IL-2 Upregulates CD86 Expression on Human CD4+ and CD8+ T Cells

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IL-2 Upregulates CD86 Expression on Human CD4+ and CD8+ T Cells

Ananta Paine,* Hartmut Kirchner,† Stephan Immenschuh,* Mathias Oelke,‡ Rainer Blasczyk,* and Britta Eiz-Vesper*

The glycoprotein CD86 is an important costimulatory molecule that has been shown to be predominantly expressed on APCs, such as dendritic cells, macrophages, and B cells. More recently, CD86 was also detected on T cells in specific pathological conditions. The mechanisms of how CD86 might be induced and its functional role in T cells are not well understood. In the present study, we showed that treatment with IL-2 markedly upregulated CD86, but not CD80, in human CD4+ and CD8+ T cells. This upregulation occurred in the absence of bystander cells, and isolated naive CD4+ or CD8+ T cells exhibited different time-dependent CD86-expression patterns in response to IL-2. Upregulation of CD86 on activated T cells was reduced by Abs that block IL-2 and IL-2R (CD25), indicating a receptor-mediated mechanism. IL-2–dependent CD86 upregulation was blocked by pharmacological inhibitors of the NFAT and mammalian target of rapamycin pathways and was largely reduced by simultaneous exposure to IFN-α. Importantly, a marked increase in CD86 on T cells was also observed in vivo in IL-2–treated patients. In conclusion, IL-2 upregulates CD86 expression on human CD4+ and CD8+ T cells via a receptor-dependent mechanism that involves the NFAT and mammalian target of rapamycin pathways.

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CD86, also known as B7.2, is a central costimulatory molecule that is considered to be mainly expressed on APCs, such as dendritic cells (DCs), macrophages, and B cells (1). CD86 is a ligand for CD28 and CTLA-4, both of which are immunologically important receptors on T cells and provide signals for the activation or inhibition of Ag-specific T cells (2). Similar to CD86, another member of the B7 family, CD80 (B7.1) also interacts with CD28 and CTLA-4 (3). Although increased levels of CD86 and CD80 on mature and activated APCs improve T cell-mediated immune responses toward pathogens and/ or malignant cells, excessive expression of these molecules has been associated with autoimmune responses (1, 4–7). Thus, expression of these costimulatory molecules is controlled via a complex network of regulatory pathways.

More recently, CD86 was shown to be expressed on T cells in specific pathological conditions (8–11). Independent groups demonstrated that CD86 was detected on allergen-specific T cells (9), tumor-infiltrating lymphocytes (11), T cells from HIV-infected patients (8, 12), and hepatitis C virus-specific CD8+ T cells (13). Collectively, these reports suggested that TCR/costimulation-dependent activation of T cells might be a prerequisite for CD86 expression on T cells (14–16). It is well known that IL-2 plays a central role for T cell activation in vitro and in vivo (17) and in T cell-mediated immune responses (17–19). Moreover, IL-2 has been widely used for therapeutic applications in patients with malignant (17, 20–22) and infectious disorders (23–26). Although most previous reports focused on the impact of IL-2 on T cell survival and proliferation, only few studies addressed IL-2–induced phenotypic changes in T cells, which are particularly relevant in cytokine therapy. Because the role of IL-2 in regulating CD86 expression in T cells is not well understood, the goal of the current study was to investigate the specific effects of IL-2 on CD86 in human T cells.

It is shown that IL-2 upregulates the expression of CD86, but not that of CD80, on cell cultures of CD4+ or CD8+ T cells, even in the absence of bystander cells. Although exposure to IL-2 led to comparable CD86+ CD4+ and CD8+ T cells in PBMCs, separately cultured naïve CD4+ or CD8+ T cells showed differences in their CD86-expression patterns in response to IL-2. Studies with pharmacological inhibitors showed that CD86 upregulation by IL-2 is mediated via the NFAT and mammalian target of rapamycin (mTOR) pathways. Moreover, IL-2–dependent CD86 upregulation was largely reduced by IFN-α. Finally, an increased expression of CD86 was also observed on T cells in vivo in patients during IL-2 therapy.

Materials and Methods

Isolation of PBMCs and T lymphocyte subsets

PBMCs were isolated from blood samples of healthy blood donors and patients, as indicated. Prior approval for this study was received from the local ethics committee. PBMCs were isolated by discontinuous-gradient centrifugation, washed twice in sterile PBS, and resuspended at a concentration of $1 \times 10^6$ cells/ml in M’ medium supplemented with 10% heat-inactivated human AB serum (C.C.pro, Neustadt, Germany). Standard M’ medium was prepared, as described before (27), and was used in all cell culture experiments. CD3+, CD4+, and CD8+ T cells from PBMCs of healthy donors were enriched by magnetic cell sorting (MACS), using negative selection kits (Miltenyi Biotec, Bergisch Gladbach, Germany), or FACS, using a FACSARia (BD Biosciences, Heidelberg, Germany). Unless specified, the purity of the respective cell populations was 90–99.8%, as determined by flow cytometry.
Abs, reagents, flow cytometry, and intracellular staining

The following Abs were used for cell surface marker staining: anti-CD3-FITC (UCHT1), anti-CD4-FITC (13B2), anti-CD8-PE/FITC (B9.11), anti-CD80-PE/FITC (HA3-2B7), anti-CD86-PE/FITC (MAB104), and anti-CD122-FITC (RB518), all of which were purchased from Beckman Coulter (Krefeld, Germany). 

mRNA quantification by real-time RT-PCR

Total RNA was extracted using an RNeasy kit (Qiagen), and cDNA was synthesized using 1 μg RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). 

Generation of human mature DCs from primary monocytes

Primary monocytes were isolated from PBMCs of healthy donors using MACS magnetic separation system with CD14 MicroBeads (Miltenyi Biotec). Purified mononuclear cells (1 × 10^6 cells/ml) were cultured in M² medium supplemented with 2% heat-inactivated human AB serum (C. C.pro), 500 U/ml IL-4, and 800 U/ml GM-CSF for 5 d. Media change and addition of fresh media, IL-4, and GM-CSF was done after 2 d. On day 5, DCs were cultured with anti-CD3/CD28–conjugated beads (Dynabeads CD3/CD28 T Cell Expander; Invitrogen, Karlsruhe, Germany), with or without anti–IL-2 and anti-CD25 mAb (each 10 μg/ml). For blocking experiments, purified mouse anti-CD3 (clone UCD-1), anti-CD25 (clone PC61), and anti–IL-2Rα (clone 313, clone L307.4), anti–CD62L-PE (SK11), anti–CCR7-PE–Cy7 (3D12), anti–CD127-PE–Cy7 (HB7), anti–CD71-FITC (M-A712), anti–CD98-FITC (UM7F8), anti–p-STAT5–PE (pY694), anti–CD44-FITC (G44-26), anti–CD38-FITC (2RB), and anti–CD86-allophycocyanin (FUN-1), anti–CD80-PE (MAB104), and anti–CD122–PE (HA5.2B7), anti–CD80-FITC (MAB104), and anti–CD122–PE (TC13D12) were used as positive control. The purity, as determined by flow cytometry, was up to 99%. Isolated CD68+ T cells were fixed with 1% paraformaldehyde (PFA) for 10 min and washed three times before use. Naïve CD45RA-CD4+ T cells were purified from PBMCs of healthy blood donors by FACS sorting using anti-CD4, anti–CD8, anti-CD45RA, and anti-CD45RO mAbs (purity up to 99%). Isolated CD4+ T cells were stained with CFSE. For CFSE staining, 0.5–2 × 10^6 naïve CD4+ T cells were labeled with 1 μM CFSE (Invitrogen). Thereafter, 2.5 × 10^5 CFSE-stained naïve CD4+ T cells from healthy donors (n = 8) were cultured with anti-CD3 Ab-conjugated beads (Dynabeads human CD3; Invitrogen) in combination with 2.5 × 10^5 PFA-fixed CD86+ or CD86− T cells/well in a 96-well round-bottom culture plate. Each experiment was performed in triplicates. CFSE-stained naïve CD4+ T cells, cultured alone or with only anti-CD3-conjugated beads, were used as negative control. Naïve CD4+ T cells, cultured with anti-CD3/CD28–conjugated beads (Dynabeads human CD3/CD28 T Cell Expander; Invitrogen), were used as positive control. After 6 d, cell proliferation was measured as CFSE dye dilution by flow cytometry (29). The results are given as the percentage of cells undergoing proliferation in response to stimulation with anti-CD3–conjugated beads in combination with either CD86+ or CD86− T cells or anti-CD3/CD28–conjugated beads.

Results

IL-2 upregulates CD86 on T cells

We studied the phenotypical changes of T cells in IL-2-treated PBMCs from healthy blood donors (Fig. 1). An unexpected upregulation of CD86, also termed B7.2, on T cells was observed by flow cytometry studies (Fig. 1A). As a control, levels of CD80 (also known as B7.1), another member of the B7 family, were not altered in response to IL-2 (Fig. 1B). We also determined the levels of CD86 on CD4+ and CD8+ T cell subsets separately. CD86 levels increased in both T cell subsets and reached a maximum at day 10 (Fig. 1C, 1D). After day 10, a gradual decrease in CD86+ was observed in CD4+, but not in CD8+, T cells (Fig. 1D). We also performed IL-2 dose-dependency studies and found a statistically significant correlation between the dose of IL-2 and CD86 expression on CD4+ and CD8+ T cells.

IL-2-induced CD86+ T cells are of effector/memory phenotype

We further characterized IL-2–treated CD86+ T cells by flow cytometry with Abs against various cell surface markers. As summarized in Table I, CD86+ positivity was mainly observed on CD45RA-CD45RO+ effector/memory T cells. In addition, we found that CD86+ T cells expressed higher levels of the activation markers CD25, CD38, CD69, CD71, and CD98 compared with CD86− T cells (Table I). However, the expression of these activation markers was mainly transient. Interestingly, higher levels of the costimulatory molecules CD70 and CD30 were also observed on a fraction of in vitro IL-2–induced CD86+ T cells but not on CD86− T cells (Table I). We also analyzed expression level of FOXP3, CD127, CD62L, CR7, and CD44 on CD86+ and CD86− T cells, and fractions of both subsets expressed FOXP3, CD127, CD62L, CC7, and CD44 (Table I).
IL-2 upregulates CD86 expression on naive CD8+ T cells but not on naive CD4+ T cells

Next, we examined the levels of CD86 on naive or effector/memory T cells. Naive and effector/memory CD4+ and CD8+ T cells were separated by cell sorting, based on the presence of the phenotypic markers CD45RA and CD45RO. Our experiments showed that naive CD4+ T cells did not express CD86, even after 10 d of IL-2 treatment, whereas CD8+ naive T cells exhibited CD86 upregulation similar to its effector/memory counterparts (Fig. 2A). Increased levels of CD86 in response to IL-2 on CD4+ and CD8+ T cells were clearly visible in effector and memory T cells. Thus, our experiments indicated that naive CD4+ and CD8+ T cells have a different pattern of IL-2–dependent CD86 upregulation. We further determined the expression of the three IL-2R chains (IL-2Rα [CD25], IL-2Rβ [CD122], and IL-2Rγ [CD132]) in naive and memory CD4+ and CD8+ T cells. Our experiments showed that expression levels of these three subunits were higher in memory subsets of CD4+ and CD8+ T cells than in their naive counterparts (Fig. 2B). Furthermore, levels of IL-2Rα, IL-2Rβ, and IL-2Rγ in naive CD8+ T cells were higher compared with their naive CD4+ counterparts (Fig. 2B). Thus, the differences in IL-2–dependent CD86 upregulation in naive and memory CD4+ and CD8+ T cells might be due to different expression levels of IL-2R subunits on these cells.

CD86 expression on CD4+ and CD8+ T cells does not require the presence of bystander cells

Because CD86 is predominantly expressed on professional APCs (31), the presence of this protein on T cells might be a consequence of membrane transfer from CD86-expressing bystander cells (32, 33) or the uptake of CD86-containing exosomes (34). Alternatively, the T cell phenotype might be affected by bystander cells through cell–cell interactions or secreted cytokines. To examine how CD86 might be upregulated on IL-2–activated T cells, we purified CD4+ and CD8+ T cell subsets from PBMCs and cultured them with IL-2 in the absence of other cells. As shown in Fig. 3A, upregulation of CD86 was also observed on isolated CD4+ and CD8+ T cells in our in vitro experiments, >90% of T cells became CD86+ when cultured with 100 IU/ml IL-2 for 10 d (Fig. 3B), suggesting that CD86 expression on T cells is due to increased synthesis rather than to protein transfer from bystander cells.

To further investigate whether CD86 expression on CD4+ and CD8+ T cell subsets might be interdependent of each other, we...
performed additional experiments with isolated CD4+ and CD8+ T cells. In donor 1, treatment with IL-2 caused CD86 upregulation of 54% in CD4+ T cells and 93% in CD8+ T cells, independent of whether they were cocultured or cultured separately (Fig. 3B). Moreover, in two donors, exposure to IL-2 resulted in a higher percentage of CD8+CD86+ T cells when CD8+ T cells were cultured in the presence of the CD4+ counterpart compared with cell culture alone (donors 2 and 3). In these cases, percentages of CD4+CD86+ T cells were comparable (∼45% and 55% of CD86+CD4+ in donor 2 and 3, respectively) (Fig. 3B).

Expression of CD86 mRNA by real-time RT-PCR was analyzed in purified CD3+ T cells before or after exposure to IL-2. We observed that IL-2 caused a marked increase in CD86 mRNA synthesis in T cells (Fig. 3C). These results further indicated that CD86 expression on T cells is due to increased synthesis of CD86. In conclusion, these results demonstrated that CD86 expression on CD4+ and CD8+ T cells is not dependent on the presence of bystander cells.

Exposure to IL-2 upregulates CD86 expression on CD25−CD86− T cells

We also investigated whether CD86+ T cells would occur as a result of the expansion of initially present CD86+ T cells or as a result of CD86− T cells acquiring a CD86+ phenotype in response to IL-2 exposure. To this end, we selectively purified CD25−CD86− T cells by flow cytometry-based cell sorting and cultured them with IL-2. Our experiments showed that, even in isolated CD25−CD86− T cells (purity > 99.9%), 69% of those cells acquired a CD86+ phenotype (after 10 d, Fig. 3D). As demonstrated in Fig. 3D, 63% cells were CD86+CD25−, whereas ∼5% were CD86+CD25+ (day 10).

Upregulation of CD86 on activated T cells is mediated via secreted IL-2

Activation of T cells was shown to cause an increase in CD86 on T cells (14–16). Because this upregulation might be due to an autocrine mechanism via secreted IL-2, we performed studies with anti–IL-2– and anti–IL-2R–blocking Abs in T cells. More spe-

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**Table 1. Phenotypic characteristics of CD86+ and CD86− T cells**

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The phenotype of the CD86− and CD86+ T cells was compared by flow cytometric analysis with fluorescent-labeled mAbs against the markers specified above. Patient and in vitro data are summarized in the table above (n = 4–10).

+ Transient expression.
−, Notable/high level of expression; −, low expression/undetectable.

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**FIGURE 2.** IL-2 upregulates CD86 expression on naive CD8+ T cells but not on naive CD4+ T cells. CD45RA+ (naive) and CD45RO+ (effector/memory) CD4+ and CD8+ T cells were isolated from PBMCs by cell sorter and were cultured with 100 IU/ml of IL-2 for 10 d. Thereafter, cells were stained with different fluorescent-labeled Abs against different phenotypic markers. One representative example is shown (n = 3). Results are expressed as the percentage of CD86+ cells among CD4+ (A) or CD8+ T cells (B). C, Expression levels of different receptor chains of IL-2R (i.e., CD25 [IL-2Rα], CD122 [IL-2Rβ], and CD132 [IL-2Rγ]) on naive and memory CD4+ and CD8+ T cells were analyzed by flow cytometry, and the average of respective mean fluorescence intensity (MFI) values obtained from four different independent experiments are presented as bar graphs (n = 4).
specifically, isolated T cells were stimulated with anti-CD3/anti-CD28 Ab-coated cell-sized beads in the presence or absence of anti–IL-2 and anti–IL-2Rα (anti-CD25)-blocking Abs. Although incubation of T cells with anti-CD3/anti-CD28–coated beads resulted in a significant increase in CD86 expression on T cells (Fig. 4), we noted that addition of anti–IL-2– and anti–IL-2Rα–blocking Abs inhibited the upregulation of CD86 expression on activated T cells (Fig. 4). Therefore, the results suggested that CD86 upregulation on activated T cells might be primarily due to an autocrine mechanism that is mediated via secreted IL-2.

**CsA and Rapa block IL-2–dependent CD86 upregulation on T cells**

CsA and Rapa are immunosuppressants, which are commonly used in the context of transplantation, and are known to prevent T cell-mediated graft rejections. Importantly, both compounds interfere with IL-2 secretion or IL-2–dependent signaling in T cells. To examine whether these compounds might have an impact on CD86 upregulation by IL-2 on T cells, we cultured T cells with anti-CD3/anti-CD28–coated beads in the presence of CsA or Rapa. Although incubation of T cells with anti-CD3/anti-CD28–coated beads resulted in a significant increase in CD86 expression on T cells (Fig. 4), we noted that addition of anti–IL-2– and anti–IL-2Rα–blocking Abs inhibited the upregulation of CD86 expression on activated T cells (Fig. 4). Therefore, the results suggested that CD86 upregulation on activated T cells might be primarily due to an autocrine mechanism that is mediated via secreted IL-2.

**IFN-α has an inhibitory effect on IL-2–dependent upregulation of CD86 on T cells**

IFN-α is frequently used in combination with IL-2 for the treatment of cancer patients (21, 35). To determine the effects of IFN-α on the IL-2–dependent CD86 upregulation in T cells, we cultured PBMCs with IL-2 (100 IU/ml) alone or in combination with IFN-α (IFN-α–2a, 100 IU/ml). IFN-α alone was used as a negative control.
monitored levels of STAT5 in CD86+ and CD86
STAT5 was shown to mediate IL-2 signaling in T cells. We
CD86+ T cells exhibit higher levels of p-STAT5
IL-2–mediated upregulation of CD86 on T cells.
Thus, IFN-
both cytokines together, the percentage of CD86+ T cells was
shown). Thus, IFN-
by flow cytometry. Only IL-15 was able to upregulate CD86 on
T cells, PBMCs from healthy donors were cultured with IL-7,
cytokines, IL-7 and IL-15, might also affect CD86 expression on
T cells. For this purpose, CD3+CD86+ and CD3+CD86– T cells were sorted, fixed, and cocultured with CFSE-labeled naive CD4+ T cells in combination with anti-CD3–conjugated beads. In our assays, CD86 expressed on IL-2–treated T cells showed no apparent costimulatory effect (Fig. 7).

CD86 levels on T cells are upregulated in vivo by IL-2
To investigate the impact of IL-2 in vivo, we collected PBMCs from IL-2–treated patients and studied the levels of CD86 on T cells. Increased levels of CD86 on T cells from IL-2–treated patients appeared to correlate with our in vitro experiments (Tables II, III). CD86 expression increased from 1.8% on day 0 to 41.2% on day 7 in patient 1 (Tables II, III). Similarly, >32% of the second patient’s T cells expressed CD86 during treatment (Tables II, III). In patients 3 and 4, after 7 d of therapy, up to 48.7% of T cells were CD86+ (Tables II, III). In patients 1 and 2, the upregulation of CD86 expression was more evident in CD8+ T cells than in CD4+ T cells (Tables II, III). After 7 d of stimulation, up to 42% of CD8+ T cells were CD86+ compared with up to 22% of CD4+ CD8+ T cells. In patients 3 and 4, initially the expression of CD86 was more evident on CD4+ T cells; however, on day 7, percentage of CD86+ T cells was equal or slightly higher in the CD8+ T cell subset compared with the CD4+ counterpart. Taken together, these results indicated that IL-2 upregulates expression of CD86 (B7.2) on human CD4+ and CD8+ T cells in vivo.

Discussion
The costimulatory molecules CD80 and CD86 are predominantly expressed on professional APCs (i.e., DCs, macrophages, and activated B cells) (2). Both molecules, which belong to the B7 family of proteins, either interact with the activating receptor CD28 or with the inhibitory receptor CTLA-4 on T cells and modulate the activation of these cells. However, expression of CD80 and CD86 on nonprofessional APCs is not well studied. Because CD86 was also recently detected on allergen-specific T cells (9), tumor-infiltrating lymphocytes (11), and T cells from HIV-infected (12) and hepatitis C virus-infected individuals (13), we examined the role of IL-2 in the upregulation of CD86 on T cells in this study. We noted that exposure to IL-2 alone markedly increased expression of CD86 on T cells. Furthermore, IL-2 upregulated CD86 expression on T cells in a time- and dose-dependent manner, and it was restricted to T cells with an effector/memory phenotype.

Uregulation of CD86 by IL-2 in CD4+ and CD8+ T cell subsets
IL-2-dependent upregulation of CD86 in CD4+ and CD8+ T cell subsets exhibited different time-dependent expression patterns (Fig. 1). Although a decreased percentage of CD86 positivity on CD4+ T cells was noted after 10 d, CD8+ T cells appeared to have sustained CD86 expression for up to 21 d. Our study also showed that isolated naive and effector/memory CD4+ and CD8+ T cells had clear differences in terms of IL-2–dependent CD86 upregulation (Fig. 2). IL-2 exposure to naive CD8+, but not naive CD4+, T cells led to a marked increase in CD86 expression. However, in the case of effector/memory CD4+ and CD8+ T cells,
exposure to IL-2 caused a marked increase in CD86 expression on both of these T cell subsets. This observation showed additional differences within CD4+ and CD8+ T cell subsets in terms of IL-2–dependent CD86 upregulation. Results from our experiments indicated that these differences might be due to the differences in the expression levels of IL-2Rα, IL-2Rβ, and IL-2Rγ.

Because exposure to IL-2 resulted in a similar or higher percentage of CD86+CD8+ T cells compared with CD8+ T cells, which were cultured alone without CD4+ cells (Fig. 3B), the effect of IL-2 on CD86 in isolated CD4+ or CD8+ T cells appeared to be independent of the presence of both T cell subsets. This finding is in contradiction to earlier work with HIV patients, in which it was suggested that the upregulation of CD86 expression on CD8+ T cell might depend on the presence or absence of CD4+ T cells (8). Earlier studies suggested that T cells can acquire CD86 and other surface proteins simply by membrane exchange with APCs (32, 33) or by exosome uptake (34). To rule out such bystander effects, we performed supplementary in vitro experiments to assess the expression of CD86 on CD8+ T cells using highly purified CD4+ and CD8+ T cells (Fig. 3B). A notable increase in CD86+ T cells was observed within 10 d (Fig. 3), suggesting that, although some acquisition of CD86 molecules from adjacent cells may occur in vivo, the IL-2–dependent increase in CD86 expression does not seem to be explained by such a mechanism.

Mechanisms of IL-2–dependent CD86 upregulation in T cells

Experiments with purified T cells showed that CD86 expression on CD4+ and CD8+ T cells is not dependent on the presence of bystander cells; rather, it resulted from increased expression of CD86 mRNA in IL-2–treated CD86+ T cells (Fig. 3C). We also evaluated whether the occurrence of the high percentage of CD86+ T cells after 7–10 d of IL-2 exposure resulted from the expansion of the initially existing CD86+ T cells. Our experiments with purified CD25+CD86+ T cells showed that IL-2 exposure can upregulate CD86 expression, even on T cells, despite the absence of CD25+ or CD86+ T cells (Fig. 3D).

To further investigate the mechanism of IL-2–mediated CD86 upregulation in T cells, we applied the pharmacological inhibitors CsA and Rapa, which are known to interfere with IL-2 signaling in T cells. CsA is known to prevent the dephosphorylation of NFAT by binding to cyclophilin. Our experiments showed that CsA prevented IL-2–induced CD86 expression on T cells, suggesting that NFAT is involved in CD86 induction. Unlike CsA, Rapa is known to inhibit IL-2 signaling and to block T cell activation by inhibiting the mTOR pathway. A significant reduction in IL-2–
induced CD86 expression on T cells by Rapa also indicated the involvement of mTOR in CD86 upregulation in T cells. Moreover, in our studies, we noted that STAT5 levels in IL-2–treated CD86+ T cells were significantly higher compared with their CD86+ counterparts, indicating a role for STAT5 proteins in IL-2–mediated CD86 upregulation in T cells. However, the exact interactions of the STAT5 pathway with the NFAT and/or mTOR cascades in this regulation are unclear. IFN-α was shown to affect IL-2–induced changes in human T cells (36, 37). Our current observations indicated that IFN-α negatively modulates the IL-2–induced upregulation of CD86 expression on T cells, which correlates with these earlier reports (36, 37). However, because of the pleiotropic nature of IFN-α and differential effects on different cell types, further studies are necessary to understand the detailed cross-talk of the signaling cascades by IL-2 and IFN-α in T cells and the potential relevance.

Clinical implications of the IL-2–dependent CD86 upregulation on T cells

Increased expression of CD86 on T cells might have far-reaching implications for the T cell-mediated immune response. It is well known that interactions of peptide-MHCs with TCRs are modulated by CD28-CD80/CD86 binding, which can augment the upregulation of Bcl-xL (intrinsic cell survival factor) and IL-2 in T cells (38–41). Increased and/or uncontrolled expression of CD86 and CD80 in specific pathological conditions may cause autoimmune responses (4–7). Similarly, Stephan et al. (42) demonstrated that the expression of costimulatory molecules, such as CD80 and 4-1BB, can cause auto- and trans-costimulation in T cells, which significantly improved immune responses against tumors in experimental conditions. IL-2 is known to be important for T cell activation and proliferation in vivo and in vitro; therefore, it was initially termed “T cell growth factor” (18, 19). Because of the present observations, it is tempting to suggest that IL-2 may not only act as a T cell growth factor, but may also indirectly enhance the immune response by inducing the expression of CD86 on T cells, which, in turn, could support T cell activation.

Our in vitro costimulation assays indicated that, in the absence of any supplementary cytokines, CD86 expressed on T cells did not show apparent costimulatory effects. Two reasons can be postulated: 1) our results from the RT-PCR experiments and flow cytometric analysis showed that CD86 mRNA and protein expression on T cells was markedly lower compared with mature DCs or monocytes (Figs. 1A, 3C). Therefore, it is also likely that CD86 expressed on T cells was unable to provide the necessary costimulatory signal simply because of their lower expression levels on T cells. 2) In contrast, it is possible that the interactions of CD86 expressed on T cells with CTLA-4 is stronger compared with binding with CD28. However, binding to CTLA-4 is not likely the main cause, because we did not note an inhibition of basal proliferation of responder cells (Fig. 7). Preliminary experiments showed that CD86 on IL-2–treated T cells did not bind to CD28, but it had significant binding affinity for CTLA-4 (data not shown). Our experiments showed that CD82-Ig and CTLA-4–Ig bind equally to monocyctic cells. Moreover, a polyclonal goat anti-CD86 Ab blocked the binding of CTLA-4–Ig to the CD86+ T cells, indicating that only CD86 on these T cells contributed to CTLA-4 binding (data not shown). CTLA-4 plays a critical role in the regulation of activated T cells by providing inhibitory effects (43), and it possesses greater binding affinity toward CD86 than CD28. The level of CTLA-4 expression in naive T cells is known to be significantly lower, and it increases significantly after successful activation (44, 45). Especially at sites of ongoing inflammation densely populated by activated T cells, CD86 expressed on T cells might play a regulatory role simply by interacting with CTLA-4 expressed on activated T cells (45). Therefore, it is likely that IL-2–mediated overexpression of CD86 on T cells might play a role in controlling the IL-2–mediated autoimmune and increased inflammatory response, and it may help to prevent tissue damage. Future in-depth studies should reveal more on this issue.

It is important to note that, in addition to our in vitro data, we noted a marked upregulation of CD86 on T cells in all IL-2–treated patients tested (Table II). Similar to our in vitro observations, we were unable to detect significant changes in the expression of CD80 on T cells in these IL-2–treated patients. Because our study showed that IL-2 upregulates CD86 on T cells in vitro, as well as in vivo, this event is likely to have major physiological significance and might have an important overall impact on the clinical outcome. In this regard, it is also remarkable that IL-2 was the first cytokine to be approved by the U.S. Food and Drug Administration (in 1992) for the treatment of advanced kidney cancer (46). Since then, this cytokine has been widely used for the treatment of different malignant and infectious diseases (17, 23–26, 47). However, positive clinical responses after IL-2 therapy remained limited. Recently, it was discovered that IL-2 also plays a crucial role in immunoregulation (48). Thus, a so-called “yin-and-yang effect” of this cytokine (48) has added new dimensions and raised concerns about the therapeutic use of IL-2. However, the existing evidence of its effectiveness (22, 46, 49–51) cannot be completely ignored. It is also noteworthy that moderate, reversible autoimmunity during cytokine therapy has been reported and was associated with better cure and survival in cancer patients (51). Thus, in IL-2 therapy, it is tempting to speculate that IL-2–induced CD86 expression and its interactions with CTLA-4 expressed on activated T cells might be a mechanism involved in controlling the excessive inflammatory responses. At the same time, this potential blunting effect might be a reason for the limited curative response associated with IL-2 therapy. Further studies are necessary to evaluate such effects. In recent years, in addition to IL-2, other members of common γ-chain cytokines, especially IL-7 and IL-15, have been tested or used to boost immune responses for the treatment of various diseases (17, 52, 53). Thus, the IL-2–like effect of IL-15 on CD86 expression on T cells (Fig. 6C) might be relevant to understanding the clinical outcome of their therapeutic usage.

### Table II. Increased expression of CD86 on T cells of patients during IL-2 therapy

<table>
<thead>
<tr>
<th>Patient Information</th>
<th>CD86/CD3+ T Cells (%)</th>
<th>CD86/CD4+ T Cells (%)</th>
<th>CD86/CD8+ T Cells (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 0</td>
</tr>
<tr>
<td>Patient 1 (mRCC)</td>
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<td>41.2</td>
<td>2.0</td>
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<td>Patient 2 (melanoma)</td>
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<td>34.1</td>
<td>4.5</td>
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<td>Patient 4 (melanoma)</td>
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<td>48.2</td>
<td>1.1</td>
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</tbody>
</table>

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</tbody>
</table>

mRCC. Metastatic renal cell carcinoma.
In summary, we reported that IL-2 upregulates expression of CD86, but not that of CD80, on T cells. Our studies showed that this IL-2-dependent upregulation on CD4+ and CD8+ T cell is not dependent on bystander cells and is likely mediated via NFAT and mTOR pathways. The present study highlighted a novel aspect of IL-2 and its influence on T cell phenotype, which might have a far-reaching impact on future IL-2–based cytokine therapies.

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Disclosures

The authors have no financial conflicts of interest.

References

10 IL-2 UPREGULATES CD86 ON HUMAN CD4+ AND CD8+ T CELLS