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Anti-Ebola MAb17A3 Reacts with Bovine and Human Alpha-2-macroglobulin Proteins

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Abstract

Monoclonal antibodies (MAbs) were developed against soluble Ebola virus (EBOV) envelope glycoprotein (GP) for the study of the diversity of EBOV envelope and development of diagnostic reagents. Of the three anti-EBOV GP mouse MAbs produced, MAb 15H10 recognized all human EBOV GP species tested (Zaire, Sudan, Ivory Coast), and as well as reacted with the Reston nonhuman primate EBOV GPs. A second MAb, 6D11 recognized EBOV GP species of Sudan and Sudan-Gulu. The third MAb, 17A3, was reported originally in the same article to be EBOV GP-specific has now been found to be specific for bovine and human α -2 macroglobulin (α -2M) proteins which were contaminants in the Ebola envelope protein preparation. Thus, while MAbs 15H10 and 6D11 are indeed EBOV GP specific, MAb 17A3 is an α -2 macroglobulin MAb.

Keywords

Ebola virus; glycoprotein detection; monoclonal antibody; alpha-2-macroglobulin

The 17A3 monoclonal antibody (MAb) reported previously to be specific for EBOV GP has now been found to have an alternative specificity. In the previous report (Yu et al., 2006), MAb 17A3 reacted with an ~220kDa protein in the Ebola envelope preparation only under non-denaturing conditions in clear native gels but not in denaturing gels. The result was interpreted initially as reactivity of MAb 17A3 with a conformational determinant of the Ebola trimer. More recent studies have now found that this putative anti-EBOV GP 17A3 MAb actually does not react with EBOV GP, but rather reacts with bovine alpha-2 macroglobulin (α -2M) that was a contaminant in the Ebola envelope proteins. α -2M is a ~720kDa tetrameric glycoproteins found in blood and is able to inactivate a variety of proteinases including serine-, cysteine-, aspartic- and metalloproteinases (Blacker et al., 1998; Chu and Pizzo, 1994; Kovacs, 2000).

For transient expression of Ebola glycoproteins, plasmids coding EBOV GP of Ebola virus Zaire strain Mayinga (GeneBank No. U23187), and Ebola virus Sudan strain Gulu (Sudan-Gulu, GeneBank No. AY729654) were transfected into 293T cells and cell lysates were prepared. Recombinant soluble glycoproteins were purified from transfected-293T cell culture and analyzed with methods as described the previous report (Yu et al., 2006). Bovine

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alpha-2-macroglobulin was purified by agarose *Galanthus Nivalis* lectin column chromatography (Vector Labs, Burlingame, CA) and human alpha-2-macroglobulin was purchased from Sigma, St. Louis, MO.

The reactivity of MAb 17A3 with purified bovine (Figure 1, Lanes 3 and 7) and human α -2M (Figure 1, Lanes 4 and 8) were studied using Western blot analysis (Figure 1B). As expected, and as described previously, MAb 17A3 did not detect protein bands in the purified recombinant glycoprotein preparations of both Ebola Sudan-Gulu in SDS-PAGE reducing gels (Figure 1, lanes 1 and 5) and Zaire strains (Figure 1, Lanes 2 and 6) as shown in the original report. While MAb 17A3 did not react in denaturing Western blots with any proteins in the Ebola envelope preparation (Yu et al., 2006), it did react with a high molecular weight protein band at ($\approx$ 220 kDa) in the human alpha-2-macroglobulin preparation, that co-migrated with the Ebola envelope protein trimer in SDS-PAGE gels under reducing conditions (Figure 1B, lane 4).

MAb 17A3 detected both bovine (Figure 1 B, lane 7) and human (Figure 1B, lane 8) purified α -2M protein under non-reducing conditions (Panel B). As a control, anti-human alpha-2M MAb (Human alpha-2-Macroglobulin MAb (Catalog #: MAB1938, R & D Systems, Minneapolis, MN) (Figure 1 Panel A) detected α -2M protein bands that had co-purified in the recombinant glycoprotein preparations of both Ebola Sudan-Gulu (Figure 1, lanes 1 and 5) and Zaire strains (Figure 1, lanes 2 and 6) with similar migration patterns as α -2M proteins purified from fetal bovine serum (that was used to grow the 293T cells transfected with EBOV GP) (Figure 1, lanes 3 and 7) and purified human alpha-2-macroglobulin (Figure 1, lanes 4 and 8).

The reactivity of MAb 17A3 was further analyzed in ELISA against bovine and human alpha-2M proteins captured on ELISA plates. Anti-human alpha-2M MAb (R & D Systems, Minneapolis, MN) was used as the positive control. An irrelevant MAb, 7E5, was used as the negative control. MAb 17A3 reacted strongly with the purified bovine alpha-2M with an end-point titer of 0.2ug/ml (Figure 2A), and reacted weakly with human alpha-2M with an end-point titer of 25ug/ml (Figure 2B). The anti- α -2M MAb positive control MAb reacted with both bovine and human alpha-2M with end-point titers of 0.05ug/ml for both bovine and human alpha-2M proteins (Figure 2).

MAb 17A3, also reacted with recombinant EBOV GP preparations of both Ebola Sudan-Gulu and Zaire strains in colorless native gels (precasted 3-8% Tris-acetate gel, Invitrogen, Carlsbad, USA) using the same methods as described previously (Yu et al., 2006). Native gel Western blots showed that both MAb 17A3 and anti-human alpha-2M MAb detected protein bands in the purified recombinant glycoprotein preparations of both Ebola Sudan-Gulu and Zaire strains (Figure 3). However, anti-Ebola MAbs such as 6D11 and 15H10 did not react with purified EBOV GPs in native gel conditions as reported originally (Figure 3).

The protein bands recognized by MAb 17A3 in EBOV GP preparations were cut out of the native gels and assessed for identity by mass spectrometry (indicated by the arrow in Figure 3). The protein bands in EBOV GP preparations that were recognized by MAb 17A3 were indeed confirmed to be bovine α -2M based on the mass spectroscopy analysis (Species: *Bos taurus*; NCBI database ID: gi: 157954061, MW: 167 kDa). In addition, as expected, also present in this MW range was also EBOV GP the precursor of Sudan-Gulu strain (NCBI database ID: gi:8479509) and the precursor of Zaire strain (NCBI database ID: gi:8479503).

Thus, MAb 17A3 is not an anti-EBOV GP antibody as reported originally, but rather is an alpha-2M MAb that reacts best with bovine alpha-2M and weakly with human alpha-2-macroglobulin. The cause of this mis-identification comes from the ability of lentil lectin columns to co-purify viral glycoproteins and alpha-2-macroglobulin (Arnold et al., 2006).

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Figure 1. Detection of α -2M in the purified recombinant glycoprotein preparations of both Ebola Sudan-Gulu and Zaire strains

The recombinant glycoprotein preparations of both Ebola Sudan-Gulu and Zaire strains that were prepared as described in the original report and the purified bovine and human α -2M proteins were fractionated on 4-20% gradient SDS-PAGE under reducing and non-reducing conditions. Shown are Western blots under reducing (Lanes 1 to 4) and non-reducing conditions (Lanes 5-8); M= Molecular markers; lanes 1 and 5= purified Ebola envelope protein of Sudan-Gulu strain; lanes 2 and 6= purified Ebola envelope protein of Zaire strain; lanes 3 and 7= purified bovine α -2M proteins; and lanes 4 and 8= human α -2M proteins. Anti- α -2M MAb reactivity with the EBOV GP preparations and bovine and human α -2M proteins is shown in panel A, while MAb 17A3 lack of reactivity is shown in panel B. While the anti-alpha-2-macroglobulin antibody reacted with alpha-2-macroglobulins in both Ebola GP preparations as well as with purified bovine and human alpha-2-macroglobulin, MAb 17A3 only reacted weakly with human alpha-2-macroglobulin.

Figure 2. Reactivity of MAb17A3 with bovine and human α -2M proteins
 MAb 17A3 reacted the purified bovine (Figure 2A) and human (Figure 2B) α -2M proteins in ELISA. Anti-human α -2M MAb was used as the positive control. An irrelevant MAb, 7E5, was used as the negative control. Open circle indicates the negative MAb 7E5, closed square indicates MAb 17A3, and closed circle is anti-human α -2M MAb.

Figure 3. Native gel analysis of glycoproteins of Ebola virus Sudan-Gulu and Zaire

Ebola glycoproteins were fractionated on 3-8% gradient Tris-acetate gel under non-reducing conditions. Ebola virus Sudan-Gulu and Zaire glycoproteins are shown in both Coomassie staining gels and western blot analysis. Similar to Western blot analysis in SDS-PAGE, anti-α 2M MAb detected Ebola glycoproteins bands that MAb 17A3 also detected while MABs 6D11 and 15H10 did not detect any protein bands. The arrow indicates those bands cut for finding the identity by mass spectroscopy analysis. Lane S indicates the purified Ebola envelope protein of Sudan-Gulu, and Z denotes purified Ebola envelope protein of Zaire strain.