CD47 ANTIBODY THERAPY PROTECTS CIRCULATING RED BLOOD CELLS AND PLATELETS FROM IMMUNE DESTRUCTION

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Background Red blood cells circulate for ~120 days in humans and ~50 days in mice before they are phagocytosed by macrophages in the splenic red pulp at the end of their lifespans.1 2 The red blood cells are protected from non-specific premature destruction by macrophages by their expression of CD47, a ‘don’t eat me’ signal that binds to macrophage expressed SIRPa to inhibit phagocytosis.3 However, as monoclonal antibodies have been developed to block the CD47-SIRPa interaction for cancer immunotherapy, it has been necessary to overcome the toxicity associated with the off-target destruction of red blood cells.4 It was found that a small loading dose of CD47 antibody confers to red blood cells protection against much greater subsequent dosing.5 Here we identify the mechanism of this protection in mice and show that it can be leveraged therapeutically against autoimmune anemia and thrombocytopenia (figure 1a-b).

Methods Female C57/B6 and BALB/c mice were treated with a pre-clinical murine CD47 antibody [clone MIAP410] or an isotype control antibody. Flow cytometry was used to quantify CD47 expression by red blood cells and their progenitors from murine blood and bone marrow at various timepoints after CD47 antibody therapy and to quantify the expression of the four principal antibody-binding Fc-gamma receptors (FcgR) in myeloid and NK cell populations from the bone marrow, spleen, liver, and peritoneum of these mice. Fluorescently-labeled red blood cells were infused into mice to measure their survival in the circulation and phagocytosis by red pulp macrophages.

Results We find that a loading dose of CD47 antibody protects red blood cells by inhibiting antibody binding and phagocytosis by red pulp macrophages. We begin by demonstrating that CD47 antibody induces FcgR-mediated pruning of erythroid CD47, with global concomitant FcgR loss by reticuloendothelial myeloid populations. We then show that CD47 antibody therapy impairs red pulp macrophage phagocytosis of red blood cells. Finally, we show that CD47 antibody therapy protects red blood cells and platelets from antibody-mediated destruction and thus may have therapeutic potential for autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP).

Conclusions We demonstrate that CD47 antibody therapy prevents macrophage-mediated destruction of red blood cells and platelets in mice, both by reducing myeloid FcgR expression organism-wide and by directly impairing macrophage phagocytosis. We then show that this mechanism can be leveraged therapeutically to protect mice from antibody-mediated anemia and thrombocytopenia, and thus has therapeutic potential for these disorders.

REFERENCES