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Contribution of cytokines to the etiology and progression of primary myelofibrosis

Resident self-tissue of pro-inflammatory cytokines rather than their systemic levels correlates with development of myelofibrosis in *Gata1*^{low} mice

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Aim. Demonstrate that the megakaryocytes(MKs) in the bone marrow(BM) of *Gata1*^{low} mice, a mouse model of myelofibrosis, express high levels of TGF-β1, CXCL1 (the murine counterpart of human IL-8), LCN-2 (CXCL1 promoter), and of the CXCL1 receptors, CXCR1 and CXCR2, compared to wild-type (WT) littermates.

Materials and methods. Femurs of WT and *Gata1*^{low} mice were fixed in formaldehyde, included in paraffin and cut in 3µm thick section to further study TGF-β1, LCN2, CXCL1, CXCR1 and CXCR2 expression by immunohistochemistry.

Results. TGF-β1, LCN2, CXCL1, CXCR1 and CXCR2 were highly expressed mainly in the MKs of *Gata1*^{low} mice compared to WT littermates (***Figure 1***).

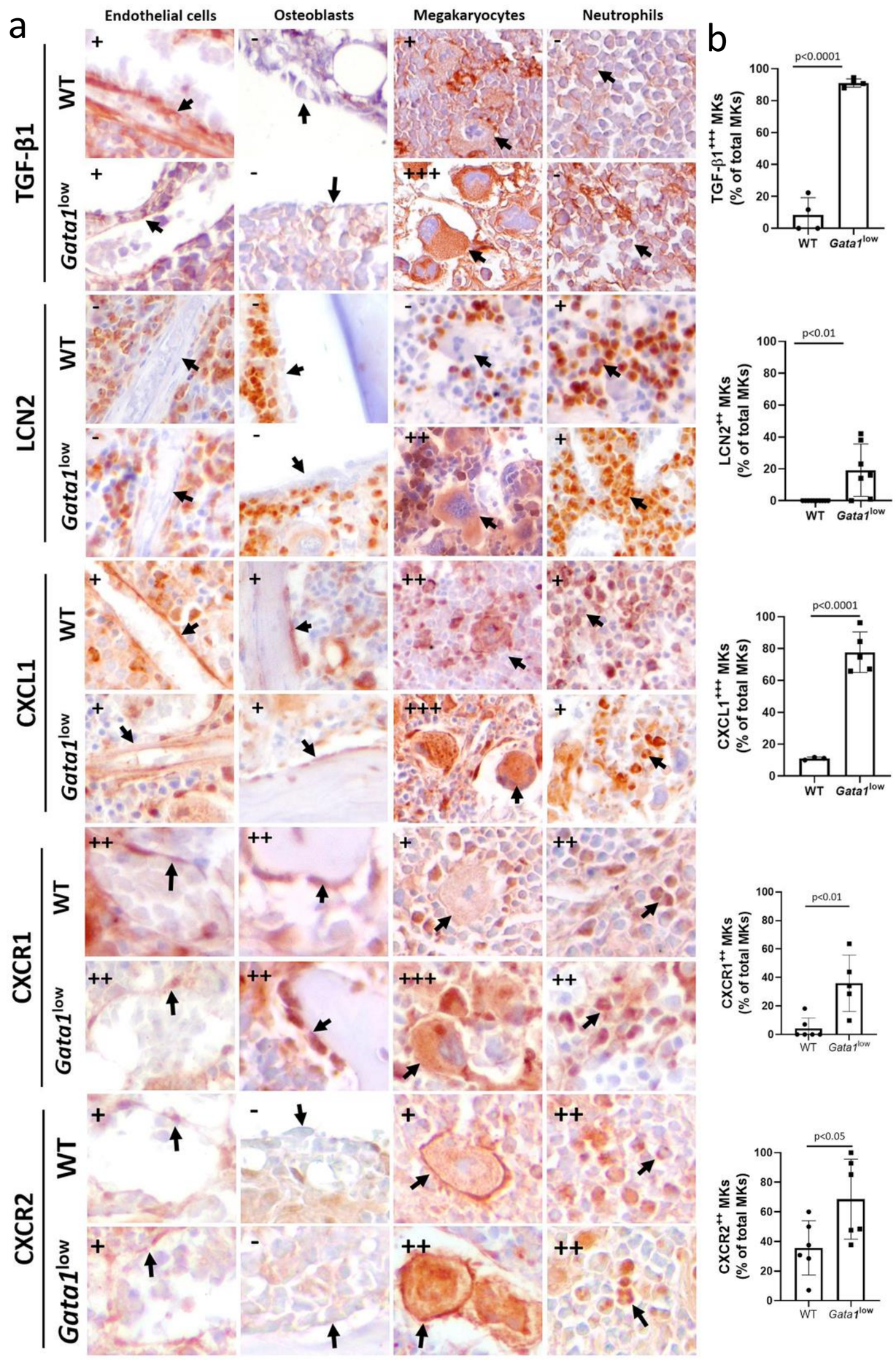

Figure 1. Megakaryocytes from the BM of *Gata1*^{low} mice contain more TGF-β1, LCN2, CXCL1, CXCR1 and CXCR2 than the corresponding cells from the wild-type littermates. **(a)** Representative sections from the BM of wild-type and *Gata1*^{low} littermates immuno-stained with antibodies per TGF-β1, LCN2, CXCL1 and its receptors CXCR1 and CXCR2 showing the level of these cytokines in endothelial cells, osteoblasts, megakaryocytes and neutrophils (all indicated by arrows) as indicated. Semi-quantitative estimates (-, +, ++ or +++) of the intensity of the staining in each population is indicated on the top left. Original magnification 400X. **(b)** Percentage of TGF-β1, LCN2, CXCL1, CXCR1 and CXCR2 expressing MKs of the BM of wild-type and *Gata1*^{low} mice. Each dot represents an individual mouse.

Ethics Statement. The animal study was reviewed and approved by the Institutional animal care committee according to the European Directive 86/609/EEC.
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The CXCR1/CXCR2 inhibitor Reparixin alters the development of myelofibrosis in the *Gata1*^{low} mice

Verachi P, Gobbo F, Martelli F, Martinelli A, Sarli G, Dunbar A, Levine RL, Hoffman R, Massucci MT, Brandolini L, Giorgio C, Allegretti M, Migliaccio AR

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Aim. To test the effect of CXCR1/R2 inhibitor Reparixin in *Gata1*^{low} mice on the myelofibrosis expressed by this model.

Treatment. *Gata1*^{low} mice (8-month old) were treated either with vehicle (3 males and 3 females) or with Reparixin (5 males and 5 females) for either 20 or 37 days. The drug was administered by minipumps implanted subcutaneously in the dorsal region set to deliver 7.5mg of drug/hr/kg of body weight. The mice receiving the drug for 37 days had the minipumps replaced by day 17.

Results. The efficiency of drug delivery decreased over time since the plasma levels of Reparixin were 13.90±4.18 and 6.71±4.18ug/mL at day 20 and 37, respectively. Great reductions in the fibrosis of the BM and spleen was observed in mice that had been treated with Reparixin for 20 days compared to vehicle and the levels of fibrosis were inversely correlated with the plasma levels of Reparixin (***Figure 2***). Reparixin restores the GATA1 content of the CD42b positive MKs in the BM. Of note, the number of MKs expressing GATA1 was positively correlated with the plasma levels of Reparixin observed in the same mice (***Figure 4***).

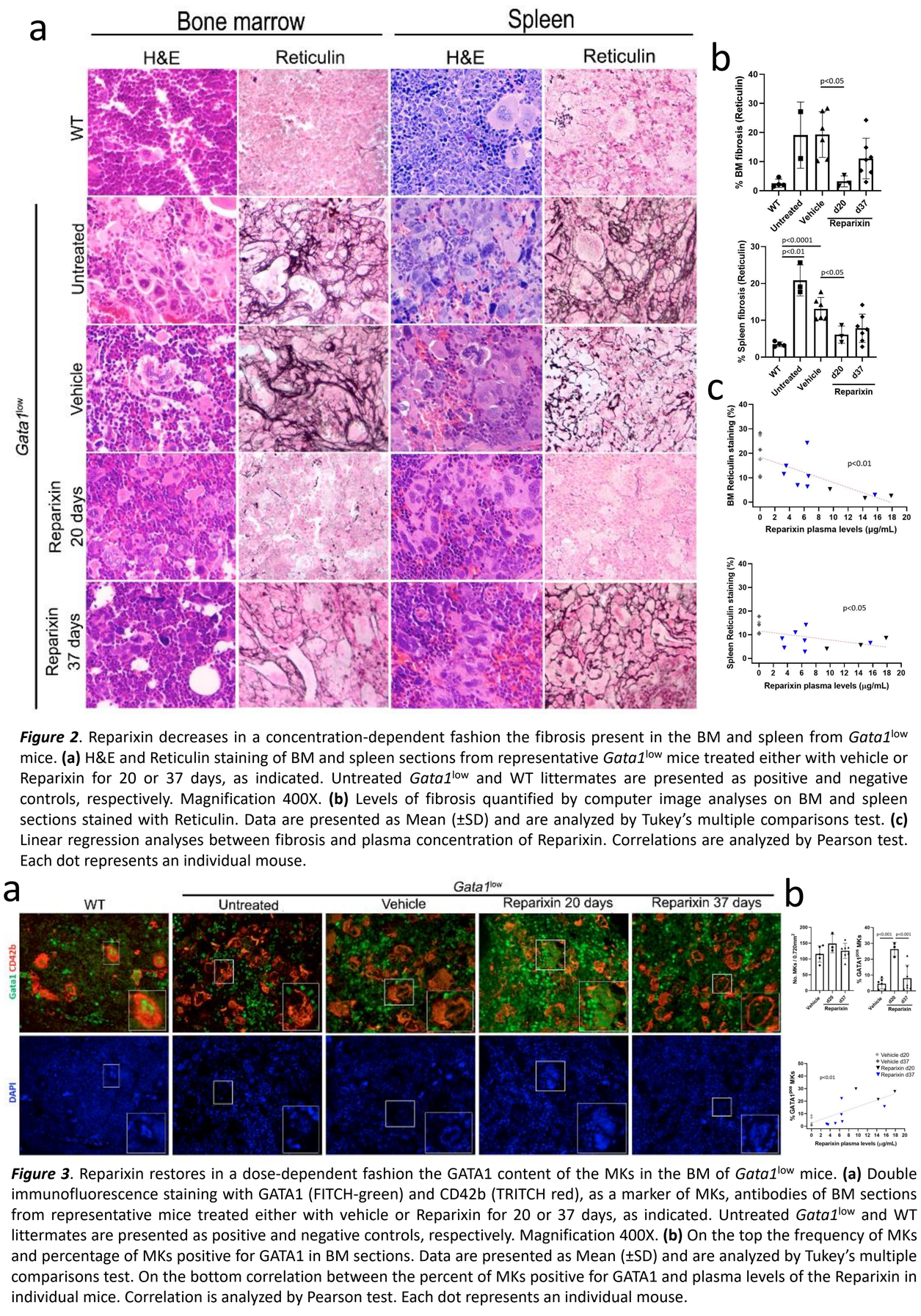

Figure 2. Reparixin decreases in a concentration-dependent fashion the fibrosis present in the BM and spleen from *Gata1*^{low} mice. **(a)** H&E and Reticulin staining of BM and spleen sections from representative *Gata1*^{low} mice treated either with vehicle or Reparixin for 20 or 37 days, as indicated. Untreated *Gata1*^{low} and WT littermates are presented as positive and negative controls, respectively. Magnification 400X. **(b)** Levels of fibrosis quantified by computer image analyses on BM and spleen sections stained with Reticulin. Data are presented as Mean (±SD) and are analyzed by Tukey's multiple comparisons test. **(c)** Linear regression analyses between fibrosis and plasma concentration of Reparixin. Correlations are analyzed by Pearson test. Each dot represents an individual mouse.

Figure 3. Reparixin restores in a dose-dependent fashion the GATA1 content of the MKs in the BM of *Gata1*^{low} mice. **(a)** Double immunofluorescence staining with GATA1 (FITCH-green) and CD42b (TRITCH red), as a marker of MKs, antibodies of BM sections from representative mice treated either with vehicle or Reparixin for 20 or 37 days, as indicated. Untreated *Gata1*^{low} and WT littermates are presented as positive and negative controls, respectively. Magnification 400X. **(b)** On the top the frequency of MKs and percentage of MKs positive for GATA1 in BM sections. Data are presented as Mean (±SD) and are analyzed by Tukey's multiple comparisons test. On the bottom correlation between the percent of MKs positive for GATA1 and plasma levels of the Reparixin in individual mice. Correlation is analyzed by Pearson test. Each dot represents an individual mouse.

Conclusions. In *Gata1*^{low} mice, Reparixin, an inhibitor of CXCR1/R2, reduces fibrosis in BM and spleens while increasing GATA1 in megakaryocytes in concentration-dependent fashion.