GENETIC BACKGROUND DETERMINES MURINE PRIMARY MESANGIAL CELL RESPONSE TO TGF-ß

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BACKGROUND

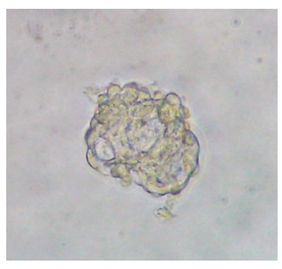
C57Bl6/J (B6) mice have been reported as resistant to experimental renal fibrosis. Alb/TGF-beta1 transgenic mice on B6 background develop only mild renal fibrosis compared to TGF-ß transgenic mice on CBA background, despite the comparable elevated circulating TGF-ß levels (1).

We therefore aimed to investigate the effects of TGF-ß administration at the cellular level on primary mesangial cells isolated from B6 and CBA mice.

METHODS

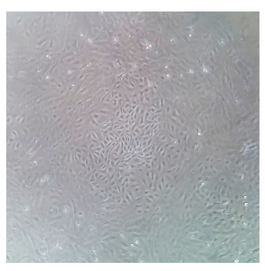
Mesangial cell isolation and characterization

Mesangial cells were isolated from a male 6 week-old B6 and a CBA mice using the magnetic bead glomerular separation method (2).



Freshly isolated glomerulus (400x magnification)

Glomerular outgrowths were maintained in selective RPMI medium for 21 days.
P2 cells were seeded (20000 cells/cm2) on gelatin-coated 8-well chamber slides and 6-well plates, grown for 24 hours and characterized for mesangial markers using immunocyto-chemmistry and immunoblot, respectively.



Glomerular outgrowth of spindle-like cells (day 21)

Cell treatment protocol

Primary cells from P5 to P8 were seeded on 24-well plates and analyzed upon PBS or TGF-ß treatment (10 ng/ml) for 48 hours.

Experimantal groups:
1) B6 control (n=6)
2) B6 + TGFß (n=6)
3) CBA control (n=6)
4) CBA + TGFß (n=6)

Performed analyses:

- Immunostaining and immunoblot
  Antibodies:
  -rabbit Fibronectin /Sigma/,
  -rabbit vimentin (CST),
  -rabbit E-cadherin (CST),
  -mouse tubulin (Millipore)
- qPCR for TGF-ß, Egr-1, Type I Collagen and TIMP-1

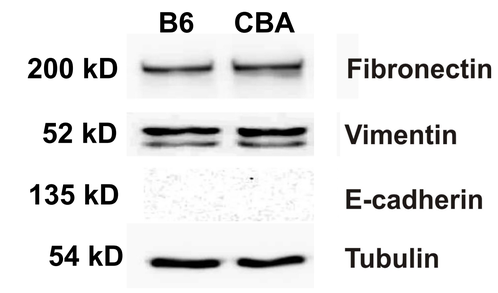
Statistics:

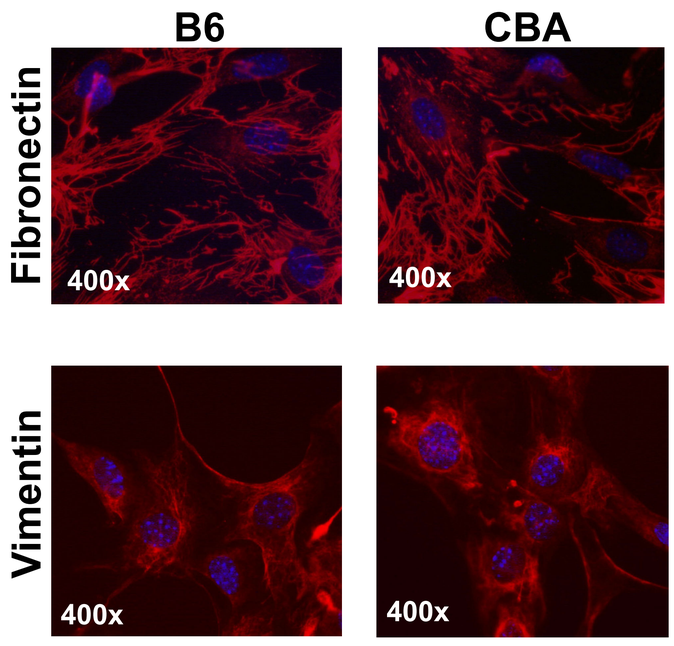
Data are presented as mean±SD. Kruskal-Wallis test was performed to test statistical significance.

RESULTS

Characterization of primary cells

On immunoblot, both B6 and CBA primary cells were positive for mesangial markers fibronectin and vimentin, but negative for the epithelial marker E-cadherin.

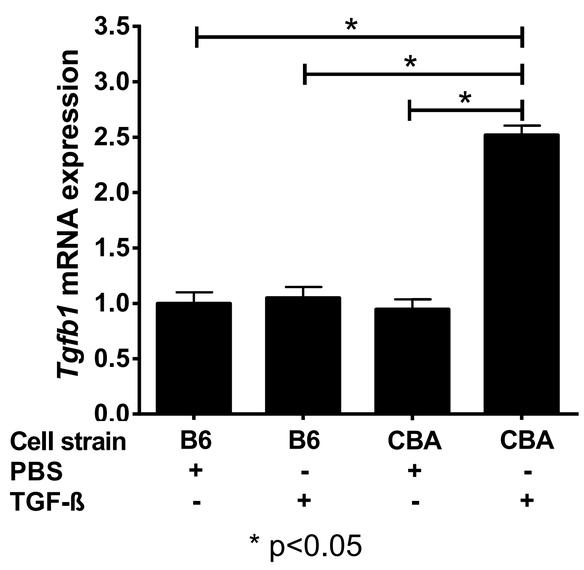




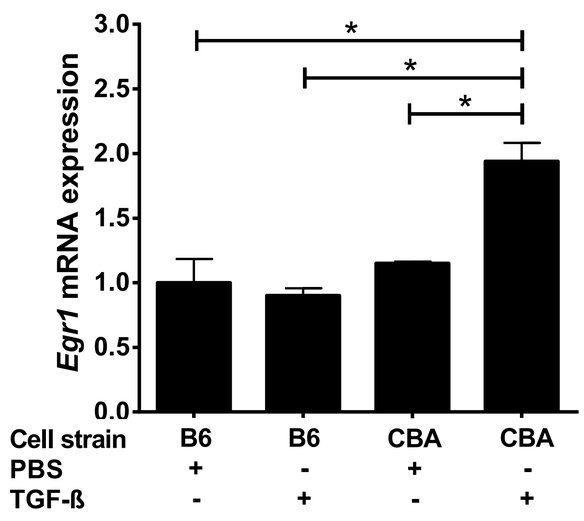
Nuclei are stained with DAPI (blue), specfic primary antibodies and Cy3-conjugated secondary antibody (red)

Results of TGF-ß treatment: overexpression of profibrotic genes

Compared to B6 cells, TGF-ß treated CBA cells showed significant mRNA overexpression of profibrotic TGF-ß and Egr-1.



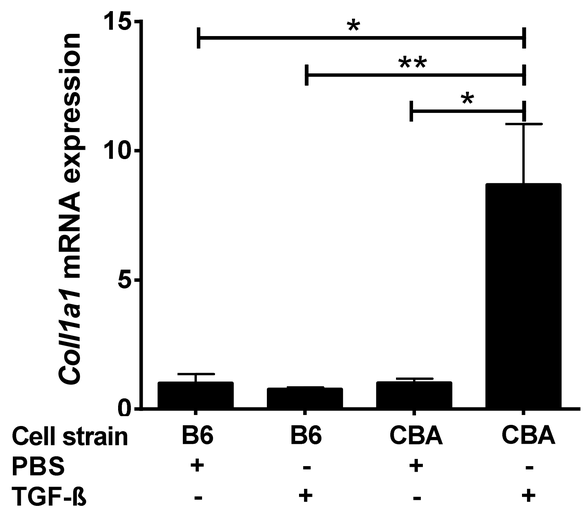
* p<0.05



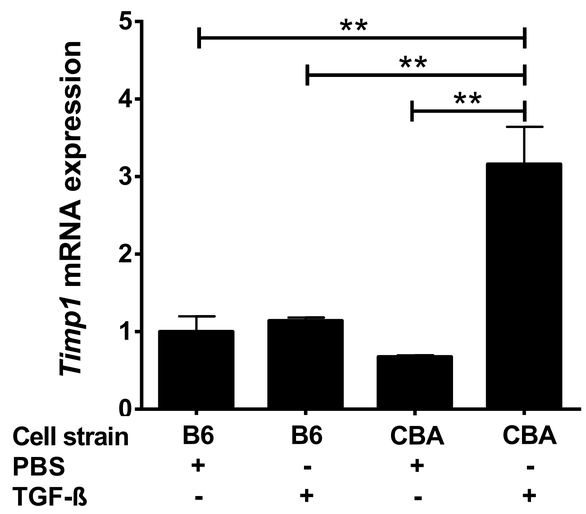
* p<0.05

Results of TGF-ß treatment: expression of collagen and TIMP-1

As compared to B6 cells, TGF-ß treated CBA cells showed increased type-I collagen and TIMP-1mRNA expressions.



* p<0.05, ** p<0.01



* p<0.05, ** p<0.01

CONCLUSION

Our in vitro results on primary murine mesangial cells isolated from CBA/J and C57Bl6/J mice have further confirmed the previous reports on renal fibrosis resistance of C57Bl6/J mouse strain over other inbred strains. Based on our observations we postulate that this relative resistance to renal fibrosis might be related to strain-dependent expression response of profibrotic genes like Egr-1 or TIMP-1.

REFERENCES

1. Kokeny et al, Clin Kidney J 2011;4(S2):421-429.

2. Takemoto et al. Am J Pathol. 2002 Sep; 161(3): 799–805.

FUNDING

Hungarian Scientific Research Fund (OTKA PD 112960 to GK).