Effects of the Administration of High-Dose Interleukin-2 on Immunoregulatory Cell Subsets in Patients with Advanced Melanoma and Renal Cell Cancer

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Abstract

Purpose: High-dose recombinant human interleukin-2 (IL-2) therapy is of clinical benefit in a subset of patients with advanced melanoma and renal cell cancer. Although IL-2 is well known as a T-cell growth factor, its potential in vivo effects on human immunoregulatory cell subsets are largely unexplored.

Experimental Design: Here, we studied the effects of high-dose IL-2 therapy on circulating dendritic cell subsets (DC), CD1d-reactive invariant natural killer T cells (iNKT), and CD4+CD25+ regulatory-type T cells.

Results: The frequency of both circulating myeloid DC1 and plasmacytoid DC decreased during high-dose IL-2 treatment. Of these, only a significant fraction of myeloid DC expressed CD1d. Although the proportion of Th1-type CD4+ iNKT increased, similarly to DC subsets, the total frequency of iNKT decreased during high-dose IL-2 treatment. In contrast, the frequency of CD4+CD25+ T cells, including CD4+Foxp3+ T cells, which have been reported to suppress antitumor immune responses, increased during high-dose IL-2 therapy. However, there was little, if any, change of expression of GITR, CD30, or CTLA-4 on CD4+CD25+ T cells in response to IL-2. Functionally, patient CD25+ T cells at their peak level (immediately after the first cycle of high-dose IL-2) were less suppressive than healthy donor CD25+ T cells and mostly failed to Th2 polarize iNKT.

Conclusions: Our data show that there are reciprocal quantitative and qualitative alterations of immunoregulatory cell subsets with opposing functions during treatment with high-dose IL-2, some of which may compromise the establishment of effective antitumor immune responses.

Interleukin (IL)-2 was first identified in 1976 and entered clinical trials in cancer patients after it was shown to exert potent antitumor activity in several murine tumor models (1, 2). In highly selected patients with metastatic melanoma or renal cell cancer (RCC), treatment with high-dose bolus IL-2 has now been shown to produce durable responses (3, 4). Although IL-2 is well known as a T-cell growth factor and has been shown to result in the early depletion and subsequent expansion of lymphoid subsets after in vivo administration (5), no data are available on the effects of high-dose IL-2 therapy on immunoregulatory natural killer (NK) T cells and CD4+CD25+ regulatory T cells.

CD1d-restricted invariant NK T cells (iNKT) display a semi-invariant T-cell receptor (Vα24/Jα18 in human and Vα14/Jα18 in mouse) and are characterized by their capacity to rapidly produce large amounts of cytokines on triggering. iNKT cells have been shown to promote antitumor immune responses by rapidly producing copious amounts of IFN-γ, resulting in the activation of NK cells, dendritic cells (DC), and conventional CD4+ and CD8+ T cells as well as in the inhibition of tumor angiogenesis (reviewed in refs. 6, 7). Importantly, the circulating pool of iNKT cells shows quantitative and qualitative defects in cancer patients (8, 9), which seem to be clinically relevant, as increased numbers of intratumoral or circulating iNKT cells are associated with improved prognosis (10, 11).

In contrast to iNKT cells, CD4+CD25+ regulatory T cells, a population of CD4+ T cells constitutively expressing the IL-2 receptor α-chain, have been shown to suppress immune responses, including antitumor immune responses, via both contact-dependent (CTLA-4 and CD30) and contact-independent

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(IL-10 and transforming growth factor-β) pathways (reviewed in refs. 6, 12, 13). As elevated numbers of CD4+CD25+ regulatory T cells have been observed in melanoma and various other types of cancer (14, 15) and are associated with reduced survival (16), a recent study evaluated whether depletion of CD4+CD25+ regulatory T cells using recombinant IL-2 diphtheria toxin (DAB389IL-2) would enhance tumor-specific T-cell responses in patients with RCC, as indicated by earlier reports in mice (17), and found that this was indeed the case (18).

As discussed above, both iNKT cells and CD4+CD25+ regulatory T cells seem to play important roles in the regulation of antitumor immune responses. As CD4+CD25+ regulatory T cells constitutively express the IL-2 receptor α-chain (CD25) and IL-2 is an important growth factor for both iNKT cells and CD4+CD25+ regulatory T cells, both these regulatory T-cell subsets could be important cellular targets during high-dose IL-2. Here, we evaluated the in vivo effects of high-dose IL-2 on circulating iNKT cells, CD4+CD25+ regulatory T cells, and the DC with which they interact.

Materials and Methods

Patients. Patients (mean age, 53 years; range, 30-68 years; 15 males; 10 females) in this study had been diagnosed with either advanced melanoma (n = 15) or RCC (n = 10). All were treated with high-dose IL-2 as described (3). Peripheral blood was obtained on four occasions: before treatment, after the first week of high-dose IL-2, after the second week of high-dose IL-2, and after the second week of high-dose IL-2. Informed consent was obtained from all patients, and the study was approved by the Committee for Clinical Investigation of Beth Israel Deaconess Medical Center (Boston, MA). Clinical responses were assessed using previously reported criteria (19).

Flow cytometry. Peripheral blood mononuclear cells were obtained by Ficol-Faque (Amer sham Pharma cia, Uppsala, Sweden) density gradient centrifugation of heparinized peripheral blood. Peripheral blood mononuclear cells were stained using combinations of the following reagents after an initial incubation with 10% human pooled serum to reduce nonspecific binding. FITC-labeled CD3, FITC-labeled CD25, phycoerythrin (PE)-labeled CD56, PE-labeled CD1d, PE-labeled CD8α, PE-labeled HLA-DR, PE-labeled CD86, PE-labeled CD80, PE-Cy5-labeled CD19, PE-Cy5-labeled CD25, PE-Cy7-labeled CD4, PE-Cy7-labeled CD14, FITC-labeled anti-invariant Vα24Jα18 T-cell receptor monoclonal antibody (mAb) 6B11 (9), and the appropriate isotype controls were obtained from BD Pharmingen (San Jose, CA). PE-labeled Vα24 and FITC-labeled Vβ11 were obtained from Immuno tech (Marseille, France). FITC-labeled BDCA-1, BDCA-2, and BDCA-3 were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). FITC-labeled anti-CTLA-4, FITC-labeled CD30, and PE-labeled anti-Gr1 were obtained from R&D Systems (Minneapolis, MN). The anti-human Foxp3 polyclonal antibody was obtained by repeated immunization of rabbits (20). Flow cytometry was done on a Cytomics FC 500 (Beckman Coulter, Fullerton, CA).

Generation of iNKT cell lines. iNKT cells were enriched from peripheral blood mononuclear cells of healthy adult volunteers by positive selection using biotinylated anti-invariant T-cell receptor mAb (6B11) in combination with anti-biotin microbeads (Miltenyi Biotec) and subsequently expanded using α-galactosylceramide (α-GalCer)-loaded (KR7N000, kindly provided by the Pharmaceutical Research Laboratory, Kirin Brewery, Ltd., Gunma, Japan) and lipopolysaccharide-matured monocyte-derived DCs and 100 units/mL recombinant human IL-2 (National Biological Response Modifier Program, National Cancer Institute, Frederick, MD). In some cases, iNKT cell lines were further purified using high-speed sorting (Modular Flow FACS; Cytomation, Fort Collins, CO).

Results

Effect of high-dose IL-2 therapy on T cells, NK cells, and B cells. First, we evaluated the effect of high-dose IL-2 therapy on the main peripheral blood lymphocyte subsets (Fig. 1). The frequency of both CD4+ and CD8+ T cells decreased during the first week of treatment with high-dose IL-2 (P = 0.0011 and 0.009, paired Student’s t test), but levels recovered before the second week of treatment (P = 0.003 and 0.02). The second week of high-dose IL-2 induced no significant changes in the frequency of either CD4+ or CD8+ T cells. High-dose IL-2 induced a substantial increase in the frequency of NK cells during the first week of IL-2 (P = 0.0005), and their frequency remained at higher than pretreatment levels thereafter. In contrast to NK cells, B cells substantially decreased in frequency during both the first week (P = 0.006) and the second week (P = 0.02) of high-dose IL-2 treatment.

Effect of high-dose IL-2 therapy on circulating DC subsets. We analyzed both the frequency and CD1d expression of circulating myeloid DC (mDC) 1, mDC2, and plasmacytoid DC (pDC), which can each be readily identified using either BDCA-1 (mDC1), BDCA-2 (pDC), or BDCA-3 (mDC2) expression, respectively, within CD14+CD19- peripheral blood mononuclear cells (22). No statistically significant differences in the frequency of circulating mDC1, mDC2, or pDC (P = 0.06, 0.10, and 0.21, respectively) nor in their expression of CD1d (P = 0.67, 0.57, and 0.34) were found between patients with RCC and melanoma. During high-dose IL-2 therapy, mDC1 and pDC behaved in the same fashion: their frequency decreased during the first week of high-dose IL-2 (P = 0.001 for mDC1 and P = 0.024 for pDC), after which there was a significant recovery (P = 0.009 for mDC1 and P = 0.0089 for pDC) followed by a trend toward a further decrease during the second week of high-dose IL-2 (P = 0.097 for both mDC1 and pDC; Fig. 2). No significant changes were observed in the frequency of circulating mDC2. CD1d was primarily expressed by circulating mDC1, although both mDC2 and pDC were found to express some CD1d. However, the expression of CD1d...
on mDC1, mDC2, or pDC did not change significantly during treatment with high-dose IL-2 (Fig. 2).

**Effect of high-dose IL-2 therapy on circulating iNKT cells.** As expected from previous reports (8, 9), both the frequency [median, 0.007% of lymphocytes; interquartile range (IQR), 0.002-0.37%] and absolute number (median, 69/mL peripheral blood; IQR, 19-245) of iNKT cells were low before treatment. The frequency of circulating iNKT cells was comparable in patients with RCC and melanoma (P = 0.16). During both the first and the second week of treatment with high-dose IL-2, the

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**Fig. 1.** Frequency of CD4⁺ T cells, CD8⁺ T cells, NK cells, and B cells during high-dose IL-2 therapy. Columns, mean frequency of CD4⁺ T cells, CD8⁺ T cells, NK cells, and B cells as a percentage of total lymphocytes during high-dose IL-2 therapy; bars, SD. Frequencies were assessed before (pre) and after (post) the first (1) and second (2) week of high-dose IL-2.

**Fig. 2.** Frequency and CD1d expression of circulating DC subsets during high-dose IL-2 therapy. Columns, mean frequency (top row) and CD1d expression (bottom row) of circulating mDC1, pDC, and mDC2 during high-dose IL-2 therapy; bars, SD. Analyses were done before (pre) and after (post) the first (1) and second (2) week of high-dose IL-2.
iNKT cell frequency decreased further ($P = 0.01$ and $0.007$, Wilcoxon matched pairs test; Fig. 3A) with a recovery between the two cycles ($P = 0.02$). A similar trend was observed when analyzing changes in the absolute number of circulating iNKT cells. Importantly, the absolute number of iNKT cells increased to higher than pretreatment levels 1 week after the first cycle of high-dose IL-2 ($P = 0.02$; Fig. 3B). As different subsets of iNKT cells have been shown to preferentially produce either Th1-type cytokines (CD4$^+$ CD8$^-$ double-negative iNKT cells) or Th2-type cytokines (CD4$^+$ iNKT cells; refs. 23, 24), we evaluated the contribution of these subsets to the total circulating iNKT cell pool during high-dose IL-2. Of interest, we found that there was a significant increase in the proportion of double-negative iNKT cells after the first week of high-dose IL-2 ($P = 0.02$; Fig. 3C). This increase was not sustained, however, as the proportion of double-negative iNKT cells decreased in the week thereafter ($P = 0.03$) and did not increase significantly during the second week of high-dose IL-2. To evaluate whether the increase in the proportion of double-negative iNKT cells corresponded with an increased production of IFN-$\gamma$ by iNKT cells, we additionally purified iNKT cells from peripheral blood of several individuals using biotinylated 6B11 mAb and magnetic bead sorting with anti-biotin IgG beads (Miltenyi Biotec) for a subsequent overnight culture in the presence of CD1d-transfected HeLa cells and $\alpha$-GalCer. Although we found low-level IFN-$\gamma$ secretion in most cases, only in one case could we detect a clear increase in the percentage of IFN-$\gamma$-secreting iNKT cells during high-dose IL-2 therapy (data not shown).

**Effects of high-dose IL-2 therapy on CD4$^+$CD25$^+$ T cells.** Patients with RCC and melanoma were found to have a similar frequency of CD4$^+$CD25$^+$ T cells ($P = 0.39$) before treatment. High-dose IL-2 resulted in a substantial increase in the frequency of CD4$^+$CD25$^+$ T cells during both the first and the second week of therapy ($P = 0.0023$ and 0.002). However, the increased frequency was not sustained as it decreased to pretreatment levels when IL-2 was discontinued ($P = 0.0009$; Fig. 4A). The absolute numbers of circulating CD4$^+$CD25$^+$ T cells similarly increased during the first week of high-dose IL-2 ($P = 0.008$) and remained at higher levels throughout further treatment (Fig. 4B). As CD25 can be expressed by any T cell on activation, the combination of CD4 and CD25 does not unequivocally identify CD4$^+$CD25$^+$ regulatory T cells. As especially CD4$^+$CD25$^{high}$ T cells have been reported to be most suppressive (25), we evaluated whether the intensity of CD25 expression changed during high-dose IL-2 therapy. The intensity of CD25 expression closely followed the increase in the frequency of CD4$^+$CD25$^+$ T cells, suggesting an increase in regulatory CD4$^+$CD25$^+$ T cells (Fig. 4C). In five patients, we subsequently analyzed the expression of the transcription factor Foxp3, which to date is the most specific marker of CD4$^+$CD25$^+$ regulatory T cells (6). In each case, pretreatment levels of

**Fig. 3.** Response and phenotype of circulating iNKT cells during high-dose IL-2 therapy. Columns, circulating iNKT cell frequency ($A$, median ± IQR), absolute number of circulating iNKT cells ($B$, median ± IQR), and proportion of double-negative iNKT cells of the total circulating iNKT cell pool ($C$, mean and SD) during high-dose IL-2 therapy. iNKT cells were quantitated as double positive for V$\alpha$24 V$\delta$11 T-cell receptor mAb. Similar results were obtained with anti-invariant V$\nu$24 A$\nu$18 T-cell receptor mAb 6B11. Analyses were done before (pre) and after (post) the first (1) and second (2) week of high-dose IL-2.
CD4+Foxp3+ T cells were very low (data not shown; Fig. 5). In three patients in whom we observed an increase in CD4+CD25+ T cells, we also observed a substantial increase in CD4+Foxp3+ T cells to comparable levels during high-dose IL-2 therapy (representative dot plots are shown in Fig. 5). In contrast, in two patients in whom the CD4+CD25+ T-cell frequency did not substantially change during high-dose IL-2, the frequency of CD4+Foxp3+ T cells similarly remained in the same range (data not shown).

High-dose IL-2–induced changes in the cell surface expression of several molecules that have been implicated in the function of CD4+CD25+ T cells (i.e., GITR, CD30, and CTLA-4) are depicted in Fig. 6. GITR, CD30, and CTLA-4 were all expressed by a minority of CD4+CD25+ T cells. Compared with pretreatment values, the expression of GITR was significantly increased after the second cycle of high-dose IL-2 (P = 0.009; Fig. 6A). The expression of CD30 increased during the first cycle of high-dose IL-2 (P = 0.0007; Fig. 6B), decreased before the second cycle (P = 0.003), and tended to increase again during the second cycle (P = 0.10). Surface expression of CTLA-4 tended to increase during both the first and the second cycle of high-dose IL-2 (P = 0.07 and 0.05; Fig. 6C).

Further experiments addressed the ability of CD25+ T cells from cancer patients to suppress CD25+/CD4+ T-cell responses. Purified CD25+ cells were coincubated with autologous CD25+/CD4+ cells in the presence of CD3 mAb, and cytokine responses were measured. The presence of higher levels of healthy donor CD25+ cells (1:1:1 relative to CD25- cells) reduced the levels of both IFN-γ and IL-4 produced (Fig. 7A). In contrast, cancer patient CD25+ cells from the peak of the high-dose IL-2 CD25+ cell response (after week 1) did not significantly influence CD25+/CD4+ T-cell IFN-γ and IL-4 responses (Fig. 7B).

We next evaluated whether the failure of CD25+ T cells from cancer patients tested to suppress the CD3 response of CD25- cells represented a general suppressive defect. The Th1-suppressive properties of the expanded pool of CD4+CD25+ T cells on iNKT were assayed (Fig. 7C). CD4+CD25+ and CD4+CD25- T cells were isolated from peripheral blood mononuclear cells by high-speed sorting and subsequently added to cocultures of α-GalCer–loaded mature monocyte-derived DCs and healthy donor–derived iNKT cell lines (iNKT cell to CD4+CD25+ and iNKT cell to CD4+CD25- T-cell ratio = 2:1) over a period of 2 days. A clear Th2 deviation of the IFN-γ/IL-4 ratio was observed in one of seven donors. In the other donors, no evidence of suppression or Th2 polarization of iNKT cells could be observed.

Relation between clinical response and iNKT cells and CD4+CD25+ T cells during high-dose IL-2 therapy. Finally, we evaluated whether the pretreatment frequency of iNKT cells or CD4+CD25+ T cells or whether changes in the frequency of CD4+CD25+ T cells could be related to clinical outcome. Clinical responses were assessed at 6 and 12 weeks after initiation of high-dose IL-2 therapy. The pretreatment
CD4+CD25+ T-cell frequency was comparable in patients with a partial response (7.4 ± 6.1% [mean ± SD]), mixed response (3.6 ± 1.9%), stable disease (4.2 ± 2.6%), or progressive disease (12.8 ± 12.0%) at 6 weeks ($P = 0.26$, one-way ANOVA). Similarly, the pretreatment iNKT cell frequency was not predictive of the clinical outcome ($P = 0.58$ and 0.55 for response assessment at 6 and 12 weeks, respectively). As high-dose IL-2 resulted in an increase in the frequency of CD4+CD25+ T cells in most, but not all, patients, we also evaluated whether the fold change in CD4+CD25+ T-cell frequency (pretreatment versus 1 week after high-dose IL-2) could be used to predict disease outcome. We found that the fold change in the frequency of CD4+CD25+ T cells was similar in patients with partial response ($3.7 ± 4.0$-fold), mixed response ($3.1 ± 0.6$-fold), stable disease ($2.8 ± 1.6$-fold), or progressive disease ($2.7 ± 2.1$-fold) at 6 weeks ($P = 0.91$). Similarly, neither the pretreatment CD4+CD25+ T-cell frequency nor the fold change in CD4+CD25+ T cells during the first week of high-dose IL-2 was statistically significantly different in patients with a partial response, mixed response, stable disease, or progressive disease at 12 weeks ($P = 0.49$ and 0.40, respectively; data not shown).

**Discussion**

Treatment with high-dose bolus IL-2 can be used in selected patients with metastatic melanoma or RCC (3, 4). IL-2, best known as a T-cell growth factor, plays a dominant role in the biology of iNKT cells and CD4+CD25+ regulatory T cells, both of which have been shown to play important roles in the regulation of antitumor immune responses. Here, we evaluated the in vivo effects of high-dose IL-2 therapy on iNKT cells, CD4+CD25+ regulatory T cells, and the DC with which they interact.

As professional antigen-presenting cells, DCs play critical roles in the initiation of immune responses that, depending on the microenvironment and DC subtype (e.g., myeloid versus lymphoid), can support either Th1- or Th2-type immune responses (26). We analyzed the frequency and CD1d expression of three types of circulating DC that can be readily identified by expression of BDCA-1 (mDC1), BDCA-2 (pDC), and BDCA-3 (mDC2; ref. 22). We found that CD1d was predominantly expressed by mDC1, confirming previous data (27), and that this mDC1-specific expression did not fluctuate during high-dose IL-2 therapy. The frequency of mDC1 and pDC, but not mDC2, temporarily decreased during treatment with high-dose IL-2. Because of the very low frequency of circulating mDC1 and pDC during high-dose IL-2, we were unable to assess whether the decreases in DC frequencies were accompanied by functional changes.

As expected from previous reports (8, 9), we found low pretreatment circulating iNKT cell numbers in the evaluated patients. Interestingly, although both the frequency and the absolute number of iNKT cells decreased further during treatment with high-dose IL-2, the absolute number of iNKT cells thereafter recovered to higher than pretreatment levels, reflecting either redistribution of iNKT cells from tissue to peripheral blood or an actual expansion of the total iNKT cell.

![Fig. 5. Response of circulating CD4+CD25+ regulatory T cells during high-dose IL-2 therapy. Representative flow cytometric dot plots of one donor showing the association between changes in the frequency of circulating CD4+CD25+ T cells and CD4+Foxp3+ T cells during high-dose IL-2 therapy. Right of each dot plot, frequency of double-positive cells. Analyses were done before (pre) and after (post) the first (1) and second (2) week of high-dose IL-2.](image-url)
pool. As the size of the circulating iNKT cell pool predicts disease outcome in patients with squamous cell head and neck cancer (11) and immunologic responses to the iNKT cell ligand α-GalCer (28), this suggests potential value of evaluating the antitumor effects of α-GalCer after pretreatment of patients with IL-2. As CD4+ iNKT cells and CD4+CD8− double-negative iNKT cells represent distinct functional lineages of iNKT cells (23, 24), we assessed whether the relative proportion of each subset would change during high-dose IL-2. Although not sustained, the contribution of proinflammatory double-negative iNKT cells to the total iNKT cell pool did increase significantly after the first week of high-dose IL-2, potentially resulting in a more Th1-biased iNKT cell response when high-dose IL-2 therapy would be followed by α-GalCer therapy. In several patients, we found that purified iNKT cells from these patients could indeed produce IFN-γ when activated by α-GalCer in the context of CD1d, but a clear increase in iNKT cell IFN-γ secretion was only detected anecdotally.

Strikingly, high-dose IL-2 therapy resulted in an increase in both the frequency and the absolute number of CD4+CD25+ T cells. These increases of CD4+CD25+ T cells were accompanied by an increase in the level of CD25 expression, indicating the predominant expansion of CD4+CD25high T cells previously shown to exert the majority of the suppressive effects attributed to the population of CD4+CD25+ T cells (25). As CD4+CD25+ regulatory T cells are critically dependent on the X chromosome–encoded FOXP3 gene (6, 12), we similarly evaluated the levels of CD4+Foxp3+ T cells during high-dose IL-2 therapy. Importantly, the increase in the frequency of CD4+CD25+ T cells was accompanied by a marked increase in CD4+Foxp3+ T cells to levels comparable with total CD4+CD25+ T cells, strongly suggesting that high-dose IL-2 indeed results in an increase in CD4+CD25+ regulatory T cells. We found limited surface expression on CD4+CD25+ T cells of GITR, CD30, and CTLA-4, molecules implicated in the function of CD4+CD25+ regulatory T cells. Among these, the increase in surface expression during high-dose IL-2 was most prominent for CD30, although the expression of GITR and CTLA-4 also increased. As IL-2 is not only a vital cytokine for the thymic generation and peripheral maintenance and function of CD4+CD25+ regulatory T cells but also seems to reduce the CD4+CD25+ regulatory T-cell inhibitory effects at higher doses (29), we additionally tested whether the expanded pool of CD4+CD25+ T cells could still suppress α-GalCer–induced iNKT cell activation as previously shown for healthy donor–derived CD4+CD25+ T cells (21). As we found no evidence for suppressive effects of the expanded pool of CD4+CD25+ T cells, one can conclude that, although high-dose IL-2 results in the expansion of CD4+CD25+ T cells, it may also impair their suppressive effects, at least temporarily, perhaps due to activated conventional CD25+ T cells overwhelming the truly functionally suppressive CD4+CD25+ T cells.

Indeed, in patients with HIV or cancer who are treated with lower doses of IL-2, an increase in CD4+CD25+ T cells was also noted, and although these cells were Foxp3+, they were also poor functional suppressors (30–32). In line with this, we found no relation between either the pretreatment CD4+CD25+ T-cell frequency or the high-dose IL-2–induced increase in CD4+CD25+ T-cell frequency and clinical responses. As previous studies showed that patients with higher CD4+CD25+ T-cell frequencies had a worse prognosis compared with
patients with lower CD4+CD25+ T-cell frequencies, one mechanism through which high-dose IL-2 might induce effective antitumor immunity may be ironically through the (temporary) inhibition of the suppressive effects of CD4+CD25+ T cells. It is likely that the suppressive effects of CD4+CD25+ T cells are indeed only temporarily inhibited, as two recent articles showed that suppressive effects of CD4+CD25+ T cells were intact from a few days to 4 weeks after high-dose IL-2 therapy (33, 34). Interestingly, these authors confirmed the increase in the frequency of circulating CD4+CD25+ T cells in patients with metastatic melanoma and RCC after high-dose IL-2 therapy, but Cesana et al. (33) also noted a significant decrease in CD4+CD25+ regulatory T cells 4 weeks after the second cycle of high-dose IL-2 in patients achieving an objective clinical response, perhaps reflecting migration of non-suppressing CD4+CD25+ regulatory T cells to sites of antigenic stimulation.

In conclusion, our data show reciprocal effects of high-dose IL-2 therapy on circulating immunoregulatory iNKT and CD4+CD25+ T cells. Although the frequency and absolute number of iNKT cells decrease during high-dose IL-2 therapy, this is accompanied by an increase in the proportion of potentially proinflammatory double-negative iNKT cells and is followed by an increase in the total number of circulating iNKT cells. In contrast, both the frequency and the absolute number of CD4+CD25+ regulatory T cells increase during high-dose IL-2 therapy, and although the frequency returns to baseline levels, the absolute number remains elevated thereafter. Significantly, the expanded population of CD4+CD25+ T cells did not consistently exert suppressive effects, suggesting surprisingly that high-dose IL-2 therapy may also reverse CD4+CD25+ T-cell suppressive effects.

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References


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