In Vivo Hydrodynamic Delivery of cDNA Encoding IL-2: Rapid, Sustained Redistribution, Activation of Mouse NK Cells, and Therapeutic Potential in the Absence of NKT Cells

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In Vivo Hydrodynamic Delivery of cDNA Encoding IL-2: Rapid, Sustained Redistribution, Activation of Mouse NK Cells, and Therapeutic Potential in the Absence of NKT Cells

John R. Ortaldo, Robin T. Winkler-Pickett, Earl W. Bere, Jr., Morihiro Watanabe, William J. Murphy, and Robert H. Wiltrout

In the present study, we have tested the ability of hydrodynamically delivered IL-2 cDNA to modulate the number and function of murine leukocyte subsets in different organs and in mice of different genetic backgrounds, and we have evaluated effects of this mode of gene delivery on established murine tumor metastases. Hydrodynamic administration of the IL-2 gene resulted in the rapid and transient production of up to 160 ng/ml IL-2 in the serum. The appearance of IL-2 was followed by transient production of IFN-γ and a dramatic and sustained increase in NK cell numbers and NK-mediated cytolytic activity in liver and spleen leukocytes. In addition, significant increases in other lymphocyte subpopulations (e.g., NKT, T, and B cells) that are known to be responsive to IL-2 were observed following IL-2 cDNA plasmid delivery. Finally, hydrodynamic delivery of only 4 μg of the IL-2 plasmid to mice bearing established lung and liver metastases was as effective in inhibiting progression of metastases as was the administration of large amounts (100,000 IU/twice daily) of IL-2 protein. Studies performed in mice bearing metastatic renal cell tumors demonstrated that the IL-2 cDNA plasmid was an effective treatment against liver metastasis and moderately effective against lung metastasis. Collectively, these results demonstrate that hydrodynamic delivery of relatively small amounts of IL-2 cDNA provides a simple and inexpensive method to increase the numbers of NK and NKT cells, to induce the biological effects of IL-2 in vivo for use in combination with other biological agents, and for studies of its antitumor activity. The Journal of Immunology, 2005, 175: 693–699.
of pre-existing mouse renal cell carcinoma metastasis in liver, and this antitumor activity could be achieved in the absence of NKT cells. These results provide an experimental platform that can be used to dissect IL-2-dependent effects of NK cells from NKT cells in host tumor immunity.

Materials and Methods

Leukocyte isolation

Leukocytes and enriched NK cells were isolated from the organs of IL-2-treated mice as described previously (25). Animal care was provided in accordance with the procedures outlined in the “Guide for the Care and Use of Laboratory Animals.”

Flow cytometry reagents and Abs

NK1.1-PE (or allophycocyanin), DX-5-PE, CD19-F (or PE), and CD3-PcP (BD Pharmingen), as well as CD69-FITC, were used for flow cytometric analysis of various leukocyte subsets, as described previously (25). Detection of cytoplasmic IFN-γ was performed using kits purchased from BD Pharmingen as described by the manufacturer.

Cytokine measurement

Cytokines were measured using IFN-γ and chemokine ELISA kits (R&D Systems) as described previously (5, 26, 27). In vitro leukocyte stimulations were performed at concentrations of 1–5 × 10⁶ cells/ml. In all assays, the SD for cytokine production was <5 pg/ml.

Cytotoxicity assay

NK cell-mediated cytotoxicity against Yac-1 target cells was measured by a standard 4-h ⁵¹Cr-release assay as described previously (26).

Mice used in this study

Mice were obtained from the Animal Production Area, National Cancer Institute-Frederick Cancer Research and Development Center, or from our own breeding colony and were between 6 and 12 wk of age.

Bone marrow transplantation

Bone marrow transplantation was evaluated by a new method (28) that uses CFSE (Molecular Probes) to track the fate of labeled bone marrow cells after 1–4 days. Briefly, 10³ × 10⁶ CFSE-labeled autologous bone marrow cells (29) are injected into irradiated recipient mice (C57BL/6-900R), and the effects of IL-2 gene delivery on the number of labeled cells were assessed in the spleen on days 1–5. Mice were evaluated individually, and data were presented as the mean and SD for each group.

Construct used

Mouse cDNA for IL-2 was subcloned into expression plasmid pcDEF/CMV in which the transgene is driven via CMV promoter and human elongation factor-1α enhancer. The GFP encoded in pcDEF/CMV was used as a control vector.

Plasmid DNA purification and cell transformation

For hydrodynamic gene delivery, plasmid was purified from 500 ml of bacterial culture using Endofree Mega kit (Qiagen). Endotoxin level in the plasmid preparation was <0.1 endotoxin U/μg of DNA.

Renca model

The use of the BALB/c mouse renal cell carcinoma line, Renca, to establish experimental liver and lung tumors was performed as described previously (14, 20).

Gene delivery

Injection of the plasmid DNA was performed as described by Liu et al. (23). Briefly, small amounts of DNA were diluted in 1.6 ml of sterilized 0.9% NaCl solution and injected into mice through their tail vein in a 5-s push, using a 27.5-gauge needle. At various time points after gene delivery, mice were bled or euthanized, and the spleen and the lymph node were collected, weighed, and processed for single-cell suspensions.

FIGURE 2. Evaluation of liver leukocyte changes after IL-2 cDNA administration. Mice were injected with various doses of cDNA by hydrodynamic administration and flow cytometric analyses performed after 3 days. Expression of CD3 and NK1.1 (A and B) size and granularity (C and D) and lymphocyte size (E and F) for control mice (top) and IL-2 cDNA administration (bottom) were evaluated.
show that the administration of active protein-based therapeutic regimens. The data shown in Fig. 1 logic effects of IL-2 without a need for large amounts of purified levels of IFN-24 or 48 h, depending on the dose of IL-2 cDNA (Fig. 1 cases, IL-2 serum levels of IFN-24 h, whereas 0.5–1/H11022 sustained through 48 h. Doses of DNA (up to /H11022 significant toxicity and/or lethality to mice, whereas doses of 2–4 /H9262 p 0.05.

Table I. Effects of IL-2 cDNA on leukocyte subsets at 72 h after hydrodynamic deliverya

<table>
<thead>
<tr>
<th>Organ</th>
<th>cDNA</th>
<th>Cell No./Mouse (×106)</th>
<th>Fold Increase</th>
<th>Percentage Cell No. Fold Increasea</th>
<th>Cell No. (×106)</th>
<th>Fold Increaseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Control</td>
<td>2.6</td>
<td>1.0</td>
<td>NK 7.6 15.4 18.2 13.1</td>
<td>NKT 4.0</td>
<td>0.5 0.3</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>13.2</td>
<td>5.1</td>
<td>29.5 24.1 24.8 15.6</td>
<td>38.9</td>
<td>3.3 2.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>Control</td>
<td>18.3</td>
<td>1.0</td>
<td>4.6 3.3 32.9 34.5</td>
<td>8.5</td>
<td>6.1 6.0</td>
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<tr>
<td></td>
<td>IL-2</td>
<td>61.7</td>
<td>3.4</td>
<td>6.0 4.4 29.4 35.6</td>
<td>37.1</td>
<td>26.8 21.9</td>
</tr>
<tr>
<td>Blood</td>
<td>Control</td>
<td>3.3</td>
<td>1.0</td>
<td>8.0 1.3 32.6 41.8</td>
<td>2.7</td>
<td>0.4 1.1</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>4.5</td>
<td>1.4</td>
<td>15.6 3.4 29.5 36.6</td>
<td>7.0</td>
<td>1.5 1.3</td>
</tr>
</tbody>
</table>

a Data are pooled cells from five mice and are representative of more than three experiments. Bold text indicates significant difference from control tissue using T test where p < 0.05.

b Based on total subset numbers.

Results
Exogenous administration of IL-2 protein dramatically perturbs the NK and T cell compartments and can induce antitumor activity under some conditions as a single agent (2, 4, 30) or also when administered in combination with some other cytokines (5–7). However, these activities usually require large amounts of IL-2 and can be accompanied in some conditions as a single agent (2, 4, 30) or also when administered by significant toxicity. In this study, we show that hydrodynamic delivery of small amounts of IL-2 cDNA recapitulates the major biologic effects of IL-2 without a need for large amounts of purified protein and without the often substantial toxicity that accompanies active protein-based therapeutic regimens. The data shown in Fig. 1A show that the administration of IL-2 cDNA resulted in a rapid increase (up to >150 ng/ml) of mouse IL-2 in the serum by 6 h that was sustained through 48 h. Doses of DNA >4 µg/mouse resulted in significant toxicity and/or lethality to mice, whereas doses of 2–4 µg/mouse resulted in >100 ng of serum IL-2 that was sustained for 24 h, whereas 0.5–1 µg/mouse resulted in substantial but lower levels of IL-2 (15–20 ng/ml). Subsequent to the peak of IL-2 production, serum levels of IFN-γ were also detected with peak amounts seen at 24 or 48 h, depending on the dose of IL-2 cDNA (Fig. 1B). In some cases, IL-2 cDNA could sustain detectable IFN-γ beyond 6 days. Doses of cDNA <1 µg/mouse did not result in consistently detectable levels of IFN-γ in the serum. Neither IL-2 or IFN-γ were observed when cDNA encoding the control protein GFP was used (data not shown).

The substantial levels of IL-2 and IFN-γ induced by hydrodynamic gene delivery suggested that this approach would have strong immune modifying effects. To examine the effects of high dose IL-2 production, leukocyte numbers and frequency in various organs were determined after hydrodynamic delivery of IL-2 cDNA. A dramatic increase in liver-associated NK1.1+/CD3− NK cells was observed (Fig. 2, A vs B), whereas smaller relative increases in NKT, T, and B cell numbers were also observed as well in both spleen and liver (Table I). A small increase in leukocyte size (Fig. 2, E vs F) was also observed. In addition, as shown in Fig. 3, top panels, both the percentage (Fig. 3A) and the total number (Fig. 3B) of NK cells was increased dramatically by day 3 and remained elevated at 7 days. This increase was not observed when cDNA encoding for GFP was used as a control.

When subset analyses were performed comparing IL-2 cDNA to administration of IL-2 protein (at a dose and schedule previously optimized for effects on NK cell number and function), the percentage of NK cells (Fig. 3C) and the total number of NK cells (Fig. 3D) in the liver increased as much as 5- and 100-fold, respectively. These increases remained sustained above background for at least 7 days (data not shown). Both the peak levels and the
duration of increased NK cell numbers were greater for cDNA than for IL-2 protein. As shown in Table I, although spleen and blood leukocyte numbers were increased by day 3, there were no marked preferential increases in any subset in these organs. However, in the liver, there was a dramatic preferential increase in both the number and percentage of NK cells. In addition to the induced increase in NK cell numbers in the liver, the data in Fig. 4 revealed substantial increases in the ability of NK cells in the liver (Fig. 4A) and the spleen (Fig. 3B) to lyse the prototype NK cell target, Yac-1. As shown in two representative experiments, hydrodynamic delivery of IL-2 cDNA resulted in an enhancement of NK-mediated lytic activity by both liver and spleen leukocytes that exceeded levels that could be explained simply by increased frequency. Specifically, in the liver, the percentage of NK cells increased by 5-fold, whereas augmentation of total NK lytic activity was >10-fold. Similarly, in the spleen, the NK frequency increased by <2-fold, whereas the lytic activity was augmented by ~10-fold. In addition, substantively increased expression of CD69 was observed on NK cells during this phase of increased lytic potential (data not shown). Therefore, the hydrodynamic administration of IL-2 cDNA increased both the number of NK cells and the cytolytic activity per cell in both spleen and liver.

To determine whether IL-2 cDNA-induced NK cell increases were broadly applicable to other mouse strains, six different mouse inbred mice (CBA/J, C57BL/6, DBA/2, AKR, BALB/C, and C3H/HeJ) were compared for the impact of hydrodynamic administration of IL-2 cDNA on NK cell numbers in the liver. The data shown in Table II demonstrate that increases in both the frequency and total numbers of liver NK cells were observed in all strains. Similar trends were observed in splenic NK cell lysis (data not shown). Interestingly, the number of NKT cells also increased, but the degree of increase varied depending on the strain. For all strains, relatively small increases in T and B cells were also observed (data not shown).

Overall, these results show that hydrodynamic delivery of IL-2 cDNA has potent immune-modifying effects in all strains tested. Because the immune system is regulated by a complex network of interacting proteins, we also studied the effectiveness of hydrodynamic delivery of IL-2 cDNA in various gene knockout mice that are often used in preclinical cancer and infectious disease models to provide mechanistic insight into new strategies for treatment. Some gene-targeted mice have been reported to have disrupted immune systems, and thus, the expected responses to IL-2 might not be equivalent to normal wild-type mice. When mice with dysregulated TNF-α, IFN-γ, IL-12p35, MIP1α, PFP, Fas, iNos, Mig, and CD40L genes were injected with IL-2 cDNA, the mice demonstrated similar total and subset-specific increases to IL-2 (data not shown). These results demonstrate that hydrodynamic delivery of IL-2 cDNA yields predictable and consistent biological effects on the NK cell compartments of many mice with disrupted cytokine or other immune-related genes.

Because the increase in total leukocytes and NK cells was so large and occurred very rapidly, we speculated that these cells were being quickly recruited from either peripheral redistribution via rapid exportation from the bone marrow and/or through stimulation of proliferation. To address this question, CFSE labeling of bone marrow cells was used to study the impact of IL-2. CFSE-labeled cells were transferred into sublethally irradiated syngeneic mice, and the effects of IL-2 cDNA on NK cell repopulation after bone marrow transfer were evaluated (Fig. 5). Comparison of the histograms for NK cells at day 4 (selected based on absence of CD3 and presence of NK1.1) revealed very few NK cells in the livers of untreated control mice (Fig. 5A (6.2%)), and these cells exhibited little evidence of increased division (movement to the other gates).

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**Table II. Effects of IL-2 cDNA administration on NK cells in various mouse strains**

<table>
<thead>
<tr>
<th>Liver</th>
<th>Treatment</th>
<th>Cells</th>
<th>NK (CD3⁻/DX5⁺)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. (×10⁷)</td>
<td>Fold Increase</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>None</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>cDNA IL-2</td>
<td>9.8</td>
<td>8.2</td>
</tr>
<tr>
<td>BALB/c</td>
<td>None</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td>cDNA IL-2</td>
<td>3.9</td>
<td>2.8</td>
</tr>
<tr>
<td>CBA/J</td>
<td>None</td>
<td>0.7</td>
<td></td>
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<tr>
<td>CBA/J</td>
<td>cDNA IL-2</td>
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<td>5.0</td>
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<tr>
<td>DBA2</td>
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<td>1.0</td>
<td></td>
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<td>cDNA IL-2</td>
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<td>3.6</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>None</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>cDNA IL-2</td>
<td>2.5</td>
<td>3.4</td>
</tr>
<tr>
<td>AKR</td>
<td>None</td>
<td>0.9</td>
<td></td>
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<tr>
<td>AKR</td>
<td>cDNA IL-2</td>
<td>3.8</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*In IL-2 cDNA (4 μg/mouse) was injected by hydrodynamic administration into the indicated mouse strains. Livers were harvested on day 4, and the cell numbers, percentage of NK (CD3⁻/DX5⁺) cells, the number of NK cells calculated, and the LU (30%) per 10⁷ cells to measure lytic potency were measured against YAC-1 in a 4-h ⁵¹Cr release assay. Data are representative of more than three experiments, with this data being the result of one of those experiments.

*Fold increase is based on cell number (total or NK).
left). In contrast, the mice treated with IL-2 cDNA exhibited 17.5% NK cells (Fig. 5B) and dramatic evidence of cell division based on CFSE intensity shifts to the left. When the percentages (Fig. 5C) of splenic or liver NK cells were examined from three individual mice, preferential increases in NK cells induced by IL-2 cDNA could be seen at day 3 in the spleen and days 3 and 4 in the liver. This increase in incidence of NK cells correlated directly with an absolute expansion based on CFSE-positive cells (Fig. 5D), where a 2- to 3-fold increase in CFSE-positive NK cells was seen in the liver and spleen by day 3, with a 7.5-fold increase detectable by day 4 in the liver. These data show a rapid expansion and repopulation of bone marrow progenitors in the IL-2-treated mice compared with controls, suggesting that the dramatic mobilization of NK cells induced by IL-2 cDNA was derived at least partially from profound effects on bone marrow progenitors and their rapid export to peripheral organs.

The results presented to this point demonstrate that hydrodynamically delivered IL-2 provides a rapid, highly efficient approach for modulating key elements of the immune system, provides an easy, cost-effective tool for obtaining large numbers of leukocyte subsets for biological studies from the liver and spleen, and illustrates the possible efficacy of this approach in the context of tumor models.

Therefore, the therapeutic impact of hydrodynamic delivery of IL-2 cDNA was studied in an experimental metastasis model using the Renca renal cell carcinoma in BALB/c mice. Previous studies from our laboratory (14, 20) and others (13, 31, 32) have shown that NK cells can have potent antitumor effects. Previous reports (20, 30) have confirmed a role for NK cells in the regulation of metastases in the liver in that the pretreatment with the NK cell-depleting reagent anti-asialo GM1 resulted in a dramatic increase in experimentally established metastases in the absence of NKT cell modification (data not shown). Therefore, we used experimental models of liver (intrasplenic injection) and lung (i.v. injection) to compare and contrast the effects of IL-2 protein and cDNA on progression of metastases in different organ microenvironments. The data shown in Fig. 6 show results for IL-2 cDNA against...
established liver (Fig. 6A) and lung (Fig. 6B) metastases. The results showed that hydrodynamic delivery of IL-2 cDNA to mice bearing established metastases inhibited progression to about the same extent (~60%) as did an established high-dose regimen of IL-2 protein (100,000 U delivered twice daily) for 3 days. However, when the same treatment approaches were compared for effectiveness against established lung metastases, neither strategy was effective in reducing the number of metastases. These results demonstrate that hydrodynamic delivery of IL-2 cDNA is active against established metastases in the liver. However, the failure of both IL-2 protein and IL-2 cDNA against lung metastases derived from the same tumor highlights the importance of unique organ microenvironments in the success or failure of IL-2 therapy and suggests that the liver and the lung contain distinctly different constitutive or inducible effector cell subsets.

The disparity in therapeutic efficacy in liver vs lung suggests important differences in key elements of the immunological microenvironments of those two organs. One specific difference in immune cell composition of these organs is the incidence of NKT cells that are a prominent subset in the liver but not the lung. At present there are no specific Abs available with which to selectively deplete NKT cells. However, we have shown recently that β-galactosylceramide (GalCer) (C12;βGalCer) can selectively deplete NKT cells in vivo without activating NK or other leukocytes (27). Therefore, we used C12;βGalCer to selectively impair NKT cell function in the absence of NK cell activation to determine the possible role of NKT cells in the antitumorigenic effects of IL-2 cDNA in the liver (Fig. 7). The impact of C12;βGalCer was compared in these studies to the effect of αGalCer, which stimulates and depletes NKT cells, and results in downstream activation of NK cells. However, when βGalCer, which selectively binds to and depletes NKT cells was given, a small reduction (NS; Fig. 7 statistics shown in Table III) in the number of metastases was observed, whereas IL-2 cDNA achieved the expected significant (p < 0.01) decrease in metastases. However, interestingly, when both βGalCer (to remove NKT cells) and IL-2 cDNA (to activate NK cells) were coadministered (p < 0.05), a reduction in liver metastasis that was quantitatively similar to that achieved with αGalCer was observed. To further investigate the possible contribution of NKT cells to IL-2-induced metastatic effects in the liver, experiments were performed in CD1d−/− mice (Fig. 7). Interestingly, the number of metastases in untreated mice is lower but not significantly different from those seen in mice where NKT cells are depleted with αGalCer or βGalCer (Table III). In addition, IL-2 cDNA remained as effective in reducing the number of liver metastases in these CD1d−/− mice as it was in mice selectively depleted of NKT cells by βGalCer. Overall, these results show that the effectiveness of IL-2 cDNA against liver metastasis is not dependent on NKT cells, and the increased effectiveness of IL-2 cDNA in liver vs lung is most likely due to its profound effect on NK cells and NK cell progenitors. However, the significantly reduced number of metastases seen in wild-type mice treated with βGalCer (selectively removes NKT cells; Ref. 27) and CD1d−/− mice, which have no NKT cells, suggest that NKT cells may actually actively inhibit the ability of other elements of the immune system to control of newly established metastases in the liver, an observation that requires further study.

Overall, these results show that hydrodynamically administered IL-2 cDNA has profound immunoregulatory and antitumor effects in the liver microenvironment, provides a unique cost-effective approach for obtaining large numbers of NK cells for biological study, and provides a platform for proof-of-principle studies where IL-2 can be used in combination with other immune modifiers.

### Discussion

The results of this study have demonstrated that hydrodynamic-based gene delivery can provide a simple method to express biologically active IL-2 protein. The results show that IL-2 cDNA administration is accompanied by a rapid production of IL-2 protein that appeared in serum by 6 h and remained detectable for >48 h. Although the duration of proteins expressed from various genes may vary based on the nature of the cDNA construct and the specific gene, the levels of IL-2 protein achieved (>100 ng/ml serum) were active biologically. This was evident by the rapid subsequent production of IFN-γ that followed the peak and nadir of IL-2 in the serum. In addition, the in vivo translation of IL-2 protein achieved qualitatively and quantitatively similar perturbations in critical innate and adaptive immune leukocyte subsets to those induced by large amounts of highly purified rIL-2 protein. Specifically, the degree and timing of the increase in NK cell number and frequency that were observed with hydrodynamic gene delivery was similar to that induced by protein IL-2. The effects of hydrodynamic injection of IL-2 cDNA were dependent on the IL-2 gene because vectors encoding irrelevant protein did not have similar immunomodulatory effects. The hydrodynamic delivery of IL-2 cDNA resulted in a sustained activation of NK cells that was confirmed by increased cell numbers, increased cell size based on flow cytometric forward scatter, and increased expression of CD69 (an early activation Ag on lymphocytes), and an increase in NK cell-mediated lytic potential in both NK- and Ab-dependent cellular cytotoxicity assays was measurable after several hours (data not shown). The increase in NK cells is accompanied by an increase in lytic activity per cell (LU [specific activity]). The treatment with IL-2 cDNA also increases both total cell number and total lytic activity. Thus, the IL-2 cDNA treatment results in substantially increased lytic potential and an increase in the number of NK cells.

---

**Table III. Statistical comparison of treatments shown in Fig. 7**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BALB/c</th>
<th>CD1d-KO*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment</td>
<td>IL-2</td>
</tr>
<tr>
<td>No treatment</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>αGal</td>
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<tr>
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<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>βGal and IL-2</td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Statistics were performed by two-tailed t test.

KO, knockout.

Values of p are compared with **** treatments, as shown.

---

*Abbreviation used in this paper: GalCer, galactosylceramide.*
Our studies with numerous strains of wild-type and mutant mice have shown that the effects of IL-2 cDNA gene delivery are predictable and reproducible in most mice with immune defects. These results suggest that the use of hydrodynamic gene delivery in conjunction with selected knockout mice may be a powerful tool with which to rapidly study unique mechanisms of immunological response against infectious agents or cancer without the need for large amounts or repeated administration of purified proteins. In this setting, selected cytokines or other unique proteins of interest could be administered by hydrodynamic gene delivery at different times during an immune response in knockout mice specific for the same protein to determine the importance of specific cytokines or proteins in the initiation, duration, and termination of various unique immune responses.

In addition, we demonstrated that hydrodynamic delivery of IL-2 cDNA potently modified the cellular immune response profile of peripheral organs via dramatic effects in the bone marrow. Specifically, using CFSE-labeled precursors to trace the origin of cells, we detected 10-fold increases in progeny of adoptively transferred bone marrow progenitors. This finding supports the contention that most of the increases in peripheral leukocyte subsets induced by IL-2 result from increased bone marrow differentiation and redistribution to the periphery.

Finally, we were able to use a unique approach of hydrodynamic IL-2 cDNA injection combined with several different NKT cell binding ligands to analyze the role of NKT cells in tumor rejection. NKT cells have been implicated previously as a contributing element in the cytokine storm created in vivo (27). Using a renal cell carcinoma model that can selectively establish metastases in either the liver or lung, we combined hydrodynamic administration of IL-2 cDNA with βGalCer, an agent that we have shown recently to remove but not activate NKT or NK cells to elucidate the role of these effector cells. This approach demonstrated that an additive antitumor effect was achieved against metastases in the liver. In addition, treatment with βGalCer alone, which only removed NKT cells, also showed a significant ability to reduce metastases, suggesting that these cells may actually inhibit other antimetastatic mechanisms in the liver. The antitumor effects of NKT cell depletion by βGalCer were further amplified by IL-2 cDNA that potently engages the antimetastatic effects of NKT cells.

In summary, we have demonstrated that hydrodynamic delivery of naked DNA encoding secreted mouse IL-2 can dramatically increase the number and function of various leukocyte subsets and preferentially increase the number and biological functions of NK cells. These results show that hydrodynamic delivery of IL-2 cDNA provides a simple, efficient, and inexpensive way of delivering new genes in vivo, as well as an inexpensive way to dissect antitumor and immunoregulating functions of IL-2 in vivo.

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Disclosures
The authors have no financial conflict of interest.

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