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Urocanic Acid Enhances IL-10 Production in Activated CD4+ T Cells

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The immunosuppressive effects of UV radiation have been well documented. This suppression has been attributed to the action of the cis form of urocanic acid (UCA), a photoproduct of trans-UCA, a natural constituent of the skin. Here, we show that mouse spleen cells preincubated with cis-UCA have a diminished proliferative response to allogeneic cells in MLC and to stimulation with anti-CD3 mAb. Cells preincubated with cis-UCA also had a decreased ability to serve as APC and to stimulate the proliferation of allogeneic lymphocytes in MLC. Simultaneously, the production of IL-2 and IFN-γ by cells preincubated with cis-UCA was decreased. However, IL-10 gene expression and IL-10 protein secretion by spleen cells stimulated in the presence of cis-UCA were significantly enhanced. The principal cell population displaying the cis-UCA-induced elevated production of IL-10 was CD4+ T cells, which were shown to be a direct target of cis-UCA action. This was also supported by the observation that production of IL-10 by stimulated splenic non-T cells or by macrophages was not altered by cis-UCA. The enhanced production of IL-10 by activated CD4+ T cells may represent a novel pathway of UVB radiation-induced, cis-UCA-mediated immunosuppression. We suggest that the elevated production of IL-10 by activated CD4+ T cells may account for the suppressor T cell phenomena described in UV-irradiated recipients. The Journal of Immunology, 1998, 161: 3237–3241.

The immunosuppressive effects of UV radiation have been well documented in many systems (1, 2), After UV irradiation, contact hypersensitivity (CH)3 is impaired (3), delayed-type hypersensitivity (DTH) and allograft rejection (11–13). Consequently, IL-10 can inhibit the Ag-presenting function of LC in vitro. Therefore, cis-UCA does not appear to be a direct effector molecule causing immune suppression.

We present another explanation herein for cis-UCA-induced immunosuppression. We found that cis-UCA significantly enhances the production of IL-10, a cytokine with immunosuppressive actions, by direct action on activated, IL-10-producing CD4+ T cells. Consequently, IL-10 can inhibit the Ag-presenting function of LC (17) or induce Ag-specific anergy in T cell clones (18), thus suppressing the immune response.

Materials and Methods

Animals

BALB/c and C57BL/10Sn (B10) mice of both sexes, obtained from the breeding colony of the Institute of Molecular Genetics (Prague, Czech Republic) were used at the age of 8 to 12 wk.

Urocanic acid

A commercially available trans-UCA (Sigma Chemical, St. Louis, MO) was dissolved at a concentration of 2 mg/ml in NaCl solution. Aliquots of this solution were irradiated by an XeCl excimer laser beam pulsed with a wavelength of 308 nm, frequency of 10 Hz, spot diameter of 3.5 cm, and fluence of 1.8 mJ/cm2 for 14 min. While unirradiated samples contained 100% trans isomer, irradiated samples contained 50 to 56% cis-UCA and 44 to 50% trans-UCA as determined by HPLC. All samples were stored at 4°C in dark bottles to ensure their stability. Samples of irradiated UCA are referred to as cis-UCA.

Cell separation

Enriched T cell populations were prepared by two cycles of spleen cell separation on a nylon wool column according to the technique of Julius et al. (19). Any remaining B cells were removed by a “panning” technique...
using dishes coated with swine anti-mouse Ig (20). Non-T cell populations were prepared by passing spleen cells through a nylon wool column, then eliminating any remaining T cells from nylon wool-adherent cells with anti-Thy-1.2 mAb (21) and C. CD4-4, CD8-1, and Thy-1.2- negative cell populations were prepared by two cycles of treating spleen cells with cytotoxic anti-CD4 (clone GK1.5 (22)), anti-CD8 (clone TIB 150 (23)), or anti-Thy-1.2 (clone F7D5 (21)) mAb, respectively, and C.

Preincubation of cells with UCA
Spleen cells at a concentration of $3 \times 10^6$/ml were preincubated in a volume of 3 ml of RPMI 1640 medium (Sigma Chemical) containing 10% heat-inactivated FCS (Sigma), antibiotics (100 U/ml of penicillin, 100 g/ml of streptomycin), 10 mM HEPES buffer, and $5 \times 10^{-5}$ M 2-ME (hereafter referred to as complete RPMI 1640 medium) in six-well tissue culture plates (Sterilin, Feltham, U.K.) with 200 g/ml of trans- or cis-UCA. After a 2- or 24-h preincubation, cells were washed twice and resuspended at an appropriate concentration.

Proliferative assay
Freshly isolated or preincubated spleen cells at a concentration of $0.5 \times 10^6$/ml were incubated in 200 g/ml of complete RPMI 1640 medium in flat-bottomed 24-well plates (Nunclon, Roskilde, Denmark) with 2 g/ml of anti-CD3 mAb (24) in the presence or absence of 200 g/ml of cis- or trans-UCA. Cell proliferation was determined by adding 0.5 Ci of $[^{3}H]$thymidine per well for the last 6 h of the 72-h incubation period.

MLC reaction
Reactive spleen cells ($1.5 \times 10^8$/per well) were incubated in 96-well tissue culture plates (Nunc) with stimulatory spleen cells ($2 \times 10^6$/per well) in a volume of 200 g/ml of complete RPMI 1640 medium, as described elsewhere (25). Stimulatory cells were preincubated for 24 h alone or with 200 g/ml of cis- or trans-UCA, then were irradiated with 3000 rad. Neutralizing rat anti-mouse IL-10 mAb (IgG1) (Genzyme, Cambridge, MA) or normal rat IgG1 (Sevac, Prague, Czech Republic) at a concentration of 10 g/ml were added to cultures containing cells preincubated with cis-UCA. Cell proliferation was determined by adding 0.5 Ci of $[^{3}H]$thymidine for the last 6 h of the 96-h incubation period.

Cytokine production and determination
Unseparated or purified spleen cells ($1 \times 10^7$/ml) were incubated with 2 g/ml of anti-CD3 mAb in a volume of 1 ml of complete RPMI 1640 medium, with or without 200 g/ml of cis- or trans-UCA. Cell-free supernatants were harvested after 24, 48, or 72 h. After stimulation with alloantigens ($1.5 \times 10^6$ reactive cells and $2 \times 10^6$ irradiated stimulator cells in 1 ml of culture medium), the supernatants were harvested after 48, 72, and 96 h.

The presence of IL-2, IL-4, IL-10, and IFN-g in the supernatant was measured by ELISA (26) using sets of cytokine-specific capture and detection mAb purchased from PharMingen (San Diego, CA) and precisely following the manufacturer’s instructions. For quantitation of cytokine levels, standards for IL-2, IL-4, IL-10, and IFN-g (all purchased from Genzyme) were included in all ELISA determinations.

RNA preparation and RT-PCR
Using the guanidine isothiocyanate method (27), total RNA was extracted from BALB/c spleen cells that were cultivated for 20 h while unstimulated or stimulated with 2 g/ml of anti-CD3 mAb. The cultures were set with or without 200 g/ml of cis- or trans-UCA. Two micrograms of total RNA were reverse transcribed into cDNA in a 50-g reaction mixture containing 0.5 g/10 mg/ml) of BSA, 5 g/10 mM) of dNTPs, 2 g (0.5 g/ml) of p(dT)15, 1 g (27250 U/ml) of RNAsin, 1 g (15600 U/ml) of M-MuLV RT, and 10 g of 5X RT buffer. Five microliters of the cDNA preparation was amplified during 29 cycles (MJ Research, Watertown, MA) in a reaction mixture containing 5 g of 10 PCR buffer, 1 g (10 mM) of dNTPs, 2 g (25 g/ml) of 5′ and 3′ primers (Strategene, La Jolla, CA), 0.5 g (5000 U/ml) of Taq polymerase, and distilled water to a final volume of 50 g. Cycling conditions were 94°C for 30 s, 60°C for 45 s, 72°C for 1 min, and a final extension of 72°C for 10 min. After PCR, the products were electrophoresed on an ethidium bromide-stained agarose gel.

Statistics
All results are expressed as means ± SEM. The statistical significance of differences between the means of individual experimental groups was calculated using Student’s t test.

Results

Proliferative response of cells preincubated with UCA
Spleen cells from BALB/c mice were preincubated for 24 h in the presence of 200 g/ml of cis-UCA had a significantly decreased proliferative response after stimulation with anti-CD3 mAb or irradiated allogeneic cells compared with the response of cells preincubated with trans-UCA or medium alone (Fig. 1). To obtain a significant suppression of the proliferative responses, a 24-h preincubation of cells with cis-UCA was required; preincubation for 12 h or for a shorter period of time did not result in a significant decrease in the proliferative response.

Ag-presenting function of cells preincubated with UCA
Spleen cells preincubated for 24 h with cis-UCA had a significantly decreased ability to stimulate proliferation of allogeneic cells in an MLC reaction in comparison with cells preincubated with trans-UCA or medium only (Fig. 2). This immunosuppression was significantly blocked by adding neutralizing anti-IL-10 mAb, but not control rat IgG1, to the MLC reaction (Fig. 2).

Production of IL-10 by cells stimulated in the presence of UCA
Preliminary experiments showed that spleen cells preincubated for 24 h with cis- but not trans-UCA and stimulated with anti-CD3 mAb or allogeneic cells had an enhanced production of IL-10, but the production of IL-2 and IFN-g was somewhat decreased (data not shown). To test the possibility that cis-UCA enhances the production of IL-10, we measured production of IL-10 in cell cultures stimulated with anti-CD3 mAb or with irradiated allogeneic cells in the presence of cis- or trans-UCA. Figure 3A shows that the production of IL-10 was significantly increased in the cultures containing cis- but not trans-UCA. This increase in IL-10 production was apparent at all time points tested (i.e., after 48, 72, and 96 h of incubation; data not shown) and was significant in a range of cis-UCA concentrations from 50 to 400 g/ml (Fig. 3B). Neither cis- nor trans-UCA itself induced a significant IL-10 production (i.e., >5 pg/ml, which is the sensitivity limit of the assay) in spleen cells (Fig. 3A).

Effects of UCA on IL-10 production by cell subpopulations
The elimination of CD4+ cells or total T cells from the spleen cell population completely abolished the ability of the remaining cells.
to produce IL-10 after stimulation with anti-CD3 (Fig. 4). However, spleen cells treated with anti-CD8 mAb and C produced even more IL-10 than untreated cells, and this production was considerably increased in the presence of cis-UCA (Fig. 4). In addition, both spleen cells depleted of T cells and stimulated with the B cell mitogen LPS as well as purified peritoneal macrophages stimulated with LPS produced IL-10; but the production of IL-10 by these cells was not altered in the presence of cis-UCA (data not shown).

To identify a cellular target of cis-UCA action, spleen cells were separated into T cell and non-T cell populations. Neither of these populations alone produced detectable IL-10 after stimulation with anti-CD3 (Fig. 5). Separated T cell and non-T cell populations were preincubated for 2 h either alone or in the presence of 200 μg/ml of cis- or trans-UCA. Then, untreated T cells were mixed with non-T cells that were preincubated either alone, with cis-UCA, or with trans-UCA; vice versa, untreated non-T cells were mixed with T cells preincubated either alone, with cis-UCA or trans-UCA. As shown in Figure 5, enhanced IL-10 production was observed only in cultures containing untreated non-T cells and T cells preincubated with cis-UCA.

Cis-UCA enhances expression of IL-10 gene

To test whether cis-UCA acts on the level of IL-10 gene expression, spleen cells were cultured unstimulated for 20 h or were stimulated with anti-CD3 mAb and the cultures were set in the presence or absence of cis- or trans-UCA. The level of IL-10 mRNA was measured by RT-PCR. As demonstrated in Figure 6, neither cis-UCA nor trans-UCA increased production of IL-10 mRNA in unstimulated cells, but the level of IL-10 mRNA was considerably increased in cultures stimulated with anti-CD3 mAb in the presence of cis-UCA.

Discussion

The immunosuppressive effects of UV radiation have been well documented in various systems, but the molecular mechanism of the suppression has remained unclear. It has been proposed that the immunosuppressive effects of UVB radiation are mediated via cis-UCA, a photoproduct of naturally occurring trans-UCA (7). However, we demonstrate here that cis-UCA is not the effector molecule that inhibits immune system function. Rather, cis-UCA stimulates the production of IL-10 by activated CD4+ T cells through an increase in the expression of the IL-10 gene. Consequently, IL-10 can inhibit the activity of APC (17) or anergize T cells (18) and thus suppress the immune response.

We have shown that spleen cells preincubated with cis-UCA, but not trans-UCA, have a diminished ability to respond by proliferation to stimulation with allogeneic cells in a MLC reaction and to stimulation with anti-CD3 mAb. Simultaneously, the ability of cells preincubated with cis-UCA to stimulate the proliferation of allogeneic cells in MLC was decreased. Previous studies have shown the harmful effects of UV radiation on the activity of APC (28, 29). Since direct effects of cis-UCA on APC function (16) or on the expression of costimulatory molecules (15) have not been shown, another molecule mediating the effects of UV radiation and UCA on APC function has been hypothesized. Since IL-10 can inhibit Ag presentation by epidermal APC (30) and possesses an ability to inhibit the production of Th1 cytokines through modulating APC function (31), IL-10 has appeared as a candidate molecule for cis-UCA-mediated immunosuppression. Indeed, we also observed a decreased ability of spleen cells preincubated with cis-UCA to produce IL-2 and IFN-γ.
Several lines of evidence support our conclusion that \textit{cis}-UCA enhances IL-10 production by activated CD4$^+$ T cells. First, IL-10 gene expression was increased in cultures of spleen cells stimulated with anti-CD3 mAb in the presence of \textit{cis}-UCA. Second, the production of IL-10 protein by spleen cells was significantly increased in the presence of \textit{cis}-UCA. The enhanced production of IL-10 was completely inhibited by treating spleen cells with anti-Thy-1.2 or anti-CD4 mAb, but was not impaired by the elimination of CD8$^+$ cells. In addition, the production of IL-10 by stimulated splenic non-T cells or by LPS-stimulated macrophages was not altered by \textit{cis}-UCA (data not shown). Thus, activated CD4$^+$ T cells appear to be the principal cells responding to \textit{cis}-UCA by enhancing IL-10 production. These cells might be responsible for the Ts cell phenomena described after UV irradiation (32, 33). This conclusion is also supported by the findings of Rivas and Ulrich (34), who showed that the activity of UV radiation-induced Ts cells can be blocked by anti-IL-10 mAb.

The observation that \textit{cis}-UCA increases IL-10 production in activated T cells and the finding of decreased IL-2 and IFN-\gamma production in cultures pretreated with \textit{cis}-UCA suggest a shift to a Th2-type response in cells preincubated with \textit{cis}-UCA. This observation is consisitent with the report of Brown et al. (35), who showed that UV irradiation in vivo down-regulates Th1 immune responses while leaving Th2 responses intact.

To exclude the possibility that \textit{cis}-UCA enhances IL-10 production in T cells through an effect on APC, we divided spleen cells into T and non-T subpopulations and preincubated them with \textit{cis}-UCA or \textit{trans}-UCA. Then, the cells of each subpopulation were mixed with untreated cells of the opposite population, and the mixtures were stimulated with anti-CD3 mAb. This experiment showed that T cells were a direct target of \textit{cis}-UCA.

We are aware that the described pathway of \textit{cis}-UCA-mediated immunosuppression is not the only one operating after UV irradiation. UV radiation can have a direct deleterious effect on DNA (36). It has been shown that the extent of DNA damage after UV irradiation correlates with UV-induced systemic suppression of CH and DTH (36, 37). \textit{Cis}-UCA arising after UVB irradiation of naturally occurring \textit{trans}-UCA may induce a local increase in the synthesis of TNF-\alpha, which suppresses Ag presentation by LC (10). This observation is supported by findings that UVB radiation-induced suppression of contact sensitivity in mice can be blocked by anti-TNF-\alpha Abs (10, 33). However, TNF-\alpha appears as a molecule involved in the UV-induced suppression of CH, but only to a much lesser extent in the suppression of DTH (34). Ulrich and coworkers reported that UVB radiation may act directly on keratinocytes by inducing IL-10 production in these cells (38). These authors also showed that immunosuppression induced by UVB irradiation can be mimicked by the administration of IL-10 or reversed by anti-IL-10 Abs (38–40). However, it seems improbable that \textit{cis}-UCA induces a significant production of IL-10 in keratinocytes. Moodycliffe et al. (41) showed that injecting anti-cis-UCA mAb into UV-irradiated mice reversed UV-induced suppression of DTH but did not block IL-10 production in the epidermis. Similarly, we found in our system that anti-IL-10 mAb blocked the immunosuppressive effect of \textit{cis}-UCA observed in MLC. In accordance with the other authors who have shown that IL-10 inhibits allogeneic proliferative responses in vitro (42, 43), we observed that IL-10 suppressive effect of \textit{cis}-UCA observed in MLC. In accordance with the other authors who have shown that IL-10 inhibits allogeneic proliferative responses in vitro (42, 43), we observed that IL-10

\begin{figure}
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\includegraphics[width=\textwidth]{figure5.png}
\caption{UCA acts directly on T cells. Spleen cells from BALB/c mice, either unseparated or separated into T and non-T cell subpopulations, were stimulated with 2\,$\mu$g/ml of anti-CD3 mAb with or without (□) 200\,$\mu$g/ml of \textit{cis}-UCA (□) or \textit{trans}-UCA (□). In addition, each subpopulation was preincubated for 2 h either alone or with 200\,$\mu$g/ml of \textit{cis}- or \textit{trans}-UCA. After washing, each of these populations was mixed at a ratio of 1:1 with fresh, untreated cells of the opposite population (i.e., treated T cells with untreated non-T cells and vice versa) and incubated with 2\,$\mu$g/ml of anti-CD3 mAb. The level of IL-10 in the supernatants was determined after a 72-h incubation. One of three similar experiments is shown. Values marked with asterisks are significantly ($p < 0.001$) different from those of control cultures without UCA.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Expression of IL-10 mRNA by spleen cells stimulated in the presence of UCA. Spleen cells from BALB/c mice were cultivated unstimulated for 20 h alone (lane 1) or in the presence of 200\,$\mu$g/ml of \textit{cis}-UCA (lane 2) or \textit{trans}-UCA (lane 3); or were stimulated with 2\,$\mu$g/ml of anti-CD3 mAb alone (lane 4) or in the presence of 200\,$\mu$g/ml of \textit{cis}-UCA (lane 5) or \textit{trans}-UCA (lane 6). Total RNA was isolated, reverse transcribed, and amplified by PCR with 5\,'9\, and 3\,'1 primers for IL-10 and actin. PCR products were electrophoresed; the ethidium bromide-stained agarose gel is shown.}
\end{figure}
added to the cultures can mimic the immunosuppressive effects of cis-UCA (data not shown).

IL-10 secreted by CD4+ T cells activated with Ag in the presence of cis-UCA appears to be the main effector molecule responsible for UVB radiation-induced immunosuppression. It has been shown that IL-10 can decrease APC function (44) and, together with Ag, can induce anergy in responding T cell clones (18). We suggest that IL-10 secreted by CD4+ T cells in the presence of cis-UCA might be responsible for the suppressor T cell phenomenon observed after UV irradiation.

References