pharmaceutics

MDPI

# Supplementary Materials: Both IgM and IgG Antibodies against Polyethylene Glycol Can Alter the Biological Activity of Methoxy Polyethylene Glycol-Epoetin Beta in Mice

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**Table 1.** Sources of antibodies.

| Antibody | Source | Catalog No. | Assay |
|---|---|---|---|
| 6.3 anti-PEG IgG | Academia Sinica (Taipei, Taiwan) | 6.3-PABG-A | in all studies |
| AGP4 anti-PEG IgM | Academia Sinica | AGP4-PABM-A | in all studies |
| Human $IgG_1$ | Abcam (Cambridge, UK) | Ab90283 | Human $IgG_1$ negative control antibody |
| Human IgM | Rockland (Limerick, PA) | 009-0107-0001 | Human IgM negative control antibody |
| Goat IgG anti-erythropoietin | R&D system (Minneapolis, MN) | AF959 | Neutralization assay and immunoblotting |
| Peroxidase-conjugated affinipure goat $F(ab')_2$ anti-human IgG Fcγ | Jackson ImmunoResearch (West Grove, PA) | 109-036-098 | Second antibody in anti-PEG IgG ELISA |
| Peroxidase-conjugated affinipure rabbit anti-human $_{IgM}$ $Fc_{5\mu}$ | Jackson ImmunoResearch | 309-035-095 | Second antibody in anti-PEG IgM ELISA |
| Peroxidase-conjugated affinipure donkey anti-mouse IgG (H+L) | Jackson ImmunoResearch | 715-035-150 | Second antibody in anti-PEG IgG ELISA and immunoblotting |
| Peroxidase-conjugated affinipure goat anti-mouse IgM μ chain | Jackson ImmunoResearch | 115-035-020 | Second antibody in anti-PEG IgM ELISA and immunoblotting |
| Peroxidase-conjugated goat anti-mouse IgG $F(ab')_2$ | Cappel™, Organon Teknika Corporation (Durham, NC) | 55553 | Second antibody in anti-PEG IgG $F(ab')_2$ ELISA |
| Peroxidase-conjugated goat anti-mouse IgG Fc | Cappel™, Organon Teknika Corporation | 55554 | Second antibody in anti-PEG IgG $F(ab')_2$ ELISA |

**Table S2.** Concentrations of anti-PEG antibodies in human serum samples.

| Sample name | Anti-PEG IgM (μg $mL^{-1}$) | Anti-PEG IgG (μg $mL^{-1}$) |
|---|---|---|
| hM1 | 4.93 | 0 |
| hM2 | 8.01 | 0 |
| hG1 | 0 | 15.0 |
| hG2 | 0 | 34.8 |
| hMG1 | 6.66 | 4.65 |
| hMG2 | 2.52 | 5.16 |

Samples were from normal Taiwanese donors.

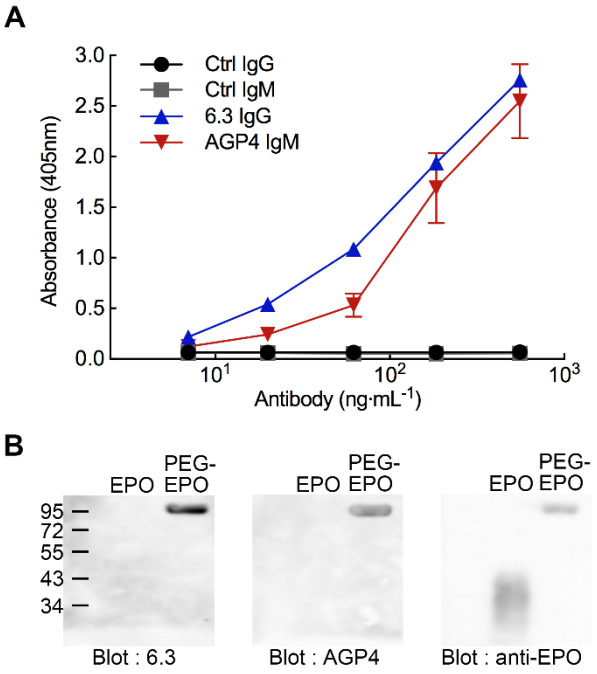


**Figure S1.** Mouse anti-PEG AGP4 IgM and 6.3 IgG antibodies bind to PEG-EPO. (**A**) Serial dilutions of AGP4 and 6.3 monoclonal antibodies were assayed for binding to PEG-EPO in 96-well plates using peroxidase-conjugated affinipure anti-mouse IgM or IgG specific secondary antibodies. Control IgM (Ctrl IgM) and control IgG (Ctrl IgG) are negative control mouse IgM and IgG antibodies, respectively. (bars, SD; $n$ = 2). (**B**) Recombinant human erythropoietin (EPO) and PEG-EPO were immunoblotted with 6.3, AGP4, or anti-EPO and specific secondary antibodies.

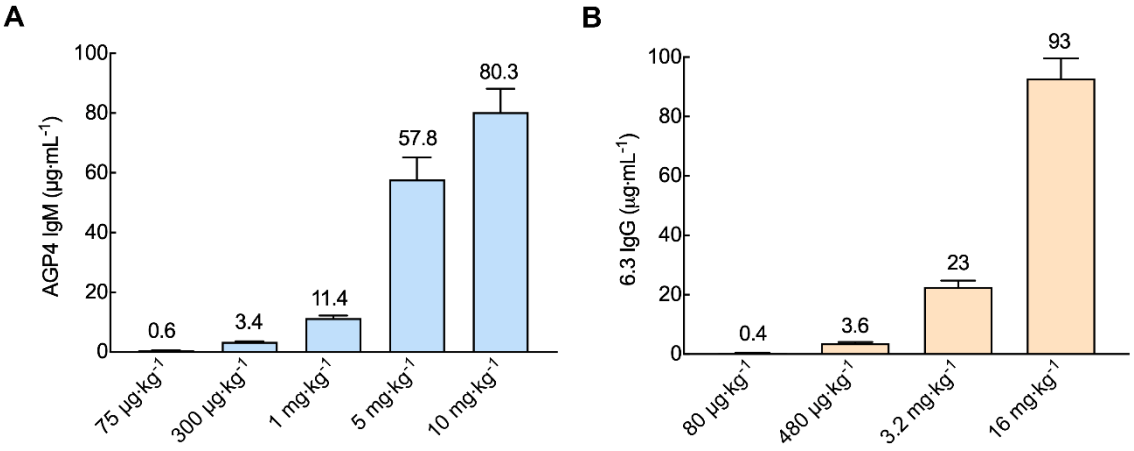


**Figure S2.** Measurement of anti-PEG antibodies in serum at 24 h after injection. BALB/c mice were intravenously injected with different doses of AGP4 (**A**) or 6.3 (**B**) antibodies. At 24 h after injection, serum samples were collected and the concentrations of anti-PEG antibodies were measured by direct ELISA on PEG-coated plates using peroxidase-conjugated affinipure anti-mouse IgM or IgG specific secondary antibodies. (bars, SD; $n$ = 4)

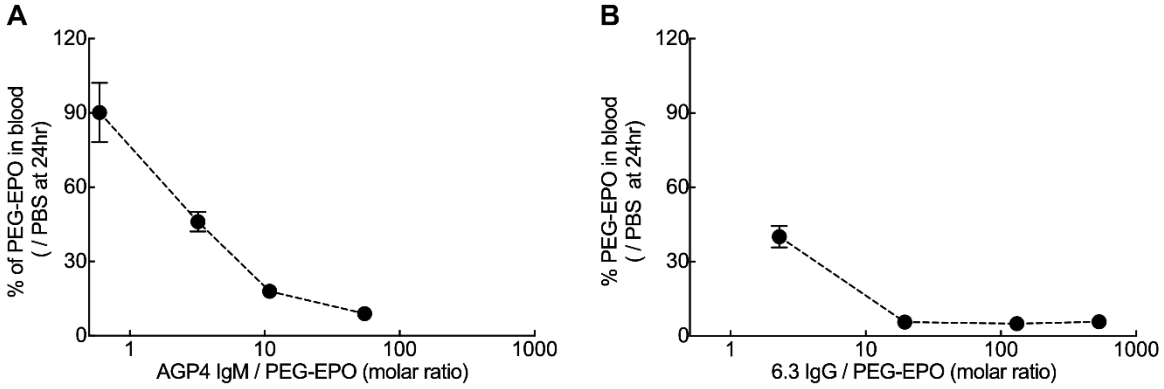


**Figure S3.** Anti-PEG antibodies accelerate clearance of PEG-EPO in mice. Female BALB/c mice were pre-injected with different doses of mouse anti-PEG AGP4 IgM or 6.3 IgG 24 h before intravenously injection of 5 μg $kg^{-1}$ $^{125}$I-PEG-EPO. Radioactivity was measured in plasma samples collected at 30 min, 2 h, 6 h and 24 h. Percentages of PEG-EPO remaining in the circulation at different molar ratios of AGP4 (**A**) or 6.3 (**B**) are shown. ($n$ = 4).

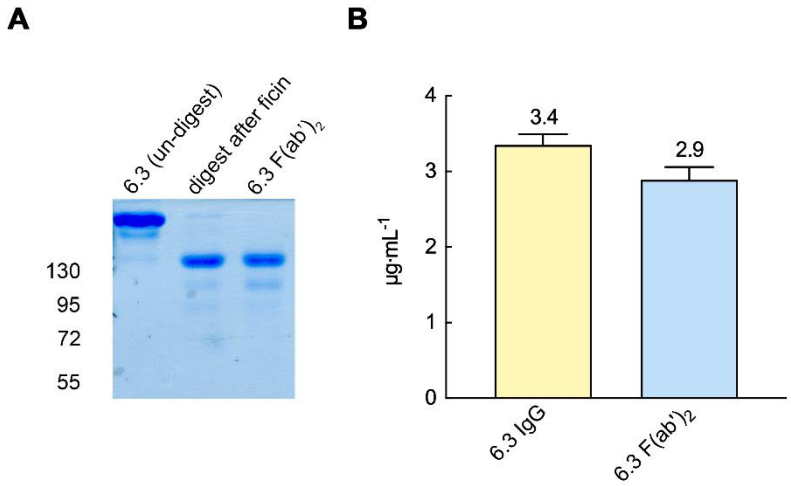


**Figure S4.** Characterization of 6.3 F(ab′)$_2$. (**A**) Non-reducing SDS-PAGE electrophoresis of 6.3 IgG and 6.3 F(ab′)$_2$ fragments. (**B**) Concentrations of 6.3 IgG and 6.3 F(ab′)$_2$ in mouse serum 1 h after i.v. injection (bars, SD; $n$ = 4).