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Intravenous immunoglobulin replacement induces an in vivo reduction of inflammatory monocytes and retains the monocyte ability to respond to bacterial stimulation in patients with common variable immunodeficiencies

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ABSTRACT

Intravenous IgG administration induces significant modifications in the innate and adaptive compartment of the immune system including the monocyte/macrophage system. We analyzed the in vivo effect of IgG administered at replacement dosages on the frequency of monocytes subsets, on the modulation of CD11b and sialic acid-binding immunoglobulin-like lectin receptor (Siglec 9) expression and on monocytes production of reactive oxygen species. We showed that patients with Common Variable Immune Deficiency have an increased frequency pro-inflammatory intermediate $CD14^{++}CD16^{+}$ monocytes and an increased expression of CD11b and Siglec 9 on monocytes. IgG administered at replacement dosages exerted an in vivo anti-inflammatory effect as shown by a reduction of circulating monocytes, of intermediate pro-inflammatory monocytes, of CD11b and Siglec 9 expression and of ex vivo monocytes oxidative burst. Nevertheless, intravenous IgG administration did not affect the monocyte functional ability to respond to a bacterial stimulation in terms of CD11b and Siglec 9 expression and reactive oxygen species production.

1. Introduction

Intravenous immunoglobulins (IVIgs) used in treatment of a broad spectrum of diseases are prepared from the collective plasma of several thousand donors. Therapeutic immunoglobulin consists mostly of human polyvalent IgG [1–4]. IgG replacement, administered at a dosage of 400–600 mg/kg every 3 or 4 weeks, is the standard therapy for primary antibody deficiencies (PAD) aiming to replace the missing antibodies and thereby to prevent recurrent infections [5,6]. Intravenous IgG is widely used also as anti-inflammatory therapy in a variety of acute and chronic autoimmune diseases. In vitro studies showed that IVIg has diverse effects on the immune system [7–9]. Poor information is available on the in vivo effects of IVIg administered at replacement dosages on human immune cells, including the monocyte/macrophage system [10]. A recent study in patients with Common Variable Immune Deficiency (CVID) showed that IVIg infusion decreased the number of non-classical pro-inflammatory monocytes in vivo and suppressed the production of pro-inflammatory cytokines such as TNF-α in response to lipopolysaccharide (LPS) in vitro [11].

Monocytes play a crucial role in the first line of host defense against invading microorganisms. In response to pathogens, after migration into inflamed tissues, monocytes undergoes to a rapid production of reactive oxygen species (ROS) also known as oxidative burst. This process is crucial for bacterial killing but can also cause tissue injury if excessive or inappropriate [12]. Few studies addressed the effects of IVIg on monocyte function, including monocytes respiratory burst and on functional monocytes receptors, such as the phagocytic receptor αMβ2 (CD11b/CD18) [13] and sialic acid-binding immunoglobulin-like lectin receptor (Siglec 9), a member of transmembrane sialic acid-binding proteins CD33-related [14,15] with a postulated inhibitory activity on the immune response through host and bacterial sialoglycans recognition [16,17]. A new subset of monocyte, named intermediate monocytes, co-expressing CD14 and CD16 was recently identified [18]. The selective gene expression suggested that the intermediate monocytes may play distinct functional roles in immune response and inflammation [19].

We analyzed the in vivo effect of IVIg administered in CVID at replacement doses on the frequency of classical, non-classical and intermediate monocytes subsets, on the modulation of CD11b and Siglec

Abbreviations: CVID, Common Variable Immune Deficiency; IVIg, intravenous immunoglobulin; HD, healthy Donors; MFI, mean Fluorescence Intensity; PAD, primary antibody deficiencies; LPS, lipopolysaccharide; Siglec 9, sialic acid-binding immunoglobulin-like lectin receptor; ROS, reactive oxygen species; LIP, lymphocytic interstitial pneumonitis.

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9 expression, on the monocyte functional ability to respond to a bacterial stimulation and on monocyte-mediated intracellular ROS production.

2. Material and methods

2.1. Patients

We studied 19 CVID patients (8 males and 11 females; age range of 15–74 years; mean age: 50.3 ± 16.8 years) diagnosed according to the criteria established by the European Society for Immunodeficiencies (http://www.esid.org). CVID patients were on replacement treatment, with a cumulative monthly dosage of 300–400 mg/kg of IVIg administered every two-three weeks (Supplementary Table 1). Nineteen healthy donors (HD) (6 males and 13 females; age range 26–65 years; mean age 41.6 ± 13.4 years) were included as controls. The infusion time ranged from 1.5 h to 3 h. The infusion speed was established according to the individual tolerability. The mean IVIg dose administered at the time of blood sampling was 28.7 ± 3.9 g. None of the patients was on steroids or immunosuppressive drugs at the time of the study. All subjects provided informed consent for blood sampling and processing, in accordance with the Institutional Review Board of the Sapienza University of Rome and with the Declaration of Helsinki.

2.2. Phenotypic analysis of monocyte subpopulations

Blood samples were collected immediately before and within 1 h after IVIg administration. Total peripheral blood monocyte count was determined from blood cell counts and white blood cell differentials. Peripheral blood monocytes were directly stained in 30 μl of whole blood after lysing erythrocytes with a fixed volume of Becton Dickinson lysing buffer. Monocyte subpopulations were phenotypically identified by a 4-color flow cytometry single platform assay using anti-CD45 APC, HLA-DR FITC, CD14 PE, and CD16 PerCp mAbs (BD, Becton-Dickinson Biosciences, Franklin Lakes, NJ). A PerCp-conjugated isotype control (IgG_1, BD) was run in parallel. Monocyte subpopulations selected by gating on $CD14^+$ $HLA\text{-}DR^+$ monocytes were analyzed in parallel for the expression of surface receptors CD11b (using HLA-DR FITC, CD14 PE, CD16 PerCp, CD11b APC) and Siglec 9 (using Siglec 9 FITC, CD14 PE, CD16 PerCp, HLA-DR APC). The corresponding APC-conjugated and FITC-conjugated isotype controls (IgG_1, BD) was run in parallel. Results were expressed as percentage of cells that stained positive for a given marker, or as GeoMean Fluorescence Intensity (MFI) within the defined population.

2.3. Stimulation with Escherichia coli

100 μl of whole blood, collected immediately before and within 1 h after IgG administration, was added to 20 μl of pre-cooled opsonized non-labeled whole *E. coli* bacteria at a concentration of $1–2 \times 10^9$/ml (Glycotope, Biotechnology). Samples were incubated in water bath for 20 min at 37 °C and lysed with a fixed volume of Becton Dickinson lysing buffer. Cells were stained with fluorochrome-conjugated antibodies to CD14, CD16, Siglec 9, and CD11b in various combinations. Flow cytometry analysis was done by a FACSCalibur instrument (Becton Dickinson) using Cell Quest (Becton Dickinson) and FlowJo (TreeStar, Ashland, Ore) software.

2.4. Monocytes oxidative burst activity

The quantitative evaluation of leukocyte oxidative burst was determined with a PHAGOBURST kit (Glycotope, Biotechnology), according to the manufacturers' instructions. Briefly, 100 μl of precooled heparinized human whole blood was added to 20 μl of Wash Solution, as a negative control or 20 μl of pre-cooled opsonized (non-labeled) *E. coli* bacteria, incubated for 20 min at 37 °C in a water bath. After incubation, 20 μl of dihydrorhodamine 123 (DHR 123), as substrate solution, was added to tubes. After 20 min incubation at 37 °C in a water bath, samples were lysed, fixed with pre-warmed lysing solution and incubated for additional 20 min at room temperature. After washing, 200 μl of DNA staining solution (propidium iodide) was added. Samples were incubated for 10 min on ice in the dark and within 30 min, analyzed by flow cytometry using the blue-green excitation light. The percentage as well as MFI of ROS producing cells was analyzed. During data acquisition, a "live" gate was set in the red fluorescence (propidium iodide) histogram on those events that had at least the same DNA content as a

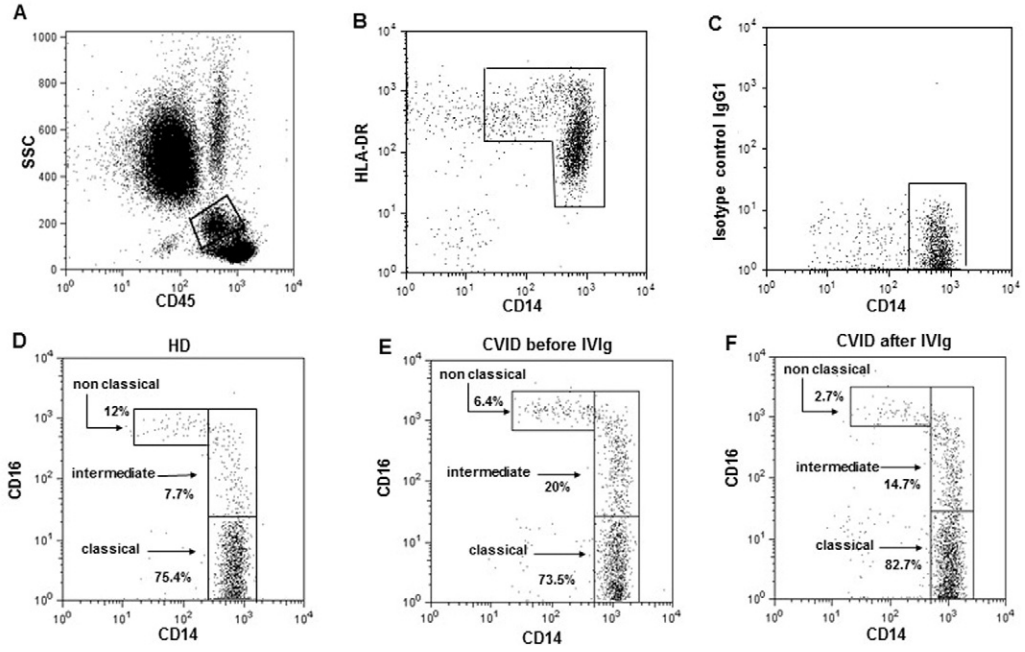

Fig. 1. Identification of monocyte subpopulations. Plot A: CD45 + monocytes and adjacent lymphocytes, including NK cells. Cells were gated to exclude CD14-/HLA-DR (plot B, cells outside the gate). To determine the boundary between intermediate and classical monocyte subsets an isotype control was used (plot C). Monocyte subsets were analyzed for CD14 and CD16 expression in a representative healthy donor (plot D) and in a CVID patient before (plot E) and after IVIg infusion (plot F). Percentages denote mean values.