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Apoptosis: A target for potentiation of UV-induced IL-1Ra synthesis by IVIg

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Abstract

IL-1Ra prevents IL-1 induced inflammatory signalling, a mechanism potentially important for several pathological conditions characterized by inflammation. When administered as a drug in the recombinant form, it displays a protective effect towards them. We postulated that this action could also be achieved by pharmacological activation of endogenous IL-1Ra production. We previously showed that photochemotherapy and UV-light increased monocyte/macrophages IL-1Ra secretion. A similar effect has been shown for IVIg. The aim of this study was to define optimal in vitro conditions for induction of IL-1Ra secretion. As both agents induce lymphocytes apoptosis, we focused our analysis on the influence of IVIg on UV-induced-IL-1Ra production on this mechanism.

After overnight preincubation at 37 °C, UV-irradiated PBL mixed with two IVIg concentrations (1 and 25 mg/ml) were cocultured with autologous PBMC. Apoptosis was measured by annexin V/PI. IL-1Ra secretion was evaluated by RT-PCR and Luminex microbead array assay.

A significant additive dose-dependent influence of IVIg (+85%; $p = 0.0005$) on UV-induced IL-1Ra secretion, involved both Fc-receptor activation at a low dose (1 mg/ml) and a potent apoptotic action on PBL reinforcing the UV effect at high concentrations (25 mg/ml).

We conclude that lymphocyte apoptosis represents an important pathway contributing to enhancement of UV-induced monocyte/macrophages IL-1Ra production by IVIg and that these findings should be considered when evaluating *in vivo* protocols for photochemotherapy and IVIg treatment, in hope of improving efficacy.

Keywords: IVIg; UV-light; Apoptosis; Monocyte/macrophages; IL-1Ra

1. Introduction

The pivotal role of IL-1 and its receptor in pathogenesis of immune conditions involving an inflammatory component has been demonstrated by investigating the natural course of animal models and human diseases [1] and its improvement by agents reducing IL-1 activity [2]. Among these, the antagonist of IL-1 receptor (IL-1Ra) usually administered in the recombinant form (rIL-1Ra) prevents IL-1 induced inflammatory signalling by competing for IL-1 receptor with its ligands on monocyte/macrophages and neutrophils [2,3]. It has been used successfully not only in autoimmune diseases such as rheumatoid arthritis [4] and lupus erythematosus [5] but also in alloimmune conditions such as GVH in stem cell transplantation [6] and rejection of organs such as pancreatic islets [7] and cornea [8]. The pleiomorphic character of the anti-inflammatory action of IL-1Ra appears from recent studies showing that beside targeting cellular mechanisms directly associated with IL-1, IL-1Ra antagonizes the strong humoral and even coagulation perturbations occurring in xenogeneic heart transplantation in rats treated with cobra venom factors [9]. In murine myasthenia gravis [10], IL-1Ra administration inhibits proinflammatory cytokine production and lowers C3

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; IVIg, intravenous immunoglobulin; LPS, lipopolysaccharide; mAb, monoclonal antibody; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; PI, propidium iodide; RT-PCR, reverse-transcriptase polymerase chain reaction; UVL, ultraviolet light

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and anti-acetylcholine receptor IgG levels. Remarkable results achieved by rIL-1Ra administration in two rare genetic diseases, Muckle–Wells syndrome [11] and familial cold induced urticaria [12] have contributed to decipher molecular defects affecting interactions between IL-1 and its receptor in their pathogenesis. Furthermore, the importance of endogenous production of IL-1Ra in the immunosuppression status is illustrated by the fact that defective IL-1Ra production in patients rejecting acutely their liver transplant renders them resistant to steroids [13]. Taken together, these observations led us to consider pharmacologically induced endogenous IL-1Ra activation as a potential mean contributing to prevention and correction of diseases in which IL-1 and its receptor are involved.

Besides classical activators of IL-1Ra production such as bacterial lipopolysaccharides [14] and GM-CSF [15], immunoglobulins G [1], intravenous (IV) IgG [16] and anti-D IgG [17] stimulate IL-1Ra secretion not only in vitro but also in vivo. Indeed, IVIg infusions have been shown to activate IL-1Ra production both in patients [18–20] and in a mouse ITP model [21]. IVIg, which are pooled IgG purified from plasma of several thousands of healthy donors display complex immunomodulatory effects through a vast array of pathways [22]. One of the modes of action proposed for IgG enhancement of IL-1Ra synthesis consists in Fc-receptor activation as indicated by abrogation of this effect through Fc portion removal by pepsin digestion [16].

More recently, the capacity of UV-irradiation to induce marked activation of IL-1Ra production has been documented on PBMC obtained by apheresis during extracorporeal photochemotherapy [23] and on in vitro UV-irradiated PBMC [24]. A mechanism consisting of lymphocyte apoptosis followed by increased phagocytosis by monocytes–macrophages [25,26] leading to production of immunosuppressive and anti-inflammatory cytokines, has been proposed [27]. The recent finding that IVIg contain natural anti-Fas antibodies able to provoke cell apoptosis, especially at high concentrations [28,29], prompted us to establish whether IVIg-induced lymphocyte apoptosis could constitute an additional mechanism contributing to IL-1Ra production and whether they could potentiate the UV effect.

The aim of the present in vitro study was to define optimal conditions for IL-1Ra production that could eventually be applied for in vivo therapy. It analyses mechanisms involved in influences of various doses of IVIg on UV-induced IL-1Ra synthesis and more precisely those involving apoptosis which represents a common action of both treatments.

2. Materials and methods

2.1. Cell purification

PBMC isolated from citrated blood of healthy donors by Ficoll density gradient centrifugation (Lymphoprep® Nycomed, Norway) were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (Bio Whittaker/Boehringer Ingelheim, Verviers, Belgium) and 40 μg/ml gentamicin (Schering-Plough, Kennelworth, NJ, USA), in a 5% CO_2 atmosphere. Purified peripheral blood lymphocytes (PBL) were obtained by monocyte depletion of PBMC by phagocytosis of iron particles as follows. PBMC adjusted at 10 millions/ml were incubated for 1 h at 37 °C with 20 mg/ml carbonyl iron powder (Carbonyl Iron Sigma, Steinheim, Germany). After monocytes removal with a magnet, the PBL suspensions contained less than 0.5% monocytes, as assessed by flow cytometry.

2.2. PBMC–PBL coculture

After overnight preincubation at 37 °C, UV (254 nm, 10 J/m^2)-irradiated PBL were cocultured for 8 h with autologous PBMC at a PBMC–PBL ratio of 3:1 as previously described [22]. To evaluate IVIg influence on IL-1Ra production, PBMC–PBL cocultures were incubated, after preliminary experiments defining optimal concentrations and timing of IVIg addition with two IVIg concentrations [28] (Multigam®, DCF-CAF Brussels, Belgium used for all experiments presented in the paper; Sandoglobuline® ZLB Behring N.V., Bern, Switzerland was also tested, in parallel with Multigam in some experiments): (1) 1 mg/ml added to PBMC-UV treated PBL at 30 min before, during and 1 h after initiation of coculture; (2) 25 mg/ml preincubated overnight with PBL followed by their washing before initiation of coculture. F(ab′)2 fragments prepared according to the manufacturer's protocol (Pierce, Rockford, IL, USA) by addition of 10 mg/ml IVIg suspended in a 20 mM sodium acetate buffer (pH 4.5) solution to 0.25 ml of the 50% slurry of immobilized pepsin were incubated 4 h at 37° in a shaker incubator. Solubilized F(ab′)2 and Fc fragments were recovered by a serum separator and Fc fractions were removed by an immobilized protein A column. Solubilized F(ab′)2 fragments were concentrated by Slide-A-Lyzer concentrating solution and dialysis cassette.

2.3. Apoptosis studies

Measurement of apoptosis by the light scatter method was performed by double staining with annexin V (FITC) and propidium iodide (PI) (Sigma, Steinheim, Germany). Annexin V binding which reveals phosphatidylserine on the outer surface of cells undergoing apoptosis was measured by a commercially available kit (Caltag Laboratories, Burlingame, CA, USA). Mortality was measured at 16 h. Data were collected on at least 40,000 cells using a FACScan flow cytometer and Cellquest software (Becton Dickinson). When specified, blocking anti-CD95 mAb (Alexis® Biochemicals, Zandhoven, Belgium) was added to the cocultures.

2.4. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) for cytokine assessment

mRNA was extracted using a MagnaPure LC RNA Isolation Kit (Roche Diagnostics, Brussels, Belgium) according to the manufacturer's instructions. Reverse transcription and real-time PCR reactions were then carried out using LightCycler-RNA Master Hybridization Probes (one-step procedure) on a Lightcycler® apparatus (Roche Diagnostics, Brussels, Bel-