3-dioxygenase is expressed in the endometrium of cycling mice throughout the oestrous cycle. J Reprod Immunol. 2009; 80:41-8.
15 Davis HM, Zurawski VR Jr, Bast RC Jr, Klug TL. Characterization of the CA 125 antigen associated with human epithelial ovarian carcinomas. Cancer Res. 1986; 46:6143-8.
16 Lloyd KO, Yin BW, Kudryashov V. Isolation and characterization of ovarian cancer antigen CA 125 using a new monoclonal antibody (VK-8): identification as a mucin-type molecule. Int J Cancer. 1997; 71:842-50.
17 Nam KS. Effective production of monoclonal antibodies against phospholipids by intrasplenic immunization method. Korean Biochem. J. 1991; 24:176-181.
18 Vyas JM, Van der Veen AG, Ploegh HL. The known unknowns of antigen processing and presentation. Nature Re v Immunol. 2008; 8:607-618.
19 Nairn R, Helbert M. Cell-Cell intraction in generating effect on lymphocytes. Immunology for Medical Students. Mosby; 2002; 133-137.
20 Shojaeian J, Jeddi-Tehrani M, Dokouhaki P, Mahmoudi AR, Ghods R, Bozorgmehr M et al. Mutual helper effect in copulsing of dendritic cells with 2 antigens: a novel approach for improvement of dendritic-based vaccine efficacy against tumors and infectious diseases simultaneously. J Immunother. 2009; 32:325-32.
21 Singleton J, Guillen DE, Scully MS, Xue J, Moffet J, Chen C et al. Characterization of antibodies to CA 125 that bind preferentially to the cell-associated form of the antigen. Tumour Biol. 2006; 27:122-32.

22 Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981; 68:1331-7.
23 Nap M, Vitali A, Nustad K, Bast RC Jr, O'Brien TJ, Nilsson O et al. Immunohistochemical characterization of 22 monoclonal antibodies against the CA125 antigen: 2nd report from the ISOBM TD-1 Workshop. Tumour Biol. 1996; 17:325-31.
24 Duffy MJ, Bonfrer JM, Kulpa J, Rustink GJ, Soletormos G, Torre GC et al. CA125 in ovarian cancer: European group on tumor markers guidelines for clinical use. Int J Gynecol Cancer. 2005; 15: 679–91.