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Profiling on Altered Productions of Cytokines or Chemokines from THP-1 Cell Line Following *in Vitro* Exposure to Cyclophosphamide, Cyclosporine, Dexamethasone, or Tacrolimus, Representative Immunosuppressants

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ABSTRACT. Although immune suppressive drugs have contributed for the cancer chemotherapy, organ transplant, but their therapeutic efficacy is limited. This study aims to evaluate the immunological modulations induced by four representative immunosuppressive on THP-1 cell line. 75% cell viabilities were determined and four test concentrations, 0.01X, 0.1X, 0.5X, and vehicle control were chosen. Culture supernatants were collected at 24 h after lipopolysaccharide (LPS1 μg/ml) activation in the presence of test substances. 27 target cytokines were measured through luminex system and relative cytokine production levels (RCPLs,%) were calculated. Cytokines with the RCPL below 100% at all the three concentrations were 21 including IL-1beta, -1ra, -2, -4, -5, -6, -7, -9, -10, -12, -13, -15, -17, Eotaxin, FGF-basic, GM-CSF, IFNγ, IP-10, MCP-1α, PDGF-BB, and VEGF in dexamethasone-treated cells. Concerning on cyclophosphamide, the RCPLs on 11 cytokines were less than 100% at all the 3 concentrations. Treatment of cyclosporine demonstrated 10 cytokines with below 100% RCPL at all the 3 concentrations. The RCPLs below 100% were observed with 22 cytokines including IL-1β, -1ra, -2, -4, -5, -6, -7, -9, -12, -13, -15, -17, Eotaxin, GFG-basic, G-CSF, GM-CSF, IFNγ, MCP-1, MIP-1α, PDGF-BB, TNFα, and VEGF from THP-1 cells treated with tacrolimus at all the 3 concentrations. The present study indicates that tacrolimus and dexamethasone are stronger immunosuppressants than cyclophosphamide and cyclosporine on THP-1 cell line.

KEY WORDS: Immunotoxicants, immunosuppression, cytokines, chemokines, THP-1 cell line

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Introduction

Immunotoxicity refers to the toxicological effect of xenobiotics on the functioning of the immune system (Lankveld et al., 2010). These immunotoxicants could dysregulate the immune homeostasis, resulting in aberrant suppression or stimulation, eventually leading to the occurrence of disease, thus considered a potent hazard (Hartung and Corsini, 2013).

The discovery of immune-suppressive drugs has aided in the treatment of several autoimmune disorders, organ transplantation and cancer therapy (Hirano, 2013). However individual variations in the clinical efficacy of these immune-suppressive drugs have been observed, resulting in an unsatisfactory solution for the therapeutic purpose. Various toxicological evaluations have been performed of such immune-suppressive drugs using the *in vivo* test method, involving qualitative and quantitative analysis of innate and adaptive immune response, immunohistopathological assessment, hematological findings, etc (Hinton, 1995). But to date, no alternative test method, especially based on the *in vitro* approach for immunotoxicity assessment has proven to be standard (Gennari et al., 2005).

In this study, we have tried to establish an *in vitro* approach to evaluate the effect of potent immunosuppressive drugs, (dexamethasone, cyclophosphamide, cyclosporine, and tacrolimus) on the human immune system. These drugs are reported to be effective for cancer chemotherapy, autoimmune disorders, and organ transplant (Cavallasca et al., 2015; Ciliao et al., 2015; Gong et al., 2020; Pointon et al., 2010). The potency to suppress the immune reaction is a key criterion for the choice of these drugs for clinical implication. This study was conducted using the THP-1 dendritic cell line, considering the antigen-presenting cell as an important target immune cell. The effect of these drugs on the production of 27 cytokines or chemokines (hereafter abbreviated as cytokines) were analyzed belonging to category inflammation-inducing, chemotactic, allergy mediated, and immune cell differentiation-related.

Materials and methods

1. Reagents

Dexamethasone (CAS No. 50-02-2), cyclophosphamide (CAS No. 6055-19-2), cyclosporine (CAS No. 59865-13), tacrolimus (CAS No. 109581-9), and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. Cell Culture

The THP-1 cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The entire cell culture process was carried out by applying the European Union Reference Laboratory for Alternatives to Anmal Testing Good Cell Culture Guideline (Coecke et al., 2005). Fetal bovine serum (10%, Gibco, Waltham, MA, USA), 0.05 mM 2-mercaptoethanol (Sigma-Aldrich), penicillin (100U/ml), and streptomycin (100 μg/ml) were added into RPMI-1640 containing 25 mM HEPES buffer and L-glutamine (Gibco), and used as a culture medium. Lipopolysaccharide (LPS, 1 μg/10^6 cells, Sigma-Aldrich) was used as an activating material.

3. Cell viability evaluation of test compound

The cells in log-phase were seeded in 96 well culture plate (1x10^4/well). The cells were stabilized for 24 hours by incubation and treated with dexamethasone and tacrolimus at concentration 0, 1, 10, 15, 22.5, 33.7, 50.6, 75.9, 100 μg/ml, and cyclophosphamide and cyclosporine at 0, 1, 10, 100, 500, 1000 μg/ml. Since the cell viability for tacrolimus dropped sharply between 10 (111.7% for tacrolimus) and 100 μg/ml (3.0% for tacrolimus) test concentration, stock solution was prepared by using a narrow dilution factor (1:1.5) from 10 to 100 μg/mlto obtain a more accurate determination of the 75% cell viability

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(Cell Viability 75%, CV75). For dexamethasone, the highest soluble concentration in DMSO was 100 ㎍/㎖ for the experiment, and 1:1.5 dilution factor was applied, similarly as tacrolimus. The experiment was conducted independently, with all treatment performed in triplicates. The chemicals were prepared by dissolving in the culture medium at a final concentration of 0.1% vehicle (DMSO for dexamethasone, tacrolimus, cyclosporin/ saline for cyclophosphamide). The CV75s were determined using the Cell Counting Kit (CCK, Dojindo Molecular Technology, Japan). The relative cell proliferation was quantified by measuring absorbance at 450 nm with a reference of 630nm using a microplate reader (Epoch, BioTek, Winooski, VT, USA). The average CV75 was determined through repeating the test twice using a different batch of the cell.

4. Determination of soluble cytokines release in the culture supernatants

CV75 described above was 1x, and test substances were added at concentrations of 0.01x, 0.1x, and 0.5x. THP-1 cells in log phase were dispensed at $1X10^6$ cells/㎖ into a 24 well culture plate. The cells were stabilized for 24 hours by incubation and treated with test substance in presence of lipopolysaccharide (LPS, 1 ㎍/㎖) as stimulus and the culture supernatant was collected after 24 hours of activation.

Quantitative analysis of cytokines in the culture was performed using a Luminex Multiplex Assay (Bio-Plex Pro Human Cytokine 27-Plex Assay Kit, Bio-Rad, Hercules, CA, USA) kit. A total of 27 cytokines involved in this analysis include interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Granulocyte-colony stimulating factor (G-CSF), Granulocyte macrophage-colony stimulating factor (GM-CSF), IFNγ, Interferon gamma-induced protein-10 (IP-10), Monocyte chemoattractant protein-1 (MCP-1), Eotaxin, Fibroblast growth factor (FGF) basic, Platelet-derived growth factor (PDGF-BB), Macrophage inflammatory protein (MIP)-1α, MIP-1β, RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted), Tumor necrosis factor (TNFα), and Vascular endothelial growth factor (VEGF). The 27-Plex Assay kit considered as the most appropriate one since this commercial kit covers all the cytokines reported for its production from THP-1 cell line, which includes IL-1β, IL-6, IL-8, IL-10, IL-13, MCP-1, MIP-1α, MIP-1β, and TNFα.

5. Analysis

Relative cytokine production level (RCPL, %) was calculated by dividing the amount of cytokine produced at each concentration (0.01X, 0.1X, 0.5X CV75%) by the amount of cytokine produced for the vehicle control (0X), and multiplied by 100. Result was interpreted by comparing the RCPL at each concentration with RCPL of the vehicle control.

Results

1. Cell viability for the 4 immunosuppressants

The CV75 value for dexamethasone, cyclophosphamide, cyclosporine or tacrolimus was determined as 100, 1000, 24.37, and 25.16 ㎍/㎖, respectively. These concentrations were considered 1X during cell treatment for cytokine analysis. Three concentrations of dexamethasone, cyclophosphamide, cyclosporine or tacrolimustest for the main experiments were as followings: dexamethasone: 1, 10, 50 ㎍/㎖, cyclophosphamide: 10, 100, 500 ㎍/㎖, cyclosporine: 0.24, 2.43, 12.18 ㎍/㎖, tacrolimus: 0.25, 2.51, 12.58 ㎍/㎖ for 0.01X, 0.1X, 0.5X CV75, respectively.

2. Effect of immunosuppressive substances on cytokine production from THP-1 cell line

In this study, we analyzed how these immuno-suppressive drugs (dexamethasone, cyclophosphamide,

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cyclosporine, and tacrolimus) modulate the cytokine production by the THP-1 cell line. Among the 27 cytokines, 2 cytokines (IL-8 and MIP-1β) could not be obtained as the value exceed the detection limit.

Fig. 1 indicates that the RCPLs of dexamethasone for IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF-basic, GM-CSF, IFN-γ, IP-10, MCP-1α, PDGF-BB, and VEGF demonstrated lower than 100% at all three test concentrations. Meanwhile, dexamethasone showed few exceptions with higher or nearly equal RCPL compared to vehicle control (100%) for G-CSF (0.01X-113.6%, 0.5X-108.1%), MIP-1α (0.01X-103.2%, 0.1X-105.6%, 0.5X-108.7%), RANTES (0.01X-114.6%, 0.1X-101.5%, 0.5X-107.6%) and TNFα (0.5X-145.8%).

Concerning on cyclosphosphamide treatment, 11 cytokines were downregulated, in that its RCPLs were 100% at all the three test concentrations, which includes IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, Eotaxin, FGF-basic, IFNγ, IP-10, and RANTES (Fig. 2). The list of cytokines for RCPL values with higher or nearly equal to 100% are as followings: IL-1β (0.01X-105%), IL-1ra (0.01X-104%, 0.1X-103%, 0.5X-100%), IL-2 (0.01X-104%), IL-12 (0.01X-117%, 0.5X-110%), IL-15 (0.01X-102%, 0.1X-101%), IL-17 (0.1X-102%), G-CSF (0.01X-102%), GM-CSF (0.01X-103%, 0.1X-104%), MCP-1 (0.01X-116%), MIP-1α (0.1X-102%, 0.5X-104%), PDGF-BB (0.01X-103%, 0.1X-105%), TNF α (0.01X-121%, 0.1X-116%, 0.5X-117%), and VEGF (0.01X-121%, 0.1X-116%, 0.5X-114%).

Cyclosporine treatment resulted in downregulation of 10 cytokines with RCPLs lower than 100% at all the three test concnetrations (IL-1ra, IL-2, IL-7, IL-12, IL-13, G-CSF, IFNγ, MCP-1, MIP-1α, and TNFα, Fig. 3). RCPLs exceeding

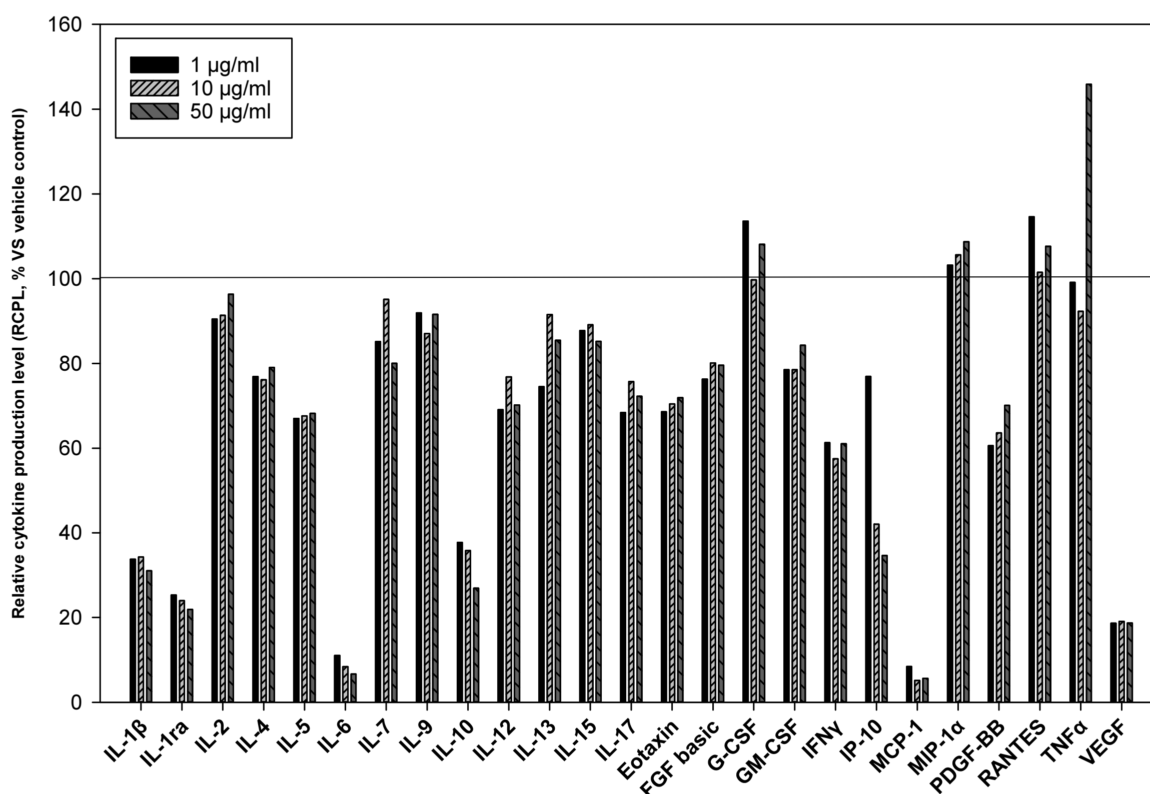

Fig 1. Relative cytokine production level (RCPL, %) of cytokines/chemokines produced from THP-1 cells treated with dexamethasone at 1, 10, and 50 μg/ml. THP-1 cells were treated with dexamethasone along with 1 μg/ml lipopolysaccharide for 24h. The RCPL was calculated by dividing the amount of cytokine produced at each concentration (0.01X, 0.1X, 0.5X CV75%) of dexamethasone by the amount of cytokine produced for the vehicle control, and multiplied with 100

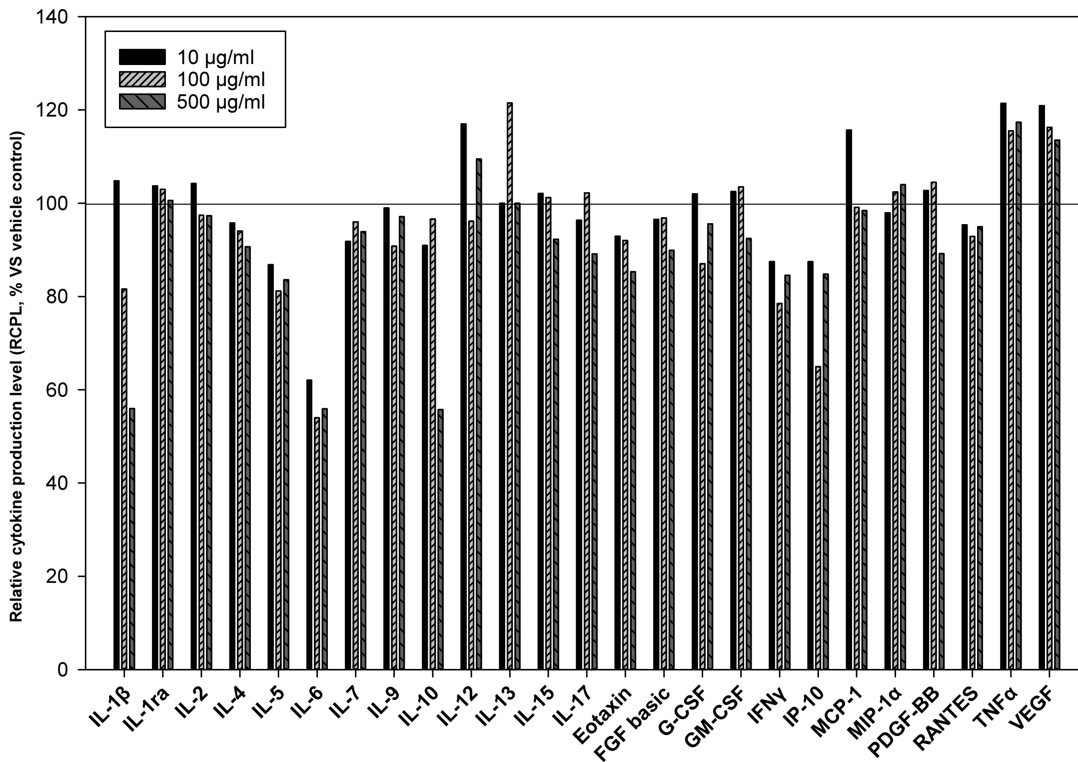

Fig 2. Relative cytokine production level (RCPL) of cytokines/chemokines produced from THP-1 cells treated with cyclophosphamide at 10, 100, and 500 μg/ml. Details on stimulation condition and calculation of RCPL are the same as Fig 1.

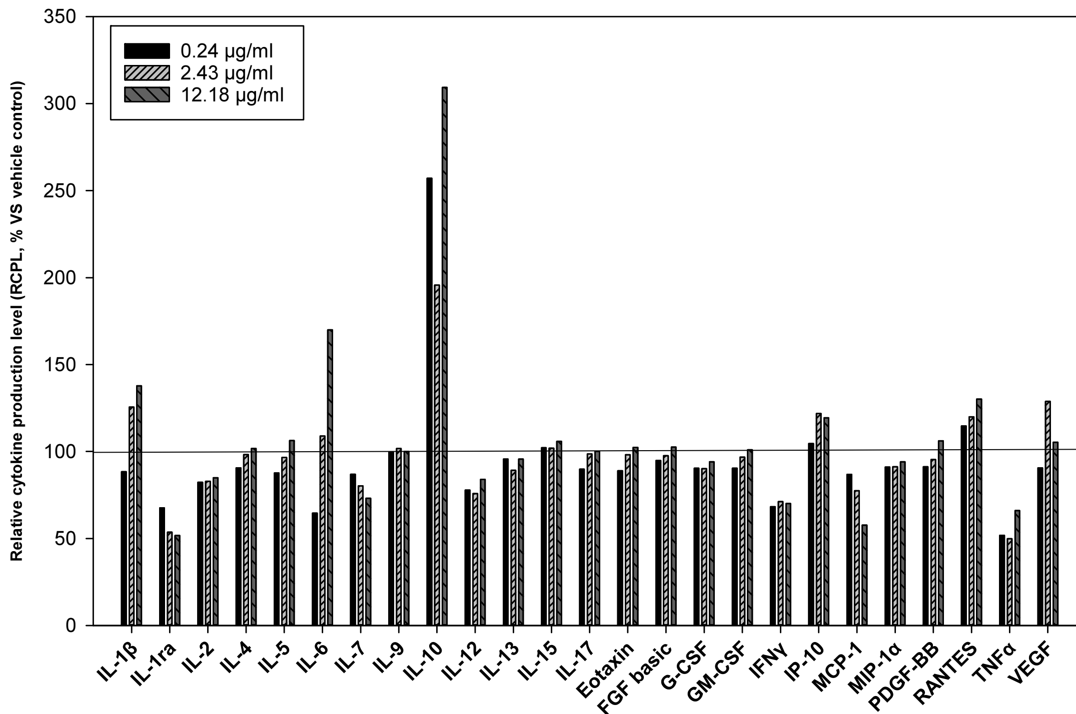

Fig 3. Relative cytokine production level (RCPL) of cytokines/chemokines produced from THP-1 cells treated with cyclosporine at 0.24, 2.43, and 12.18 μg/ml. Details on stimulation condition and calculation of RCPL are the same as Fig 1.

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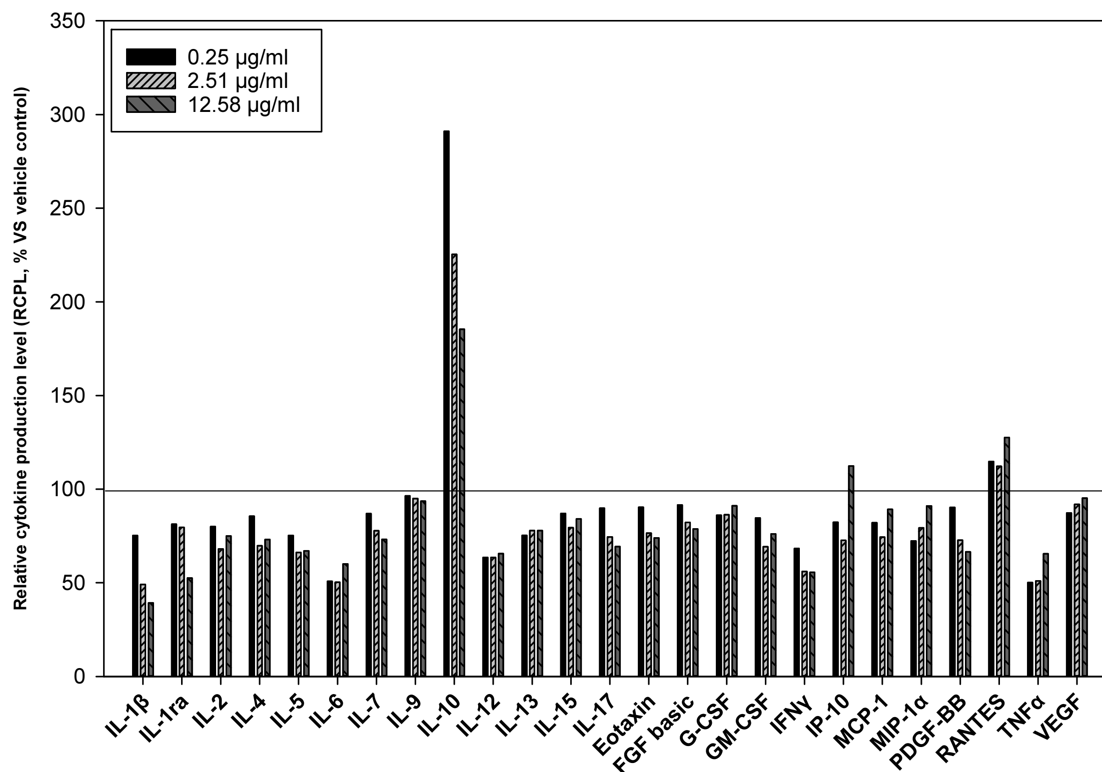

Fig 4. Relative cytokine production level (RCPL) of cytokines/chemokines produced from THP-1 cells treated with tacrolimus at 0.25, 2.51, and 12.58 μg/ml. Details on stimulation condition and calculation of RCPL are the same as Fig 1.

100% were calculated for IL-1β (0.1X-126%, 0.5X-138%), IL-4 (0.5X-101%), IL-5 (0.5X-106%), IL-6 (0.1X-109%, 0.5X-170%), IL-9 (0.1X-102%), IL-10 (0.01X-257%, 0.1X-196%, 0.5X-309%), IL-15 (0.01X-102%, 0.1X-102%, 0.5X-106%), eotaxin (0.5X-102%), FGF-basic (0.5X-103%), GM-CSF (0.5X-101%), IP-10 (0.01X-105%, 0.1X-122%, 0.5X-119%), PDGF-BB (0.5X-106%), RANTES (0.01X-115%, 0.1X-120%, 0.5X-130%), and VEGF (0.1X-129%, 0.5X-105%).

The treatment of tacrolimus led to downregulation of 22 cytokines including IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF-basic, G-CSF, GM-CSF, IFNγ, MCP-1, MIP-1α, PDGF-BB, TNFα, and VEGF, which demonstrated the RCPLs lower than 100% at all the three test concnetrations (Fig. 4). While 3 cytokines exhibited higher RCPL than 100%; IL-10 (0.01X-291%, 0.1X-225%, 0.5X-185%), IP-10 (0.5X-112%), and RANTES (0.01X-115%, 0.1X-112%, 0.5X-128%).

Discussion

The present investigation delineates the occurrence of immunological events associated with 4 potent immunosuppressant drugs, dexamethasone, cyclophosphamide, cyclosporine and tacrolimus on THP-1 cell. Dexamethasone, a corticosteroid is widely used in cancer treatment (Wang et al., 2004). It blocks the naive T cell proliferation and differentiation thus suppressing the production of cytokines by immune cells (Wang et al., 2007). This corticosteroid suppress IL-2 mediated T cell proliferation and production of cytokine (Bianchi et al., 2000). Dexamethasone inhibits naive T cell proliferation and differentiation in human T cell and murine glioblastoma by attenuating the CD28 costimulatory pathway (Giles et al., 2018). Similar findings were observed in our study with downregulation in the production of cytokines or chemokines in dexamethasone-treated cells compared to vehicle control indicated

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by 21 cytokines (IL−1β, IL−1ra, IL−2, IL−4, IL−5, IL−6, IL−7, IL−9, IL−10, IL−12, IL−13, IL−15, IL−17, Eotaxin, FGF−basic, GM−CSF, IFN γ, IP−10, MCP−1α, PDGF−BB, VEGF) with lower RCPL%, thus a potent suppressor.

A very early study revealed the immunosuppression effect of cyclophosphamide on leucocyte, lymphocyte, and antibody formation using a rat model (Winkelstein, 1973). Low dose cyclophosphamide was associated with tumor growth suppression with an increase in TNFα mRNA expression (Tongu et al., 2010). Also study demonstrated that low dose cyclophosphamide show immunomodulation of regulatory T cells, decreasing the secretion of IFNγ and IL−2 while increasing IL−4 and IL−10 in cerebrospinal fluid and peripheral blood (Ahlmann and Hempel, 2016). A similar result was followed by our study demonstrating 11 cytokines (IL−4, IL−5, IL−6, IL−7, IL−9, IL−10, Eotaxin, FGF−basic, IFNγ, IP−10, RANTES) with the lower RCPL than 100% at all the three concentrations.

Cyclosporine and tacrolimus are immunosuppressive drugs widely used in cancer treatment, organ rejection, and inflammatory disorder (Brazelton et al., 1996; Matsuda and Koyasu, 2000; Zhai et al., 2011). The mechanism of action of both drugs are similar belonging to calcineurin inhibitor. These drugs after inhibiting calcineurin suppress the dephosphorylation of nuclear factor of activated T cells resulting in suppression of cytokine production by T lymphocytes (Naesens et al., 2009). Studies have also revealed the inhibition of T lymphocyte to cause inhibition of other inflammatory cytokines which in turn downregulates the production of other cytokines as well (Shoughy et al., 2016). Similar findings were supported by this study, demonstrating lower cytokine or chemokine production in cyclosporine indicated by RCPL below 100% at all the three concentrations in 10 cytokines (IL−1ra, IL−2, IL−7, IL−12, IL−13, GCSF, IFNγ, MCP−1, MIP−1α, TNFα). Similarly, treatment of tacrolimus showed 22 cytokines (IL−1β, IL−1ra, IL−2, IL−4, IL−5, IL−6, IL−7, IL−9, IL−12, IL−13, IL−15, IL−17, Eotaxin, G−CSF, GM−CSF, IFNγ, IP−10, MCP−1, MIP−1α, PDGF−BB, TNFα, VEGF) with RCPL below 100% at all the three concentrations.

No clear distinction was observed with the patterns or characteristics of those immunosuppressants−mediated downregulation of various cytokines or chemokines evaluated in the present study. The most previous investigations for assessing immunosuppressive effects of those drugs have been focusing activation or proliferation of T lymphocytes and have not much examined for downregulatory effects on antigen presenting cells like as THP−1 cell line with dendritic cell lineage (Diehl et al., 2017). Dexamethasone, a typical glucocorticoidal immunosuppressant, and tacrolimus, calcineurin inhibitor via binding to FK506 binding protein (FKBP12), exhibited stronger immunosuppressive potency than cytostatic cyclophosphamide and direct calcineurin inhibitir cyclosporine (Zhang et al., 2019). In addition, chemotactic factors such as RANTES or IP−10 (Akdis et al., 2016) secreted from THP−1 cells seem not much affected by treatment of dexamethasone, cyclosporine, or tacrolimus since RCPLs of RANTES were higher than 100% for these three suppressants, and RCPLs of IP−10 were higher than 100% for cyclosporine and tacrolimus. In conclusion, although preliminary with limitation on multiple replication of experiment, the present results add information on the immunosuppressive potential of the representative immunosuppressant (dexamethasone, cyclophosphamide, cyclosporine, and tacrolimus) on THP−1 cell line with antigen presenting capacity. Furthemore, tacrolimus and dexamethasone was found to have stronger immune suppression potential than cyclophosphamide and cyclosporine. These results could be useful in prediction of the immunological disturbance induced by application of these drugs, hence valuable for assessing its therapeutic or toxic efficacy.

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References

Akdis M, Aab A, Alturnbulakli C, Azkur K, Costa R, Crameri R, et al. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases. J. Allergy Clin. Immunol. 138: 984-1010.

Ahlmann M and Hemperl G. (2016). The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemother. Pharmacol. 78: 661-671.

Bianchi M, Meng C, and Ivashkiv LB. (2000). Inhibition of IL-2-induced Jak-STAT signaling by glucocorticoids. PNAS. 97: 9573-9578.

Brazelton TR and Morris RE. (1996). Molecular mechanisms of action of new xenobiotic immunosuppressive drugs: tacrolimus (FK506), sirolimus (rapamycin), mycophenolate mofetil and leflunomide. Curr. Opin. Immunol. 8: 710-720.

Cavallasca JA, Costa CA, Maliandi MR, Contini LE, Fernandez de Carrera E, and Musuruana JL. (2015). Severe infections in patients with autoimmune disease treated with cyclophosphamide. Reumatol. Clin. 11: 221-223.

Cilião HL, Ribeiro DL, Camargo-Godoy RB, Specian AF, Serpeloni JM, and Cólus IM. (2015). Cytotoxic and genotoxic effects of high concentrations of the immunosuppressive drugs cyclosporine and tacrolimus in MRC-5 cells. Exp. Toxicol. Pathol. 67: 179-187.

Coecke S, Ballas M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay Robert, Price A, Merten O-W, Stokes W, Cchechtman L, and Stacey G. (2005). Guidance on good cell culture practice. A report of the second ECVAM task force on good cell culture practice. ATLA. 33: 1-27.

Diehl R, Ferrara F, Müller C, Dreyer AY, McLeod DD, Fricke S, and Boltze J. (2017). Immunosuppression for in vivo research: state-of-the-art protocols and experimental approaches. Cell. Mol. Immunol. 14: 146-179.

Gennari A, Ban M, Braun A, Casati S, Corsini E, Dastych J, Descotes J, Hartung T, Hooghe-Peters R, House R, Pallardy M, Pieters R, Reid L, Tryphonas H, Tschirhart E, Tuschl H, Vandebriel R, and Gribaldo L. (2005). The Use of *In Vitro* Systems for Evaluating Immunotoxicity: The Report and Recommendations of an ECVAM Workshop. J. Immunotoxicol. 2: 61-83.

Giles AJ, Hutchinson MKND, Sonnemann HM, Jung J, Fecci PE, Ratnam NM, Zhang W, Song H, Bailey R, Davis D, Reid CM, Park DM, and Gilbert MR. (2018). Dexamethasone-induced immunosuppression: mechanisms and implications for immunotherapy. J. ImmunoTher. Cancer. 6: 51.

Gong JH, Zheng YB, Zhang MR, Wang YX, Yang SQ, Wang RH, Miao QF, Liu XJ, and Zhen YS. (2020). Dexamethasone enhances the antitumor efficacy of Gemcitabine by glucocorticoid receptor signaling. Cancer Biol. Ther. 21: 332-343.

Hartung T and Corsini E. (2013). Immunotoxicology: Challenges in the 21st century and in vitro opportunities. ALTEX. 30: 411-426.

Hinton DM. (1995). Immunotoxicity testing applied to direct food and colour additives:US FDA'Redbook II' guidelines. Hum. Exp. Toxicol. 14: 143-145.

Hirano T. (2007). Cellular pharmacodynamics of immunosuppressive drugs for individualized medicine. Int. Immunopharmacol. 7: 3-22.

Lankveld DP, Van Loveren H, Baken KA, and Vandebriel RJ. (2010). In Vitro testing for Direct Immunotoxicity: State of the Art. Methods Mol. Biol. 598: 401-423.

Matsuda S and Koyasu S. (2000). Mechanisms of action of cyclosporine. Immunopharmacology 47:119-25.

Naesens M, Kuypers DR, and Sarwal M. (2009). Calcineurin inhibitor nephrotoxicity. Clin. J. Am. Soc. Nephrol. 4: 481-508.

Pointon JC, Eagle G, Bailey J, Evans P, Allsup D, and Greenman J. (2010). Thalidomide enhances cyclophosphamide and dexamethasone mediated cytotoxicity towards cultured chronic lymphocytic leukaemia cells. Oncol. Rep. 24: 1315-1321.

Shoughy SS, Jaroudi MO, and Tabbara KF. (2016). Efficacy and safety of low-dose topical tacrolimus in vernal keratoconjunctivitis. Clin. Ophthalmol. 10: 643-647.

Tongu M, Harashima N, Yamada T, Harada T, and Harada M. (2010). Immunogenic chemotherapy with cyclophosphamide and doxorubicin against established murine carcinoma. Cancer Immunol. Immunother. 59: 769-777.

Wang H, Li M, Rinehart JJ, and Zhang R. (2004). Dexamethasone as a chemoprotectant in cancer chemotherapy: hematoprotective effects and altered pharmacokinetics and tissue distribution of carboplatin and gemcitabine. Cancer Chemother. Pharmacol. 53: 459-467.

Wang H, Wang Y, Rayburn ER, Hill DL, Rinehart JJ, and Zhang R. (2007). Dexamethasone as a chemosensitizer for breast cancer chemotherapy: potentiation of the antitumor activity of adriamycin, modulation of cytokine expression, and pharmacokinetics. Int. J. Oncol. 30: 947-953.

Winkelstein A. (1973) Mechanism of Immunosuppression: Effects of Cyclophosphamide on Cellular Immunity. Blood 41: 273-284.

Zhai J, Gu J, Yuan J, and Chen J. (2011). Tacrolimus in the treatment of ocular diseases. BioDrugs 25: 89-103.

Zhang W, Egashira N, and Masuda S. (2019). Recent topics on the mechanisms of immunosuppressive therapy-related neurotoxicities. Int. J. Mol. Sci. 20: 3210.

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