

Epigallocatechin gallate affects human dendritic cell differentiation and maturation

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Background: Epigallocatechin gallate (EGCG), a component of green tea catechin with the strongest biological activity, has been focused in recent years because of its anti-inflammatory and immunomodulatory activities. Dendritic cells (DCs) are professional antigen-presenting cells, capable of priming naive T cells, and play the key roles in the activation of T-cell-mediated immune responses.

Objective: We aimed to investigate the effect of EGCG on human monocyte-derived DCs (MODCs) and, consequently, on the T-cell-mediated immune response.

Methods: The induction of apoptosis, and the detailed phenotypic and functional changes of MODCs, generated by culture of peripheral blood monocytes in the presence of GM-CSF and IL-4, induced by EGCG was investigated and compared with the effects of dexamethasone.

Results: Epigallocatechin gallate induced apoptosis and affected the phenotype of the developing DCs. The expressions of CD83, CD80, CD11c, and MHC class II, which are molecules essential for antigen presentation by DCs, were downregulated by EGCG. EGCG also suppressed the endocytotic ability of immature DCs, whereas dexamethasone-treated DCs had higher endocytotic ability than control DCs. Most importantly, mature DCs treated with EGCG inhibited stimulatory activity toward allogeneic T cells while secreting high amounts of IL-10. **Conclusion:** Epigallocatechin gallate induces immunosuppressive alterations on human MODCs, both by induction of apoptosis and suppression of cell surface molecules and antigen presentation.

Clinical implications: These alterations should be considered promising new immunosuppressive and anti-inflammatory agents to treat autoimmune and allergic diseases and to prevent

the graft rejection in organ transplantation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Dendritic cells, EGCG, IL-10, antigen presentation, maturation, corticosteroids

Dendritic cells (DCs), one of the most potent antigen presenting cells *in vivo*, represent a terminally differentiated stage of monocytes.¹ Peripheral blood monocytes cultured *in vitro* with GM-CSF and IL-4 differentiate into nonadherent CD1a⁺CD14^{low/-} immature DCs, which show a high ability for antigen uptake. Pathogen-related molecules such as LPS and inflammatory cytokines stimulate maturation of DCs and their subsequent migration to lymphoid organs, where they present antigens to T cells. This conversion from antigen-capturing immature DCs to an antigen-presenting mode is defined as DC maturation.^{1,2}

Polyphenols are the most significant group of tea components, especially the catechin group of the flavonols. The major tea catechins are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). The anti-inflammatory, antioxidative,^{3,4} and cancer-preventative⁵⁻⁸ properties of tea polyphenols, especially EGCG, have been well documented. However, how these inhibitory responses are modulated by EGCG is not well characterized.

Recently, we reported that EGCG had an inhibitory effect on CD8⁺ T cell-mediated inflammation⁹ and also induced apoptosis of human monocytes,¹⁰ a mechanism similar to the immunosuppressive effect of glucocorticoids. Anti-inflammatory glucocorticoids, such as dexamethasone, are also known as promising agents for developing tolerogenic DCs.¹¹⁻¹⁵ Here, we aimed to investigate the effect of EGCG on the differentiation and maturation processes of human monocyte-derived DCs (MODCs), the key cells responsible for the initiation of T cell-mediated immunity. We demonstrate that exposure of monocytes to EGCG together with GM-CSF and IL-4 inhibited their differentiation to DCs and, in addition, induced apoptosis of DC-precursors and immature DCs, an effect equivalent to the immunosuppressive alterations induced by dexamethasone. Moreover, EGCG blocked the terminal maturation process of already differentiated DCs.

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Abbreviations used

DC:	Dendritic cell
EC:	Epicatechin
ECG:	Epicatechin gallate
EGC:	Epigallocatechin
EGCG:	Epigallocatechin gallate
FITC:	Fluorescein isothiocyanate
MFI:	Mean fluorescence intensity
MLR:	Mixed leukocyte reaction
MODC:	Monocyte-derived dendritic cell
PI:	Propidium iodide
rFI:	Relative fluorescence intensity

METHODS**Reagents**

All cultures were performed in AIM-V (GIBCO, Grand Island, NY) supplemented with 10% human serum of blood group type AB. Recombinant human GM-CSF and recombinant human IL-4 were obtained from R&D Systems (Minneapolis, Minn).

Epicatechin, ECG, EGC, and EGCG were purchased from Sigma (St Louis, Mo). Dexamethasone and LPS (*Escherichia coli*; serotype 026:B6) were also from Sigma.

Culture of DCs

Approval for all experiments with human blood was provided by the Human Ethics Committees of the University of Tokyo. PBMCs from venous blood of healthy volunteers were prepared as previously described.⁹ PBMCs isolated on Ficoll-Paque (Amersham Pharmacia, Uppsala, Sweden) were subjected to plastic adherence for 2 hours (37°C) in culture medium. Adherent cells were cultured in the presence of 500 U/mL GM-CSF and 500 U/mL IL-4. On days 3 and 5 of culture, the medium was replaced, and on day 7, the generated immature DCs were treated with different concentrations of EGCG or dexamethasone for another 24 hours. To induce maturation, LPS (1 µg/mL) was added on day 7, and the generated mature DCs were treated with different concentrations of EGCG or dexamethasone for another 24 hours. DC precursors, which are cells in the differentiation process from monocytes into immature DCs, were generated by culture of human monocytes for 5 days in the presence of GM-CSF and IL-4 and treated with EGCG or dexamethasone.

Flow-cytometric analysis of cell apoptosis

Flow cytometry was used for the analysis of apoptosis, after double-staining of DCs with fluorescein isothiocyanate (FITC)-conjugated annexin V and propidium iodide (PI; Bio Vision, Inc, Mountain View, Calif). After treatment for 24 hours with different concentrations of EGCG or dexamethasone, annexin V(-)PI(-) cells were considered viable cells; annexin V(+)PI(-) cells, early apoptotic cells; annexin V(+)PI(+) cells, late apoptotic cells; and annexin V(-)PI(+) cells, necrotic cells. Both early and late apoptotic cells were analyzed together as apoptotic cells. Data collected in a FACS Calibur (Becton Dickinson, San Jose, Calif) and analyzed with the CellQuest software (Becton Dickinson). The generated immature and mature DCs were treated with various catechins (EC, ECG, EGC, and EGCG) for another 24 hours and then analyzed.

Flow-cytometric analysis of cell surface molecules

The changes in the surface expression of DCs were analyzed by flow cytometry. Cells were stained with FITC-conjugated mAbs:

anti-CD1a, anti-CD83, the specific marker of mature DCs,¹⁶ and anti-HLA-DR; or phycoerythrin-conjugated mAbs: anti-CD14, the marker of monocytes,¹⁶ anti-CD11c, anti-CD80 and anti-CD86, the costimulatory factors of the T-lymphocyte activation by DCs¹⁶ (obtained from BD Pharmingen, San Diego, Calif). Results were expressed as the relative fluorescence intensity (rFI), calculated as $rFI = (MFI_{\text{sample}} - MFI_{\text{control}}) / MFI_{\text{control}}$, where MFI is the mean fluorescence intensity.¹⁷

Determination of endocytotic ability of DCs

Endocytotic ability of DCs was measured as the uptake of FITC-dextran, as previously described,¹³ with some modifications. Briefly, 2×10^5 cells per sample were incubated at 37°C or 4°C in media containing FITC-dextran (10 mg/mL; molecular weight, 40,000; Sigma) for 1 hour. The negative control sample was incubated at 4°C, the temperature in which cells are hypoactive with low endocytotic ability.¹⁸ Then, cells were washed and fixed in cold 1% formalin, and the quantitative uptake of FITC-dextran was determined by the fluorescence-activated cell sorting. Results were expressed as rFI.

Mixed leukocyte reaction assays

The effect of EGCG on the ability of DCs to stimulate allogeneic T lymphocytes in mixed leukocyte reaction (MLR) was investigated and compared with that of dexamethasone. Nonadherent allogeneic PBMCs were placed at a density of 2×10^5 /well as the responders, and coincubated with 0.025% glutaraldehyde-fixed DCs treated with or without EGCG or dexamethasone as the stimulators. The plates were incubated at 37°C for 7 days. Then the proliferative activity of lymphocytes was measured by the MTS assay (Cell Titer 96 NonRadioactive Cell Proliferation Assay; Promega, Madison, Wis). Viability of responder cells was calculated as the ratio to the OD of control wells, containing responder cells only. All the determinations were set up in triplicate, and data were presented as mean values $\pm$ SDs.

Quantification of IL-10

The effect of EGCG on the production of IL-10, the anti-inflammatory cytokine produced during the maturation process by MODCs, was investigated. DCs (2×10^5) were cultured with or without LPS and treated with different concentrations of EGCG for 24 hours. Cytokine levels in the supernatants were measured by the ELISA kit (Becton Dickinson). All determinations were set up in triplicate, and data were presented as mean values $\pm$ SDs.

Statistical analysis

The results were analyzed by 2-tailed Student *t* test and expressed as means $\pm$ SDs. Differences were considered statistically significant at $P \leq .05$ or $P \leq .01$.

RESULTS**Effect of various catechins on the induction of DC apoptosis**

Monocyte-derived DCs were confirmed to be negative for CD14, the surface marker of monocytes, and positive for HLA-DR, CD11c, CD80, CD83, and CD86 (data not shown). At day 7 of culture, immature and mature (induced by LPS) DCs were incubated for 24 hours in the absence or presence of the various catechins, and analyzed. As shown in Fig 1, A, both EGCG and ECG, but not EC and EGC, specifically induced apoptosis of DCs. The effect of ECG, however, was significantly lower than that of EGCG, and therefore, EGCG was chosen for all the following experiments.

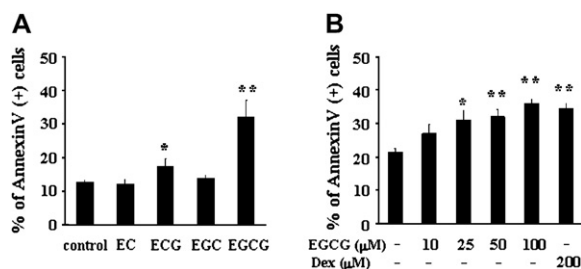


FIG 1. A, Effect of catechins on induction of apoptosis of immature DCs. MODCs were treated with EC, ECG, ECG, and EGCG at 100 $\mu\text{mol/L}$. Apoptotic cells were analyzed by annexin V binding. **B,** EGCG-induced apoptosis of immature MODC dose-dependently. EGCG and dexamethasone (*Dex*) were tested at concentrations indicated. Data are expressed as means $\pm$ SDs ($n = 5$). * $P \leq .05$; ** $P \leq .01$.

Epigallocatechin gallate was tested from physiological to high nonphysiological doses. As shown in Fig 1, B, EGCG specifically and dose-dependently induced apoptosis of immature DC, but doses as high as 25 to 100 $\mu\text{mol/L}$, hardly achievable at physiological conditions, were necessary for the effect to be significant. The effect observed with EGCG at 100 $\mu\text{mol/L}$ was equivalent to that of dexamethasone at 200 $\mu\text{mol/L}$. In addition, EGCG at 25 to 100 $\mu\text{mol/L}$ induced apoptosis of mature DCs at the same levels as immature DCs (data not shown).

EGCG induces morphologic alterations of DCs

By light microscopy, on day 2 or 3, many cell clusters loosely adhered to the bottom of the culture plate were seen. Among them, floating cells with dendritic appearance appeared. Conversion of adherent monocytes to cells with the typical morphology of DCs was observed for 6 days. At day 7, EGCG or dexamethasone was added, and DCs were incubated for another 24 hours. In the presence of EGCG at 100 $\mu\text{mol/L}$, the typical cluster formation by DCs disappeared, and cells remained adherent and developed elongated pseudopods and a fusiform body. In the presence of dexamethasone at 200 $\mu\text{mol/L}$, a few smaller aggregates of irregular cells appeared (data not shown).

EGCG suppressed the differentiation of DCs from monocytes

The immature DCs expressed HLA-DR, CD11c, and CD86, but low levels of CD80 and CD83. Mature DCs generated in the presence of LPS (1 $\mu\text{g/mL}$) expressed high levels of HLA-DR, CD11c, CD80, CD83, and CD86. EGCG significantly altered the immunophenotype of DCs, both immature and mature (Fig 2), causing a dose-dependent inhibition of the expression of HLA-DR, CD11c, CD80, CD83, markedly downregulated by EGCG at 100 $\mu\text{mol/L}$. A dose-dependent downregulation of CD86 on mature DCs was also observed, but on immature DCs, statistical significance was achieved only with EGCG at 100 $\mu\text{mol/L}$. Dexamethasone also caused downregulation of HLA-DR and CD86 on both immature and mature DCs, an effect stronger than that of EGCG at 100 $\mu\text{mol/L}$. The downregulation of CD11c and CD80

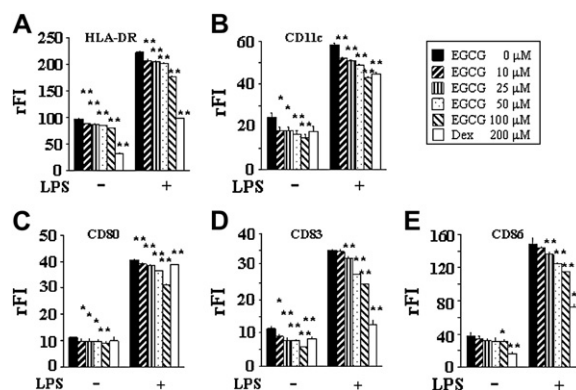


FIG 2. EGCG downregulated the expression of phenotype markers of DCs. MODCs were stimulated with LPS and/or EGCG or dexamethasone (*Dex*). MODCs were labeled with mAbs against HLA-DR (A), CD11c (B), CD80 (C), CD83 (D), and CD86 (E). Data represent the ratio between the mean fluorescence intensity of the sample and that of control ($n = 5$). * $P \leq .05$; ** $P \leq .01$.

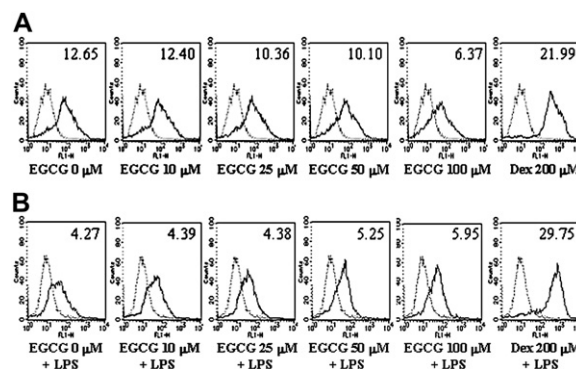


FIG 3. Endocytosis of FITC-dextran by MODCs. **A,** Immature DCs treated with or without EGCG or dexamethasone (*Dex*). **B,** Mature DCs generated in the presence of LPS (1 $\mu\text{g/mL}$) and treated with or without EGCG or Dex. The dotted line represents negative control (4°C). Data are expressed as rFI ($n = 5$).

on both immature and mature DCs, and that of CD83 on immature DCs, was more pronounced with EGCG than dexamethasone.

EGCG suppressed the endocytotic ability of DCs

Endocytosis is a feature of immature DCs, which declines during maturation. As shown in Fig 3, EGCG dose-dependently suppressed the endocytotic ability of immature DCs. EGCG at 100 $\mu\text{mol/L}$ enhanced the endocytotic ability of mature DCs, compared with control mature DCs. Dexamethasone-treated DCs, both immature and mature, had also higher endocytotic ability than control DCs ($P \leq .01$).

EGCG inhibited the allostimulatory capacity of DCs

Epigallocatechin gallate significantly impaired the allostimulatory capacity of both immature and mature DCs (Fig 4, A and B), which was dose-dependently affected

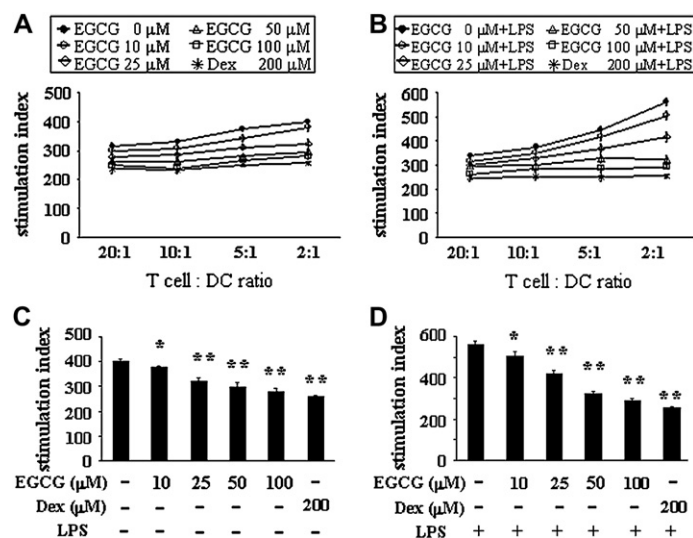


FIG 4. EGCG inhibited the allostimulatory capacity of MODCs. Immature (**A**) and mature (**B**) DCs cultured in the presence of EGCG or dexamethasone (*Dex*) were coincubated with allogeneic T cells. Allostimulatory capacity of immature DCs (**C**) and mature DCs (**D**) was affected dose-dependently by EGCG or Dex at a T-cell:DC ratio of 2:1 ($n = 5$). * $P \leq .05$; ** $P \leq .01$.

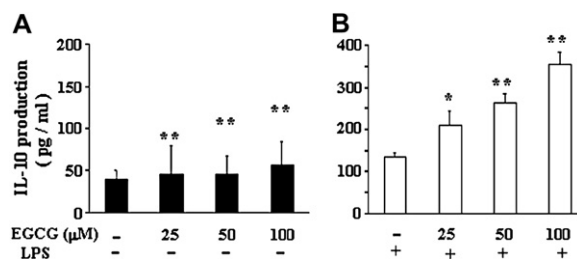


FIG 5. The effect of EGCG on IL-10 production by MODCs. Immature (**A**) and mature (**B**) DCs were cultured in the presence or absence of EGCG. After 24 hours, the culture supernatants were analyzed by an IL-10-specific ELISA. Values are expressed as means $\pm$ SDs ($n = 5$). * $P \leq .05$; ** $P \leq .01$.

when tested at a T cell:DC ratio between 2:1 and 20:1 (Fig 4, *C* and *D*). Immature DCs treated with EGCG at 10 μ M/L showed reduced allostimulatory ability at a T cell:DC ratio between 10:1 and 20:1, and that of mature DCs treated with EGCG at 10 μ M/L was reduced at a T cell:DC ratio between 5:1 and 20:1, but the effect was not statistically significant (data not shown). Similarly, dexamethasone markedly impaired the ability of both immature and mature DCs to stimulate T-cell proliferation (Fig 4).

EGCG affected the secretion of IL-10 by DCs

The IL-10 production by immature DCs was significantly upregulated by EGCG in a dose-dependent manner (Fig 5, *A*). EGCG treatment of mature DCs also resulted in significantly higher production of IL-10 compared with control mature DCs (Fig 5, *B*).

EGCG induced apoptosis of DC precursors and suppressed the differentiation into DCs

As shown in Fig 6, *A*, EGCG treatment at 50 μ M/L and 100 μ M/L specifically induced apoptosis of DC

precursors. The significant increase of apoptosis was observed with EGCG at high doses (100 μ M/L). The percentage of apoptotic DC precursor cells was higher than that of immature DCs at the same conditions (Fig 1, *B*), but the difference was not statistically significant.

Dendritic cell precursors expressed CD1a but low levels of CD14. EGCG and dexamethasone significantly altered the immunophenotype of DC precursors. As shown in Fig 6, *C*, the expression of CD1a was markedly downregulated by EGCG at 100 μ M/L. Treatment with dexamethasone resulted in upregulation of surface expression of CD14 (Fig 6, *B*). EGCG, however, did not affect the expression of CD14 on DC precursors (Fig 6, *B*) or immature DCs (data not shown).

DISCUSSION

Epigallocatechin gallate, which accounts for more than 40% of polyphenols in green tea,¹⁹ has been reported to exert a variety of biological effects, including antiproliferative and cancer chemopreventive activities.^{5,6} Antiviral effects of EGCG against HIV-1 infection by inhibiting the attachment of the HIV-1 virion gp120 to the CD4 molecule on T_H cells were also reported.²⁰⁻²² In a recent report, we demonstrated that one mechanism of the anti-inflammatory effect of EGCG is the attenuation of the adhesive and migratory properties of CD8⁺ T cells by the direct binding of EGCG to CD11b.⁹ EGCG also induced apoptosis of human monocytes, another possible mechanism of its anti-inflammatory and immunosuppressive effects.¹⁰ Currently, however, the effects of EGCG on human MODCs are poorly described.

First, we investigated the effect of various catechins on the induction of DCs' apoptosis. Although weaker than EGCG, ECG also induced apoptosis of human MODCs.

Other catechins had little or no effect on MODCs. Interestingly, both EGCG and ECG are gallate-containing compounds, which were also effective in our previous report.¹⁰ Treatment of both immature and mature DCs with EGCG resulted in lower expression levels of costimulatory molecules, namely HLA-DR, CD11c, CD80, and CD83. Maturation of DCs in the presence of LPS is known to upregulate the expression of these costimulatory molecules;^{23,24} however, addition of EGCG during the maturation process resulted in limited upregulation, suggesting that EGCG inhibits the maturation of DCs. Therefore, EGCG treatment could induce T-cell tolerance during DC–T-cell interaction, dependent on the poor signal 1 caused by low HLA-DR antigen presentation and poor signal 2 caused by low expression of costimulatory molecules.^{25,26} The allostimulatory capacity of both immature and mature MODCs in the MLR assay was significantly reduced by EGCG. A recent study has shown that EGCG had a suppressive effect on the expression and activity of indoleamine 2,3-dioxygenase, a key enzyme involved in T-cell suppression of IFN- γ -stimulated DCs; and, as a consequence, recovered the indoleamine 2,3-dioxygenase-dependent T-cell suppression.²⁷ Although through different perspectives, both studies have focused on the immunomodulatory effect of EGCG, with opposite findings. A possible explanation is the different type and origin of DCs analyzed in both studies. Whereas Jeong et al²⁷ used mouse bone marrow-derived DCs, we used human monocyte-derived DCs. The comparative studies between human and mouse models as well as DCs of various origins are necessary to confirm both findings. In addition, EGCG suppressed the endocytotic ability of immature DCs, but contrarily increased that of LPS-treated mature DCs. Similarly, during the process of DC maturation, dexamethasone is known to increase their endocytotic ability, dependent on the inhibition of DCs maturation.¹³ Because efficient endocytotic accumulation of FITC-dextran is a characteristic of immature DCs, and neither monocytes nor macrophages share this property,²⁸ it is suggestive that EGCG-treated human MODCs are functionally immature, similar to those treated with dexamethasone.

Several cytokines have been shown to regulate the maturation process of DCs. IL-10 has been identified as the major cytokine that can prevent both differentiation of DCs from monocytes and their maturation by blocking release of IL-12.²⁹ IL-10 also inhibits expression of costimulatory molecules and consequently causes inhibition of T_H1 responses.³⁰ Thus, autocrine IL-10 production can prevent maturation of DCs and, as a result, induce anergy in responder T cells.³¹ IL-10 production by MODCs may be a possible mechanism of the inhibitory effect of EGCG on DC maturation. On LPS stimulation, human MODCs cultured with EGCG produced high amounts of IL-10, dose-dependently of EGCG. Our findings suggested that the increased IL-10 production by MODCs during the differentiation from monocyte precursors is consistent with the DC phenotype described as inhibitory DCs, and IL-10 is possibly involved in the increased endocytotic

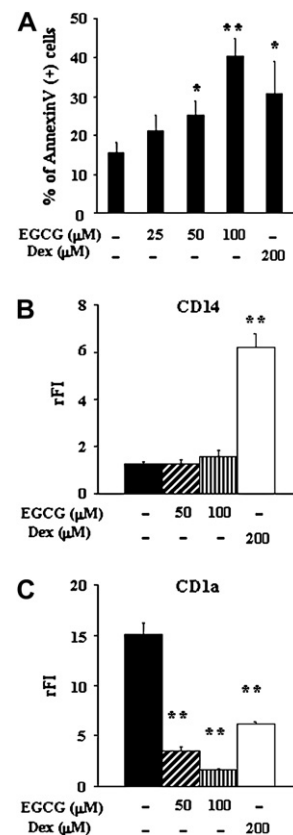


FIG 6. A, Induction of apoptosis of DC precursors by EGCG. EGCG caused a dose-dependent increase of annexin V-positive apoptotic cells. Inhibition of the differentiation of DC precursors into immature DCs by EGCG or dexamethasone (*Dex*), as shown by the relative changes of CD14(+) (**B**) or CD1a(+) (**C**) cells. Data are expressed as mean rFIs $\pm$ SDs ($n = 5$). * $P \leq .05$; ** $P \leq .01$.

ability of LPS-stimulated DCs treated with EGCG. Recently it was shown that IL-10 upregulates mannose receptor expression and mannose receptor-mediated endocytotic ability of MODCs and appears to stimulate the antigen uptake pathways through this mechanism.³² Production of high levels of IL-10 by LPS-stimulated EGCG-treated DCs may be involved in the inhibition of MODC maturation, at least in part.

Glucocorticoids, such as dexamethasone, are potent anti-inflammatory and immunosuppressive agents that, in addition to inhibition of activated T cells, also prevent activation of DCs by production of the anti-inflammatory cytokine IL-10 and suppression of the proinflammatory cytokine IL-12.^{11,12,14,15} Dexamethasone is known to inhibit the upregulation of costimulatory molecules such as CD80 and CD86 as well as MHC class II molecules.^{33,34} Dexamethasone also impaired the differentiation of DCs from monocyte precursors, thereby leading to a decrease in their T-cell-stimulatory potential.³⁵ In addition, dexamethasone significantly upregulated the endocytotic activity of DCs, which occurs via mannose receptor.²⁸ Our results also confirmed these findings.

Next we compared the immunosuppressive alterations induced by EGCG with that by dexamethasone on DCs. Our results suggested that EGCG had effects similar to dexamethasone on human MODCs by causing inhibition of their differentiation, terminal maturation, and antigen-presenting function. Interestingly, although dexamethasone significantly upregulated the endocytotic ability of both immature and mature MODCs, EGCG only partially upregulated that of mature DCs but suppressed that of immature DCs. The increase of the endocytotic ability of DCs induced by dexamethasone is known to be dependent on increased production of IL-10, and mature DCs produced high levels of IL-10, dose-dependently of EGCG. On the other hand, the production of IL-10 by immature DCs was negligible compared with mature DCs, and although not prominent, IL-10 production also increased dose-dependently of EGCG. It is suggestive that EGCG-treated mature DCs are functionally immature while secreting high amounts of IL-10, an effect similar to dexamethasone. However, EGCG seemed to inhibit the differentiation of immature DCs from monocyte precursors, probably through a mechanism independent of IL-10. Similarly, zoledronic acid, another inhibitor of DC differentiation, was shown to inhibit the endocytotic ability of immature DCs.³⁶ Alterations of the rigidity of the cell membrane, caused by either alterations of the cytoskeleton, induced by EGCG in human hepatic stellate cells,³⁷ or actin remodeling by suppression of myosin II regulatory light chain phosphorylation mediated through the binding of EGCG to the 67-kd laminin receptor,³⁸ may be responsible for the modulation of the phagocytotic ability of immature DCs.

Some previous reports have shown that the addition of IL-10 at the initiation of culture of monocytes with GM-CSF and IL-4 or IL-13 resulted in the generation of macrophagelike cells that expressed high levels of CD14 and decreased levels of CD1a and were less efficient stimulators of T cells in MLR compared with DCs.³⁹ Because EGCG induced the production of IL-10 by DCs, the effect of EGCG on DC precursors was examined. EGCG dose-dependently induced apoptosis of DC precursors. Although dexamethasone caused upregulation of the surface expression of CD14 on MODC, EGCG had no similar effect on either DC precursors or immature DCs, with neither expressing this molecule. On the other hand, both EGCG and dexamethasone induced downregulation of CD1a expression on DC precursors, corroborating the previous findings of the inhibitory effect of EGCG on the differentiation process of human MODCs.

To assess the physiologic relevance of the present findings, the knowledge on the plasma EGCG concentrations achievable *in vivo* becomes essential. The physiological concentration of EGCG is reported to be 0.1 to 0.6 $\mu\text{mol/L}$, and oral administration of 1600 mg EGCG under fasting conditions was shown to give the maximum human plasma level of 3.4 $\mu\text{g/mL}$ (approximately 7.4 $\mu\text{mol/L}$).⁴⁰ These levels are lower than the lowest concentration of EGCG used in the current study, which was 4.6 $\mu\text{g/mL}$ (10 $\mu\text{mol/L}$). EGCG at 10 $\mu\text{mol/L}$ did not affect the

endocytotic ability of DCs, nor did it induce apoptosis of DCs. However, 10 $\mu\text{mol/L}$ EGCG caused downregulation of the surface expression of costimulatory molecules of DCs and consequently impaired the ability to stimulate CTL in the MLR. For the inhibitory effect on the endocytotic ability of DCs, doses higher than 50 $\mu\text{mol/L}$ EGCG were necessary, and for induction of apoptosis, higher than 25 $\mu\text{mol/L}$. Because daily intake of EGCG from green tea is reported to give plasma concentrations of about 2.5 $\mu\text{mol/L}$,⁴⁰ it seems likely that 4 to 10 times higher doses are necessary for the immunosuppressive effects of EGCG to develop. Pharmaceutical formulations of green tea would be necessary to achieve these plasma levels.

In conclusion, here we demonstrate that EGCG is a potential immunosuppressive agent by inducing apoptosis of human MODCs and exerting strong inhibitory effects on the differentiation of and the antigen presentation by DCs, effects possibly mediated by increased production of IL-10. Therefore, EGCG treatment seems to be an effective alternative to generate tolerogenic DCs, which may be useful in the treatment of allergic and autoimmune disorders as well as allograft rejection.

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