

# Human Leukocyte Antigen-G Expression on Dendritic Cells Induced by Transforming Growth Factor- $\beta$ 1 and CD4 $^{+}$ T Cells Proliferation

Saeid Abediankenari<sup>\*1</sup>, Maryam Ghasemi<sup>2</sup> and Young-June Kim<sup>3</sup>

<sup>1</sup>Dept. of Microbiology and Immunology and <sup>2</sup>Dept. of Pathology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, <sup>3</sup>Dept. of Microbiology and Immunology, Walter Oncology Center, Indianapolis, Indiana, USA

Received 9 June 2010; revised 5 December 2010; accepted 18 December 2010

## ABSTRACT

**Background:** During antigen capture and processing, mature dendritic cells (DC) express large amounts of peptide-MHC complexes and accessory molecules on their surface. DC are antigen-presenting cells that have an important role in tolerance and autoimmunity. The transforming growth factor-beta1 (TGF- $\beta$ 1) cytokine has a regulatory role on the immune and non-immune cells. The aim of this study is to evaluate the effect of TGF- $\beta$ 1 on the induction of human leukocyte antigen-G (HLA-G) expression on the DC which is derived from monocyte. **Methods:** In this study, we evaluated the effect of TGF- $\beta$ 1 in induction HLA-G expression on the monocyte-derived DC by flowcytometry and then CD4 $^{+}$  T cell proliferative responses in the presence of DC-treated TGF- $\beta$ 1 was studied. **Results:** The results of this study showed that DC bearing HLA-G down-regulated activation of CD4 $^{+}$  T cells and production of IL-6 and IL-17 in comparison with control ( $P<0.05$ ). **Conclusion:** It is concluded that TGF- $\beta$ 1 has an important regulatory role in CD4 $^{+}$  T cell proliferation by increasing HLA-G on DC and these cells can probably prevent unexpected immune responses *in vivo*. *Iran. Biomed. J.* 15 (1 & 2): 1-5, 2011

**Keywords:** Human leukocyte antigen-G (HLA-G), Transforming growth factor-beta1 (TGF- $\beta$ 1), Dendritic cell (DC), IL-17

## INTRODUCTION

Mature dendritic cells (DC) are professional antigen-presenting cells that have an important role in the immune tolerance and autoimmunity [1]. DC express not only co-stimulatory but also co-inhibitory molecules on their surface to regulate T cell-mediated adaptive immune responses [2]. Human leukocyte antigen-G (HLA-G1), a non-classical membrane MHC class I antigen, is expressed as a co-regulatory molecule on trophoblasts that protects fetus from maternal immune response during pregnancy [3-6]. In addition, HLA-G molecules can modulate cytokine secretion from T lymphocytes [7-9].

CD4 $^{+}$  naive T cells can be differentiated to T helper (Th) 1, Th2, Th17, Th9, follicular helper T and regulatory T cells by specific cytokines. Trans-

forming growth factor beta1 (TGF- $\beta$ 1), the pleiotropic cytokine, exerts its regulatory functions both directly on immune cell and indirectly through other cell types. This cytokine is one of the key cytokines for immune tolerance and immune regulation [10]. Th17 with the capacity to produce IL-17 has a proinflammatory role [11]. In addition, IL-6 is a cytokine that was initially known as a mediator of cell proliferation and later found as a factor to exhibit a wide variety of biological effects. IL-6 promotes Th17 differentiation and suppresses regulatory T-cell generation [12, 13].

In this study, we evaluated the effect of TGF- $\beta$ 1 on HLA-G expression on DC and CD4 $^{+}$  T cell proliferation response in the presence of TGF- $\beta$ -treated DC (TGF $\beta$ -DC).

\*Corresponding Author; Tel. (+98-912) 1985667; E-mail: abedianks@razi.tums.ac.ir or abedianlab@yahoo.co.uk

## MATERIALS AND METHODS

**Isolation of monocytes and CD4<sup>+</sup> T cells from peripheral blood.** Peripheral blood mononuclear cells were isolated from heparinized blood that is donated by 5 healthy volunteers by centrifugation on Ficoll histopaque 1.077 (Lymphoprep, Norway). Cells from interphases were collected and washed three times with RPMI-1640 medium (Sigma, USA). The number of cells were counted and the cell viability was determined by trypan blue exclusion. Monocytes and CD4<sup>+</sup> T cells were respectively isolated from peripheral blood mononuclear cells by the help of CD14<sup>+</sup> monocyte and CD4<sup>+</sup> T cell isolation kit (Miltenyi Biotec, USA). Purity of CD4<sup>+</sup> T cells and CD14<sup>+</sup> monocyte was >95%, as analyzed by flow cytometry.

**Generation of DC from CD14<sup>+</sup> cells.** CD14<sup>+</sup> monocytes ( $5 \times 10^5$ ) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100  $\mu$ g/ml streptomycin for 5 days with different combinations of purified recombinant human cytokines including 10 ng/ml IL-4 (Serotec, UK), 10 ng/ml TGF- $\beta$ 1 (Serotec, UK) and 10 ng/ml granulocyte macrophage colony stimulating factor (GM-CSF, Serotec, UK) to generate immature DC. To induce maturation of DC, the immature DC was incubated with 1  $\mu$ g/ml bacterial LPS for additional 48 h before cells were harvested [14].

**Flowcytometry.** Cells were stained with FITC-conjugated anti-CD83, HLA-G and PE-conjugated anti-HLR-Dr, CD86 (eBioscience, USA) or isotype control antibodies (eBioscience, USA) at 4°C for 30 min. Surface expressions of the antigens were measured by flowcytometry (Becton Dickenson, USA).

**Co-culture of DC with CD4<sup>+</sup> T cells.** CD4<sup>+</sup> T cells ( $1 \times 10^5$ ) and allogenic TGF $\beta$ -DC ( $1 \times 10^4$ ) or control (DC) from five different donors were separately cultured in 96 U-shape bottom plates (200  $\mu$ l per well). All samples were run in triplicates and cells were incubated in a humidified 5% CO<sub>2</sub> atmosphere at 37°C for 5 days. Proliferative T cells to TGF $\beta$ -DC or DC, were measured by pulsing the cells with 200  $\mu$ l 3-[4,5-dimethylthiazolyl]-2,5-diphenyl-tetrazolium bromide (MTT, 0.5 mg/ml, Sigma, USA) for 4 h and reading the color changes at 575 nm wave length [15].

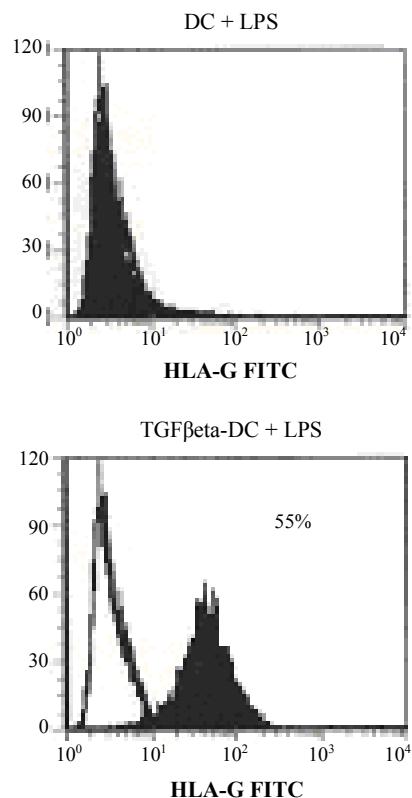
### Determination of cytokines in supernatant.

Supernatants of DC-T cell co-cultures were harvested after 5 days and frozen for subsequent cytokine analysis. Concentrations of IL-17 and IL-6 in the supernatants of culture media were measured by ELISA using respective assay kits (PeproTech, USA). The assays were performed in duplicate.

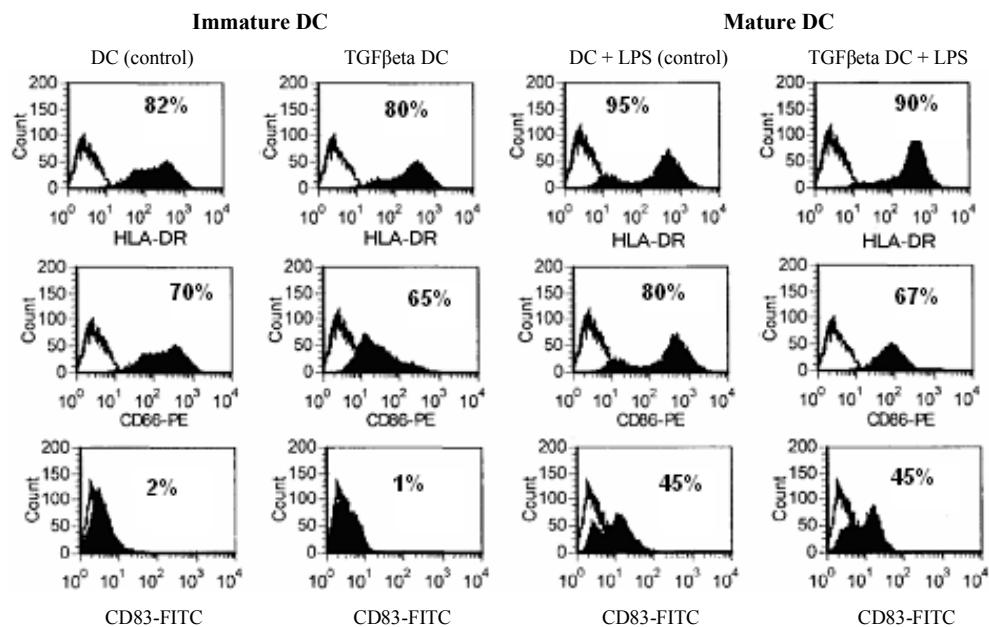
**Statistical analysis.** Data are presented as mean  $\pm$  SD. For statistical analysis, paired *t*-tests were used and the *P* values were determined in all cases.

## RESULTS

**HLA-G expression on DC by TGF- $\beta$ 1.** DC generated with GM-CSF plus IL-4 (control DC) did not express HLA-G on their surface even after LPS stimulation. Presence of TGF- $\beta$ 1 during *in vitro* DC generation substantially increased expression of HLA-G on DC (TGF $\beta$ -DC) in comparison with the DC generated without TGF- $\beta$ 1 ( $P < 0.05$ ) as observed by flowcytometry (Figs. 1 and 2). Initial



**Fig. 1.** Effect of TGF- $\beta$ 1 in expression of HLA-G on mature DC (below). TGF- $\beta$ 1 substantially raised the density of HLA-G molecules on DC *in vitro* in comparison with control ( $P < 0.05$ ).



**Fig. 2.** Analysis of DC phenotype showing the expression of HLA-DR, CD86 on the TGF $\beta$ -DC and DC before stimulation with LPS (immature DC). LPS significantly affected by up regulating surface level of HLA-DR and CD86 on two DC (mature DC).

CD14 $^{+}$  cells did not express HLA-G (data not shown). CD83 is transiently up-regulated on DC after activation and can be used as a reliable marker for matured cells.

**IL-17 and IL-6 results.** The cells significantly produced lower levels of IL-17 and IL-6 when they were co-cultured with CD4 $^{+}$  T cells (Table 1).

**TGF $\beta$ -DC led to lower allogenic CD4 $^{+}$  T cell proliferation compared to control DC upon co-culture.** We compared TGF $\beta$ -DC and control DC according to the ability to induce allogenic CD4 $^{+}$  T cell proliferation by the means of MTT proliferation assay. It was clear that TGF $\beta$ -DC and control DC showed differential ability to induce allogenic CD4 $^{+}$  T cell proliferation. CD4 $^{+}$  T cells co-cultured with TGF $\beta$ -DC proliferated at significantly lower degree (mean  $\pm$  SD of optical density,  $139 \pm 14.3$ ) compared to those with control DC (mean  $\pm$  SD,  $170 \pm 28.2$ ) ( $P = 0.02$ ). These results indicate that TGF- $\beta$ 1 during DC generation not only induces HLA-G but also suppresses allogenic CD4 $^{+}$  T cell response.

## DISCUSSION

In the present study, we generated DC with an

inhibitory role in CD4 $^{+}$  T cell proliferation *in vitro*. Many experimental systems have demonstrated that DC bearing HLA-G acquires tolerogenic potential to down-regulate various immune activities [5, 7, 16]. Our results showed that these cells down-regulated the secretion of IL-6 and IL-17 cytokines from CD4 $^{+}$  T Cell (Table 1).

We have previously reported that TGF- $\beta$ 1 induces differentiation of peripheral blood monocytes into DC with regulatory function [14]. This study demonstrated that presence of TGF- $\beta$ 1 with GM-CSF and IL-4 results in generation of DC expressing HLA-G (Fig. 1). HLA-G is an inhibitory constituent [9, 17] and therefore, it is possible that HLA-G which is expressed on DC by TGF- $\beta$ 1 exert a major effect on suppressing CD4 $^{+}$  T cell proliferative response. In the present study, the inhibition of T cells proliferation was observed only when cells

**Table 1.** IL-17 and IL-6 concentrations (pg/ml) in the supernatant of mixed leukocyte reactions (MLR) between DC and T cells after 5 days. Data are the mean  $\pm$  SD.

MLR \ Cytokine	IL-17	IL-6
TGF $\beta$ -DC + T	$41.8 \pm 7.66$	$92.6 \pm 22.66$
DC + T cell	$89.2 \pm 15.22$	$227 \pm 57.4$
P value	<0.05	<0.05

**Table 2.** Effect of TGF $\beta$ -DC and control DC on allogenic CD4 $^{+}$  T cell proliferation in MLR as evaluated by MTT assay. Data were obtained by measuring optical density at 575 nm.

MLR	mean $\pm$ SD	P value
TGF $\beta$ -DC + CD4 $^{+}$ T cell (1:10)	139 $\pm$ 14.3	
DC + CD4 $^{+}$ T cell (1:10)	170 $\pm$ 28.2	0.02

were treated with TGF $\beta$ -DC (Table 2). Thus, TGF- $\beta$ 1 and HLA-G molecules on the DC are two components contributing to this phenomenon, limiting cells proliferation, thereby reducing the potential of T-cells comparing with control. The ability of TGF $\beta$ -DC to induce allogenic T cell activation, which was leading to proliferation, markedly impaired. This inhibition can reflect levels of co-stimulatory molecules on the cell surface compared to the control DC after LPS stimulation [18]. Thus, it is also possible that the reduction of co-stimulatory molecules on DC as a result of TGF- $\beta$ 1 treatment may contribute to less allogenic CD4 $^{+}$  T cell proliferative response to TGF $\beta$ -DC (Fig. 2). IL-17 and IL-6 promote Th17 differentiation while they suppress T regulatory development [19, 20]. Toh *et al.* [21] showed that IL-17 inhibits human Th1 differentiation through IL-12R $\beta$ 2 down-regulation. Our results also suggest that tolerogenic effects of TGF- $\beta$ 1 may be accomplished by down-regulation of proinflammatory cytokines production.

According to our data, we would like to suggest that TGF $\beta$ -DC may be a critical subset of DC to limit undesirable immune response by suppressing adaptive T-cell activities.

## ACKNOWLEDGMENTS

This study was supported by a grant (No.87-116) from Mazandaran University of Medical Sciences (Iran). The authors are grateful to Rosa Abedian for technical assistance.

## REFERENCES

- Chen, L. (2004) Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol.* 4: 336-347.
- Fogel-petrovic, M., Long, J.A., Misso, N.L., Foster, P.S., Bhoola, K.D. and Thompson, P.J. (2007) Physiological concentrations of transforming growth factor beta1 selectively inhibit human dendritic cell function. *Int. Immunopharmacol.* 7: 1924-33.
- Racca, A.L., Veaute, C.M., Bailat, A.S., Gaite, L., Arriola, M., Hajos, S.E. and Malan Borel, I.S. (2009) Expression of HLA-G and MICA mRNA in renal allograft. *Transpl. Immunol.* 21: 10-12
- Selmani, Z., Naji, A., Gaiffe, E., Obert, L., Tiberghien, P., Rouas-Freiss, N., Carosella, E.D. and Deschaseaux, F. (2009) HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. *Transplantation* 87:62-66.
- Giannopoulos, K., Dmoszyńska, A., Bojarska-Junak, A., Schmitt, M. and Rolinski, J. (2008) Expression of HLA-G in patients with B-cell chronic lymphocytic leukemia (B-CLL). *Folia. Histochem. Cytobiol.* 46: 457-460.
- Levitsky, J., Miller, J., Wang, E., Rosen, A., Flaa, C., Abecassis, M., Mathew, J. and Tambur, A. (2009) Immunoregulatory profiles in liver transplantation on different immunosuppressive agents. *Hum. Immunol.* 70: 46-50.
- Abediankenari, S., Shaker, D., Abedian, F. and Mirabi, A. (2009) The effect of beta interferon on dendritic cells and cytokine synthesis by CD4 $^{+}$  T cells. *Iran. J. Immunol.* 6: 661- 666.
- Román, A., Rodríguez, M., Herraiz, M.A., Jordá, J., Cervera, I., Peñaloza, J., Vidart, J.A. and Martínez-Laso, J. (2009) Heterogeneous expression of HLA-G1, -G2, -G5, -G6, and -G7 in myeloid and plasmacytoid dendritic cells isolated from umbilical cord blood. *Hum. Immunol.* 70:104-109.
- Knafel, A., Basta, P., Pitynski, K., Mach, P., Bednarek, W., Klimek, M., Zietek, J., Zajac, K., Dancewicz, L., Iwaniec, M., Basta, A. and Wichererek, L. (2009) Soluble HLA-G changes in maternal blood serum during the progression of labor. *Neuro. Endocrinol. Lett.* 30: 67-73.
- Nasef, A., Chapel, A., Mazurier, C., Bouchet, S., Lopez, M., Mathieu, N., Sensebe, L., Zhang, Y., Gorin, N.C., Thierry, D. and Fouillard, L. (2007) Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells. *Gene. Expr.* 132: 217-226.
- Afzali, B., Lombardi, G., Lechler, R.I. and Lord, G. M. (2007) The role of T helper 17 (Th17) and regulatory T cells (Treg) in human and organ transplantation and autoimmune disease. *Clin. Exp. Immunol.* 148: 32-46.
- Wang, L., Kortylewski, T., Yi, P., Pardoll, M. D.M., Zeng, D., Yu, H. (2009) IL-17 can promote tumor growth through an IL-6-state3 signaling pathway. *J. Exp. Med.* 16: 1457-64.
- Witowski, J., Pawlaczyk, K., Breborowicz, A., Scheuren, A., Kuzlan-Pawlaczyk, M., Wisniewska, J., Polubinska, A., Friess, H., Gahl, G.M., Frei, U. and Jörres, A. (2000) IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* 165:5814-1821.

14. Abediankenari, S. and Ghasemi, M. (2009) Generation of immune inhibitory dendritic cells and CD4<sup>+</sup> T regulatory cells inducing by TGF- $\beta$ . *Iran. J. Allergy Asthma Immunol.* 8: 25-30.
15. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65:55-63.
16. Wu, J. and Horuzsko, A. (2009) Expression and function of immunoglobulin-like transcripts on tolerogenic dendritic cells. *Hum. Immunol.* 70: 353-356.
17. Baricordi, O.R., Stignani, M., Melchiorri, L. and Rizzo, R. (2008) HLA-G and inflammatory diseases, Inflamm, Allergy. *Drug Targets* 7: 67-74.
18. Wang, Q., Chen, Y., Xie, F., Ge, Y., Wang, X. and Zhang, X. (2009) A novel agonist anti-human OX40L monoclonal antibody that stimulates T cell proliferation and enhances cytokine secretion. *Hybridoma (Larchmt)* 28: 269-276.
19. Morishima, N., Mizoguchi, I., Takeda, K., Mizuguchi, J. and Yoshimoto, T. (2009) TGF-beta is necessary for induction of IL-23R and Th17 differentiation by IL-6 and IL-23, *Biochem. Biophys. Res. Commun.* 14:105-110.
20. Yamazaki, S. and Steinman, R.M. (2009) Dendritic cell as controllers of antigen-specific Foxp3<sup>+</sup> regulatory T cells. *J. Dermatol. Sci.* 54:69-75.
21. Toh, M., Kawashima, M., Zrioual, S., Hot, A., Miossec, P., Miossec, P. (2009) IL-17 inhibits human Th1 differentiation through IL-12R $\beta$ 2 down-regulation. *Cytokine* 48: 226-230.