Addition of Histamine to Interleukin 2 Treatment Augments Type 1 T-Cell Responses in Patients with Melanoma In vivo: Immunologic Results from a Randomized Clinical Trial of Interleukin 2 with or without Histamine (MP 104)

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ABSTRACT

Purpose: Preclinical investigations suggest that histamine dihydrochloride (HDC) protects T cells and natural killer cells from inhibition by monocyte-derived reactive oxygen metabolites and synergizes with interleukin (IL) 2 in inducing T-cell activation. Here, we investigate whether this mechanism is operational in patients with melanoma treated with HDC as an adjunct to IL-2.

Experimental Design: Melanoma patients having liver metastases were treated with IL-2 with or without HDC within a randomized, multicenter, phase III trial. The effect of HDC on type 1 and type 2 T-cell cytokine production was investigated in peripheral blood samples from 19 patients with the use of intracellular cytokine flow cytometry. Melanoma-specific T-cell responses were analyzed in eight HLA-A2-positive patients.

Results: Frequencies of CD3+ T cells producing IFN-γ (type 1 T cells) in response to phorbol myristate acetate/ionomycin increased (median, 1.8-fold) in patients receiving IL-2 plus HDC but not in those receiving IL-2 alone (P < 0.01 for comparison between arms). In contrast, the number of IL-13-producing type 2 T cells that increased in patients after treatment with IL-2 was not modulated by HDC.Melanoma- and tyrosinase-specific IFN-γ and IL-13-producing T cells were detected in two of four HLA-A2-positive patients with melanoma following treatment with HDC + IL-2.

Conclusions: Treatment of patients with stage IV melanoma with HDC in combination with IL-2 increases type 1 T-cell responses and may promote induction of melanoma-specific T cells. These effects are of relevance for tumor immunotherapy and provide a potential mechanism for the clinical efficacy of HDC added to IL-2.

INTRODUCTION

Interleukin (IL) 2 is highly effective in activating tumor-specific cytotoxic T cells and natural killer (NK) cells in vitro, but IL-2-based immunotherapy has been efficacious in only a small subset of patients with melanoma and renal cell carcinoma. One potential explanation for the limited efficacy of IL-2 is the presence of an immunosuppressive environment within tumors, as previously reported for various types of cancers (reviewed in refs. 1, 2). Tumor-associated macrophages contribute to the tumor-mediated immunosuppressive effect via synthesis of reactive oxygen species, leading to dysfunction of tumor-infiltrating T cells and NK cells (reviewed in ref. 2). Reactive oxygen species can inhibit the cytotoxicity of tumor-reactive T cells and NK cells, blunt the response to IL-2, and trigger lymphocyte cell death by apoptosis (3, 4).

Histamine dihydrochloride (HDC) was found to efficiently inhibit the synthesis of reactive oxygen species in monocytes and thereby protect NK and T cells from monocyte-derived inhibition and also synergize with IL-2 in inducing NK and T-cell activation (5). These properties led to a series of clinical trials evaluating HDC as an adjunct to IL-2-based therapies in cancer patients. Early clinical phase I and II trials of HDC in combination with IL-2 or IL-2 plus IFN-α in patients with metastatic melanoma or acute myeloid leukemia have indicated an unexpectedly good clinical activity for this combination therapy (6, 7), whereas others could not show improved efficacy (8). A randomized phase III study including IL-2 was recently completed in patients with stage IV melanoma, revealing a survival benefit for all enrolled patients and, in particular, patients with melanoma liver metastases receiving the combination of IL-2 and HDC (9, 10). Based on this trial, a subsequent second randomized, multicenter, phase III trial was initiated in patients with melanoma liver metastases comparing treatment with HDC + IL-2 to IL-2 alone.

We analyzed in a subset of patients randomized into this second trial within a single institution whether HDC synergizes with IL-2 in activating T-cell responses. The role of HDC in the immune system is complex (reviewed in ref. 11); both the activation (12, 13) and the inhibition (14) of type 1 T-cell responses as well as the activation of type 2 T-cell responses (15–17) have been reported in various in vitro and animal studies. Therefore, we analyzed simultaneously the modulation
of type 1 and type 2 cytokine production in T cells. In addition, we studied an induction or augmentation of melanoma-specific T-cell responses by the two treatments. Our data clearly show that the addition of HDC to IL-2 significantly increases the number of circulating IFN-γ-producing type 1 T cells, whereas IL-2 treatment alone has no effect. In two patients receiving treatment with HDC + IL-2, induction of melanoma-specific T-cell responses was also observed.

MATERIALS AND METHODS

Patients and Treatment. Patients with histologically proven melanoma of the skin or with uveal melanoma who had progressed to American Joint Committee on Cancer stage IV melanoma with liver metastases were included in the trial after written informed consent. The patients had WHO performance status of 0 or 1 (Karnofsky score of ≥70) and an anticipated life expectancy of >3 months when included in the trial. After stratification for serum lactate dehydrogenase, visceral metastases outside the liver, and treatment center, patients were randomly assigned to receive HDC + IL-2 or IL-2 alone, administered s.c. The treatment schedule and timing of blood draws are indicated in Fig. 1. For the purpose of the investigation reported here, a subset of patients included into the open-label international multicenter trial at a single clinical site (Charité Berlin) was asked for participation in the translational research project under a separate informed consent. The clinical trial and the translational research project had been approved by the Institutional Ethics Committee.

Preparation of Mononuclear Cells. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density gradient centrifugation using Ficoll-Hypaque 1.077 (Biochrom, Berlin, Germany). Cells were washed twice with PBS and stored in liquid nitrogen.

HLA Typing. Expression of HLA-A2 was determined by using fluorescein-conjugated monoclonal mouse antihuman immunoglobulin G antibodies specific for HLA-A2 (One Lambda, Drefeld, Germany) and compared to isotype control staining (Becton Dickinson, Heidelberg, Germany). Cells were washed twice with PBS and stored in liquid nitrogen.

Tumor Cell Lines and Peptides. The following cell lines were used for analyzing specific T-cell responses: the HLA-A*0201–positive melanoma cell lines SK-Mel 24 and SK-Mel 30 (American Type Culture Collection, Rockville, MD) and the HLA-A*0201–transfected K562 cell line, which was kindly provided by Dr. Cedrik Britten (University of Mainz, Germany). The peptides were kindly provided by Dr. Stefan Stevanovic (University of Tuebingen, Germany) and had been synthesized using an Applied Biosystems (Foster City, CA) 432 A peptide synthesizer according to the published sequences: tyrosinase 368 to 376 (370D, YMDGTMSQV), cytomegalovirus (CMV) 495 to 504 (NLVPMVATV) and influenza matrix protein 58 to 66 (GILGFVFTL), and HIV reverse transcriptase 476 to 484 (ILKEPVHG). The peptides were purified by reverse-phase high-performance liquid chromatography and checked by mass spectrometry. They were dissolved in DMSO (Merck, Darmstadt, Germany) at a concentration of 5 mg/mL and further diluted in PBS.

Analysis of T-Cell Cytokine Production and Specific T-Cell Responses by Four-Color Flow Cytometry. For assessment of T-cell cytokine production, 2 × 10^6 PBMCs were incubated with 100 ng/mL phorbol myristate acetate (PMA) and 1 ng/mL ionomycin in Iscove’s medium supplemented with 10% human AB serum and 2% L-glutamine at 37°C and 5% CO2. After 1 hour, 15 μg/mL of brefeldin A (Sigma, Steinheim, Germany) were added. After five additional hours of stimulation, PBMCs were washed once and incubated in PBS containing 1 mmol/L EDTA for 10 minutes. A further washing step with PBS containing 2% human immunoglobulin G (Endoglobuline, Baxter Hyland-Immuno Division, Unterschleissheim, Germany) was done. CD8 and CD3 were stained by incubation with fluorescence-conjugated monoclonal antibodies (Becton Dickinson) for 15 minutes on ice in the dark. Afterward, cell lysing and permeabilization solution (Becton Dickinson), respectively, were added according to the manufacturer’s instruction. IFN-γ and IL-13 were stained by incubation with fluorescein-conjugated monoclonal antibodies (Becton Dickinson) for 30 minutes on ice in the dark. After another washing step with PBS supplemented with human immunoglobulin G, cells were fixed with 1% formaldehyde in PBS. Data acquisition was done on a FACSCalibur and data were analyzed using CellQuest software (Becton Dickinson).

Fig. 1  Treatment schedule and timing of blood draw. To minimize influence of transient compartment shift phenomena, posttreatment blood samples were drawn 2 weeks after end of study medication.
For analysis of melanoma-specific T-cell responses $2 \times 10^6$ PBMCs from HLA-A*0201-positive patients were either incubated with 10 µg/mL of peptide or with tumor cells in a ratio of 10:1. After 1 hour, 15 µg/mL of brefeldin A (Sigma, Steinheim, Germany) was added and the same intracellular cytokine staining protocol was done as described above.

**In vitro Analysis of HDC Effect on IFN-γ Production.** Separated T-cell subpopulations (CD3+, CD3+CD8+, or CD3+CD8−) from healthy subjects were incubated alone or with monocytes in various ratios and stimulated with PMA/ionomycin for 16 hours as described above. Either HDC (100 µmol/L), diphenylene iodonium (an inhibitor of the NADPH oxidase), catalase, ranitidine and its chemical control, AH 20239AA, were added as indicated. T-cell IFN-γ production was analyzed by intracellular flow cytometry as described above.

**Statistical Analysis.** The Wilcoxon test was calculated to test whether a change in the percentage of cytokine-producing T cells before and after therapy was significant. The Mann-Whitney U-test was calculated to compare between both patient groups the ratios of the percentage of cytokine-producing T cells before and after therapy. The effect of addition of HDC on IFN-γ production in vitro was analyzed with the paired-sample t test.

A T-cell response to peptides was considered positive if the percentage of peptide-specific IFN-γ-producing CD3+CD8+ T cells was ≥2-fold higher as compared with the percentage of IFN-γ-producing CD3+CD8+ T cells incubated with an irrelevant peptide and if there was a minimum of 0.05% peptide-specific IFN-γ-producing CD3+CD8+ T cells (after subtracting the percentage of IFN-γ-producing CD3+CD8− T cells incubated with an irrelevant peptide).

T-cell responses to peptides or cell lines were considered relevant peptide and if there was a minimum of 0.05% IFN-γ-producing CD3+CD8+ T cells incubated with an irrelevant peptide).

**RESULTS**

**Augmentation of Type 1 T-Cell Responses by HDC + IL-2 Treatment.** The effect of IL-2 in combination with HDC compared with IL-2 treatment alone on T-cell cytokine production capacity was examined. Intracellular cytokine staining for IFN-γ (type 1 T cells) or IL-13 (type 2 T cells) was done simultaneously on cryopreserved pre- and posttreatment PBMCs stimulated by PMA/ionomycin. PBMCs were available from eight patients treated with HDC + IL-2 before and after one cycle (n = 6) or two cycles (n = 2) and from 11 patients treated with IL-2 alone before and after one cycle (n = 10) or two cycles (n = 1). In both patient groups, treatment resulted in a modest increase of total numbers of lymphocytes after one cycle (1.6-fold in both groups, data not shown). In the PBMC preparation, the percentages of IFN-γ-producing CD3+ T cells remained similar before (median, 23.0%; range, 12.1-70.4%; SD, ± 18.7%) and after (median, 29.3%; range, 9.7-53%; SD, ± 14.2%) treatment in patients receiving IL-2 alone (P = 0.93). In contrast, following treatment with HDC + IL-2 the median percentage of IFN-γ-producing CD3+ T cells increased 1.8-fold from median 19.8% (range, 0.8-36.4%; SD, ± 12.0%) before to median 35.8% (range, 3.5-60.3%; SD, ± 19.0%) after treatment (P = 0.01). In 11 of these patients (n = 7 in the IL-2 arm, n = 4 in the HDC + IL-2 arm) in whom samples after one and after two cycles were available, similar percentages of IFN-γ-producing CD3+ T cells were observed after one and two cycles (data not shown).

Because of the considerable pretreatment variation in the percentage of IFN-γ-producing T cells, ratios of post/pretreatment values were calculated for statistical comparison of both patient groups. The comparison of the ratios of the percentages of IFN-γ-producing T cells showed a highly significant difference between the two treatment arms (P < 0.01, Fig. 2A). This difference was seen for both CD3+CD8+ T cells (P = 0.01) and the CD3 + CD8− (P = 0.04) compartment, presumably consisting of CD4+ T cells. All patient samples were simultaneously analyzed for IL-13 production. The percentage of IL-13-producing CD3+ T cells increased 2.3-fold from median 1.8% (range, 0.6-8.3%; SD, ± 2.3%) before to median 4.1% (range, 2.1-9.0%; SD, ± 2.3%) after IL-2 treatment (P = 0.05). In patients receiving treatment with HDC + IL-2, the percentage of IL-13-producing CD3+ T cells increased 1.4-fold from median 1.2% (range, 0.1-2.2%; SD, ± 0.7%) before to

A T-cell response to peptides was considered positive if the percentage of peptide-specific IFN-γ-producing CD3+CD8+ T cells was ≥2-fold higher as compared with the percentage of IFN-γ-producing CD3+CD8+ T cells incubated with an irrelevant peptide and if there was a minimum of 0.05% peptide-specific IFN-γ-producing CD3+CD8+ T cells (after subtracting the percentage of IFN-γ-producing CD3+CD8− T cells incubated with an irrelevant peptide).

T-cell responses to peptides or cell lines were considered enhanced by treatment if the percentage of specific IFN-γ-producing CD3+CD8+ T cells was ≥2-fold higher posttreatment as compared to pretreatment.

![Fig. 2](https://example.com/f2.png) Ratios of the percentages of type 1 (IFN-γ producing) (A) and type 2 (= IL-13 producing) (B) T cells. PBMC of 11 patients in the IL-2 arm and 8 patients in the HDC + IL-2 arm were analyzed after incubation with PMA/ionomycin. The ratio post/pretreatment values were calculated to eliminate baseline variations in cytokine-producing T cells. The comparison of the ratios of the percentages of IFN-γ-producing T cells showed a highly significant difference between the two treatment arms (P < 0.01). In contrast, no significant difference of the post/pretreatment ratios in the percentages of IL-13-producing T cells was observed between the two treatment arms (P = 0.34).
median 1.7% (range, 0.5-2.9%; SD, ± 0.7%) after treatment ($P = 0.03$). No significant difference of the post/pretreatment ratios in the percentages of IL-13-producing T cells was observed between the two treatment arms ($P = 0.34$; Fig. 2B). An example of the flow cytometric analysis of IFN-$\gamma$- and IL-13-producing T cells in one patient is shown in Fig. 3 illustrating the increase of cytokine-producing T cells in all CD3$^+$ T-cell compartments after one cycle of HDC + IL-2 treatment.

**Augmentation of Type 1 T-Cell Response In Vitro Is Mediated by HDC Rescue of Monocyte-Induced Inhibition of IFN-$\gamma$ Secretion.** We next evaluated the effect of HDC on the production of IFN-$\gamma$ by T cells in vitro. T cells from healthy subjects were incubated with medium alone or with HDC for 16 hours. Thereafter, the same short-term stimulation with PMA/ionomycin for induction of IFN-$\gamma$ was done as with the patient samples. Under these assay conditions HDC did not affect IFN-$\gamma$ accumulation in CD3$^+$, CD8$^+$, or CD8$^-$ cells in the absence of monocytes ($P > 0.6$ for all comparisons). Addition of increasing numbers of monocytes at a ratio of 0.25:1, 0.5:1, or 1:1 (monocyte/lymphocyte ratio = 0.25, 0.5, and 1, respectively), however, induced a significant down-regulation of intracellular IFN-$\gamma$ accumulation in cells cultured in medium alone. The addition of HDC significantly restored the IFN-$\gamma$-producing capacity of T cells from monocyte-induced down-regulation at all monocyte/lymphocyte ratios tested ($P = 0.046$-$0.0005$ over control cells) and for both subsets of CD3$^+$CD8$^+$ as well as CD3$^+$CD8$^-$ T cells ($P = 0.043$ to $< 0.01$ over IL-2; Fig. 4). Taken together, these data show that in vitro HDC does not alter the induction of IFN-$\gamma$ in isolated T cells, but that HDC protects T cells from monocyte-induced inhibition of IFN-$\gamma$ production.

**Melanoma-Reactive Type 1 and Type 2 T Cells before and after Therapy.** To analyze a potential induction or augmentation of melanoma-specific T-cell responses by treatment with IL-2 alone or in combination with HDC, T-cell

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**Fig. 3** Example of intracellular accumulation of IFN-$\gamma$ and IL-13. IFN-$\gamma$ (A) and IL-13-producing (B) CD3$^+$CD8$^+$ T cells induced by PMA/ionomycin and simultaneously detected by intracellular cytokine flow cytometry from a patient treated with HDC + IL-2 is shown. Percentage of cytokine-producing CD3$^+$ T cells are indicated and also frequencies of CD3$^+$CD8$^+$ and CD3$^+$CD8$^-$ T cells producing IFN-$\gamma$ or IL-13 are given, demonstrating that an increase in cytokine-producing T cells occurred in both lymphocyte-producing T cells compartments.
responses toward melanoma cell lines and tyrosinase peptides were analyzed in all eight HLA-A*0201 patients \((n = 4, \text{HDC + IL-2}; n = 4, \text{IL-2})\) before and after two treatment cycles. PBMC obtained from these patients were exposed to two HLA-A*0201–positive melanoma cell lines (SK-Mel 24 and SK-Mel 30) for 6 hours and the number of IFN-\(\gamma\)– or IL-13-secreting T cells was determined by intracellular cytokine staining (Table 1). The HLA-A*0201–transfected leukemia cell line K562 was used as nonmelanoma control. Following therapy, none of the patients in any of the two arms showed a more than 2-fold increase in the numbers of IFN-\(\gamma\)-secreting T cells in response to the A*0201-transfected K562 cell line. In two of four HLA-A2+ patients treated with HDC + IL-2, a ≥2-fold increase in CD3+CD8+ T cells secreting IFN-\(\gamma\) in response to SK-Mel 24 after two cycles of therapy was observed (patient 1, from 0.09% to 0.18%; patient 5, from 0.12% to 0.62%; Fig. 5). In both patients, an increase in T cells secreting IL-13 in response to SK-Mel 24 was also found (patient 1, from 0.24% to 1.38%; patient 5, from 0.05% to 0.12% after therapy; Fig. 5). Both patients had received adjuvant vaccination with the HLA-A*0201–binding tyrosinase 368 to 376 peptide 3 and 4 years prior to inclusion into this trial, respectively. Whereas no T-cell response to the tyrosinase peptide could be detected prior to HDC + IL-2 treatment, both patients had a clear population of tyrosinase-specific IFN-\(\gamma\)-secreting as well as IL-13-secreting T cells after therapy (Table 1; Fig. 5). In contrast, memory T-cell responses to influenza, CMV, and HIV peptide, which served as negative control, were in both patients not enhanced after therapy (Table 1; Fig. 5). None of the four HLA-A*0201–positive patients treated in the IL-2 arm showed an increase in specific IFN-\(\gamma\)-producing T cells in response to the HLA-A*0201 melanoma cell lines after therapy. In two of these four patients who had received previous vaccination with the tyrosinase 368 to 376 peptide 1.5 to 2.5 years prior to IL-2 treatment (patients 13 and 18), a clear population of tyrosinase-specific IFN-\(\gamma\)-producing T cells was detectable before treatment, but not after therapy with IL-2. In one of these patients (patient 13), however, IL-13-producing tyrosinase-specific T cells could be detected after therapy. Remarkably, in several patients (patients 8, 11, 13, and 18) from both treatment arms, a decline (>50%) of melanoma- or tyrosinase-specific IFN-\(\gamma\)-producing T cells was observed after therapy, whereas IFN-\(\gamma\) production in response to K562 (patients 8, 11, and 13) and to CMV (patient 13) remained quite stable.

**DISCUSSION**

HDC exerts pleiotropic effects on T-cell cytokine production. Various investigators have suggested that HDC favors a
damage. The relevance of this mechanism on T-cell cytokine production, however, had not been studied in vitro thus far. We show that corresponding to the findings in NK cells, HDC also in T cells significantly reverses the inhibitory effect of monocytes on PMA/ionomycin-induced IFN-γ production. This protective effect of HDC on T-cell functional capacity is therefore a putative mechanism for the observed increase in the number of T cells capable of producing IFN-γ in patients treated with HDC + IL-2.

Several investigators have shown that HDC favors a type 2 T-cell immune response (15–17), but also direct induction of IFN-γ secretion has been shown (11, 12). Others report that HDC reduces lectin-induced production of IFN-γ in human CD4+ T cells, an effect presumably mediated by inhibition of endogenous IL-2 production in these cells (18). Another important effect of HDC is the suppression of monocyte-derived reactive oxygen species, which can result in restoration of T and NK cell functions especially in the microenvironment of malignant tumors (19). Because of these pleiotropic effects and the partially contradictory results observed for HDC on T-cell function in preclinical studies, it is of major relevance to analyze its in vitro influence on the T-cell response in patients with melanoma. This type of analysis is best done by investigating a subset of patients treated within a randomized clinical trial with or without HDC. The most prominent finding in our study was a sustained increase of functional type 1 T cells following treatment of patients with HDC in combination with IL-2 but not with IL-2 alone. This effect is of significant relevance for tumor immunotherapy because the induction of a type 1 T-cell immunity is considered critical for tumor rejection. In tumor patients with advanced disease a type 2 shift is frequently observed (20), which has been associated with suppression of CTL responses and progressive tumor growth in various animal models (21).

The observed increase of functional type 1 T cells in patients treated with HDC + IL-2 is in accordance with in vitro studies showing a synergistic effect of HDC on IL-2-induced activation of T cells and NK cells. Following exposure to monocyte-derived reactive oxygen species, T cells and NK cells became anergic to IL-2, which could be reversed by HDC. Analysis of cytokine production in NK cells has shown enhancement of IL-2-induced IFN-γ production by HDC mediated through the prevention of monocyte-induced oxidative damage. The relevance of this mechanism on T-cell cytokine production, however, had not been studied in vitro thus far. We show that corresponding to the findings in NK cells, HDC also in T cells significantly reverses the inhibitory effect of monocytes on PMA/IONOMYcin-induced IFN-γ production. This protective effect of HDC on T-cell functional capacity is therefore a putative mechanism for the observed increase in the number of T cells capable of producing IFN-γ in patients treated with HDC + IL-2.

Several investigators have shown that HDC favors a type 2 T-cell immune response and have showed its capacity to enhance IL-5, IL-10, and IL-13 production by Th2 cells (15–17). Unexpectedly, treatment of patients with IL-2 alone already resulted in an increase in the number of type 2 T cells as assessed by their IL-13-producing capacity. Addition of HDC to IL-2 had no influence on this increase in IL-13-producing T cells. Again, the absence of a type 2–inducing or –potentiating effect of HDC in vivo is important for tumor immunotherapy as a type 2 T-cell polarization was found to be associated with tumor progression (20, 21) and IL-13 is considered to be the main cytokine responsible for down-regulation of T-cell-mediated tumor immunosurveillance (22).

Of major relevance is the question whether the increase in type 1 T cells observed by addition of HDC would be associated with an induction of tumor-reactive T cells. This question cannot be sufficiently answered by our study because we could only analyze the melanoma-specific T-cell response in the eight HLA-A2–positive patients. It is of interest, however, that in two of four patients IFN-γ-producing melanoma and tyrosinase-specific T-cell responses could be shown following therapy with HDC + IL-2. Both patients had received previous adjuvant vaccination with tyrosinase peptide as reported elsewhere (23). This prior vaccination may have resulted in an increased precursor frequency of tyrosinase-specific T cells. Interestingly, in both patients also IL-13-producing melanoma- and tyrosinase-specific

<table>
<thead>
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<th>Patient No.</th>
<th>Treatment</th>
<th>Previous vaccination</th>
<th>Time of sample</th>
<th>% IFN-γ+ CD3+CD8+ T cells</th>
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<td>1</td>
<td>IL-2 + HDC</td>
<td>Yes</td>
<td>Before therapy</td>
<td>SK Mel 24: 0.09, SK Mel 30: 0.12, TYR: 0.01, IMP: 0.02, CMV: 0.03, HIV: 0.02, IL-2: 0.11</td>
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<td>5</td>
<td>IL-2 + HDC</td>
<td>Yes</td>
<td>After 2 cycles</td>
<td>SK Mel 24: 0.18+, SK Mel 30: 0.06, TYR: 0.08+, IMP: 0.02, CMV: 0.06, HIV: 0.02, IL-2: 0.13</td>
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NOTE: T-cell responses to tyrosinase, CMV, or influenza peptide antigens, which exceeded 2-fold or more the frequency in PBMCs stimulated with irrelevant peptide (HIV) were considered as antigen-specific. T-cell responses to SK-Mel 24 or SK-Mel 30 melanoma cell lines, which were 2-fold or more increased in comparison to pretreatment values were considered to be augmented by therapy. Abbreviations: ND, not done; TYR, tyrosinase 368 to 376; CMV, cytomegalovirus epitope 495 to 504; IMP, influenza matrix protein 58 to 66; HIV, HIV reverse transcriptase epitope 476 to 484; +, positive T-cell responses to antigen.
Table 1  Frequencies of CD3+CD8+ T cells reactive with various antigens in HLA-A*0201 positive patients (Cont’d)

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<th>IMP</th>
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Fig. 5  Frequencies of melanoma- and peptide-specific type 1 (IFN-γ) and type 2 (IL-13) secreting T cells before and after two cycles of treatment with HDC + IL-2 in two HLA-A*0201–positive patients. Analysis was done using intracellular cytokine staining following coincubation with the tumor cell lines or peptides as indicated. Specific IFN-γ- or IL-13-producing T cells are shown as percentage of CD3+CD8+ T cells. Induction of both IFN-γ- and IL-13-producing T cells in response to the melanoma cell line SK-Mel 24 and the tyrosinase peptide was seen in both patients. IMP A2, influenza matrix protein A2.
T cells were found following treatment. Whereas tumor-specific cytotoxic T cells typically produce IFN-γ, the role of tumor-specific type 2 T-cell responses in tumor control is controversial. Although tumor-specific type 2 T cells can promote tumor rejection and mediate long-term tumor immunity (24, 25), it has been shown in other models that type 2 T cells enhanced tumor growth (21). Because IL-2 treatment alone has induced an increase in the total number of type 2 T cells, it is possible that IL-2 may also have played a role in the induction of a type 2 melanoma-specific T-cell response in vivo. This hypothesis is supported by the finding that IL-13-producing but not IFN-γ-producing tyrosinase-specific T cells were observed in one patient following treatment with IL-2 alone. It is also noteworthy that in four patients a marked decline of melanoma- or tyrosinase-specific IFN-γ-producing T cells but not of IFN-γ-producing T cells in response to the leukemia cell line K562 or to viral peptides or to PMA/ionomycin (data not shown) was observed after treatment. This observation is in accordance with a recent study showing a transient decline of melanoma antigen-specific but not viral T-cell responses in peripheral blood during IL-2 therapy (26).

Taken together, our data show that the use of HDC as adjunct to IL-2 therapy results in a shift toward a type 1 T-cell immunity, providing a possible mechanism of action leading to the favorable clinical results observed in patients treated with HDC + IL-2. Furthermore, our observations could be used as a rationale to investigate potential cooperation of HDC with cancer vaccines.

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Addition of Histamine to Interleukin 2 Treatment Augments Type 1 T-Cell Responses in Patients with Melanoma \textit{In vivo}: Immunologic Results from a Randomized Clinical Trial of Interleukin 2 with or without Histamine (MP 104)

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