IFN-γ–Dependent Reduction of Erythrocyte Life Span Leads to Anemia during Mycobacterial Infection

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Background

Anemia of Chronic Disease (ACD) usually develops during mycobacterial infections, such as Tuberculosis (TB). Moreover, anemic TB patients are at a higher risk of death. However, the exact mechanisms triggering anemia during these infections remain unknown.

Erythrocytes are produced continuously in the bone marrow (BM) from Hematopoietic Stem Cells in a tightly regulated stepwise process. At later stages of development, erythroblasts enucleate and become reticulocytes. Reticulocytes egress from the BM to the circulation, where they mature into erythrocytes within a week. Mature erythrocytes remain in circulation for about 120 days in humans and 40 days in mice. Under homeostatic conditions, 1% of circulating erythrocytes are removed from circulation daily. The engulfment and degradation of damaged or aged RBC by macrophages is named erythrophagocytosis and occurs mostly in the spleen. However, in stress conditions, the liver becomes the major site of RBC degradation.

Our results have shown that *Mycobacterium avium* infected mice developed microcytic anemia with lower serum iron availability and reduced iron stores in BM. Iron redistribution did not block erythropoiesis but resulted in increased erythropoiesis in BM with resulting reticulocytosis and mobilization of erythroid progenitors to the spleen.

Here, we addressed how anemia established the role of IFN-γ during mycobacterial infection, comparing different mouse models of intravenous infection by *M. avium:* WT mice produce IFN-γ, which is sensed by the cells expressing its receptor; *Ifng*$^{-/-}$ mice are not capable of producing the cytokine; and MIIG mice produce IFN-γ but CD69-expressing cells (mainly macrophages) cannot signal through the IFN-γ receptor.

During mycobacterial infection, RBC have a shorter half-life

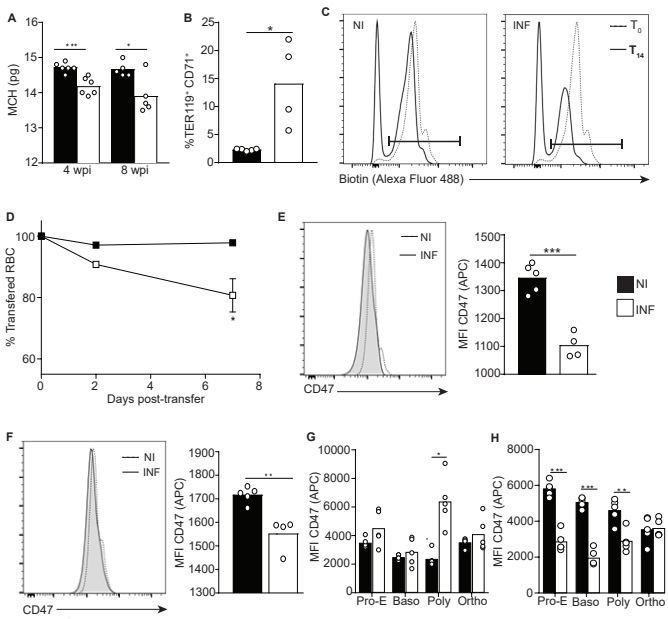


FIGURE 1. During mycobacterial infection, RBC have a shorter half-life. (A) Mean corpuscular hemoglobin (MCH) in peripheral blood. (B) Percentage of developing erythrocytes (defined as TER119+CD71+ cells) in peripheral blood. (C) Percentage of biotinylated TER119+ cells in the blood at 2 h (T0, dotted lines) and 14 d (T14, filled lines) after biotin i.v. injection in noninfected (left panel) and 8-wk *M. avium*–infected (right panel) mice. Black bars denote noninfected mice (NI). (D) Percentage of RBC transferred from infected and noninfected donors in the circulation of noninfected recipients at different time points after the adoptive transfer. (E–H) CD47 MFI in circulating mature erythrocytes (D), circulating developing erythrocytes (TER119+CD71+) (E), erythroid progenitors in BM (F), and erythroid progenitors in spleen (G). Black bars denote NI. White bars denote infected mice (INF). Bars represent the average of the experimental group, and dots represent each analyzed mouse. Each group contained at least three animals. *p , 0.05, **p , 0.01, ***p , 0.001 (unpaired, two-tailed Student t test).

Infection induces erythrophagocytosis in the liver

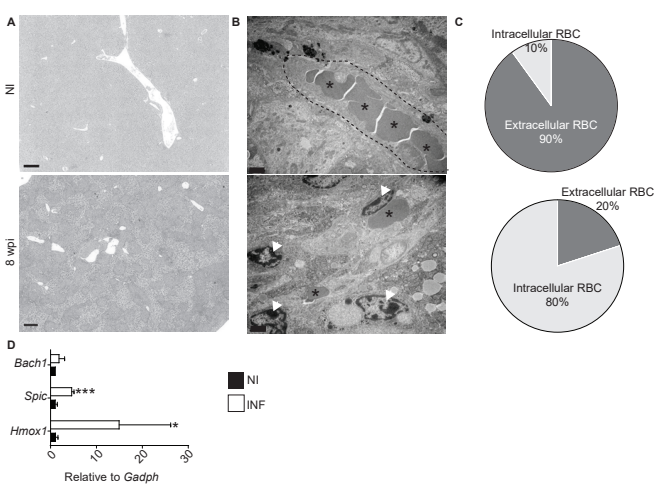


FIGURE 2. Infection by mycobacteria increases erythrophagocytosis. (A) Low-magnification micrographs of H&E-stained liver sections of noninfected (upper panel) and 8-wk-infected mice (lower panel). Scale bar, 500 mm. (B) Ultrathin liver sections showing erythrocytes (denoted by an asterisk [*]) predominantly inside vessels (labeled by the dash lines) in noninfected mice (upper panel) and predominantly inside cells in *M. avium*–infected mice at 8 wk of infection (lower panel), as shown by the arrowheads labeling nuclei. Scale bar, 2 mm (C) Percentage of intracellular versus extracellular erythrocytes in livers of noninfected (upper plot) and 8-wk-infected mice (lower plot). (D) Expression of *Bach1*, *Spic*, and *Hmox1* genes relative to *Gapdh* in the livers of noninfected mice (NI, black bars) and infected mice (INF, white bars) at 8 wk p.i. Bars indicate the average of each group, and error bars represent SD. Ten mice were analyzed per condition. Bars represent the average of the experimental group, and dots represent each analyzed mouse. *p , 0.05, ***p , 0.001 (unpaired, two-tailed Student t test).

IFN-γ-dependent reticulocytosis and erythrophagocytosis are key to infection-induced anemia

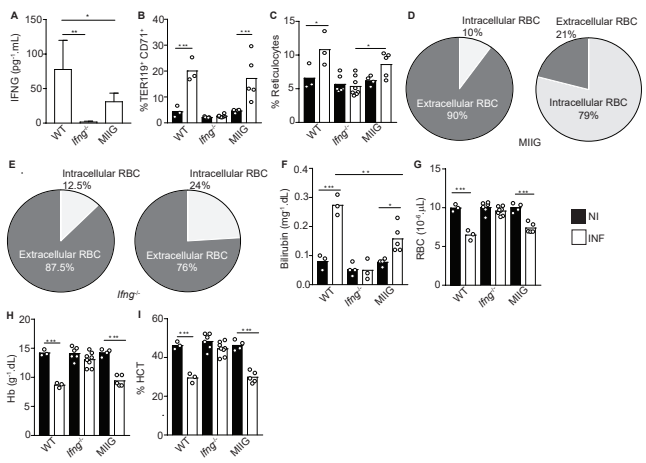
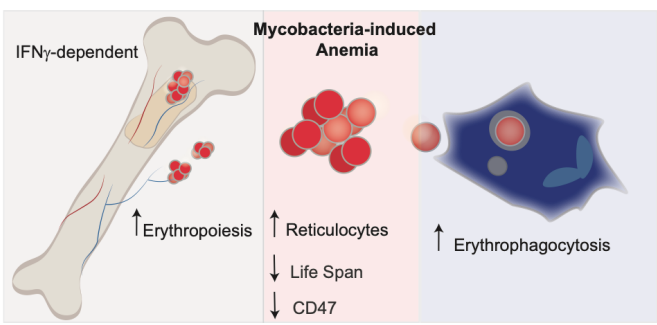


FIGURE 3. IFN-γ induces reticulocytosis, premature release of immature erythrocytes to circulation, and increased erythrophagocytosis, independently of its sensing by macrophages. (A) Amounts of serum IFN-γ in WT, *Ifng*$^{-/-}$, and MIIG mice at 8 wk p.i. (B) Percentage of TER119+ CD71+ cells in peripheral blood. (C) Percentage of circulating reticulocytes. (D) Percentage of intracellular versus extracellular erythrocytes in livers of noninfected (left plot) and in 8-wk-infected MIIG mice (right plot). (E) Percentage of intracellular versus extracellular erythrocytes in livers of noninfected (left plot) and in 8-wk-infected *Ifng*$^{-/-}$ mice (right plot). (F) Amount of total bilirubin in the serum. (G–I) Enumeration of circulating RBCs (G), hemoglobin (H), and hematocrit (I) in peripheral blood of *M. avium*–infected (INF, white bars) and noninfected (NI, black bars) WT, *Ifng*$^{-/-}$, and MIIG mice. Bars indicate average, and each dot corresponds to each analyzed sample. At least three mice were used in each experimental group. *p , 0.05, **p , 0.01, ***p , 0.001 (one-way ANOVA).

Conclusion



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