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Inhibitory and Stimulatory Effects of IL-10 on Human CD8$^+$ T Cells[1]

Hervé Groux,[2] Mike Bigler, Jan E. de Vries,[3] and Maria-Grazia Roncarolo[4]

IL-10 is a well-documented immunosuppressant that inhibits macrophage-dependent Ag presentation and CD4$^+$ T cell proliferation in vitro. We report that IL-10 inhibits alloantigen-specific proliferative responses and induces a long lasting anergic state in human purified CD8$^+$ T cells when added concomitantly with the Ag in the presence of APC. Moreover, the generation of allospecific cytotoxic activity is inhibited by IL-10. These effects are indirect and are mediated through inhibition of the costimulatory functions of APC. In contrast, IL-10 has no direct inhibitory effects on the proliferation of purified CD8$^+$ T cells activated by anti-CD3 mAb and promotes the growth of activated CD8$^+$ T cells in combination with low doses of IL-2. Taken together, these results indicate that IL-10 has differential effects on CD8$^+$ T cells depending on their state of activation, which may explain both the enhancing and inhibitory effects observed after IL-10 treatment in different in vivo experimental models. ***The Journal of Immunology,*** **1998, 160: 3188–3193.**

Interleukin-10, which is produced by a variety of cells, including T lymphocytes, B lymphocytes, and monocytes, has been identified as a cytokine with important antiinflammatory and immunosuppressive properties. In vitro studies have shown that IL-10 inhibits Ag-specific activation and proliferation of human peripheral blood T cells and T cell clones belonging to the Th0, Th1, or Th2 subsets (1, 2). This inhibition is associated with a reduction of the Ag-presenting capacity of monocytes. IL-10 down-regulates monokine production (3, 4) and the expression of CD54 (ICAM-1, the ligand for LFA-1), CD80, and CD86, which function as important costimulatory molecules for T cell activation (5–7). Furthermore, IL-10 down-regulates constitutive and IFN-γ- or IL-4-induced class II MHC expression on monocytes, dendritic cells, and Langerhans cells (2, 8, 9). It has also been shown that IL-10, in the absence of professional APC, has direct effects on CD4$^+$ T by suppressing IL-2 and TNF-α secretion (10, 11). IL-10 also strongly reduces the proliferation and cytokine production of alloreactive T cells in MLR. Moreover, the generation of allospecific cytotoxic activity is inhibited by IL-10 (12). We have recently shown that in addition to inhibiting Ag-specific responses, IL-10 induces long lasting Ag-specific anergy in human CD4$^+$ T cells (13).

On the other hand, IL-10 also has immunostimulatory effects on B and T cells. IL-10 augments the proliferation and the differentiation into Ab-secreting cells of splenic activated B cells (14, 15). IL-10 also rescues T cells from apoptotic cell death (16). In addition, it has been reported that IL-10 enhances the proliferative responses of murine thymocytes (17) and IL-2- and IL-4-driven proliferation of murine CD8$^+$ T cells in vitro (18). IL-10 also has immunostimulatory effects in vivo. Injection of high doses of IL-10 (200 μg/mice/day) in mice with graft-vs-host disease, due to both major and minor MHC disparities, resulted in a exacerbation of the disease, which was probably mediated by IFN-γ production (19). In addition, in an IL-10 transgenic model, IL-10 enhances the accumulation of CD8$^+$ T cells in the pancreata (20), leading to an earlier onset of diabetes in nonobese diabetic mice (21). These data suggest that IL-10 may have differential effects on CD4$^+$ and CD8$^+$ T cells.

In the present study, we demonstrate that IL-10 has a dual effect on human CD8$^+$ T cells. IL-10 suppresses the proliferative responses and induces alloantigen-specific unresponsiveness in human CD8$^+$ T cells activated in the presence of professional APC. These effects are indirect and are mediated through inhibition of the costimulatory functions of APC. In contrast, IL-10 has no direct inhibitory effect on the proliferation of CD8$^+$ T cells activated via their TCR in the absence of APC and displays a growth-promoting activity on activated CD8$^+$ T cells in combination with low doses of IL-2.

Materials and Methods

Cells

PBMC were isolated on Ficoll-Hypaque. CD8$^+$ T cells were purified by negative selection using a mixture of Abs directed against non-CD8$^+$ cells: CD4, CD14, CD16, CD19, CD20, CD56, and HLA-DR. Cells were incubated with saturating amounts of Abs for 20 min at 4°C. After washing, Dynabeads (Dynal, Oslo, Norway) were added at a ratio of 10 beads/target cell and incubated for 1 h at 4°C. Beads and contaminating cells were removed by magnetic field. The remaining cells were resuspended with the same amount of beads, and a second incubation period for 1 h at 4°C was performed. After removal of contaminating cells, CD8$^+$ T cells were analyzed by cytofluorometry and showed >90 to 95% positive. In some experiments, CD8$^+$ T cells were purified (>98% pure) by positive selection using magnetic beads coated with anti-CD8 mAb according to the manufacturer's instructions (Dynal). Monocytes were purified by negative selection using the procedure described above with an Ab mixture containing CD2, CD3, CD8, CD16, CD19, CD20, and CD56. These monocytes were >95% CD14$^+$ as shown by cytofluorometry.

Immunobiology Department, DNAX Research Institute of Molecular Immunology and Cellular Biology, Palo Alto, CA 94304

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[1] DNAX Research Institute of Molecular and Cellular Biology is supported by Schering-Plough Corporation.

[2] Address correspondence and reprint requests to Dr. Hervé Groux, DNAX Research Institute of Molecular Immunology and Cellular Biology, 901 California Avenue, Palo Alto CA 94304-1104. E-mail address: groux@dnax.org

[3] Current address: Novartis Research Institute, Brunner Strasse 59, A-1235 Wien, Austria.

[4] Current address: University of Torino, Department of Pediatrics-Immunology Unit, Regina Margherita Children's Hospital, Piazza Polonia 94, 10126 Turin, Italy.

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Reagents

Purified recombinant IL-10 was provided by Schering-Plough Research Institute (Bloomfield, NJ), and rIL-2 was a gift from Dr. S. Menon (DNAX Research Institute, Palo Alto, CA). The anti-CD3 mAb SPV-T3 (22), anti-IL-2 (BG-5), anti-IL-2R (B-B10) (23) or anti-IL-10 (9D7) mAbs were previously described. Nonconjugated, phycoerythrin-conjugated, or FITC-conjugated CD2, CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD28, CD54, CD56, CD80, HLA class I, HLA-DR, and controls mAbs of the appropriate isotypes were purchased from Becton Dickinson (Mountain View, CA) except for CD86 (PharMingen, San Diego, CA).

Proliferation assays

All proliferation assays were conducted in Yssel's medium (24) supplemented with 10% FCS and 1% human serum. MLR were performed by stimulating purified $CD8^+$ T cells (10^5 cells/well) with purified irradiated (4000 rad) monocytes (10^5 cells/well) in 200-μl round-bottom 96-well plates (Linbro, ICN, Aurora, OH). $CD8^+$ T cell proliferation was measured after 5 days of incubation at 37°C and 5% CO_2 and a subsequent 12-h pulse with [^{3}H]TdR, after which the cells were harvested as described (25).

For plate-bound anti-CD3 mAbs activation, 100 ng/ml of anti-CD3 mAbs diluted in 0.1 M Tris buffer, pH 9.5, were incubated for 1 week at 4°C in flat-bottom 96-well plates. After the plates were washed, $CD8^+$ T cells were added at a concentration of 5×10^4 cells/well. $CD8^+$ T cell proliferation was measured after 3 days of incubation followed by a 12-h pulse with [^{3}H]TdR. All tests were conducted in triplicate.

Induction of unresponsiveness

Ag-specific unresponsiveness was induced by culturing $CD8^+$ T cells at 10^6 cells/ml with purified irradiated allogeneic monocytes (10^6 cells/ml), in the presence of IL-10 (100 U/ml) in 24-well plates. After 10 days, cells were collected, layered on a Ficoll gradient, and centrifuged for 30 min at 1200 rpm to remove dead cells. The viable cells were collected from the interface, washed twice, and restimulated at 5×10^5 cells/ml with irradiated allogeneic monocytes (5×10^5 cell/ml) from the same donor in 96-well plates.

Immunofluorescence analysis

Cell surface Ag expression on monocytes incubated with IL-10 for different period times was determined by immunofluorescence. Monocytes (10^5) were labeled with phycoerythrin- or FITC-conjugated mAbs. Cells were incubated with the appropriate Ab for 30 min at 4°C in PBS with 0.1% BSA and 0.02 mM NaN_3. After three washes, the labeled cell samples were analyzed on a FACScan (Becton Dickinson, San Jose, CA).

Cytotoxicity assays

The cytotoxic activity was measured as described previously (12). In brief, 2×10^3 ^{51}Cr-labeled target cells (PHA blasts) were incubated with graded numbers of $CD8^+$ T effector cells in 200 μl of medium in round-bottom microtiter plates (Linbro, ICN). The plates were centrifuged and incubated for 4 h at 37°C. The supernatants were harvested with a Skatron (Sterling, VA) harvesting system and counted in a gamma counter. All test samples were measured in triplicate. The percentage of specific ^{51}Cr release was calculated as described (12).

Results

IL-10 inhibits the monocyte-dependent proliferation of human $CD8^+$ T cells

IL-10 inhibited in a dose-dependent manner the proliferation of purified $CD8^+$ T cells in response to purified allogeneic irradiated monocytes in primary MLR. Strong inhibitory effects were observed at IL-10 concentrations as low as 10 U/ml, whereas maximal inhibition was observed at 100 U/ml (Fig. 1*A*). These inhibitory effects of IL-10 on the proliferation of purified $CD8^+$ T cells were completely restored by exogenous IL-2 (Fig. 1*A*).

To analyze whether the inhibition of proliferation by IL-10 reflected a direct effect on $CD8^+$ T cells, highly purified $CD8^+$ T cells were activated by cross-linked anti-CD3 mAbs, in the presence or absence of IL-10. No inhibitory effects of IL-10 on the proliferative responses of $CD8^+$ T cells stimulated with different doses of anti-CD3 mAbs were observed (Fig. 1*B*) even after addition of up to 10,000 U/ml of IL-10 (Fig. 1*A*). These results suggest that the inhibitory effect of IL-10 on alloantigen-specific $CD8^+$ T cell proliferation is indirect and is caused by inhibition of the APC function of monocytes. This was confirmed by the observation that IL-10 inhibited specifically the costimulatory functions of autologous monocytes on anti-CD3 mAb-induced proliferation of $CD8^+$ T cells (Fig. 1*B*). Moreover, the inhibitory effect of IL-10 was observed only after preincubating with IL-10 allogeneic monocytes before coculture (Fig. 1*C*). No inhibition was observed after preincubation of $CD8^+$ T cells with IL-10 (Fig. 1*C*). Finally, transwell experiments where $CD8^+$ T cells were stimulated with immobilized anti-CD3 mAb and cocultured with autologous monocytes either on close contact or separated by a semipermeable membrane have shown that IL-10 inhibited both the secretion of stimulatory monokines and costimulatory cell surface molecules (Fig. 1*D*) and did not induced the secretion of a suppressive factor.

IL-10 induces Ag-specific unresponsiveness in $CD8^+$ T cells

Recently, we showed that $CD4^+$ T cells stimulated by alloantigens in the presence of IL-10 for prolonged periods of time failed to respond when restimulated with the same specific Ag (13). To analyze whether IL-10 had the same effect on $CD8^+$ T cells, purified $CD8^+$ T cells were stimulated by allogeneic monocytes in the presence or absence of IL-10 for 10 days. After this culture period, alloantigen-stimulated $CD8^+$ T cells were restimulated with the same allogeneic irradiated monocytes in secondary MLR or with third-party allogeneic monocytes in primary MLR. $CD8^+$ T cells that had been cultured in the absence of IL-10 proliferated strongly in response to the allogeneic stimuli (Fig. 2). In contrast, $CD8^+$ T cells activated and incubated in the presence of IL-10 showed a strong reduction in their secondary proliferative responses to the same allogeneic monocytes (Fig. 2). However, they retained their capacity to proliferate to third-party alloantigens in primary MLR (Fig. 2). Comparable levels of cell death were observed in both culture conditions, and comparable cell numbers were harvested at termination of the assays (data not shown). These results indicate that IL-10 induces an Ag-specific unresponsive state in $CD8^+$ T cells in a manner similar to that described for $CD4^+$ T cells (13).

IL-10 down-regulates HLA-class I, CD80, CD86, and ICAM-1 expression on monocytes

To determine whether the induction of the unresponsive state in $CD8^+$ T cells following activation by allogeneic monocytes in the presence of IL-10 was due to a down-regulation of costimulatory signals, monocytes were incubated with IL-10 for different time periods, and the expression of costimulatory cell surface molecules was analyzed. Flow cytometric analysis by direct staining with a mAb against nonpolymorphic determinants of HLA class I indicated that IL-10 down-regulates HLA class I expression, but to a lesser extent than HLA class II expression (Fig. 3). In addition, IL-10 down-regulated the expression of the costimulatory molecules CD80 and CD86 and the adhesion molecule ICAM-1 (Fig. 3), as demonstrated previously (6, 26). However, no change in CD58 expression was observed (Fig. 3). Collectively, these results indicate that IL-10-induced alloantigen-specific unresponsiveness in $CD8^+$ T cells is indirectly mediated by APC and is associated with down-regulation of costimulatory molecules and to a lesser extent of class I MHC Ags.

IL-10 inhibits allogeneic cytotoxic responses by $CD8^+$ T cells

To test whether IL-10 also inhibits the generation of alloantigen specific cytotoxic T cells, purified $CD8^+$ T cells were stimulated

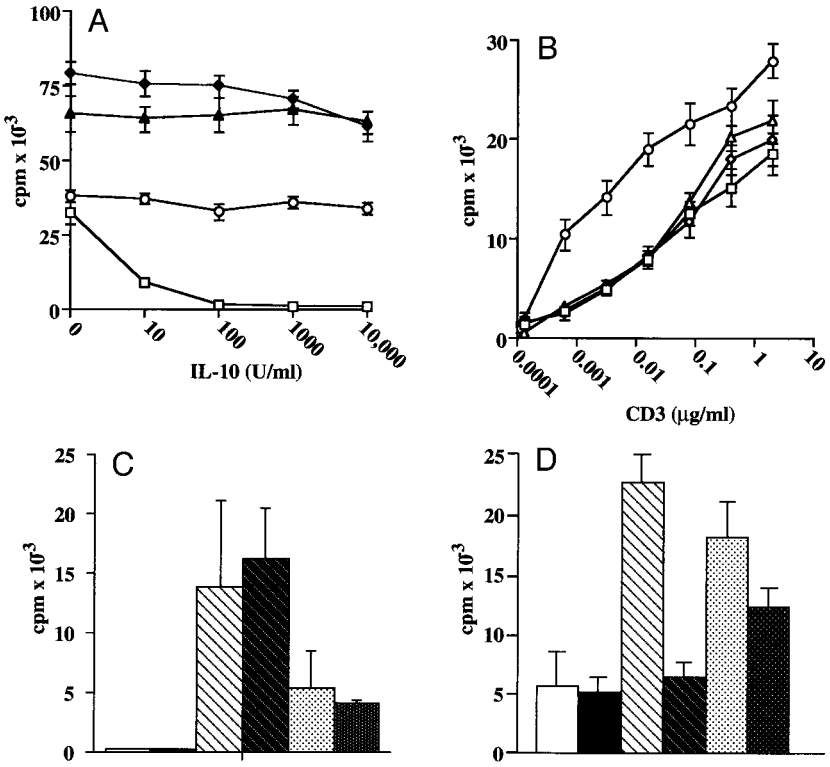

FIGURE 1. IL-10 inhibits proliferation of human CD8$^+$ T cells activated with monocytes but not with anti-CD3 mAbs. *A*, Purified CD8$^+$ T cells were stimulated with allogeneic purified monocytes in the absence (□) or presence (◆) of IL-2. Purified CD8$^+$ T cells were also stimulated with cross-linked anti-CD3 mAbs (100 ng/ml) in the absence (○) or presence (▲) of IL-2. As indicated, rIL-10 was added in the cell culture at different concentrations. *B*, Purified CD8$^+$ T cells were stimulated with different doses of immobilized anti-CD3 mAb either alone (□) or with 100 U/ml of IL-10 (◇), with autologous purified monocytes (1×10^6 cell/ml; ○), or with both IL-10 and autologous monocytes (△). *C*, Purified CD8$^+$ T cells and purified allogeneic monocytes were incubated overnight in the presence or absence of IL-10 (100 U/ml), washed, and cocultured. White bars, CD8$^+$ T cells alone; black bars, IL-10-treated CD8$^+$ T cell alone; white stripped bars, CD8$^+$ T cells and allogeneic monocytes; black stripped bars, IL-10-treated CD8$^+$ T cells and allogeneic monocytes; white dotted bars, CD8$^+$ T cells and IL-10-treated allogeneic monocytes; black dotted bars, IL-10-treated CD8$^+$ T cells and IL-10-treated allogeneic monocytes. *D*, Purified CD8$^+$ T cells were cultured in the lower compartment of a transwell system in the presence of immobilized anti-CD3 mAb (20 ng/ml). The CD8$^+$ T cells were also cocultured with autologous monocytes either on close contact (white and black stripped bars) or contained in the upper transwell compartment (white and black dotted bars). IL-10 was added at 100 U/ml (black, black stripped, and black dotted bars). Cell proliferation was measured by a pulse of [^{3}H]TdR during the last 12 h of a 5-day incubation for stimulation with allogeneic monocytes or a 3-day incubation for stimulation with anti-CD3 mAbs.

with purified allogeneic monocytes on primary MLR in the presence of different doses of IL-10. Five days later, the CD8$^+$ T cells were collected and used as effector cells against autologous or allogeneic PHA blasts. CD8$^+$ CTL killed the allogeneic T-cell blasts in a dose-dependent manner (Fig. 4), whereas no significant cytotoxic activities were observed against autologous T cell blasts (not shown). The alloantigen-specific cytotoxicity by CD8$^+$ T cells that had been activated in the presence of IL-10 was strongly reduced (Fig. 4). Significant inhibition of specific cytotoxicity was already observed at 10 U/ml of IL-10, whereas >90% reduction in cytotoxic activity was obtained at an IL-10 concentration of 100 U/ml.

IL-10 acts as a cofactor with IL-2 to promote the proliferation of activated CD8$^+$ T cells

To determine whether IL-10 has direct effects on the proliferation of human CD8$^+$ T cells, human purified CD8$^+$ T cells were stimulated with cross-linked anti-CD3 mAb in the presence of anti-IL-2 or anti-IL-2R mAbs or both and IL-10. Anti-IL-2 mAbs strongly inhibited the proliferation of purified CD8$^+$ T cells activated with anti-CD3 (Fig. 5*A*). Similar results were obtained by adding anti-IL-2R α-chain (CD25) mAbs or both Abs, demonstrating that this proliferation is IL-2 dependent. Interestingly, addition of high concentrations of IL-10 (100 U/ml) restored CD8$^+$ T cell proliferation, indicating that IL-10 may act as a growth factor for CD8$^+$ T cells in the absence of IL-2. However, the proliferation of CD8$^+$ T cells activated with immobilized anti-CD3 mAbs in the presence of saturating amounts of anti-IL-2 and/or anti-IL-2 R mAbs was never completely blocked even in the presence of a blocking anti-IL-10 mAb (Fig. 5*A*), suggesting that IL-10 may act only as a cofactor for CD8$^+$ T cell proliferation in the presence of low concentrations of IL-2. To test this possibility, IL-10 was added to CD8$^+$ T cells preactivated by immobilized anti-CD3 mAb and cultured in the presence of IL-2. IL-10 enhances IL-2 driven proliferation of preactivated CD8$^+$ T cells, especially when low IL-2 and high IL-10 concentrations were used (Fig. 5*B*). IL-10 alone, in the absence of IL-2, consistently failed to induce significant levels of CD8$^+$ T cell proliferation. This is because CD8$^+$ T cells that have been preactivated for 5 days failed to produce detectable amounts of IL-2 (not shown). These data suggest that IL-10 synergizes with a low concentration of IL-2 in enhancing CD8$^+$ T cell proliferation.

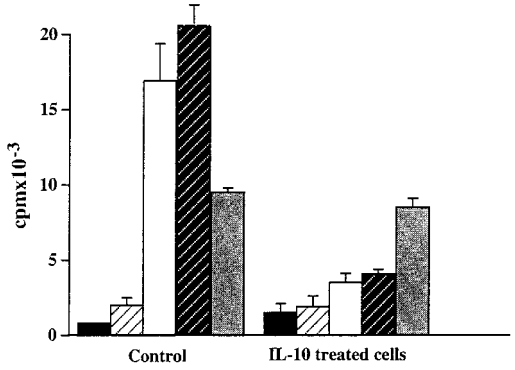

FIGURE 2. IL-10 induces an Ag-specific unresponsiveness state in CD8$^+$ T cells. Purified CD8$^+$ T cells were stimulated in a primary MLR with allogeneic irradiated purified monocytes in the presence or absence of IL-10 (100 U/ml) as indicated. After 10 days of culture, cells were collected, washed, and restimulated with medium alone (black bars), autologous monocytes (white cross-hatched bars), allogeneic monocytes purified from the same donor used in the primary MLR in the absence (white bars) or presence of exogenous IL-2 (20 U/ml; black cross-hatched bars), or allogeneic monocytes purified from an unrelated donor (third-party; gray bars). Proliferation was measured after 5 days.

Discussion

IL-10 inhibits monocyte-dependent, Ag-specific proliferation and cytokine production by human and murine CD4$^+$ T cells (27, 28).

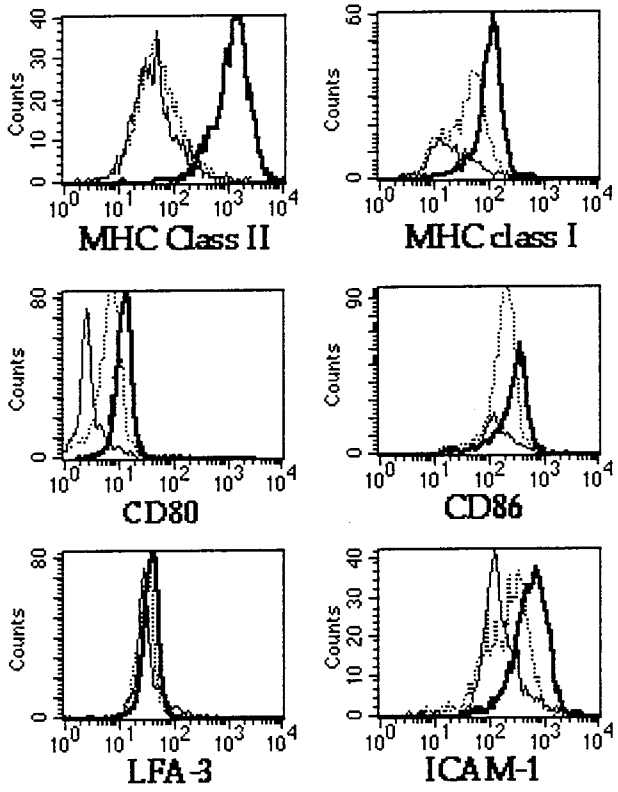

FIGURE 3. Effects of IL-10 on the expression of CD54 (ICAM-1), HLA-DR (MHC class II), MHC class I, CD80 (B7-1), CD86 (B7-2), and CD58 (LFA-3) Ags by human monocytes. Human monocytes, isolated by negative selection using magnetic beads, were stained with the different mAbs either immediately (straight lines) or following incubation in medium alone (large lines) or in the presence of IL-10 (100 U/ml; dotted lines) for 72 h and then analyzed for the expression of MHC class I (W6/32), HLA-DR (L243), CD54 (ICAM-1-LB-2), CD80 (L307), CD86 (IT2.2), or CD58 (L308) Ags.

Furthermore, IL-10 inhibits alloantigen-induced T-cell proliferation and allo-specific cytotoxic T cell generation (12). In addition to its suppressor activity on T cell proliferation, IL-10 has potent antiinflammatory effects (29) and inhibits the generation of reactive nitrogen intermediates by mouse macrophages (29). IL-10 also suppresses the production of proinflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6) and chemokines (IL-8, MIP-1α) by activated human monocytes (3, 29). These suppressive and antiinflammatory activities suggest a potential clinical use of IL-10 as a potent immunosuppressant in allogeneic transplantation and autoimmune diseases. The results of experimental (30) and clinical studies (31) support this hypothesis. High levels of endogenous IL-10 were observed in successfully transplanted SCID patients in whom tolerance was established (25). Furthermore, a correlation between high survival rates and high concentrations of endogenous IL-10 has been described in patients with malignancies transplanted with allogeneic bone marrow (32). These findings suggest that the concentrations of IL-10 may be important to prevent graft-vs-host disease and determine the outcome of the transplant. Moreover, in vivo injection of IL-10 in humans has been shown to have inhibitory effects on T cells and to suppress the production of the proinflammatory cytokines TNF-α and IL-1β (31).

However, other in vivo studies have shown that IL-10 was not able to suppress an immune response. IL-10 was not able to prevent graft-vs-host disease (33) and in some situations even had exacerbating effects (19) when administered either at the same time as or after bone marrow transplant. In addition, IL-10 transgenically expressed in β cells did not prevent or modify rejection of allogeneic fetal pancreata or adult β cells (34). The discrepancy between these in vivo effects of IL-10 in humans and mice may be due to the fact that under certain conditions IL-10 may act as an immunostimulant instead of as an immunosuppressant. In this study, we demonstrate that IL-10 has differential immunosuppressive or stimulatory effects on CD8$^+$ T cells depending on their stage of activation.

IL-10 decreases proliferation and cytotoxic functions of CD8$^+$ T cells when added before or at the time of activation. Moreover, human CD8$^+$ T cells activated by allogeneic monocytes in the presence of IL-10 for 10 days were rendered unresponsive to a secondary stimulation with the same Ag. However, these T cells proliferated normally in response to third-party alloantigens. Similar results have been obtained with CD4$^+$ T cells (13). In contrast to the results obtained with CD4$^+$ T cells, IL-10 failed to decrease

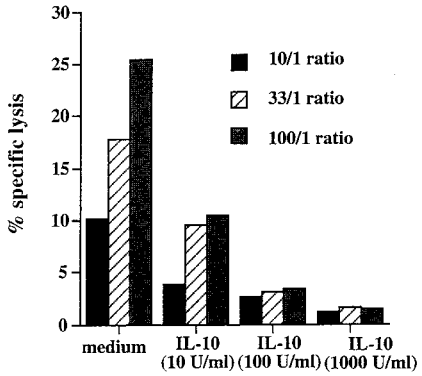

FIGURE 4. Preincubation with IL-10 inhibits cytotoxic activity of CD8$^+$ T cells. Purified CD8$^+$ T cells were stimulated with purified irradiated allogeneic monocytes in the presence of different doses of IL-10, as indicated. After 5 days, cells were collected and washed, and their cytotoxic activity was tested against allogeneic PHA-T cell blasts loaded with ^{51}Cr at different effector to target cell ratios as indicated.

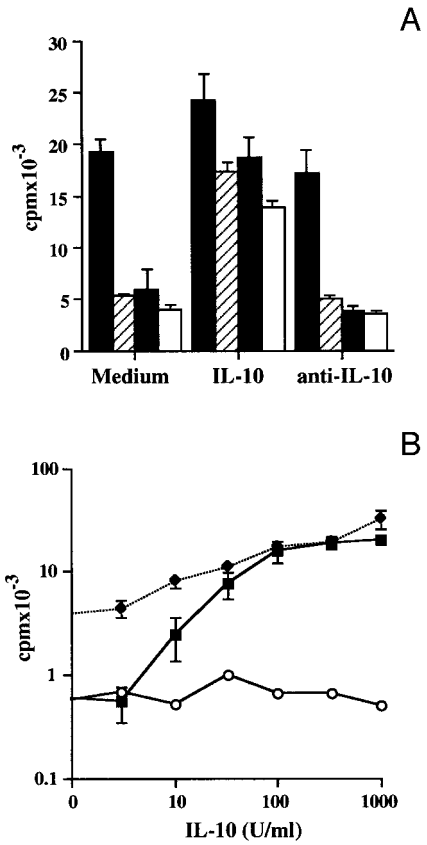

FIGURE 5. IL-10 act as a cofactor for IL-2 to induce $CD8^+$ T-cell proliferation. *A*, Purified $CD8^+$ T cells were stimulated with cross-linked anti-CD3 mAb alone (gray bars) or in the presence of anti-IL-2 mAb (BG-5, 10 μg/ml; white stripped bars) or anti-IL-2 R α-chain (B-B10, 10 μg/ml; black bars) or both anti-IL-2 and anti-IL-2R α-chain (white bars). Where indicated, IL-10 was added at 100 U/ml and anti-IL-10 (9D7) at 10 μg/ml. *B*, Purified $CD8^+$ T cells were stimulated with cross-linked anti-CD3 mAbs. After 5 days, cells were collected, washed, and cultured with different concentrations of IL-10 either alone (○) or in the presence of 2 U/ml (■ or 20 U/ml (♦) of rIL-2. Cell proliferation was measured by a pulse of [^{3}H]TdR in the last 12 h of day 2. No IL-2 secretion was detectable by ELISA on preactivated $CD8^+$ T cells.

the proliferative responses of $CD8^+$ T cells induced by activation with cross-linked anti-CD3 mAbs. Furthermore, no unresponsive state was achieved after incubation of $CD8^+$ T cells with IL-10 for 10 days following stimulation with cross-linked CD3 mAb. Taken together, these data indicate that T cell unresponsiveness induced by IL-10 is not related to a direct effect on $CD8^+$ T cells but to inhibition of Ag-presenting and accessory function of monocytes, which is associated with down-regulation of monokine secretion and expression of class I MHC and accessory molecules on these cells (Figs. 1 and 3).

It is well established that mouse $CD4^+$ and $CD8^+$ T cell clones lose their ability to proliferate and to secrete IL-2 and that they become anergic when stimulated with fixed APC (35, 36). Additional experiments have shown that these activation conditions allow TCR engagement in the absence of the costimulatory signals mediated by APC. Indeed, it has been shown, mostly for $CD4^+$ T cell clones, that anergy is induced when T cells are stimulated by APC in which the costimulatory signals are blocked by Abs directed against key costimulatory molecules such as CD80, CD86, CD54, or CD58 (37–39). However, $CD4^+$ and $CD8^+$ T cells may have different requirements for costimulation and induction of anergy, which could explain the differences in their susceptibility to be rendered unresponsive by IL-10. In $CD4^+$ T cells expressing CD80, costimulatory signals provided by T-T cell interaction can be sufficient to reach the threshold required to induce cell proliferation after CD3 cross-linking (40). Il-10-mediated down-regulation of these costimulatory signals provided by $CD4^+$ T cells may explain the observed anergic state of $CD4^+$ T cells. $CD8^+$ T cells seem to be less dependent on costimulatory signals since these cells could be induced to proliferate in response to stimulation by fibroblasts, whereas $CD4^+$ T cells failed to do so. Therefore, it may be speculated that because of this relative independence of costimulatory signals, IL-10 is not effective in inhibiting proliferation and inducing unresponsiveness of purified $CD8^+$ T cells activated in the absence of professional APC.

The failure of $CD8^+$ T cells to proliferate in response to allogeneic monocytes in the presence of IL-10 correlated with a reduced capability of these $CD8^+$ T cells to lyse allogeneic target cells. These results may reflect a decrease in the frequency of effector cells due to IL-10 inhibition of T cell activation in primary MLR. Alternatively, it is possible that a functional impairment of the $CD8^+$ T cells is induced by in vitro priming in the presence of IL-10, resulting in lack of both proliferation and cytotoxic activity. This latter hypothesis is supported by a recent study in which it is shown that IL-10 is responsible for the lack of cytotoxic activity by specific $CD8^+$ T cell clones and that blocking of endogenous IL-10 production restored the cytotoxic activity (41). In contrast to the suppressive effects of IL-10 on the proliferative and cytotoxic responses of $CD8^+$ T cells activated in the presence of APC, IL-10 enhances the proliferation of preactivated human $CD8^+$ T cells induced by IL-2. Therefore, IL-10 has a stimulatory function on activated $CD8^+$ T cells by acting as a cofactor for IL-2. The mechanism by which IL-10 acts as a cofactor for IL-2 in promoting the proliferation of activated $CD8^+$ T cells is unclear. However, in contrast to a previous report on $CD4^+$ T cells (42), IL-10 does not enhance the IL-2R α-chain on activated $CD8^+$ T cells (not shown). Moreover, the observation that IL-10 sustains $CD8^+$ T cell proliferation in the presence of an anti-IL-2R mAb suggests that the synergy between IL-10 and IL-2 is not mediated through IL-2R expression.

Overall, these data suggest that IL-10 may inhibit or stimulate $CD8^+$ T cells, depending on the time at which the cells are exposed to IL-10. These differential effects may explain some discrepancies observed in vivo in different experimental models. It can be hypothesized that IL-10 is effective in vivo in preventing and inhibiting undesirable immune responses mediated by $CD8^+$ T cells, if present at the same time as Ag exposure, but that it fails to suppress ongoing responses mediated by activated $CD8^+$ T cells. This should be taken into account for the future potential clinical use of IL-10 in autoimmune diseases and organ transplantation.

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References

1. Yssel, H., R. de Waal Malefyt, M. G. Roncarolo, J. S. Abrams, R. Lahesmaa, H. Spits, and J. E. de Vries. 1992. IL-10 is produced by subsets of human $CD4^+$ T cell clones and peripheral blood T cells. *J. Immunol. 149:2378.*
2. de Waal Malefyt, R., J. Haanen, H. Spits, M. G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med. 174:915.*

3. de Waal Malefyt, R., J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med. 174:1209.*
4. Fiorentino, D. F., A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol. 147: 3815.*
5. Ding, L., P. S. Linsley, L. Y. Huang, R. N. Germain, and E. M. Shevach. 1993. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J. Immunol. 151:1224.*
6. Willems, F., A. Marchant, J. P. Delville, C. Gerard, A. Delvaux, T. Velu, M. de Boer, and M. Goldman. 1994. Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur. J. Immunol. 24:1007.*
7. Chang, C. H., M. Furue, and K. Tamaki. 1995. B7-1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is down-regulated by interferon-gamma or by interleukin-10. *Eur. J. Immunol. 25:394.*
8. Peguet-Navarro, J., C. Moulon, C. Caux, C. Dalbiez-Gauthier, J. Banchereau, and D. Schmitt. 1994. Interleukin-10 inhibits the primary allogeneic T cell response to human epidermal Langerhans cells. *Eur. J. Immunol. 24:884.*
9. Caux, C., C. Massacrier, B. Vanbervliet, C. Barthelemy, Y. J. Liu, and J. Banchereau. 1994. Interleukin 10 inhibits T cell alloreaction induced by human dendritic cells. *Int. Immunol. 6:1177.*
10. de Waal Malefyt, R., H. Yssel, and J. E. de Vries. 1993. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells: specific inhibition of IL-2 production and proliferation. *J. Immunol. 150:4754.*
11. Taga, K., H. Mostowski, and G. Tosato. 1993. Human interleukin-10 can directly inhibit T-cell growth. *Blood 81:2964.*
12. Bejarano, M. T., R. de Waal Malefyt, J. S. Abrams, M. Bigler, R. Bacchetta, J. E. de Vries, and M. G. Roncarolo. 1992. Interleukin 10 inhibits allogeneic proliferative and cytotoxic T cell responses generated in primary mixed lymphocyte cultures. *Int. Immunol. 4:1389.*
13. Groux, H., M. Bigler, J. E. de Vries, and M. G. Roncarolo. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells. *J. Exp. Med. 184:19.*
14. Rousset, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D. H. Hsu, R. Kastelein, K. W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA 89:1890.*
15. Defrance, T., B. Vanbervliet, F. Briere, I. Durand, F. Rousset, and J. Banchereau. 1992. Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J. Exp. Med. 175:671.*
16. Taga, K., B. Cherney, and G. Tosato. 1993. IL-10 inhibits apoptotic cell death in human T cells starved of IL-2. *Int. Immunol. 5:1599.*
17. MacNeil, I. A., T. Suda, K. W. Moore, T. R. Mosmann, and A. Zlotnik. 1990. IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol. 145:4167.*
18. Chen, W. F., and A. Zlotnik. 1991. IL-10: a novel cytotoxic T cell differentiation factor. *J. Immunol. 147:528.*
19. Blazar, B. R., P. A. Taylor, S. Smith, and D. A. Vallera. 1995. Interleukin-10 administration decreases survival in murine recipients of major histocompatibility complex disparate donor bone marrow grafts. *Blood 85:842.*
20. Wogensen, L., X. Huang, and N. Sarvetnick. 1993. Leukocyte extravasation into the pancreatic tissue in transgenic mice expressing interleukin 10 in the islets of Langerhans. *J. Exp. Med. 178:175.*
21. Wogensen, L., M. S. Lee, and N. Sarvetnick. 1994. Production of interleukin 10 by islet cells accelerates immune-mediated destruction of beta cells in nonobese diabetic mice. *J. Exp. Med. 179:1379.*
22. Spits, H., G. Keizer, J. Borst, C. Terhorst, A. Hekman, and J. E. de Vries. 1983. Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma 2:423.*
23. Herve, P., J. Wijdenes, J. P. Bergerat, P. Bordigoni, N. Milpied, J. Y. Cahn, C. Clement, R. Beliard, B. Morel-Fourrier, E. Racadot, X. Troussard, E. Benz-Lemoine, C. Gaud, M. Legros, M. Attal, M. Kloft, and A. Peters. 1990. Treatment of corticosteroid resistant acute graft-versus-host disease by in vivo administration of anti-interleukin-2 receptor monoclonal antibody (B-B10). *Blood 75:1017.*
24. Yssel, H., J. E. De Vries, M. Koken, W. Van Blitterswijk, and H. Spits. 1984. Serum-free medium for generation and propagation of functional human cytotoxic and helper T cell clones. *J. Immunol. Methods 72:219.*
25. Bacchetta, R., M. Bigler, J. L. Touraine, R. Parkman, P. A. Tovo, J. Abrams, R. de Waal Malefyt, J. E. de Vries, and M. G. Roncarolo. 1994. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J. Exp. Med. 179: 493.*
26. de Waal Malefyt, R., C. G. Figdor, and J. E. de Vries. 1994. Regulation of human monocyte functions by interleukin-10. In *Interleukin-10*. J. E. de Vries, and R. de Waal Malefyt, eds. R. G. Landes Company, Austin, TX, pp. 37–52.
27. de Waal Malefyt, R., H. Yssel, M. G. Roncarolo, H. Spits, and J. E. de Vries. 1992. Interleukin-10. *Curr. Opin. Immunol. 4:314.*
28. Fiorentino, D. F., A. Zlotnik, P. Vieira, T. R. Mosmann, M. Howard, K. W. Moore, and A. O'Garra. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol. 146:3444.*
29. Moore, K. W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T. R. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol. 11:165.*
30. Bean, A. G. D., R. A. Freiberg, S. Andrade, S. Menon, and A. Zlotnik. 1993. Interleukin 10 protects mice against staphylococcal enterotoxin B-induced lethal shock. *Infect. Immun. 61:4337.*
31. Chernoff, A. E., E. V. Granowitz, L. Shapiro, E. Vannier, G. Lonnemann, J. B. Angel, J. S. Kennedy, A. R. Rabson, S. M. Wolff, and C. A. Dinarello. 1995. A randomized, controlled trial of IL-10 in humans: inhibition of inflammatory cytokine production and immune responses. *J. Immunol. 154:5492.*
32. Holler, E., M. G. Roncarolo, and R. Hintermeier-Knable. 1994. Low incidence of transplant-related complications in patients with high spontaneous cellular IL-10 production prior to conditioning: evidence for a protective role of IL-10 in allogeneic BMT. In *Annual Meeting of the EBMT.*
33. Krenger, W., K. Snyder, S. Smith, and J. L. Ferrara. 1994. Effects of exogenous interleukin-10 in a murine model of graft-versus-host disease to minor histocompatibility antigens. *Transplantation 58:1251.*
34. Lee, M.-S., L. Wogensen, J. Shizuru, M. B. A. Oldstone, and N. Sarvetnick. 1994. Pancreatic islet production of murine interleukin-10 does not inhibit immune-mediated tissue destruction. *J. Clin. Invest. 93:1332.*
35. Jenkins, M. K., and R. H. Schwartz. 1987. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J. Exp. Med. 165:302.*
36. Otten, G. R., and R. N. Germain. 1991. Split anergy in a $CD8^+$ T cell: receptor dependent cytolysis in the absence of interleukin-2 production. *Science 251:1228.*
37. Boussiotis, V. A., J. G. Gribben, G. J. Freeman, and L. M. Nadler. 1994. Blockade of the CD28 co-stimulatory pathway: a means to induce tolerance. *Curr. Opin. Immunol. 6:797.*
38. Jendrisak, G., J. Gamero, T. Mohanakumar, and M. Jendrisak. 1993. Clonal anergy induction by monoclonal antibodies to CD4, LFA-1, and ICAM-1. *Transplant. Proc. 25:828.*
39. Boussiotis, V. A., G. J. Freeman, J. D. Griffin, G. S. Gray, J. G. Gribben, and L. M. Nadler. 1994. CD2 is involved in maintenance and reversal of human alloantigen-specific clonal anergy. *J. Exp. Med. 180:1665.*
40. Viola, A., and A. Lanzavecchia. 1996. T cell activation determined by T cell receptor number and tunable thresholds. *Science 273:104.*
41. Rohrer, J. W., and J. H. Coggin, Jr. 1995. CD8 T cell clones inhibit antitumor T cell function by secreting IL-10. *J. Immunol. 155:5719.*
42. Cohen, S. B., P. D. Katsikis, M. Feldmann, and M. Londei. 1994. IL-10 enhances expression of the IL-2 receptor alpha chain on T cells. *Immunology 83:329.*