

Characterization of a Novel Specific Mouse Monoclonal Antibody Targeted to Envelope Protein E1/E2 of Hepatitis C Virus

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HEPATITIS C virus has been proven to be a major disease over the world and widely present in Egypt. The aim of this study was to propagate, characterize and test the reactivity of the hybridoma cell line (7G9) which produces a novel mouse monoclonal antibody (MoAb) targeting HCV E1/E2 envelope protein. The propagation of this hybridoma cell line (7G9) was generated using standard hybridoma technology. Isotyping of the generated mouse antibody was done by commercial ELISA kit. The reactivity of (7G9) was assessed by ELISA plates coated by HCV E1/E2 derived from cell lysate transfected by plasmid expressed HCV E1/E2. The generated monoclonal antibody (7G9) was found to be an IgM antibody with a kappa light chain. ELISA showed that (7G9) reacted with cell lysate expressed HCV E1/E2 and (7G9) presented no cross-reactivity with different antigens such as *Brucella abortus*, *Salmonella typhi* and Hepatitis B Virus (HBV) antigens. ELISA revealed high reactivity of (7G9) with HCV E1 (a.a. 315-323) antigen. Thus, the mouse monoclonal antibody (7G9) can be used for immunodiagnosis of HCV infection by detection of HCV E1/E2 antigens.

Keywords: Hepatitis C Virus - Envelope Protein E1/E2 - Hybridoma cell line (7G9)

Hepatitis C virus (HCV) is a major cause of liver disease and 3% of the global population are estimated to be infected (Vieyers *et al.*, 2014). In Egypt, HCV infections were recorded (Abdel Hamid *et al.*, 2007) and remain a major health problem (Mohamoud *et al.*, 2013). The serologic diagnosis of HCV is usually difficult due to the lack of a gold standard procedure (Araujo *et al.*, 2011). In addition, the differentiation between acute and chronic cases for HCV is difficult depending on the present available serological assays as well as upon the symptoms and the clinical history of the patients. Also, the majority of acute infections are asymptomatic which develop to a chronic case unnoticed. Even detection of IgM is not sufficient to differentiate between acute and chronic HCV cases, sometimes produced in chronic cases and not always produced in acute cases (Lange and Sarrazin, 2010). The aim of this study was the propagation, characterization and evaluation of the reactivity of novel hybrid cell line (7G9) producing mouse monoclonal antibody, targeting HCV E1/E2 envelope protein to improve diagnostics.

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Materials

Hybridoma cell line

The hybridoma cell line (7G9) targeting HCV E1/E2 was generated by Dr. Ashraf Tabll (2010) and was used in this study.

Antigens

- 1- *Brucella abortus* antigen and 2- *Salmonella typhi* antigen (Sal. O Group D Ag and Sal. Flagellar d Ag) by Becton, Dickinson and Company (Sparks, Maryland 21152 USA).
- 3- HBV antigen (HBc Ag coated plate with genetically engineered hepatitis B core antigen) by Enzygnost (Siemens Healthcare Diagnostics Products GmbH Germany).

Methods

Revival and propagation of hybridoma cell line producing mouse monoclonal antibody (MoAb)

The hybridoma cell line producing mouse MoAb (7G9) was generated by hybridoma technology (Köhler and Milstein, 1975), using C57Bl/6 mice immunized with a plasmid expressing the E1/E2 proteins of the HCV AD78 strain which was provided by Dr. Sergei Viazov (Essen Virology Institute, Germany). The plasmid was used to express the E1/E2 proteins of the HCV AD78 strain in HEK293 cells and the lysate from these cells were used for the in-house ELISA experiments (Tabll *et al.*, 2013). The monoclonal antibody producing cells (Fig. 1) were propagated in DMEM medium supplemented with 20% fetal bovine serum, (1%)L- glutamine, penicillin/streptomycin and HEPES. The supernatant of the culture was collected and tested for the presence of the HCV targeted monoclonal antibodies using in-house ELISA and the producing cells were cryopreserved (Ryan, 2004).

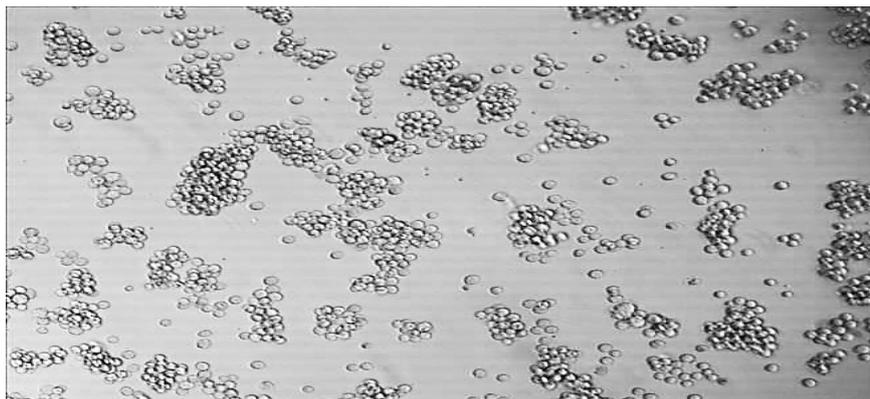


Fig. 1. The hybridoma cell line (7G9) producing monoclonal antibodies targeting HCV E1/E2 (100 x)

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Evaluation of the reactivity of the generated mouse MoAb (7G9) against the HCV E1/E2 transfected cell lysates by ELISA:

The in-house ELISA was conducted as previously described (Tabll *et al.*, 2013). Briefly, ELISA plates were coated with lectin in carbonate/bicarbonate buffer and incubated overnight at room temperature. The plates were then blocked with blocking buffer and incubated overnight at 4°C, and washed (all washes were performed 4 times using PBS/T). Positive HCV E1/E2 cell lysate was added at 1:50 dilution in PBS (100 µl/well) and incubated overnight at 4°C. The plates were washed and the generated (7G9) mouse monoclonal antibody supernatant was added (100 µl/ well). Human sera positive for HCV antibodies at 1:100 in dilution buffer were included as positive control and human sera negative for HCV antibodies and culture medium were used as negative controls. Incubations were carried out for 3 hr at 37°C followed by washing and addition of conjugated anti-human peroxidase (KPL Inc, Gaithersburg, Maryland, USA) (100 µl/ well) for the human controls at 1: 5000 in dilution buffer and conjugated anti-mouse peroxidase (Sigma, USA) at 1: 5000 for mouse antibody and incubated for 1 hr at 37 °C. After another washing step, the substrate ortho-phenylene diamine (one tablet OPD / 10 ml citrate buffer with 10 µl H₂O₂) was added (100 µl/ well) and incubated at room temperature for 5-15 min. The reaction was stopped using 3 M HCl (100 µl/well) and the plate was read at 450 nm by ELISA reader (Labsystems, Model Multiskan EX)

Isotype determination of the mouse MoAb (7G9)

The isotype was identified using the Pierce Rapid ELISA Isotyping Kit #37503(3747N Meridian Rd, Rockford, IL 61101/USA).

Evaluation of the specificity of the mouse MoAb (7G9)

The evaluation was done using ELISA (Engvall and Perlmann 1971, El Awady *et al.*, 2009) by coating ELISA plates with different antigens (*Brucella abortus*, *Salmonella typhi* and Hepatitis B Virus (HBV)) followed by blocking (0.1% non-fat dry milk in carbonate / bicarbonate buffer) and incubation for 1 hr. at 37°C, washing 3 times with PBS/T then addition of the monoclonal antibody (7G9) undiluted to the plates. Following a 2 hr. incubation at 37°C, and washing, conjugated anti-mouse IgM labelled alkaline phosphatase (KPL/Kirgegaard and Perry Laboratories INC. 2 Cessna Court, Gaithersburg, Maryland 20879) was added at a dilution of 1:100 in 0.1% non-fat dry milk/ PBST and incubated for 1 hr. at 37°C. The substrate para-nitrophenylphosphate (p-NPP) # S0942(Sigma Aldrich /USA) was then added at a dilution of 1 mg/ml in diethanolamine buffer and incubated for 30 min at 37°C. The reaction was ended by using the stopping buffer 3M NaOH and the plates were measured at 405 nm using the ELISA reader.

Evaluation of the sensitivity of the mouse MoAb (7G9)

The sensitivity of the monoclonal antibody (7G9) was tested by coating an ELISA plate with serial dilutions (15 µg/ml - 7.5 µg/ml - 3.75 µg/ml - 1.88 µg/ml

- 0.94 $\mu\text{g/ml}$ - 0.47 $\mu\text{g/ml}$ - 0.24 $\mu\text{g/ml}$) of the peptide (HCV a.a. 315-323) E1 antigen, followed by using the in-house peptide-ELISA (Tabll *et al.*, 2013)

Results

Detection of mouse MoAb in supernatants of cell line by ELISA

The tested monoclonal antibody (7G9) gave a high reactivity with an Optical Density (OD) value (2.6). The HCV human positive control gave an OD value of (1.7) whereas the HCV human negative control had an OD of (0.4) and the negative control (complete medium) had an OD value (0.08) (Fig 2.).

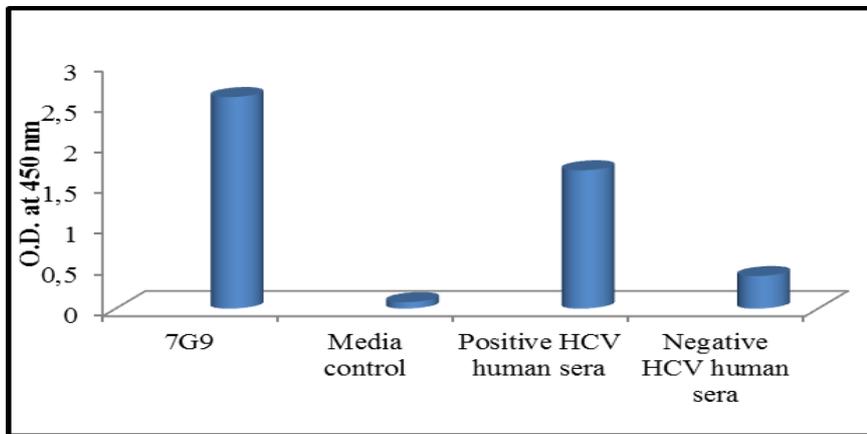


Fig. 2. Reactivity of the generated mouse monoclonal antibody (7G9)

Isotyping of the mouse MoAb

The tested mouse monoclonal antibody (7G9) had a heavy chain IgM and a kappa light chain (Fig. 3)

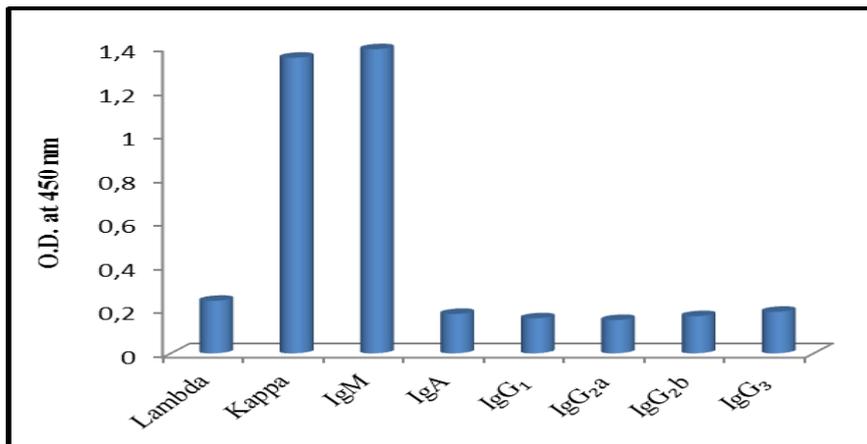


Fig 3 Isotyping of the generated mouse monoclonal antibody (7G9)

Specificity

Brucella abortus, *Salmonella typhi* and HBV antigens were used to coat wells. No reactivity with both bacteria organisms and HBV was obtained. The positive control was a pool of HCV positive samples, the negative control was DMEM medium and the blank was PBS (Fig. 4.).

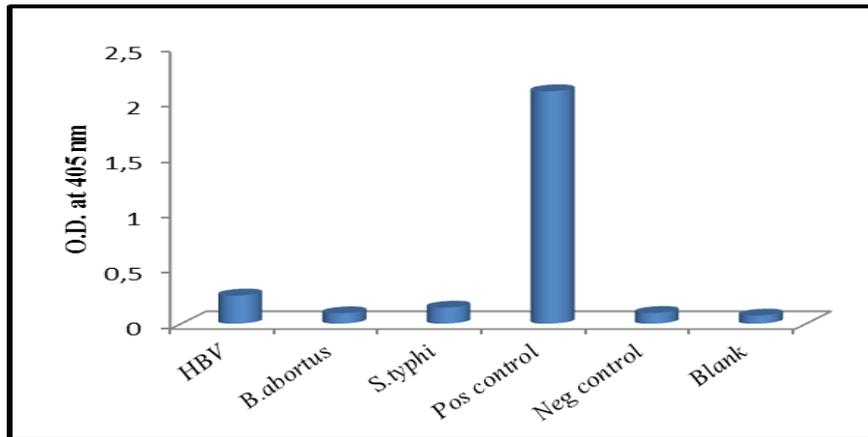


Fig. 4 Reactivity of the novel monoclonal antibody (7G9) towards *Brucella abortus*, *Salmonella typhi* and HBV antigens

Sensitivity limit of the mouse MoAb (7G9)

The sensitivity of the mouse monoclonal antibody (7G9) was detected till 2.00 µg/ml of the coated antigen, which was HCV E1 (a.a. 315-323) antigen (Fig. 5). This was a high dilution meaning that it was very sensitive.

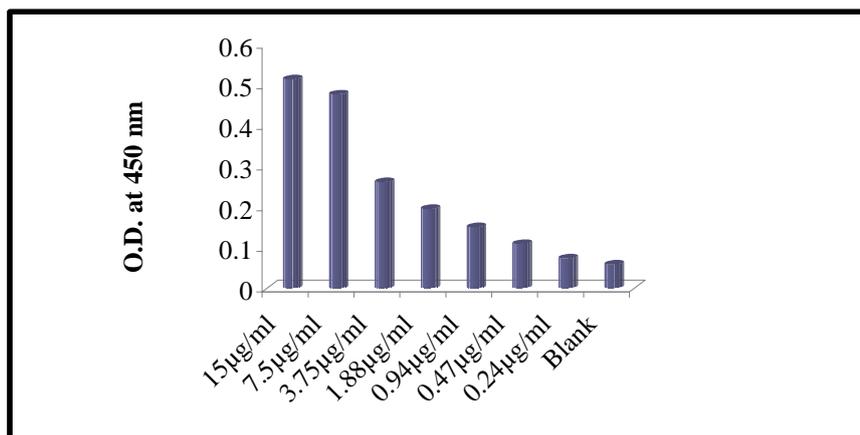


Fig. 5. Reactivity of the mouse monoclonal antibody (7G9) towards serial dilution of E1 antigen (envelope glycoproteins E1).

Discussion

Production of mouse monoclonal antibodies by the hybridoma method (Köhler and Milstein 1975) showed the possibility of the fusion of antibody producing cells from mice with tumor cells to generate monoclonal antibodies. This was the first time to obtain unlimited production of a specific monoclonal antibody, which led to an immense benefit of therapeutic and diagnostic use of monoclonal antibodies. Although, the production of monoclonal antibodies started many years ago, still there are several hundred monoclonal antibodies to treat a broad range of conditions and others under evaluation, and a handful of therapeutics on the market (Madorsky Rowdo *et al.*, 2015).

Hepatitis C virus (HCV) is a major health problem worldwide. This virus infects more than 180 million people across the whole world (2-3% of the world's total population) (Moradpour and Müllhaupt, 2015). HCV (genotype 4) is one of the main health problems particularly in Egypt. It infects 22% of its general population (El-Shabrawi and Hassanin, 2014). In the present study, we generated and propagated a hybrid cell line to produce mouse monoclonal antibody (7G9) targeting HCV envelope region. Isotyping of this antibody showed it to be isotype IgM and kappa chain. The generated antibody (7G9) showed good specificity where it did not react with *Brucella abortus*, *Salmonella typhi* or HBV antigens. The tested monoclonal antibody (7G9) showed also good sensitivity with HCV peptides only. The peptide used to test the sensitivity of the monoclonal antibody (7G9) was the envelope glycoprotein E1 (a.a. 315-323) antigen. The high sensitivity was detected till 2.00 µg/ml of the coated antigen. Several mouse monoclonal antibodies targeting HCV core and envelope regions are already known and these antibodies are mainly used for antigen detection (Cagnon *et al.*, 2004, Tabll *et al.*, 2008 and Shi *et al.*, 2014). The monoclonal antibody (7G9) is targeted to the envelope glycoproteins E1 and E2. These envelope glycoproteins E1 and E2 are essential in the attachment and entry of the virus (Sabo *et al.*, 2011). E2 causes not only viral attachment to the cell membrane at its entrance but enhances also the neutralizing antibodies in the host. The envelope proteins play a major role in attachment and entry of virions into the body (Mazumdar *et al.*, 2011) and (Park *et al.*, 2013). Moreover, although E2 is immunologically dominant and can hide the E1 epitopes, E1 causes specific antibody responses leading it to be a candidate to vaccine trials (Garrone *et al.*, 2011). Further studies will be done on the evaluation of ELISA or Dot-ELISA for diagnosis of HCV based on detection of HCV envelope glycoprotein E1-E2 antigen in sera samples. Thus, this mouse monoclonal antibody (7G9) can be useful for HCV diagnosis based on antigen detection, as well, for studying the HCV vaccine development.

Acknowledgment: Our special thanks are due to Andreas Walker, Milena Lange and Sina Luppus, Essen virology institute, for their technical assistant. The production of hybrid cell line was carried out at lab of Dr. Sergei Viazov, Essen virology institute, Germany and was funded by DAAD for Dr. Ashraf Tabll

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(Received 7 / 2 / 2016;
accepted 13/ 3 / 2016)

التشخيص للجسم المضاد الخاص وحيد النسيلة الموجه للغلاف البروتيني E1/E2 لفيروس التهاب الكبدى الوبائى سي

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فى هذه الدراسة تم احياء و اكاثر خلايا الهجين (7G9) المنتجة للجسم المضاد احادى النسيلة الموجه ضد الغلاف البروتيني (E1/E2) لفيروس التهاب الكبدى الوبائى سي.

تم اختبار معلقات الهجين (7G9) و ادت نتائج اختبار الاليزا الى وجود الجسم المضاد احادى النسيلة الموجه ضد الغلاف البروتيني (E1/E2) لفيروس التهاب الكبدى الوبائى سي. اعطى الهجين (7G9) اعلى قراءة (٢,٦) بينما القراءة للعينة الحاكمة الموجبة (١,٧) والعينة الحاكمة السالبة (٠,٤).

زيادة فى الوصف تم ايجاد نوع الجسم المضاد احادى النسيلة (7G9) وقد تبين انه من نوع (IgM/Kappa) عن طريق الاختبار السريع.

ثم تم اثبات مدى النوعية لهذا الجسم المضاد احادى النسيلة (7G9) فوجد عدم نشاطه مع فيروس التهاب الكبدى الوبائى بي و البروسىلا ابورنيس والسلمونيليا تايفى.

كما تم اختبار مدى الحساسية عن طريق تخفيفات متتالية لمولد المضاد (HCV a.a. 315-323) وقد تم الكشف عنها حتى التخفيف 2.00µg/ml

بالتالى يمكن فى دراسات أخرى استخدام الجسم المضاد احادى النسيلة (7G9) فى الاختبارات المناعية للكشف على الغلاف البروتيني (E1/E2) لفيروس التهاب الكبدى الوبائى سي عبر استحداث اختبار الاليزا و اختبار الاليزا النقطية وكذلك، يمكن دراسة تطوير لقاح لفيروس التهاب الكبدى الوبائى سي.