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Anti-D (WinRho SD™) Treatment of Children With Chronic Autoimmune Thrombocytopenic Purpura Stimulates Transient Cytokine/Chemokine Production

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Intravenous anti-D is often used in the treatment of autoimmune thrombocytopenic purpura (AITP), but little is known about its mechanisms of action. To investigate anti-D's potential in vivo mechanism(s) of action, a small group ($N = 7$) of children with chronic AITP was studied. The children initially received either 25 or 50 μg/kg of WinRho-SD in a four-cycle cross-over trial, and peripheral blood samples from the first and third cycles were assessed for cytokine levels at pre-treatment, 3 hr, 1 day, and 8 days post-treatment. Results showed that platelet counts significantly increased in all the children by day 8 post-treatment. Analysis of serum by ELISA showed that there was a significant but transient rise in both pro- and anti-inflammatory cytokine/chemokine levels (e.g., IL1RA, IL6, GM-CSF, MCP-1α, TNF-α and MCP-1) by 3 hr post-treatment in both cycles which returned to baseline levels by 8 days post-treatment. These results suggest that anti-D administration may initially activate the RES in the form of cytokine/chemokine secretion, which is subsequently followed by an increase in platelet counts. It is possible that the induced cytokine/chemokine storm may have an effect on several physiological processes such as those mediating either adverse effects or potentially RES phagocytic activity. Am. J. Hematol. 69:225–227, 2002.

Key words: autoimmune thrombocytopenia; anti-D; cytokines

INTRODUCTION

Autoimmune thrombocytopenic purpura (AITP) is an immune-mediated disorder in which platelets are opsonized by auto-antibodies and prematurely destroyed by the reticuloendothelial system [1]. Although intravenous immunoglobulin (IVIg) therapy has been shown to be effective in treating this disease [2], the mechanism of action of IVIg is not yet fully elucidated. The three major theories to explain IVIg's mechanism of action are reticuloendothelial Fc receptor (R) blockade, anti-idiotypic regulation, and alteration of cytokine networks [1]. Intravenous anti-D (WinRhoSD) has also been shown to be effective in raising the platelet counts of patients with AITP [3], but little is known about the mechanisms of action of anti-D, although the above theories may apply; anti-D opsonizes the patient's D^+ red blood cells (RBC) and blocks phagocytosis of platelets within the spleen [4].

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Recently, a new theory of how IVIg and anti-D may function in vivo has been termed Fc-mediated inhibition [5]. It was shown in a murine model of ITP that IVIg changes the composition of Fcγ receptors on the surface of monocytes; it downregulates the phagocytic-stimulatory FcγRIIIa and upregulates the inhibitory FcγRIIb [5]. Alternatively, it has been suggested that anti-D may cause immunosuppression by either interacting with receptors other than FcγR or stimulating the production of immunosuppressive cytokines [6]. To examine the latter possibility, we designed a prospective anti-D dose cross-over study in 7 children with chronic AITP to compare the clinical findings with in vivo serum cytokine/chemokine levels.

MATERIALS AND METHODS

Seven children with primary chronic AITP were studied. Chronic AITP was defined as thrombocytopenia (platelet count $<150 \times 10^9$/L) persisting for greater than 6 months, normal or increased marrow megakaryocytes, and no secondary immune or non-immune abnormality that could account for the thrombocytopenic state. AITP patients were enrolled if their platelet count was $<30 \times 10^{-9}$/L, they were blood group Rh_0(D) positive, and had not received any therapy for their AITP for at least 1 month prior to enrolment. Patients were randomized to one of two 4-dose (cycle) anti-D cross-over regimens; either 25, 25, 50, and 50 μg/kg or 50, 50, and 25, 25 μg/kg. Anti-D was administered by bolus infusion when the platelet count fell below 30×10^{-9}/L. Blood was drawn, and serum was prepared and frozen at pre-treatment, 3 hr, and 8 days post anti-D treatment. Serum cytokines were measured with ultra-sensitive commercial solid-phase ELISA kits.

RESULTS

A good platelet response, defined as a platelet count $>50 \times 10^9$/L and at least double the pre-treatment platelet count, occurred at day +8 in 100% of evaluable cases who received 50 μg/kg and 73% of cases treated with 25 μg/kg. Anti-D administration significantly increased the levels of IL1 receptor antagonist (RA), IL6, granulocyte/macrophage (GM)-colony stimulating factor (CSF), monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)-α, monocyte inhibitory peptide (MIP)-1-α, IL8, and IL10 at 3 hr following anti-D treatment (Fig. 1). By day 8 post-treatment, the majority of the cytokines were within normal or pre-treatment ranges. The most affected cytokine was IL1RA, which increased from normal pre-treatment ranges to approximately 4,000 pg/mL by 3 hr post-treatment ($P < 0.001$, Fig. 1D). Taken together, these results suggest that anti-D administration induces a significant, early (3 hr post-treatment) modulation in inflammatory and anti-inflammatory cytokines.

DISCUSSION

The mechanism of how anti-D stimulates the production of the cytokines is unknown; however, several of the cytokines are interrelated and may be associated with anti-D's mechanism of action. For example, studies have shown that adult patients with chronic AITP have altered levels of GM-CSF [7], and our results suggest that anti-D's modulation of GM-CSF levels may be an important event. Furthermore, two of the major acute phase response-associated cytokines (IL6 and TNF-α) and pro-inflammatory chemokines (MIP-1-α and MCP-1) were significantly elevated concomitantly with the anti-inflammatory cytokine IL1RA. IL1RA synthesis is enhanced and regulated particularly by IL6 and GM-CSF whereas GM-CSF is a potent stimulator of TNF-α production by monocytes/macrophages [8]. In a larger group of adult patients with chronic AITP ($N = 28$), Bussel et al. [9] demonstrated that transient increases in macrophage-derived MCP-1, IL10, and IL6 levels occurred at 2 hr post anti-D treatment which lasted to 1 day post-administration. The authors suggested that the elevated cytokines reflected a substantial interaction of anti-D coated RBC with macrophages [9]. The transient presence of cytokines may suggest that DAT^+ RBC within the spleen initially stimulates a potential "danger" signal, as originally proposed by Matzinger et al. [10], which might affect a number of physiological processes. For example, IL6 and TNF-α may be responsible for some of the clinical side effects seen with anti-D administration such as fever, chills, and nausea. Alternatively, IL1RA can significantly inhibit macrophage activation via downregulation of IL1 that may either immunomodulate or downregulate phagocytosis [8].

CONCLUSIONS

In summary, it appears that anti-D treatment causes an early and transient activation of the RES, leading to an enhanced cytokine cascade response which is followed by increased platelet counts by day 8 post-treatment. Our data suggest that anti-D may initially stimulate a cytokine/chemokine "storm", which could have an effect on RES function.

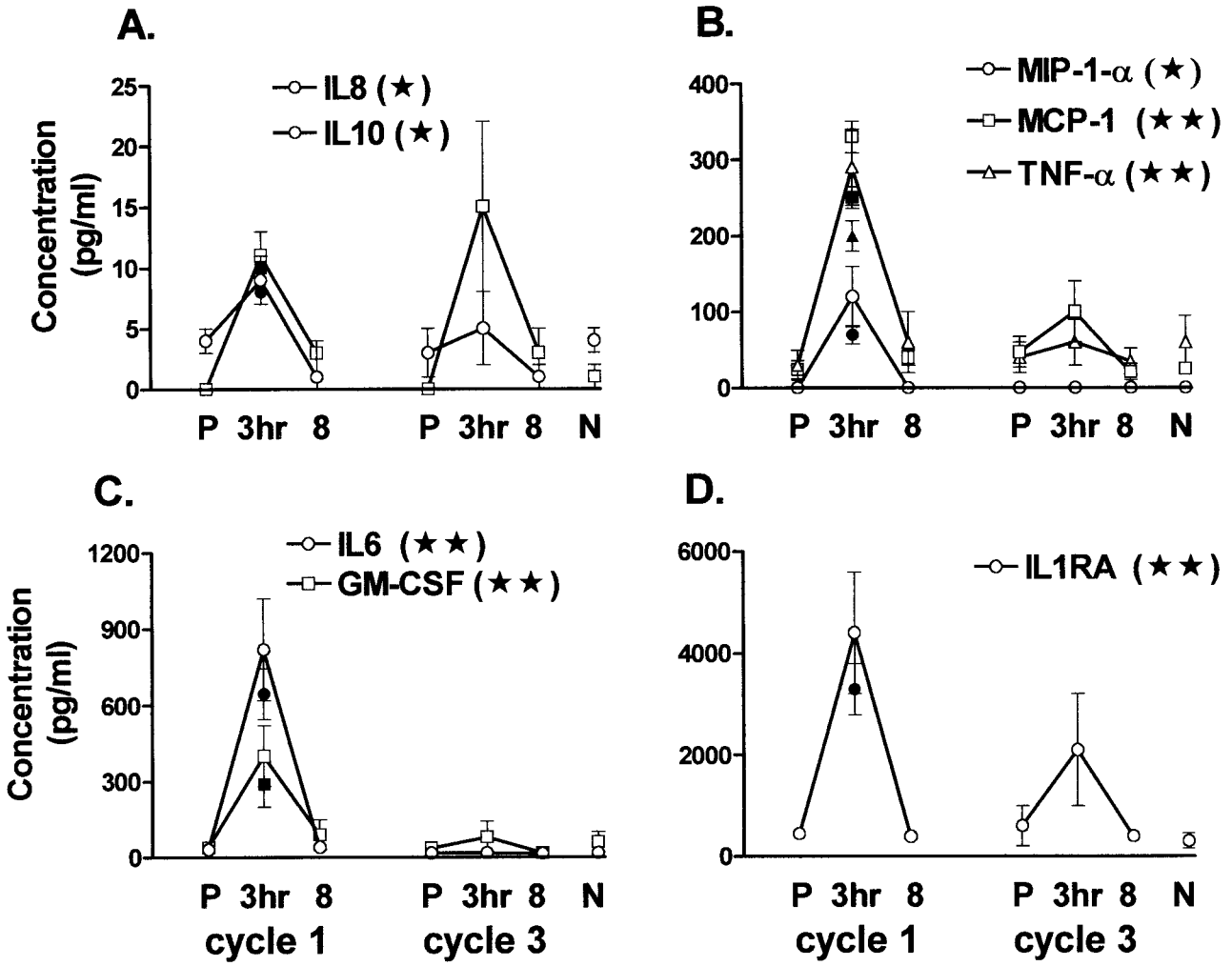

Fig. 1. Serum cytokine levels in patients at pre-treatment (P), 3 hr post-treatment (3h), and 8 days (8) post-treatment from cycle 1 ($N = 7$) and cycle 3 ($N = 7$). Normal serum cytokine values (N) from healthy children ($N = 8$) are shown. For dosage comparison, in the first cycle of each panel, the open symbols represent those patients who received 50 μg/kg ($N = 5$) and the closed symbols are those that received 25 μg/kg ($N = 2$). The data are expressed as the mean (±SD) concentration (pg/mL) of the indicated cytokines. Panels A–D were generated based on the concentration ranges of the various cytokines as indicated on the y axes. One star (★) or two stars (★★) represent significance at $P < 0.05$ or $P < 0.001$ (Student's t-test), respectively, compared to baseline, which did not differ from the normal ranges.

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