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1α,25-Dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in Human Monocytes

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ABSTRACT

CD40 ligand (CD40L) stimulation induces proinflammatory and immunomodulatory activity in monocytes. Here, we report on the effects of the steroid hormone 1α,25-dihydroxyvitamin D3 (1,25D3) on human blood monocytes that have been stimulated with the CD40L ligand. Co-treatment of CD40L-stimulated monocytes with 1,25D3 resulted in reduced production and secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-1β, as well as in reduced expression of the surface co-stimulatory molecules CD80 and CD86. In addition, costimulation of CD4+ T lymphocytes by monocytes co-treated with CD40L and 1,25D3 resulted in reduced cell proliferation and diminished interferon (IFN)-γ but enhanced IL-10 production by CD4+ T cells. Finally, 1,25D3 interfered with the ability of CD40L to rescue monocytes from apoptosis induced by serum withdrawal. These findings suggest that 1,25D3 may regulate the interaction of monocytes with T cells or other cell types that express CD40L, thus influencing the outcome of the immune or inflammatory response.

1. Introduction

In addition to its classical role in calcium homeostasis, the hormonal, active form of vitamin D3, 1,25-dihydroxyvitamin D3 (1,25D3), displays immunomodulatory properties in vitro as well as in vivo [1,2]. Its actions on the immune system are exerted via intracellular receptors (VDR), present in several immunological cells such as lymphocytes and monocytes [3]. When added to mitogen-stimulated human peripheral blood lymphocytes in vitro, 1,25D3 inhibits their proliferation, Ig synthesis, and accumulation of transcripts for interleukin (IL)-1, IL-2, IL-6, tumor necrosis factor (TNF)-α and -β and interferon (IFN)-γ [4–6]. 1,25D3 also affects monocyte phenotype and function. Müller et al. showed that 1,25D3 dose-dependently inhibited the production of IL-1α, IL-6, and TNF-α by lipopolysaccharide stimulated monocytes [7]. Moreover, 1,25D3 treatment reduced the basal expression HLA-DR, CD4, and CD86 and the induction of CD80 by TNF-α by monocytes [8–10]. Finally, a defect in accessory cell function which resulted in impaired capacity of monocytes to promote lectin-induced T cell activation was found in 1,25D3-treated monocytes, which was postulated to be the consequence of reduced MHC and CD80 expression [8,11].

Monocytes occupy a central position in many inflammatory processes. As a consequence, these cells are a target for many activating cytokines/chemokines that are involved in the regulation of the inflammatory response. However, proinflammatory cytokines/chemokines share many redundant activities so that monocyte activation by these substances is mostly aspecific. More specific activation of monocytes may be achieved via processes that involve the CD40 ligand (CD40L)-CD40 pair, members of the tumor necrosis factor (TNF)/TNF receptor (TNFR) superfamilies of molecules, respectively [12–16]. Engagement of CD40 with CD40L leads to the production of pro-inflammatory cytokines such as TNF-α and IL-1β and up-regulation of surface molecules (CD40, CD80, and CD86) [17–19]. Moreover, CD40 stimulation of monocytes plays a major role in the control of intracellular killing of pathogens, because large amounts of IL-12 are produced [20]. However, the induction of pro-inflammatory cytokines by CD40 triggering on monocytes has been suggested to be relevant to the pathogenesis of many autoimmune diseases such as systemic lupus erythematosus (SLE) [21,22], rheumatoid arthritis (RA) [23–25], multiple sclerosis (MS), [26,27] inflammatory bowel diseases (IBD) [28], glomerular inflammatory diseases, [29] and in graft-versus-host [30] and allograft rejection [31]. Blockage of the interaction of this ligand–receptor pair has resulted in a decrease in both the inci-

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dence and the severity of chronic inflammatory diseases [32–34] graft-versus-host-disease [35] and allograft rejection [36,37]. Although it has been previously shown that 1,25D3 may interfere with CD40L-induced CD40 expression in fully differentiated monocyte-derived DC [38], there are no reports on the effect of this compound on CD40L-activated fresh monocytes.

In this study, we analyzed the effect of 1,25D3 on the CD40–CD40L stimulatory pathway of human monocytes. 1,25D3 reduced both the CD40L-induced production of pro-inflammatory cytokines, such as TNF-α and IL-1β, and the expression of the surface co-stimulatory molecules CD80 and CD86. Co-stimulation of CD4+ T lymphocytes by monocytes stimulated by CD40L in the presence of 1,25D3 resulted in reduced cell proliferation and interferon (IFN)-γ production but increased IL-10 production. Finally, 1,25D3 proved able to interfere with the ability of CD40L to rescue cultured monocytes from apoptosis induced by serum withdrawal.

2. Matherials and methods

2.1. Cells

Peripheral blood obtained from healthy donors was enriched for PBMC by centrifugation over Ficoll Hypaque. PBMC were then further enriched for monocytes by counter flow centrifugal elutriation as previously described [39]. Cells obtained by this method are >90% monocytes as determined by morphological criteria and by the expression of the CD14 antigen. Monocytes were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 20% heat-inactivated fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, referred to as complete medium. The cells were kept at 37 °C in a humidified atmosphere of 5% CO_2 in air.

2.2. Compounds

Immunex (Seattle, WA) provided soluble trimeric recombinant CD40L (CD40L). Vitamin D (Sigma–Aldrich) was dissolved in absolute ethanol at a stock concentration of 10 μM before being added to cultures. ^{3}H-thymidine, specific activity 80 mCi/mmol, was purchased from Amersham, Little Chalfont, UK.

2.3. Limulus amebocyte lysate test

All the compounds and media used in this study were analyzed for endotoxin contamination by the limulus amebocyte lysate test (QCL-1000, BioWhittaker Inc., Walkersville, MD). All the samples analyzed were found free of endotoxin contamination (less than 0.1 EU/ml).

2.4. Antibodies

For FACS analysis, the following monoclonal antibodies were used: anti-CD80, anti-CD86, CD40, anti-TNF-α, anti IL-1β, anti-IL-10, and anti-IFN-γ (all from PharMingen, San Diego CA). Staining was performed with FITC-, PE-, and Quantum Red™ (QR)-conjugated antibodies.

2.5. ELISA immunoassay

Commercially available sandwich ELISA kits (R & D Systems, Minneapolis MN) were used to determine the concentration of TNF-α and IL-1β in cell free supernatants. According to the manufacturer specifications, these ELISAs are specific for the relative interleukin. All the samples were determined in duplicate, in a single analytical set. Intra-series variation coefficient was <20%.

2.6. Cell stimulation

To evaluate the ability of 1,25D3 to suppress the cytokine response of macrophages to CD40L, elutriated monocytes were cultured at a concentration of 2×10^5 cells/well/250 μl for 18 h in 96-well V bottom plates (Corning Incorporated, Corning, NY) in complete medium with 0.5 μg/ml CD40L, in the presence or in the absence of 10 nM 1,25D3. Thirty minutes after plating, 1 μg/ml of the protein transport inhibitor brefeldin A was added. At the end of the incubation period the cells were analyzed by FACS for intracellular cytokine production. For the measurement of cytokines in supernatants, the cells were cultured and stimulated as described above, with the exception of brefeldin A addition. At the end of the incubation period 200 μl of supernatant from each well were collected and stored at −20 °C for subsequent ELISA testing.

To evaluate the effect of 1,25D3 on the ability of CD40L to up modulate the expression of CD40, CD80, and CD86, elutriated monocytes were cultured for 72 h in complete medium, supplemented with 0.5 μg/ml CD40L in the presence or in the absence of 10 nM 1,25D3. At the end of the incubation period the cells were collected and analyzed by FACS for CD80, CD86, and CD40 expression.

The experiments to evaluate the capacity of 1,25D3 to interfere with the co-stimulatory ability induced by CD40L were carried out as follow. Monocytes were obtained by elutriation. From the PBMC of the same donor, purified CD4+ T cells were obtained by negative selection using a T cell negative isolation kit (Dynal) (>95% CD4+ T lymphocytes by FACS analysis) and frozen just after purification. Monocytes were cultured for 72 h in complete medium, supplemented with 0.5 μg/ml CD40L in the presence or in the absence of 10 nM 1,25D3. At the end of the incubation period, the cells were extensively washed to remove CD40L and 1,25D3, plated at the concentration of 2×10^5/ml in a flat bottom 96 wells plate coated with anti-CD3 (10 μg/ml) (Beckton–Dickinson, San Jose, CA) and mixed at the ratio of 1:1 with thawed autologous CD4+ T cells. Alternatively, monocytes were activated as described above and plated at the concentration of 5×10^5 on the top of a micro porous membrane (0.45 micron in diameter) suspended in well of a 24 well plate (Falcon 3095, Becton Dickinson). Thawed autologous CD4+ T cells (5×10^5) were plated in the bottom of the same well, coated with anti-CD3 (10 μg/ml), in the presence of 1 μg/ml soluble anti-CD28 (Beckton–Dickinson). For intracellular cytokine detection, co-cultures were incubated for 18 h, with 1 μg/ml brefeldin A added 30 min after plating. For evaluation of T cell proliferation, the co-cultures were incubated for 72 h. Then, 0.25 mCi/well of ^{3}H-thymidine was added. Eighteen hours after ^{3}H-thymidine addition the cultures were harvested using a multi-channel harvester. The amount of incorporated ^{3}H-thymidine was determined by liquid scintillation spectroscopy (β-counter, Canberra Packard Ltd., Pangbourne, UK).

2.7. Surface marker and intracellular cytokine staining

After incubation, the cells were washed and stained by incubation with the appropriate surface marker for 30 min in the dark on ice. Cells were then washed 2 times and either analyzed by FACS to determine cell surface antigen expression or suspended in Cytofix/Cytoperm solution (Pharmingen) for 20 min in the dark on ice. The permeabilized cells were washed 2 times and stained for intracellular cytokines in the dark on ice for 30 min. After intracellular cytokine staining, the cells were washed and suspended in 200 μL