

## Chemical compounds that target thiol-disulfide groups on mononuclear phagocytes inhibit immune mediated phagocytosis of red blood cells

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**BACKGROUND:** Patients having immune cytopenias produce antibodies that target hematopoietic cells resulting in their phagocytosis and intracellular destruction. Early reports suggested that phagocytosis could be inhibited by interfering with membrane thiol (SH) groups on phagocytes. Thus, whether chemical compounds that interact with SH or disulfide (SS) groups on mononuclear phagocytes can inhibit phagocytosis of antibody-coated cells was examined.

**STUDY DESIGN AND METHODS:** A monocyte monolayer assay (MMA), which examines the in vitro monocyte-macrophage (M $\phi$ ) interaction with anti-Rh(D)-coated red cells (RBCs), was used to study the ability of different SH and SS chemicals to inhibit the Fc receptor-mediated phagocytosis of sensitized RBCs. The compounds examined included thimerosal, dithiothreitol (DTT), pentane-1-thiol, and two recently described SH and two SS chemicals that have been synthesized.

**RESULTS:** All compounds were found to be able to inhibit phagocytosis to varying degrees correlating to the structure of the molecule. In general, those compounds that interact with free SH groups to inhibit phagocytosis were found better than SH-containing compounds that interact with SSs. Thimerosal and p-nitrophenyl methyl disulfide were the most effective compounds inhibiting phagocytosis. Both chemicals showed greater than 50 percent inhibition at concentrations as low as  $10^{-9}$  mol per L. DTT was the least effective compound tested. Only thimerosal showed significant toxicity, as determined by decreased cell viability and increased apoptosis, but only at concentrations of  $10^{-8}$  mol per L. The effect of chemical treatment was on attachment rather than on phagocytosis itself. Fc $\gamma$  receptor-independent endocytosis was not affected by the chemical treatment.

**CONCLUSION:** These studies indicate that pharmacologic strategies that target SH groups on mononuclear phagocytes may have future efficacy for the treatment of immune cytopenias.

Immune cytopenias are pathologic conditions where patients make antibodies to specific hematopoietic cells in the blood.<sup>1-4</sup> In these conditions, the cells become coated with antibodies and are subsequently recognized by the Fc- $\gamma$  receptors (Fc $\gamma$ Rs) on the mononuclear phagocyte membrane.<sup>4</sup> Current therapies for the treatment of severe cases of immune cytopenias include splenectomy and administration of steroids or immunoglobulins.<sup>5-9</sup> Intravenous immunoglobulin (IVIG) and RhIG are both used with varied success to treat immune cytopenias.<sup>5,6,10</sup> Both IVIG and anti-D, however, are a limited resource<sup>11</sup> owing to their acquisition from human donations. Treatment with IVIG is more costly than with anti-D<sup>12</sup> primarily because of the amount of IVIG required for effective therapy; the usual induction dose is 2000 mg IVIG per kilogram of body weight, which may be followed

**ABBREVIATIONS:** F-B = p-toluenesulfonylmethyl mercaptan; Fc $\gamma$ R(s) = Fc- $\gamma$  receptor(s); G-B = p-nitrophenyl methyl disulfide; M $\phi$  = monocyte macrophage; MMA = monocyte monolayer assay; SH = thiol; SS(s) = disulfide(s).

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