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Inhibitory effect of pooled human immunoglobulin on cytokine production in peripheral blood mononuclear cells

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Human intravenous immunoglobulins (IVIG) are widely used as immunomodulators because of their ability to modify the course of various immune-mediated diseases. We investigated the mechanisms responsible for the regulatory effects of IVIG on *in vitro* human peripheral blood mononuclear cell (PBMC) cytokine production. Pre-incubation of PBMCs with IVIG inhibited lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulated cytokine secretion. Pre-incubation of PBMCs with IVIG induced a significant inhibition of LPS-stimulated (IL-6) secretion ($p = 0.045$); the effect on tumor necrosis factor-α (TNF-α) secretion was not significant ($p = 0.234$). Pre-incubation of PBMCs with IVIG inhibited IL-6 secretion ($p = 0.033$) stimulated with anti-CD14 antibody cross-linking but had no significant effect on TNF-α secretion ($p = 0.125$). PBMC pre-incubation with anti-CD14-blocking antibody induced a significant reduction ($p = 0.042$) in LPS-stimulated TNF-α secretion in comparison with a non-significant reduction ($p = 0.256$) noted with IVIG pre-treatment. In contrast, pre-incubation of PBMCs with anti-CD14 antibody did not induce a significant reduction in LPS-stimulated IL-6 secretion ($p = 0.166$) in comparison with a significant reduction ($p = 0.001$) induced with IVIG pre-treatment. Our data suggest that the immunoregulatory properties of IVIG may rely on several mechanisms, some of which may be independent of CD14. Our data also showed that cross-linking cell membrane-bound IVIG with anti-human kappa- and lambda-chain antibodies resulted in cytokine secretion levels similar to those elicited by LPS. In addition, intracellular DNA staining results did not support the involvement of apoptosis in the regulatory mechanisms of IVIG. This data may further our understanding of the immunoregulatory effects exerted by IVIG on the production of inflammatory-response mediators.

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Intravenous immunoglobulins (IVIG) are preparations of normal polyspecific immunoglobulins G (IgG), obtained from as many as 20,000 donors, and with demonstrated *in vivo* immunoregulatory properties (1). IVIG preparations have shown efficacy in the management of primary and secondary humoral immune deficiencies, and have been investigated as immunomodulators in the management of neonatal sepsis and various autoimmune dysfunctions (2). Recently, IVIG preparations have demonstrated efficacy in a wider range of clinical conditions including atherosclerosis and malignancy (3). However, the effectiveness of IVIG in the treatment and prevention of neonatal sepsis and other autoimmune conditions in children remains to be established.

Although several theories have been postulated, the mechanisms through which IVIG

preparations exert their immunoregulatory properties in the therapy of these clinical conditions are poorly understood (4). Proposed mechanisms include functional Fc receptor blockade (2, 5), inhibition of complement-mediated damage (2, 5), modulation of cytokine production (5, 6), idiotype–anti-idiotype interactions (5), and induction of apoptosis (7).

Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), secreted by monocytes in response to inflammatory stimuli, most notably bacterial endotoxins such as lipopolysaccharide (LPS) (8, 9), are implicated in mediating the tissue damage and other clinical symptoms, such as sepsis, associated with bacterial infections (10). Furthermore, elevated pro-inflammatory cytokine levels have been implicated in the pathogenesis of various autoimmune dysfunctions (10). In some circumstances, IVIG preparations have been shown to inhibit levels of secreted pro-inflammatory cytokines (11, 12). However, the mechanisms through which IVIG preparations block the production of these pro-inflammatory cytokines remain unclear.

The objective of this study was to examine the potential mechanisms through which IVIG preparations modulate LPS-stimulated pro-inflammatory cytokine secretion, with special emphasis placed on the role of CD14.

Materials and methods

Isolation of PBMCs

Blood samples from healthy adult volunteers were collected in heparin and peripheral blood mononuclear cells (PBMCs) isolated with Ficoll-Hypaque centrifugation (Sigma, Chemical Co., St Louis, MO, USA). Prepared PBMCs were washed twice with phosphate-buffered saline (PBS) and resuspended in RPMI 1640 (GIBCO, Grand Island, NY, USA) containing 2 mM L-glutamine, 10^4 U/l penicillin G, 10^4 μg/l streptomycin, and 10% heat-inactivated human-type AB serum. Cells were cultured at 2×10^6 cells/ml (37°C, 5% CO_2). All work carried out in this study was approved by the ethics committee of Wan-Fang Hospital. Informed consent was obtained from all volunteers prior to study participation.

Isolation of B cells

Ten-week-old Balb/c mice were purchased from the Animal Facility at the College of Medicine, National Taiwan University. All animal experiments were approved by the ethics committee of Wan-Fang Hospital. Animals were sacrificed by cervical dislocation. The spleen was removed, crushed into a single cell suspension, and red blood cells lyzed with Tris-buffered ammonium chloride before washing (three times) in Hank's Balanced Salts Solution (HBSS; Atlanta Biologics, Norcross, GA, USA). B cells were isolated using magnetic cell sorting (MACS) with Thy1.2 microbeads for T-cell depletion.

Reagents

Freeze-dried IVIG (Pasteur Merieux, Paris) was diluted with culture medium and added to PBMCs (6 mg/ml). Anti-CD14 (Mouse IgG_{2a}, κ) (PharMingen, San Diego, CA, USA), anti-mouse IgG_{2a}, anti-human kappa- and lambda-chain antibody (PharMingen) were all used at final concentrations of 10 μg/ml.

Determination of IVIG concentrations

Human intravenous immunoglobulins infusions of 500 mg/kg body weight are reported to result in an average IgG-serum increment of 5 mg/ml (13). Other studies have reported the increase in IgG serum level elicited by a single dose of IVIG (500 mg/kg) to be 10 mg/ml (14, 15). A dose of 6 mg/ml was used in the culture medium of our experiments after the titration of various doses (12, 6, 1, and 0.5 mg/ml) of IVIG. A concentration of 10 μg/ml was used in cross-linking experiments (16).

Time course experiments for TNF-α and IL-6 production

Time course experiments were carried out to determine the optimal time for TNF-α and IL-6 cytokine production by LPS-stimulated PBMCs. PBMCs were stimulated with a pre-determined concentration (1 μg/ml) of LPS (*Escherichia coli* serotype 0128:b12, Sigma Chemical Co.). Supernatants were collected from stimulated cells at designated time-points (1–12 h) and analyzed by ELISA. Supernatants from PBMCs cultured in tissue culture media alone were used as negative controls. This experiment was repeated three times with PBMCs isolated from three different volunteers.

Effect of IVIG on LPS-stimulated PBMC cytokine production

To investigate the effect of IVIG on LPS-stimulated PBMC cytokine production, IVIG (6 mg/ml) was pre-cultured with LPS (1 μg/ml) for 1 h

(37°C, 5% CO_2) before addition to PBMC in suspension (2×10^6 cells/ml). PBMCs were then incubated for 6 h (37°C, 5% CO_2). Cell-free supernatants were harvested and stored at −20°C until the analysis by ELISA for cytokine levels. In subsequent experiments, PBMCs were pre-incubated with IVIG (6 mg/ml) for 2 h. PBMCs were then washed in HBSS before stimulation with LPS.

Effect of IVIG on antibody cross-linking stimulated cytokine production

In order to study the effect of cell-bound cross-linking antibody on cytokine secretion, anti-CD14 or IVIG was added to PBMCs (2×10^6/ml) at a final concentration of 10 μg/ml and incubated for 2 h. Anti-mouse IgG_{2a} or anti-human kappa- and lambda-chain antibodies were then added (10 μg/ml) and cultures incubated for a further 6 h. Cell-free supernatants were harvested and stored at −20°C until analysis by ELISA for cytokine levels.

Cytokine assays

Tumor necrosis factor-α and IL-6 levels in PBMC supernatants were determined using sandwich-ELISA kits (PharMingen) with sensitivities of 15 pg/ml. In brief, 96-well plates were pre-coated with mouse anti-human TNF-α or IL-6 monoclonal antibodies in duplicate. Supernatant samples and ELISA kit standards (recombinant cytokine) were added to the 96-well plates and incubated prior to the addition of the secondary antibody and followed by the substrate. The color development is stopped and the intensity of the color was measured at 450 nm within 30 min. Supernatant samples were diluted before analysis by ELISA. All experiments were repeated three times.

Effect of IVIG on mitogen-stimulated PBMC cytokine production and proliferation

Peripheral blood mononuclear cells (2×10^6/ml) were pre-incubated with IVIG (6 mg/ml) or media alone for 2 h. PBMCs were then washed and stimulated with 1 μM ionomycin (ATC31005; Calbiochem, La Jolla, CA, USA) in combination with 20 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) from 48–96 h. Cell supernatants were collected from cells at 48, 72 and 96 h for analysis of IL-6 and TNF-α content. To assess the effect of IVIG on mitogen-stimulated PBMC proliferation, cultures were set up in triplicate (2×10^6/ml), and incubated (5% CO_2, 37°C) for 48, 72 and 96 h. Tritiated thymidine ([^{3}H]thymidine) was added to the culture medium 18 h prior to cell harvesting using a scintillation counter yielding data in counts per minute (cpm) (Packard Instrument Co., Inc., Meridan, CT, USA).

Analysis of apoptosis by flow cytometry

The PBMCs (2×10^6/ml) were cultured with IVIG (6 mg/ml) or cultured only with RPMI 1640 tissue culture media for 20 h. Cells were then stained with FITC-conjugated anti-human-CD14 antibody (Becton Dickinson, Mountain, CA, USA). Stained cells were washed with 0.05% Tween-20 (Sigma) in PBS buffer at 37°C for 15 min, further treated with 100 μg/ml RNase (Sigma) in PBS and followed with 50 μg/ml propidium iodide (Sigma) staining. Samples were analyzed by flow cytometry (FACScan; Becton Dickinson). B cells isolated from Balb/c mice (2×10^6/ml) and incubated with 10^{-7} M dexamethasone (Sigma) for 20 h were used a positive control for apoptosis.

Statistical analysis

Cytokine data were not normally distributed. This data were transformed (Log_{10}) and are presented as mean ± s.d. PBMC proliferation data are also presented as mean ± s.d. Comparison of transformed cytokine data was performed using paired *t*-test. Probability values ≤ 0.05 were considered to represent statistically significant differences. Statistical analysis was conducted using the commercially available SPSS 11.5 statistical software.

Results

Effect of IVIG on LPS-stimulated TNF-α and IL-6 secretion

Time course experiments showed the optimal incubation time for the detection of TNF-α and IL-6 secretion from LPS-stimulated PBMCs to be 6 h (data not shown). Fig. 1a shows that pre-incubation of IVIG with LPS for 1 h prior to culture with PBMCs for 6 h had a significant inhibitory effect on IL-6 secretion ($p = 0.027$) but not on TNF-α secretion ($p = 0.254$), with levels of secreted TNF-α similar to those secreted by PBMCs stimulated with LPS in the absence of IVIG. Similarly, the pre-incubation of PBMCs with IVIG for 2 h prior to stimulation with LPS for 6 h exerted a significant inhibitory effect on IL-6 ($p = 0.045$) secretion but not on TNF-α ($p = 0.234$) secretion

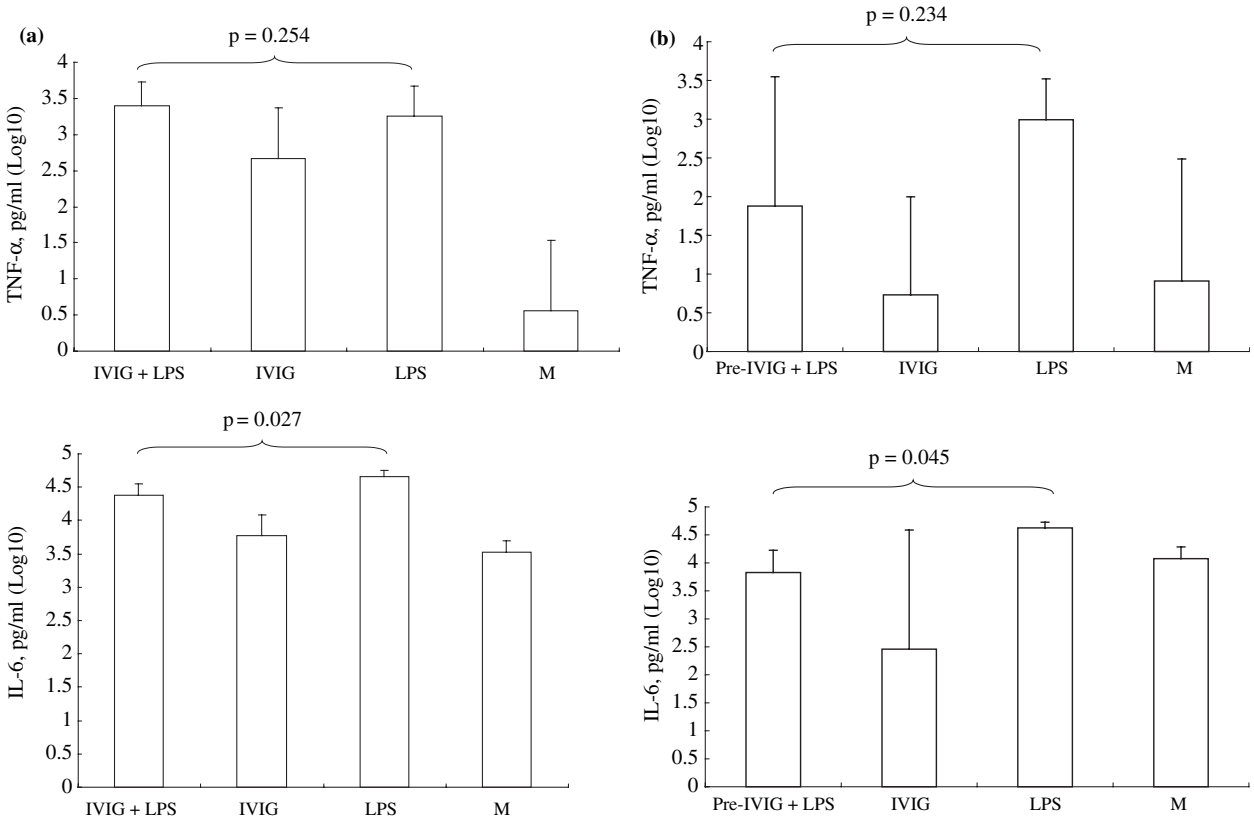

Fig. 1. Effect of IVIG on LPS-stimulated TNF-α and IL-6 secretion. (a) Pre-incubation of IVIG with LPS prior to the addition of PBMCs. IVIG + LPS, pre-incubation of IVIG with LPS before addition to PBMCs; IVIG, PBMCs in IVIG; LPS, PBMCs stimulated with LPS; M, PBMCs cultured in media alone. (b) Pre-incubation of IVIG with PBMCs for 2 h prior to the washing of cells and addition of LPS for a further 6 h of culture. IVIG + LPS, PBMCs pre-incubated with IVIG before LPS stimulated; IVIG, PBMCs in IVIG; LPS, PBMCs pre-incubated in media alone before LPS stimulation; M, PBMCs cultured in media alone. Data are representative of three experiments and are presented as mean ± s.d.

(Fig. 1b). In both conditions (Fig. 1a and b), mean values of IL-6 were lower than the corresponding positive controls (LPS alone). However, when IVIG was pre-incubated with LPS for 1 h prior to culture with PBMCs, the mean value of TNF-α secretion was higher than LPS alone. When PBMCs were pre-incubated with IVIG for 2 h prior to LPS stimulation for 6 h, the mean value of TNF-α secretion was much lower than the corresponding positive control (LPS alone).

Effect of IVIG on PMA-stimulated cytokine secretion and proliferation

Pre-incubation of PBMCs with IVIG for 2 h prior to stimulation with PMA/ionomycin resulted in the inhibition of both TNF-α and IL-6 release, although reductions in IL-6 secretion were not statistically significant (Fig. 2). However, when compared PBMCs stimulated with PMA/ionomycin, there was a significant reduction in TNF-α secretion cytokine levels from PBMCs pre-incubated with IVIG prior to PMA/ionomycin stimulation at 24 h ($p = 0.0460$) and 48 h ($p = 0.021$).

The proliferative response of PBMCs pre-incubated with IVIG to mitogenic stimulation is shown in Fig. 3. There was a trend toward the reduced levels of proliferation when IVIG was pre-incubated with PBMCs prior to PMA/ionomycin stimulation; however, the differences were not statistically significant at 48, 72 and 96 h.

Comparison of inhibitory effect of anti-CD14 antibody and IVIG on LPS-stimulated TNF-α and IL-6 secretion

To investigate whether the inhibitory effect of IVIG on LPS-stimulated cytokine secretion occurs via blocking of the CD14 receptor, PBMCs were pre-incubated with either anti-CD14 antibody or IVIG for 2 h prior to LPS stimulation. The results revealed that both IVIG and anti-CD14 antibody exerted a somewhat different degree on inhibition on LPS-stimulated TNF-α secretion (Fig. 4). When compared with PBMCs pre-incubated in media

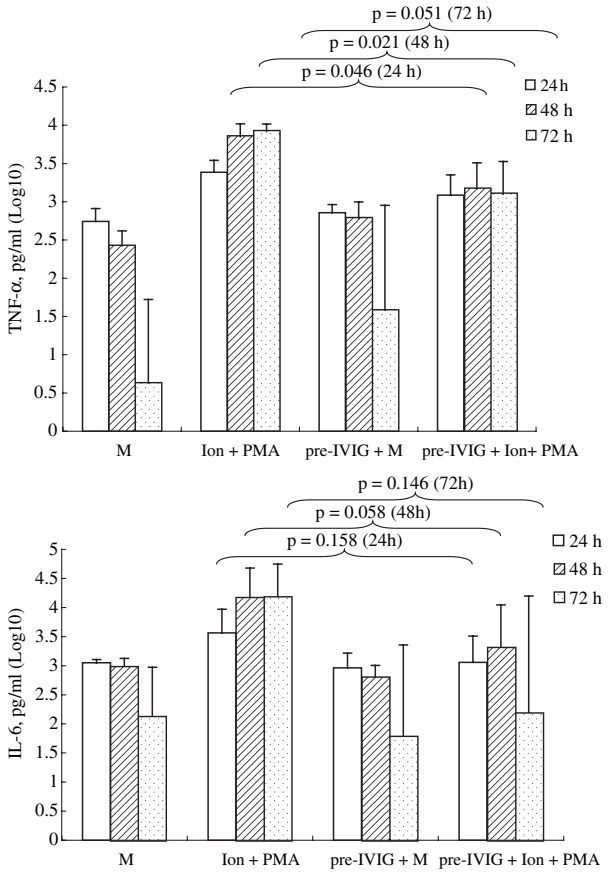

Fig. 2. Effect of IVIG on PMA-stimulated TNF-α and IL-6 production. Secretion of TNF-α and IL-6 by PBMCs pre-incubated with IVIG for 2 h before PMA/ionomycin stimulation for 24, 48 and 72 h. M, PBMCs maintained in media alone; ION + PMA, PBMCs stimulated by PMA/ionomycin; pre-IVIG + M, PBMCs pre-incubated with IVIG in media alone; pre-IVIG + ION + PMA, PBMCs pre-incubated with IVIG before PMA/ionomycin stimulation.

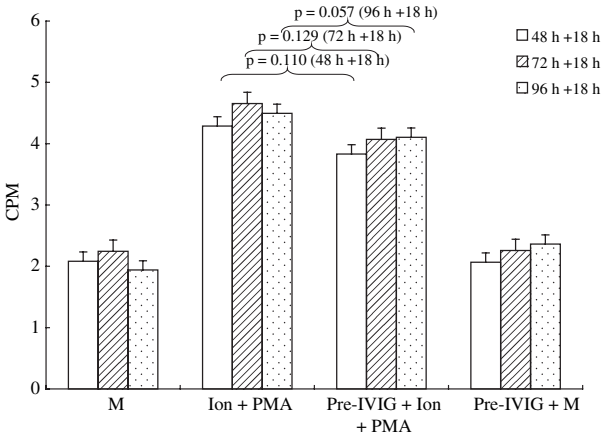

Fig. 3. Effect of IVIG on mitogen-stimulated PBMC proliferation. Proliferation of PBMCs pre-incubated with IVIG prior to PMA/ionomycin stimulation at 48, 72 and 96 h, as determined by [^{3}H]thymidine incorporation. Data are representative of three separate experiments and are presented as the mean ± s.e.m. M, media alone; Ion + PMA, PBMCs stimulated with PMA/ionomycin; pre-IVIG + Ion + PMA, PBMCs pre-incubated with IVIG prior to PMA/ionomycin stimulation; pre-IVIG + M, PBMCs pre-incubated in IVIG.

alone before LPS stimulation, pre-incubation of PBMCs with anti-CD14 antibody had a significant inhibitory effect on LPS-stimulated TNF-α secretion ($p = 0.042$). There was no statistically significant difference between the degree of TNF-α inhibition induced by anti-CD14 antibody and IVIG ($p = 0.413$). In contrast, pre-incubation of PBMCs with IVIG exerted a more profound effect on LPS-stimulated IL-6 secretion than for observed with anti-CD14 antibody. When compared with PBMCs pre-incubated in media alone before LPS stimulation, pre-incubation of PBMCs with IVIG induced a significant reduction in IL-6 secretion ($p = 0.001$). However, pre-incubation with anti-CD14 antibody did not exert a significant inhibitory effect on IL-6 secretion ($p = 0.166$). Indeed, there was a statistically significant difference between the degree of IL-6 inhibition induced by IVIG and anti-CD14 antibody pre-incubation ($p = 0.018$).

Effect of cross-linking antibody on TNF-α and IL-6 production by PBMCs

We investigated the effect of cross-linking cell membrane-bound IVIG with anti-human kappa- and lambda-chain antibodies when compared with the analogous cross-linking of anti-CD14 antibody. Cross-linking anti-CD14 antibody on PBMCs resulted in a significant secretion of in TNF-α ($p = 0.016$) and IL-6 ($p = 0.005$) secretion, in comparison with PBMCs maintained in media alone (Fig. 5). The levels of cytokine secretion induced by anti-CD14 cross-linking were greater than those induced by LPS stimulation. However, no significant differences were noted in the cytokine secretion levels induced by cross-linking with anti-human kappa- and lambda-chain antibodies in comparison with LPS-stimulated cytokine secretion (Fig. 5).

Effect of IVIG on cross-linking anti-CD14 elicited TNF-α and IL-6 secretion

When compared with PBMCs stimulated by anti-CD14 cross-linking, the pre-incubation of PBMCs with IVIG for 2 h prior to the addition of anti-CD14 cross-linking exhibited no inhibitory effect on TNF-α secretion, $p = 0.125$,

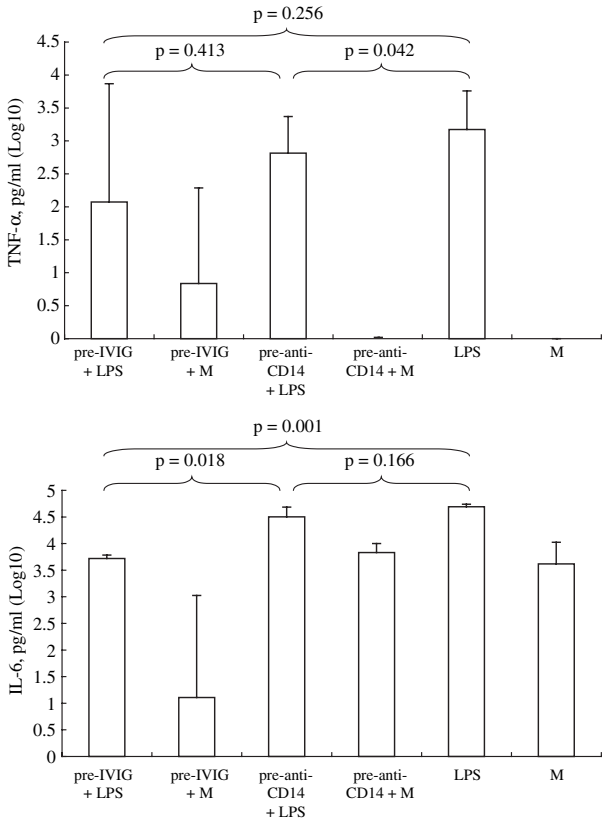

Fig. 4. Effect of anti-CD14 antibody on LPS-stimulated TNF-α and IL-6 secretion. PBMCs were pre-incubated with either IVIG or anti-CD14 antibody for 2 h prior to LPS stimulation. M, PBMCs cultured in media alone; pre-IVIG + LPS, PBMCs pre-incubated with IVIG before LPS stimulation; pre-IVIG + M, PBMCs pre-incubated in IVIG and cultured in media alone; pre-anti-CD14 + LPS, PBMCs pre-incubated with anti-CD14 antibody before LPS stimulation; pre-anti-CD14 + M, PBMCs pre-incubated with anti-CD14 and cultured in media alone; LPS, PBMCs pre-incubated in media alone and stimulated with LPS. Data are representative of three experiments conducted using PBMCs from three different healthy volunteers and are presented as mean ± s.d.

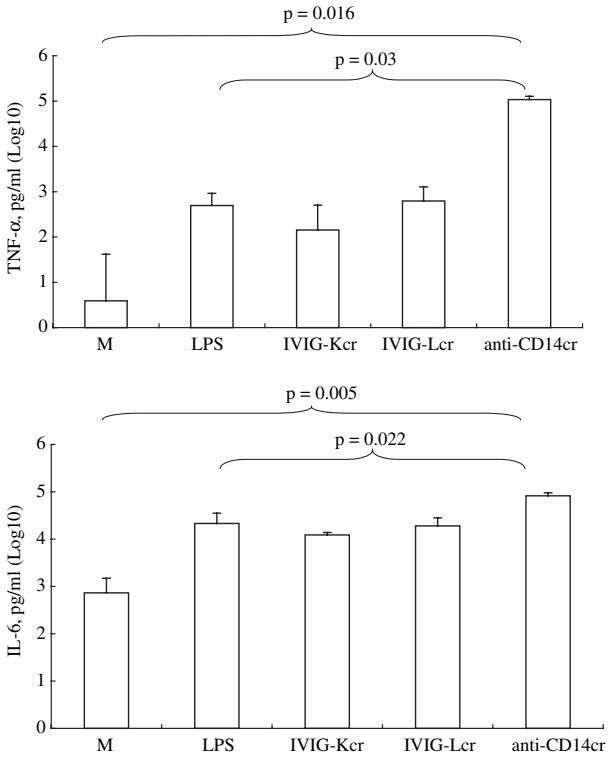

Fig. 5. Effect of cross-linking anti-CD14 antibody on TNF-α and IL-6 secretion. M, PBMCS cultured in media alone; LPS, PBMCs stimulated with LPS; IVIG-Kcr, PBMCs stimulated by cross-linking IVIG with anti-human kappa antibody. IVIG-Lcr, PBMCs stimulated by cross-linking IVIG with anti-human lambda antibody. Anti-CD14-cr, PBMCs stimulated by cross-linking anti-CD14 antibody with anti-mouse IgG_{2a} antibody.

(Fig. 6). However, there was a significant inhibition in IL-6 ($p = 0.033$) secretion.

Effect of IVIG on PBMCs apoptosis

In addition, the incidence of apoptosis in PBMCs pre-incubated with IVIG, as determined by flow cytometry, was 0.41% in comparison with that of 41.9% in B cells, isolated from Balb/c mice and pre-incubated with dexamethasone (10^{-7} M) for 20 h (data not shown).

Discussion

TNF-α, a polypeptide originally discovered as a cause of hemorrhagic necrosis in some tumors in mice (17), exerts a wide variety of effects on numerous non-malignant cells and is a major mediator of inflammatory processes (18). Furthermore, IL-6, produced largely by macrophages, stimulates the production of acute-phase proteins such as C-reactive protein (CRP) and α_2-macroglobulin by hepatocytes (19). TNF-α and IL-6 are the major pro-inflammatory cytokines implicated in the pathogenesis of many disease processes (18, 19). Our study has demonstrated that pooled human immunoglobulin inhibits the release of TNF-α and IL-6 *in vitro* when exposed to PBMCs at a concentration corresponding to levels achieved *in vivo* during the therapeutic administration.

The presence of natural anti-cytokine antibodies, mainly anti-IL-1 and anti-TNF-α, has been proposed as an explanation of the immunoregulatory properties of IVIG (10). However, our data failed to support the presence of anti-TNF-α antibodies in IVIG. In our study, IVIG could only significantly inhibit IL-6, not the TNF-α, secretion whether it was mixed with LPS for 1 h prior to PBMC stimulation, or mixed with PBMCs for 2 h prior to LPS stimulation.

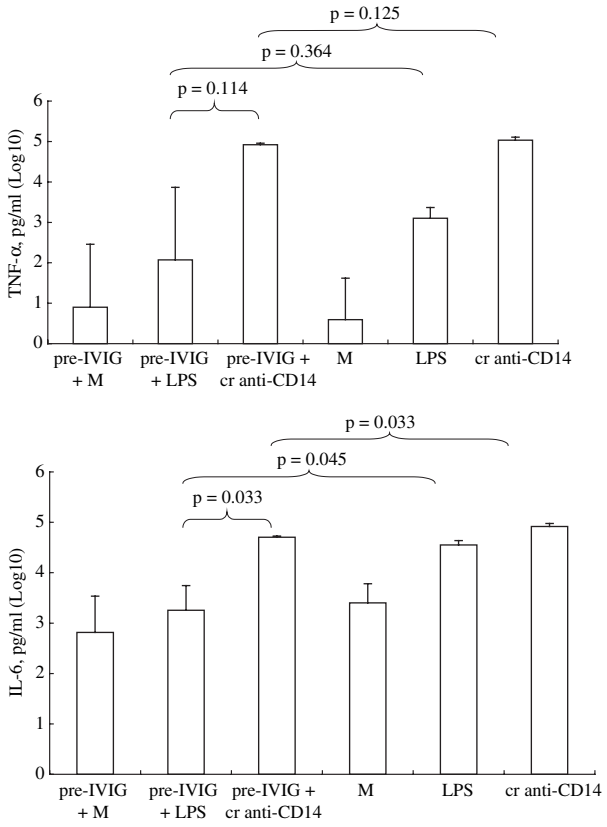

Fig. 6. Effect of IVIG on cross-linking anti-CD14 stimulated TNF-α and IL-6 production. pre-IVIG + M, PBMCs pre-incubated with IVIG and cultured in media alone; pre-IVIG + LPS, PBMCs pre-incubated with IVIG and stimulated with LPS; pre-IVIG + cr anti-CD14, PBMCs pre-incubated with IVIG and stimulated by anti-CD14 cross-linking. Data are representative of three separate experiments using PBMCs from three healthy adult volunteers and are presented as mean ± s.d.

It has been suggested that IVIG may suppress LPS-stimulated TNF-α and IL-1 secretion through an increase in intracellular levels of cAMP following the interaction of IVIG with the Fcγ receptors on monocytes (11, 12). As a testament to IVIG Fcγ receptor blockade, peripheral blood monocytes from IVIG-treated idiopathic thrombocytopenic purpura (ITP) patients exhibit a diminished ability to form rosettes with IgG-coated erythrocytes (20). The Fc domain of immunoglobulins is involved in the regulation of various monocytes and macrophage immune functions, including phagocytosis, antibody-dependent cell-mediated cytotoxicity, the clearance of immune complexes, and secretion of inflammatory mediators such as IL-1, IL-6, and TNF-α (16). Therefore, IVIG binding to Fcγ receptors on PBMCs appears to provide a reasonable explanation for the inhibitory effect of LPS-stimulated cytokine secretion.

CD14, the glycosylphosphatidylinositol-linked glycoprotein expressed on monocytes and neutrophils (21), triggers monocyte activation, and is a high-affinity cell-surface receptor for complexes of LPS and the serum LPS-binding protein, LPB (22). CD14 thus plays a central role in LPS-stimulated cytokine release in gram-negative bacterial infections (23). Our experiments demonstrated that IVIG pre-incubation and anti-CD14 antibody exerted different degrees of inhibition on LPS stimulated TNF-α secretion, when compared with the controls; however, the differences between IVIG pre-incubation and anti-CD14 pre-incubation were not significant. Our data suggest that IVIG preparations may utilize several mechanisms, some of which may be independent of CD14.

Toyoda et al. (7) reported that IVIG preparations do not contain significant quantities of blocking antibodies against CD14 and other surface molecules and suggested that IVIG modulated the expression of CD19, CD20, and CD40 on B cells and induces apoptosis. Our data do not support this mechanism, as levels of apoptosis in PBMCs pre-incubated with IVIG were low.

The effects of IVIG may be mediated through the interaction of immune complexes or IgG with monocytes Fc-receptors (15). IgGs, either soluble or coated on solid phase, activate the release of various cytokines including TNF-α (16) and IL-6 (24) upon cross-linking with monocytes Fc-receptors. The abrogation of the stimulatory activity of IgGs by digestion of the Fc portion of the IgG molecule indicates that a cross-linking mechanism is involved. Furthermore, Debets et al. (16) demonstrated a rapid secretion of TNF-α when monocyte Fc receptors bound with cytophilic human IgG were cross-linked by the addition of anti-human IgG. Our results show that cross-linking IVIG with anti-human kappa- and lambda-chain antibody stimulates cytokine secretion similar to that induced by LPS. Indeed, anti-CD14 cross-linking demonstrated elevated TNF-α and IL-6 secretion from PBMCs. In contrast to IL-6, elevated TNF-α levels stimulated by anti-CD14 cross-linking were not inhibited by pre-treatment with IVIG. A possible explanation may be that, for TNF-α secretion, pre-treatment with IVIG might not completely block the CD14 receptor, and thus cannot inhibit cytokine secretion stimulated by anti-CD14 cross-linking.

A large variety of mononuclear cells produce cytokines in response to ionomycin/PMA stimulation, an activation process that bypasses all normal cell-surface receptors (25). Activation-

induced apoptosis has been noted for human monocytes challenged with zymosan and phorbol esters after the differentiation of monocytes into macrophages was achieved by stimulation of monocytes with M-CSF and IFN-γ (26). Further, studies of the induction of apoptosis in monocytes have shown that these macrophage-precursor cells spontaneously undergo apoptosis unless given a signal to survive, these signals having been described to be LPS, TNF-α, IL-1β, and chemotactic factors (27, 28). We thus speculate that a high concentration of IVIG may induce apoptosis of monocytes and subsequently result in the decreased cytokine production by PBMCs. Although the degree of cell proliferation assessed by [^{3}H]thymidine incorporation was reduced in IVIG-pre-incubated PBMCs, no evidence of apoptosis could be detected by flow cytometry in IVIG-pre-treated PBMCs suggesting that induction of apoptosis was not a mechanism utilized by IVIG in the inhibition of PBMC cytokine secretion.

Human intravenous immunoglobulin is now widely used to treat many clinical diseases; however, the clinical use of IVIG can be associated with side-effects such as fever and hypotension have been related to the associated release of noxious mediators such as TNF-α (29). Further understanding of the regulatory mechanisms involved in any given disease will provide an effective preparing IVIG infusions tailored for the treatment of each specific diseases.

Acknowledgments

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