IMPACT OF INTRAVENOUS INFUSIONS OF LOW AND HIGH DOSES OF GAMMA GLOBULINS (IVIG) ON PHAGOCYTIC FUNCTIONS IN ADULTS WITH PRIMARY HUMORAL IMMUNODEFICIENCY

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Abstract—Twelve adult patients with primary humoral immunodeficiency were treated for at least six months with IVIG 200 mg/kg/mo and then crossed over to a high dose of 600 mg/kg/mo. Polymorphonuclear and mononuclear cells of these patients were tested after the third infusion in the low-dose cycle and then after the third infusion in the high-dose cycle, each time a day before, four days after, and 14 days after intravenous infusion. Each time, patients' cells and normal cells were tested using normal sera and patients' sera. IVIG infusions led to a significant increase in the level of circulating IgG, which was much more prominent in the high-dose group. Phagocytosis, phagocytic index, intracellular bactericidal activity and chemotaxis of polymorphonuclear cells (PMNs) were at least as active as in healthy controls. Actually in both cycles patients' PMN's had slightly higher phagocytic activity than normal cells. Patients' serum in the high dose cycle supported chemotaxis better than normal serum. Efficient phagocytic activity was maintained throughout the cycle; however, it was more active ($P < 0.0125$) in the midcycle in the high-dose cycle. Superoxide generation was normal in all conditions. Monocytic function was also normal in all conditions tested. It may be concluded that as far as cellular phagocytic functions are concerned, the high dose of IVIG does not protect the host more efficiently than the low dose.

INTRODUCTION

Intravenous infusion of gamma globulins (IVIG), is an established therapy of adult primary humoral immunodeficiencies. Although IVIG has definite therapeutic value (1-3), the optimal dose of IVIG has not been established (1). It has been suggested, however, that high doses of IVIG (400-500 mg/kg/mo) are more beneficial than low doses (100-150 mg/kg/mo) (4, 5). We investigated
prospectively 12 adults with primary humoral immunodeficiency, treated with IVIG. These patients consented to participate in an extensive study of phagocytic activity in order to detect whether low (200 mg/kg/mo) and high (600 mg/kg/mo) doses of IVIG have different impact on various phagocytic activities of polymorphonuclear and mononuclear cells. It has been reported that in vitro IVIG has complex impact on phagocytosis and bactericidal activity of normal PMN's and monocytes (6). However, to our knowledge, extensive studies of patients' cells and sera using various dosages of IVIG have not been reported.

MATERIALS AND METHODS

A group of 12 adult patients, receiving monthly intravenous infusions of gamma globulin (Iveegam, Immuno AG, Vienna, Austria) had the following assays done a day before the infusion of gamma globulin, four days after infusion, and 14 days after infusion: phagocytosis, phagocytic index, and intracellular bactericidal activity (ICBA) of polymorphonuclears (PMNs) and monocytes, as well as chemotaxis, and superoxide generation of PMNs.

In each patient, the above study was repeated twice, once on a low dose (200 mg/kg/mo) of IVIG and once on a high dose (600 mg/kg/mo), each time performing the assays after the third infusion of a given dosage. None of the patients had acute infection at the time of study. Each time sex- and age-matched healthy individuals were used as controls. All samples were tested blind without the technician knowing whether patient's or control's blood was tested. All the assays were done in the following four combinations: normal cells with normal serum (NC/NS); normal cells with patient's serum (NC/PS); patient's cells with normal serum (PC/NS); and patient's cells with patient's serum (PC/PS). Serum IgG concentration was quantitated by radial immunodiffusion using Behring NOR Partigen IgG plates.

Purification of Polymorphonuclear Cells. PMNs from patients and control individuals were separated by the previously described method (6). Briefly, venous blood (20-30 ml) was drawn into heparinized tubes, diluted 1:1 with phosphate-buffered saline (PBS), and then 5% dextran (10% by volume) was added to sediment the red blood cells. After 1 hr, 30 ml of the leukocyte-enriched plasma was layered onto 15 ml of Ficoll-Hypaque, 600 ml of 9% Ficoll (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden), and 170 ml 50% Hypaque (Winthrop Laboratories, Div. of Sterling Drugs, Inc., New York, New York).

The mixture was centrifuged at 1200g for 40 min at 4°C. The pellet containing the PMNs was collected and RBCs were lysed with 0.84% ammonium chloride. The PMNs were washed twice in PBS, resuspended in medium appropriate for each test and counted. The purity of PMN suspensions was 92–95% and the viability was 98–100%.

Purification of Peripheral Blood Monocytes. Monocytes were separated from venous blood as described (6). Venous blood (40-60 ml) was collected in heparinized tubes and diluted 1:2 with PBS, 30 ml were layered onto 15 ml Ficoll-Hyphaque (as above). The mixture was centrifuged at 1200g for 40 min at room temperature. The mononuclear cell layers were removed, washed twice in PBS, and resuspended in a 1:1 mixture of autologous serum and minimum essential medium (MEM) (Gibco, Grand Island, New York). Aliquots (0.6 ml) of the cell suspension were placed in the walls of Mackaness chambers. The sealed chambers were placed in moist, covered trays and incubated at 37°C, 5% CO₂–95% air for 30 min to allow adherence of the cells to the cover slips. After incubation, the nonadherent cells were gently washed off with three rinses of Hanks' balanced salt solution (HBSS, Gibco) at 37°C. Adherence was checked using an inverted microscope (magnification × 100). Adherent cells were used for the tests.