Profile of interleukin-2 production in human mononuclear cells and T-cell lines

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ABSTRACT

Background and Objective: Interleukin-2 (IL-2) as a T helper type 1 (Th1) cytokine has an important role in activation, growth and differentiation of several immune cells. Moreover, IL-2 is known as a pro-inflammatory and anti-tumoral cytokine. In addition, deysregulation of IL-2 in some diseases such as autoimmunity has been shown. The present study was conducted to evaluate the patterns of IL-2 production in human peripheral blood mononuclear cells (PBMCs) and human leukemic T cells lines (Molt-4 and Jurkat).

Materials and Methods: Human PBMCs and leukemic T cells were cultured in complete RPMI-1640 medium. The cells were then seeded at a density of 10^6 cells/ml and were incubated with different concentrations of phorbol myristate acetate (PMA) (1-25 ng/ml) or phytoheamagglutinin (PHA) (2-10 μg/ml) for 48 hours. Then, the cell-conditioned media were collected and used for IL-2 assay. Statistical comparisons between groups were made by analysis of variance (ANOVA).

Results: PHA/PMA significantly and dose-dependently increased IL-2 level in human PBMCs and leukemic T cells (Molt-4 and Jurkat) after 48 hours of incubation compared with untreated control cells.

Conclusion: We conclude that human PBMCs and leukemic T cells (Jurkat and Molt-4) could potentially secrete IL-2 with different degrees. Thus, these cells could offer a proper system to study the mechanisms regulating IL-2 production in diseases in which IL-2 production is disregulated. Also, these cells could be useful for screening the IL-2 modulators.

1. Introduction

Interleukins (ILs) are a large group of cytokines produced by a variety of cells and have an important role in growth and differentiation of immunocompetent cells (1, 2). Moreover, ILs are divided into pro- inflammatory, anti-inflammatory and chemoattractant groups (3). Interleukin-2 (IL-2) is a T helper type 1 (Th1) cytokine that has a key role in activation, growth and differentiation of several immune cells (4). Moreover IL-2 has anti-tumor and anti- parasitic properties (5, 6). Furthermore, IL-2 plays a key role in the immunopathogenesis of several disorders such as Kawasaki disease and prolapsed lumbar intervertebral disc (7, 8). In addition, deysregulation of IL-2 in some diseases including common variable immunodeficiency and autoimmunity has been shown (9-11).

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Regulation of IL-2 has been useful for treatment of some diseases including chronic graft-versus-host-disease (GVHD) and influenza viral pneumonia (12, 13). Also, IL-2, is known as as a proinflammatory cytokine that has an essential role in inflammation (14, 15). Moreover, down-regulation of pro-inflammatory cytokines has beneficial effect in treatment of some disorders such as active crohn's disease, experimental inflammatory bowel disease and heart mulfunction (16-18). In this study, the profile of IL-2 production in human peripheral blood mononuclear cells (PBMCs) and leukemic (Jurkat and Molt-4) T cell lines has been evaluated *in vitro*.

2. Materials and Methods

2.1. Materials

RPMI-1640 medium, penicillin, streptomycin, phytoheamagglutinin (PHA), phorbol myristate acetate (PMA) and trypan blue (TB) were from Sigma (USA). Fetal calf serum (FCS) was from Gibco (USA). Interleukin-2 (IL-2) standard ELISA kit was obtained from R&D Company (USA). Microtiter plates, flasks and tubes were purchased from Nunc (Falcon, USA).

2.2. Cell lines

Human leukemic T cells [Molt-4 (NCBI C149) and Jurkat (NCBI C121)], were obtained from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran). The cells were maintained in RPMI-1640 medium supplemented with 10% FCS in 5% CO_2 at 37°C.

2.3. PBMCs isolation

Human peripheral blood mononuclear cells (PBMCs) from the venous blood of healthy adult volunteers were isolated by ficoll-hypaque-gradiant centrifugation. Subsequently, the cells were washed three times in phosphate buffer saline (PBS). Then, the cells resuspended in RPMI-1640 medium supplemented with 10% FCS and were incubated in 5% CO2 at 37°C.

2.4. Cell culture and treatment

The method has been described in detail elsewhere (19). Briefly, the human PBMCs and leukemic cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO_2. The cells were seeded at a density of 2×10^6 cell/ml and then incubated with different concentrations of PHA (1-10 µg/ml) or PMA (5-25 ng/ml) for 48 hours. The supernatants of cell culture media were collected and used for IL-2 quantification. All experiments were done in triplicate.

2.5. IL-2 assay

The amount of IL-2 secreted in the cell culture supernatants by human PBMCs and leukemic T cell lines (Molt-4 and Jurkat) was measured with the Quantikine human enzyme-linked immunosorbent assay (ELISA) kits (R&D systems) according to the manufacturer's instructions. This assay uses the quantitative sandwich enzyme immunoassay technique. Complete RPMI medium was used as control and human recombinant IL-2 was employed as standard for drawing the standard curves.

2.6. Statistical analysis

IL-2 quantification in cell-conditioned media was performed in three independent experiments and the results were expressed as mean ±SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA). Meanwhile, $p < 0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. The SPSS 11.5 and Excel 2003 softwares were used for statistical analysis and graphs making, respectively.

3. Results

3.1. Profile of PHA-induced IL-2 production

3.1.1. Profile of PHA-induced IL-2 production in human PBMCs

IL-2 production was relatively low in unstimulated human PBMCs but PHA markedly and dose-dependently increased IL-2 production in human PBMCs after 48 hour incubation time, compared with untreated control cells (Figure 1) (p<0.05).

3.1.2. Profile of PHA-induced IL-2 production in Molt-4 cells

IL-2 production was low in unstimulated human leukemic Molt-4 cells, but PHA considerably and dose-dependently increased IL-2 production in Molt-4 cells after 48 hour incubation time compared with untreated control cells as illustrated in Figure 1(p<0.05).

3.1.3. Profile of PHA-induced IL-2 production in Jurkat cells

IL-2 production was very low in unstimulated human leukemic Jurkat cells but PHA markedly increased IL-2 production in Jurkat cells after 48 hour incubation time compared with untreated control cells. The PHA-induced IL-2 production in Jurkat cells was dose-dependent as depicted in Figure 1 (p<0.05).

3.2. Profile of PMA-induced IL-2 production

3.2.1. Profile of PMA-induced IL-2 production in human PBMCs

IL-2 production was rather low in unstimulated human PBMCs but PMA markedly increased IL-2 production in human PBMCs after 48 hour incubation time, compared with untreated control cells in a dose-dependent manner (Figure 2) (p<0.05).

3.2.2. Profile of PMA-induced IL-2 production in Molt-4 cells

IL-2 production was low in unstimulated human leukemic Molt-4 cells, but PMA considerably and dose-dependently increased IL-2 production in Molt-4 cells after 48 hour incubation time compared with untreated control cells, as shown in Figure 2 (p<0.05).

3.2.3. Profile of PMA-induced IL-2 production in Jurkat cells

IL-2 production was very low in unstimulated human leukemic Jurkat cells but PMA significantly increased IL-2 production in Jurkat cells after 48 hour incubation time compared with untreated control cells. The PMA-induced IL-2 production in Jurkat cells was dose-dependent as depicted in Figure 2 (p<0.05).
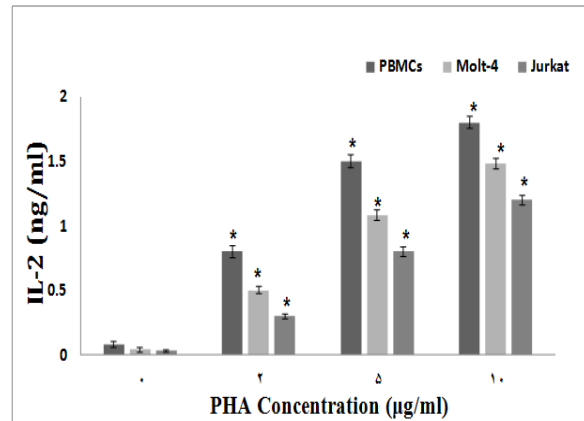

Figure 1. Effect of PHA on IL-2 secretion by human peripheral blood mononuclear cells and human leukemic Molt-4 and Jurkat T-cell lines. The cells $(1\times10^{6}$ cells/ml) were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of phytoheamagglutinin (PHA) (1-10 µg/ml) for 48 hours. At the end of incubation, Il-2 concentration in conditioned medium was quantified by ELISA. Data are mean ± SEM of three independent experiments.*P<0.05 was considered significant.
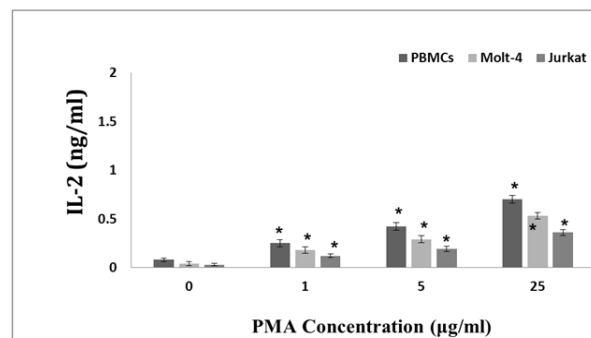

Figure 2. Effect of PMA on IL-2 secretion by human peripheral blood mononuclear cells and human leukemic Molt-4 and Jurkat T-cell lines. The cells $(1\times10^{6}$ cells/ml) were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of phorbol myristate acetate (PMA) (5-25 ng/ml) for 48 hours. At the end of incubation, Il-2 concentration in conditioned medium was quantified by ELISA. Data are mean ± SEM of three independent experiments.*P<0.05 was considered significant.

4. Discussion

In the present study, it was found out that human PBMCs and leukemic cells (Jurkat, and Molt-4) could potentially produce IL-2. As it was shown in Figures 1 and 2, all the unstimulated cells secreted low to tiny levels of IL-2. In this study, we used PMA or PHA as inducers of IL-2 production. We found that PHA/ PMA increased IL-2 production in human PBMCs and leukemic (Jurkat and Molt-4) cells dose-dependently. The IL-2 production by PHA/PMA stimulated cells was several folds more than that in unstimulated cells. Furthermore the level of IL-2 production by the stimulated cells was in this order: PBMCs > Molt-4 > Jurkat. Moreover according to our results, PHA was a more potent inducer of IL-2 production than PMA. This difference between PHA and PMA effects on IL-2 production may be due to different concentrations of PHA or PMA that was used. We used PHA at 1-10 µg/ml concentration whereas PMA was used at 5-25 ng/ml. Thus the concentrations of PHA was several times more than PMA.

Different patterns of proinflammatory cytokines such as Il-2 /TNF mRNA are expressed in different normal and cancerous lymphocytes (20, 21). The results of these studies are in accordance with our data. In one of these studies, stimulation of leukemic (Jurkat and Molt-4) cells and peripheral T-lymphocytes by PMA (30 ng/ml for 4 hours) induced the expression of IL-2 and TNF mRNA (20). In another study, PMA (50 ng/ml) or PHA (1 µg/ml) induced the IL-2, IL-8 and TNF mRNA expression in Jurkat and THP-1 leukemic cells (21). It should be noted that we used ELISA technique for quantification of IL-2 secreted in cell cultures but they used northern blot analysis for IL-2 mRNAs expressed in leukemic cell lines (20, 21).

A correlation between the increase of IL-2 level and some disorders such as autoimmunity has been reported (22, 23). Furthermore, tolerogenic actions of IL-2 at low doses via boosting the endogenous Treg cells in autoimmunity or inflammation has been shown (24). Also, a relation between the decrease of IL-2 level and a number of diseases such as oral squamous cell carcinoma and Omenn Syndrome has been reported (25, 26). In addition, deysregulation of IL-2 in some disorders including common variable immunodeficiency and autoimmunity

has been shown (9- 11). Regulation of IL-2 has been useful for treatment of some diseases including chronic graft-versus-host-disease (GVHD) and influenza viral pneumonia (12, 13). Moreover, the anti-tumor and anti- parasitic effects of IL-2 have been revealed (5, 6). Besides, the anti-tumor effects of many substances has been ascribed to their stimulatory effects on IL-2 production (27-29). Profile of interleukin-2 production may change in different diseases and IL-2 production may increase in particular carcinomas (30-32). Identifying the patterns of particular biomarkers in distinct cancers has potential benefit in anti-cancer therapy (33). Besides the prognostic and diagnostic values of IL-2 have been demonstrated (34, 35). A negative correlation between IL-2 expression and tumor invasion and metastasis has been shown (36). Also IL-2, has an essential role in inflammation (14, 15). In this regard, a positive correlation between IL-2 expression and some inflammatory diseases has been reported (37) and the anti-inflammatory effects of some drugs/medicinal plants has been attributed to their inhibitory effects on Il-2 secretion (38, 39).

Taken together, human PBMCs and leukemic T cells (Jurkat and Molt-4) could potentially secrete IL-2 with different degrees. Moreover, PHA/PMA induces IL-2 production in these cells. Also, PHA seems to be a more potent stimulator of IL-2 production than PMA in all of the studied cells. Furthermore, the stimulated PBMCs produced IL-2 much more than the stimulated Molt-4 and Jurkat leukemic cells. Thus, these cells could provide an appropriate system to study the mechanisms regulating IL-2 secretion in diseases in which IL-2 production is disregulated. Also, they could be useful to study the mechanisms regulating IL-2 expression in related disorders as well as screening the IL-2 modulators.

Acknowledgements

Conflict of interest

The authors have no conflict of interest in this research study.

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