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CTLA-4 expressed by human dendritic cells modulates their cytokine secretion and induction of T cell proliferation

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Abstract

CTLA-4 is the major negative regulator of T cell responses. We have analyzed the expression of CTLA-4 in human monocytes and monocyte-derived DCs and the effects of its engagement on cytokine production and T cell stimulatory activity by mature DCs (mDCs). We found that CTLA-4 was highly expressed on freshly isolated monocytes, then down-modulated on the immature DCs (iDCs) and upregulated on mDCs. Treatment of mDCs with an agonistic anti-CTLA-4 mAb enhanced secretion of IL-10 but reduced secretion of IL-8 and IL-12, as well as autologous CD4+ T-cell proliferation in response to stimulation with PPD recall antigen-loaded-DCs. Neutralization of IL-10 with an anti-IL-10 antibody partially restored the ability of anti-CTLA-4-treated mDCs to stimulate T cell proliferation in response to PPD. Our data provide the first evidence that CTLA-4 receptor is expressed by human mDCs and exerts immune modulatory effects in these cells.

Introduction

CTLA-4 (cytotoxic T lymphocyte antigen-4) is the most important negative regulator of T cell proliferation and function. Initially identified in conventional and regulatory T cells, its expression has been extended to a variety of non-T cells, either normal or neoplastic, including activated B cells, monocytes, placental fibroblasts, muscle cells [Reviewed in ref. 1], leukemic, breast and melanoma tumour cells [2], although its role in these cell types has not yet been clarified. CTLA-4 inhibitory function in T cells mainly occurs upon engagement with the B7 ligands (CD80/CD86) expressed on antigen presenting cells, leading to inhibition of both cytokine production and T cell proliferation through either interference with CD28 positive costimulation or association with signaling molecules (PI3K, SHP2, PP2A) [Reviewed in ref. 1].
In this study, we have analyzed the expression of CTLA-4 in human monocytes and monocyte-derived DCs and the functional effects of its engagement with an agonistic anti-CTLA-4 antibody on cytokine production and T cell stimulatory activity by mature DCs.

Materials and Methods

Generation of DC. Human monocytes (MOs) were purified from peripheral blood mononuclear cells (PBMCs) of voluntary healthy donors upon informed consent. Monocytes, immunoselected with a CD14 antibody (Miltenyi Biotec), were cultured with IL4+GM-CSF (Euroclone), at 25 ng/ml and 20 ng/ml, respectively, to differentiate into immature DCs (iDCs). iDCs were further stimulated with different maturation stimuli including lipopolysaccharide (LPS; Sigma) at 100 ng/ml, Polyl:C (Calbiochem, DBA) at 25 mg/ml, or cytokine cocktail (Euroclone) at 10 ng/ml, to generate mature DCs (mDCs).
Flow Cytometry. CTLA4 expression was evaluated on MOs, iDcs and mDcs by surface staining with the FITC-conjugated mAb 48815 and the PE-conjugated anti-CTLA-4 polyclonal antibody (both from R&D Systems) and by cytoplasmic staining of permeabilized cells with the anti-CTLA-4 mAb 14D3 (eBioscence) followed by a FITC-conjugated isotype-specific secondary antibody (Southern Biotechnology). *Transcriptional analysis.* RT-PCR was performed on MOs, iDcs and mDcs using a set of primers specific for membrane (CTLA4 TM, 348bp) and soluble (CTLA4 delTM, 238bp) CTLA-4 isoforms.
Functional analysis. Functional studies were performed after culturing iDCs in the presence of an agonistic anti-CTLA4 mAb (3D5 clone) [3] or an isotype-matched control mAb.

Culture supernatants were tested after 24 and 48h for the presence of IL-8, IL-10 and IL-12p70 cytokines by ELISA (Bender MedSystems). The ability of mDCs to stimulate T cell proliferation was evaluated by co-culturing $CD4^+$ autologous T lymphocytes with mDCs pretreated with anti-CTLA-4 or IgG1 isotype control mAb (CTR1) and pulsed with antigen purified protein derivative (PPD) (Statens Serum Institut). The experiment was performed in the presence or absence of neutralizing anti-IL-10 mAb (10 mg/ml, 23738 clone, IgG2b, R&D Systems) or isotype control mAb (CTR2) (MOPC-141, Sigma-Aldrich). All cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (Amersham Biosciences) on day 4, processed and counted in a gamma counter (Beckman).

Statistical analysis. The paired two-tailed Student's t test was used with *P* values (significance level <0.05) further adjusted with Bonferroni correction for multiple comparisons (*P* indicated as "*Pc*").

Results

CTLA-4 expression, analyzed by flow cytometry, was found on the surface of freshly isolated MOs, then down-modulated upon differentiation toward iDCs and markedly upregulated on mDCs from 7 independent donors, upon different stimulations (LPS, Poly I:C, cytokine cocktail). Representative data show surface expression on LPS-stimulated DCs (mDCs *vs* iDCs, *Pc*=0.002) (fig. 1A) and high levels of cytoplasmic CTLA-4 expression in all cellular types analyzed (fig. 1B). RT-PCR analysis showed that MOs express both transcript variants, whereas iDCs and mDCs express high levels of CTLA-4TM, but barely detectable levels of CTLA-4delTM transcript (fig. 1C).

Treatment of mDCs with the agonistic anti-CTLA-4 mAb significantly enhanced secretion of regulatory IL-10 but reduced secretion of IL-8 and IL-12 pro-inflammatory cytokines as shown for 3 donors (tab. 1).

	Cytokines		
Antibody	**IL-8**	**IL-12p70**	**IL-10**
CTR1-24h (isotype)	56.0 ± 9.8	144.1 ± 13.3	31.8 ± 8.1
anti-CTLA-4 24h	11.3 ± 7.3	94.5 ± 18.3	73.5 ± 6.8
Pc	*0.007*	*0.144*	*0.126*
CTR1-48h (isotype)	91.1 ± 27.4	268.1 ± 48.9	64.0 ± 25.9
anti-CTLA-4 48h	57.2 ± 22.4	201.2 ± 29.4	135.7 ± 16.3
Pc	*0.280*	*0.462*	*0.008*

Table 1. Cytokine secretion by anti-CTLA-4-treated mDCs, as detected by ELISA.

Moreover, we observed a significant reduction of autologous $CD4^+$ T-cell proliferation in response to stimulation with anti-CTLA-4-pretreated and PPD-loaded-mDCs (tab. 2). T-cell proliferation was partially restored by neutralization of IL-10 with an anti-IL-10 antibody (tab. 2).

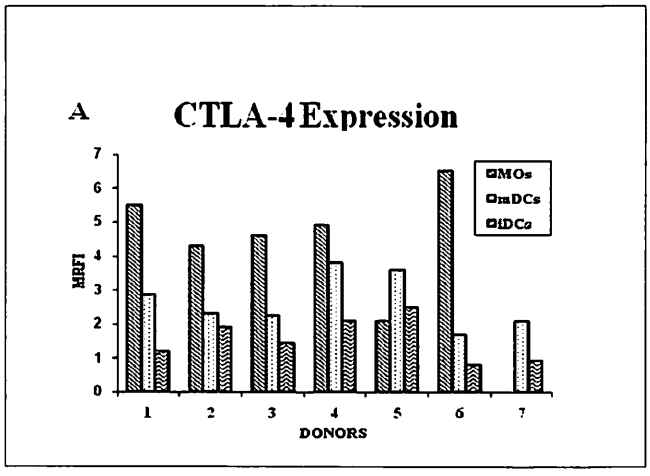

Figure 1. CTLA-4 expression in human peripheral monocytes (MOs), monocyte-derived immature DCs (iDCs) and LPS-matured DCs (mDCs), analyzed by flow cytometry (A, B) and RT-PCR (C)

mDCs + $CD4^+$T cells +	Kcpm	
isotype CTR1/2+PPD	26.7 ± 0.3	
anti-CTLA-4+isotype CTR2+PPD	15.6 ± 0.6	*Pc=0.034*
anti-CTLA-4+anti-IL10+PPD	20.9 ± 1.3	
medium	2.5 ± 0.2	
mDCs alone	1.6 ± 0.0	
$CD4^+$T cells alone	1.8 ± 0.2	

Table 2. Proliferation of autologous $CD4^+$ T cells induced by anti-CTLA-4-pretreated and PPD-loaded-mDCs, as detected by [^{3}H]thymidine uptake.

Discussion

Our data provide the first evidence that CTLA-4 receptor is expressed at protein and transcriptional level by human monocyte-derived mDCs upon their full activation and exerts immune modulatory effects. CTLA-4 signal in mDCs could play an active role in modulating the immune response, by reducing pro-inflammatory and chemoattractant factors such as IL-8 and IL-12 and by increasing the secretion of IL-10. In addition, CTLA-4 binding down-modulates $CD4^+$ T cell proliferation induced by LPS-matured DCs in response to PPD recall antigen. This event might be in part mediated by upregulation of IL-10 in synergy with other factors, as the addition of neutralizing anti-IL-10 mAb only partially restored mDC-induced T cell proliferation. Thus, CTLA-4 expressed by matured DCs might represent a negative regulatory mechanism to prevent the excessive activation of T cells. We propose that this mechanism could result from the signaling delivered by CTLA-4 to mDCs upon engagement with B7 molecules expressed by mDCs themselves, or alternatively, with B7 molecules expressed by activated T cells. In fact, T cell proliferation and survival might be reduced through activation of the indoleamine 2,3-dioxygenase enzyme responsible for tryptophan catabolism [4], in the former case and through transduction of an apoptotic signal to T cells [5], in the latter case.

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References

[1] Teft W.A., Kirchhof M.G., Madrenas J. 2006. A molecular perspective of CTLA-4 function. Annu. Rev. Immunol., 24: 65-97.

[2] Contardi E., Palmisano G.L., Tazzari P.L., Martelli A.M., Falà F., Fabbi M., Kato T., Lucarelli E., Donati D., Polito L., Bolognesi A., Ricci F., Salvi S., Gargaglione V., Mantero S., Alberghino M., Ferrara G.B., Pistillo M.P. 2005. CTLA-4 is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction. Int. J. Cancer., 117: 538-550.

[3] Merlo A., Tenca C., Fais F., Battini L., Ciccone E., Grossi C.E., Saverino D. 2005. Inhibitory receptors CD85j, LAIR-1, and CD152 down-regulate immunoglobulin and cytokine production by human B lymphocytes. Clin. Diagn. Lab. Immunol., 12: 705-712.

[4] Fallarino F., Grohmann U., Hwang K.W., Orabona C., Vacca C., Bianchi R., Belladonna M.L., Fioretti M.C., Alegre M.L., Puccetti P. 2003. Modulation of tryptophan catabolism by regulatory T cells. Nat. Immunol., 4: 1206-1212.

[5] Greenwald R.J., Freeman G.J., Sharpe A.H. 2005. The B7 family revisited. Annu. Rev. Immunol., 23: 515-548.