Recombinant Interleukin-2 Infusions and Decreased IgG2 Subclass Concentrations

By Robert J. Soiffer, Christine Murray, Jerome Ritz, Nichole Phillips, David Jacobsohn, Steven Chartier, and Donna M. Ambrosino

The administration of low doses of recombinant interleukin-2 (rIL-2) in vivo to patients with malignant neoplasms has been demonstrated to selectively increase the number of circulating natural killer (NK) cells in these patients. Recent evidence from SCID mouse models suggests that IgG subclass levels can be influenced by the presence and activity of NK cells. Therefore, we sought to examine the effect of rIL-2 infusions on human serum IgG subclass concentrations. We determined serum IgG2 subclass concentrations in 27 cancer patients receiving low-dose rIL-2 by daily continuous intravenous infusion. Eleven of these patients had active, metastatic, nonhematologic tumors; 16 patients had received IL-2 when they were in a minimal residual disease state after autologous or allogeneic bone marrow transplantation. Samples obtained before beginning IL-2 therapy and 8 to 10 weeks into therapy were tested. Treatment with IL-2 resulted in an increase in the percentage of CD56+ NK cells from 18% to 54% (P = .0001). A significant decrease in geometric mean IgG2 concentration from 2,017 μg/mL to 1,655 μg/mL was noted over this time interval (P = .03). Furthermore, the geometric mean IgG2 concentration after treatment was significantly lower than that of healthy controls (P = .026). In contrast, no significant changes in serum IgG1, IgG3, or IgG4 were noted during rIL-2 infusions. Our data suggest that rIL-2 treatment selectively decreases serum IgG2 concentrations. We speculate that increased NK cells mediate downregulation of human serum IgG2.

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Cytokines are known to influence IgG subclass concentrations in murine systems. Both γ-interferon (IFN-γ) and interleukin-4 (IL-4) play critical roles in regulating isotype switching. IFN-γ induces IgG2a immunoglobulin both in vitro and in vivo, and IL-4 selectively stimulates IgG1 and IgE secretion.1,5 The recognition that IL-4 and IFN-γ are involved in murine IgG subclass regulation and isotype selection has led investigators to focus on the cell types that elaborate these cytokines. Although significant attention has been paid to helper T-cell subsets, other cells producing cytokines (macrophages, activated B cells, and natural killer (NK) cells) may contribute to IgG subclass selection. Indeed, NK cells have recently been demonstrated to play a role in regulating murine immunoglobulin production.6

Although many data exist on regulation of murine IgG subclasses, the role of cytokines in human IgG subclass regulation is less well understood. We have been studying the role of T cells and NK cells in regulating human IgG subclass production using severe combined immunodeficiency (SCID) mice reconstituted with human peripheral blood mononuclear cells (huPBMC). In this SCID-huPBLC model, we have noted that NK cells downregulate human IgG2 subclass concentrations.7 These findings suggest that NK cells may regulate IgG2 subclass concentrations in humans.

We had an opportunity to examine this issue in patients receiving low-dose recombinant IL-2 (rIL-2) at Dana-Farber Cancer Institute (Boston, MA). Low-dose rIL2 induces a selective increase in the number of activated NK cells without significantly affecting other cellular subsets.8 We hypothesized that the increase in NK cell number and activity would be associated with a decrease in serum IgG2 concentrations. Furthermore, based on our SCID mouse model, we predicted that the effect would be isolated to IgG2 subclass concentrations. Therefore, we examined whether rIL-2 therapy affected IgG2 subclass concentrations in this study population.

MATERIALS AND METHODS

Patient eligibility. Samples for immunophenotyping and IgG subclass determinations were obtained from two distinct groups of patients. The first group consisted of 11 patients with metastatic solid tumor. Patients were eligible for entry into this study if they had evidence of metastatic cancer demonstrable either by physical exam or a radiographic imaging study. Eligibility criteria also included age greater than 18 years; ECOG performance status 0 to 2; normal or near normal parameters of renal, hepatic, and pulmonary function; and life expectancy exceeding 3 months. Patients were not receiving concurrent chemotherapy or radiotherapy while undergoing treatment with IL-2. The second group of patients consisted of 16 individuals who had undergone either autologous, syngeneic, or allogeneic bone marrow transplantation (BMT) for hematologic or nonhematologic malignancy. Patients were eligible to receive IL-2 therapy if at least 6 weeks had elapsed since marrow infusion. Patients could have no evidence of grades 2 to 4 graft-versus-host disease (GVHD) and could be taking no immune suppressive medication during IL-2 therapy. No patients received intravenous immunoglobulin either before or during IL-2 therapy. Again, patients were required to have ECOG performance status 0 to 2 and normal or near normal parameters of renal, hepatic, and pulmonary function before initiating IL-2.

IL-2 treatment. Both groups of patients received rIL-2 (Hoffmann-LaRoche, Nutley, NJ) by continuous intravenous infusion through an indwelling central catheter in an outpatient setting. Drug was administered by a portable computerized ambulatory pump (Pharmacia/Deltene Model 5100 HF; Pharmacia/Deltene, St Paul, MN). The supply of IL-2 was renewed every 7 days by the outpatient pharmacist. The group of 11 patients with metastatic cancer received IL-2 for 8 consecutive weeks at a dose of 4.5 × 10^6 U/m²/d.9 After 4 weeks of treatment had elapsed, patients were given weekly bolus infusions of IL-2.
Immunophenotypic effects of low-dose IL-2. Phenotypic analysis of PBMC on 26 of the 27 study patients pre- and post-rIL-2 infusions is shown in Table 1. One study patient did not have pre-–rIL-2 phenotype data and, thus, was not included in the evaluation of rIL-2 effects on phenotype. As previously published in these patients, a significant increase in cells staining for NHIK1 (CD56) and FcyIII (CD16) occurred while on rIL-2 treatment.8,9 The geometric mean percentage of NK cells, as defined by cells expressing CD56, increased from 18% to 54% for all study patients (P = .0001). An increase in cells expressing CD16 (another marker of NK cells) was also noted from 21% to 41% (P = .0001). The increase in NK cells during rIL-2 treatment occurred for both transplant and nontransplant patients (Table 1). Similar results were noted if absolute number of CD56- or CD16-expressing lymphocytes (instead of percentage of cells) were compared. For all study patients the geometric mean ± SEM absolute number of CD56-expressing cells increased from 155 ± 40 to 861 ± 234 during IL-2 therapy (P < .01), and the absolute number of CD16-expressing cells increased from 168 ± 286 to 647 ± 183 (P < .01). During IL-2 therapy, a concurrent significant decrease in the percentage of CD3-positive cells was observed. The percentage of CD8-positive cells actually increased due to expression of CD8 on the majority of IL-2–stimulated CD56-positive NK cells. No significant change in the number of CD4-positive cells was noted in these patients.

Immunoglobulin IgG subclass concentrations during rIL-2 therapy. IgG subclass concentrations were compared in serum samples obtained from study patients before the initiation of rIL-2 therapy and after approximately 8 to 10 weeks of treatment. In addition, sera from 16 healthy plasma donors were selected as controls, as normal values of IgG subclass concentrations can be assay-dependent.8 To minimize effects due to intraassay variations, all sera were run within two assays, and pretreatment and posttreatment samples were run within the same assay.

Before the initiation of rIL-2 treatment, the geometric mean serum IgG1, IgG2, IgG3, and IgG4 concentrations for the 27 study patients were not significantly different from those of healthy controls (Table 2). When the nontransplant and transplant patients’ pretreatment values were analyzed separately, neither significantly differed from IgG subclass concentrations in the control subjects.

We next examined IgG subclass concentration changes during rIL-2 infusion treatment. A significant decrease in geometric mean IgG2 concentration from 2.017 µg/mL to 1.655 µg/mL was noted (P = .03; Table 2). The IgG2 decrease occurred in both patient populations: a decrease from 2.065 µg/mL to 1.717 µg/mL for transplant patients and from 1.949 µg/mL to 1.574 µg/mL for the nontransplant patients. Furthermore, the post–rIL-2 geometric mean IgG2 concentration was significantly lower for the study population than for normal healthy controls (P = .026). In contrast, no significant changes in serum IgG1, IgG3, or IgG4 concentrations were noted from pre– to post–rIL-2 infusion for the entire study population. In fact, the geometric mean concentration for IgG1, the predominant IgG subclass, increased from 4.704 µg/mL to 5.280 µg/mL over the treatment period. The increase of IgG1 was confined to the transplant patients, as their geometric mean IgG1 concentration was 3.989 µg/
ml before treatment and 4,855 µg/ml after rIL-2 treatment. The nontransplant patients had little change in IgG1 concentrations from 5,979 µg/ml to 5,921 µg/ml after infusion.

We also compared total IgG, IgM, and IgA concentrations before and 7 to 10 weeks after initiation of rIL-2 therapy. Of the 27 study patients, 23 had values available. The geometric mean total IgG, IgM, and IgA serum concentrations were 1,095 mg/dL, 119 mg/dL, and 165 mg/dL, respectively, before treatment. After rIL-2 infusion, the geometric mean values were not significantly different at 1,042 mg/dL, 122 mg/dL, and 161 mg/dL, respectively.

We examined the correlation of absolute number of cells expressing CD20, CD3, CD4, CD8, CD56, and CD16 of individuals with their serum IgG1, IgG2, IgG3, and IgG4 concentrations after 8 to 10 weeks of rIL-2 treatment. However, no particular lymphoid subset was correlated with any IgG subclass concentration (data not shown). Interestingly, although rIL-2 therapy resulted in a decrease in IgG2 levels and an increase in the number of circulating NK cells in most of our patients, there did not appear to be a direct inverse correlation of NK cell number with serum IgG2 concentrations after treatment.

**DISCUSSION**

In this study we examined the effect of rIL-2 treatment on serum IgG subclass concentrations in 27 cancer patients. Serum IgG2 subclass concentrations decreased from 2,017 to 1,655 µg/ml (P = .03) after the 8- to 10-week treatment course and resulted in a lower geometric mean concentration than in healthy controls (P = .026). The effect of rIL-2 was selective for IgG2, as no significant change occurred in concentrations of IgG1, IgG3, or IgG4. In fact, the predominant IgG subclass, IgG1, increased over the study interval.

The decrease in serum IgG2 concentration occurred in both the BMT and non-BMT patients. This is significant, as low serum IgG2 concentrations without rIL-2 infusions have been demonstrated previously at 12 to 24 months after BMT.12,13 Notably, the serum IgG2 concentrations of our transplant patients decreased while IgG1 concentrations were increasing. This argues against natural waning of antibody after transplantation as an explanation of the IgG2 decrease. We suggest that the significant decreases in serum IgG2 concentrations for both patient populations were due to rIL-2 treatment.

It is possible that decreased serum IgG2 concentrations were the result of increased NK cells. In a model using SCID mice reconstituted with huPBMC, we recently noted that depletion of CD16-positive cells resulted in significant increases in human serum IgG2 concentrations. This datum suggests that CD16-positive cells (natural killers) downregulate human IgG2 production. Unfortunately, in our present analysis the absolute number of CD16- or CD56-positive cells induced by low-dose IL-2 did not significantly correlate with IgG2 levels. The absence of a significant inverse relationship may be merely due to sensitivity, or, perhaps, the actual number of NK cells is not as critical as the quantity of specific cytokine these NK cells elaborate.

One such cytokine produced by natural killers is IFN-γ. Patients receiving rIL-2 treatment in vivo have been shown to have increased IFN-γ production,14 and IFN-γ has been shown to affect specific IgG subclass concentrations in murine B cell in vitro studies.15 IFN-γ causes a decrease in IgG1 and IgG3 production by lipopolysaccharide (LPS)-activated B cells while increasing IgG2a production.16 In contrast, when murine B cells are activated with anti-IgD conjugated to dextran, IFN-γ results in increased IgG3 production.4 These data suggest that the nature of the B-cell stimulation also affects murine IgG subclass selection. In humans, IFN-γ has been shown to inhibit polyclonally induced in vitro B-cell activation16 and inhibit IgE production by rIL-417.

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**Table 1. Effect of rIL-2 Therapy on Cellular Phenotypes of PBMC**

<table>
<thead>
<tr>
<th>Cell Surface Antigen</th>
<th>All Patients</th>
<th></th>
<th></th>
<th>BMT Patients</th>
<th></th>
<th></th>
<th>Non-BMT Patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre (n = 26)</td>
<td>Post (n = 26)</td>
<td>Pre (n = 16)</td>
<td>Post (n = 16)</td>
<td>Pre (n = 10)</td>
<td>Post (n = 10)</td>
<td></td>
</tr>
<tr>
<td>(B1) CD20</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>3</td>
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<tr>
<td>(T3) CD3</td>
<td>51*</td>
<td>28*</td>
<td>48†</td>
<td>21†</td>
<td>55</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>(T4) CD4</td>
<td>16</td>
<td>14</td>
<td>9</td>
<td>7</td>
<td>33</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>(T8) CD8</td>
<td>30†</td>
<td>49†</td>
<td>35†</td>
<td>59†</td>
<td>23†</td>
<td>36†</td>
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<tr>
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<td>18†</td>
<td>54†</td>
<td>23†</td>
<td>63†</td>
<td>12†</td>
<td>42†</td>
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<tr>
<td>(FCyIII) CD16</td>
<td>21†</td>
<td>41†</td>
<td>24†</td>
<td>47†</td>
<td>15†</td>
<td>31†</td>
<td></td>
</tr>
</tbody>
</table>

Values are geometric mean percentages of positive cells.

**Abbreviations:** Pre, pre-rIL-2 therapy; Post, post-rIL-2 therapy.

*P < .01 for Post v Pre value.
† P < .005 for Post v Pre value.
‡ P < .05 for Post v Pre value.

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**Table 2. Effect of rIL-2 Therapy on IgG Subclass Concentrations in 27 Patients**

<table>
<thead>
<tr>
<th>IgG Subclass</th>
<th>Pre-rIL-2 (n = 27)</th>
<th>Post-rIL-2 (n = 26)</th>
<th>Normal Controls (n = 16)</th>
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</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>4,704</td>
<td>5,280</td>
<td>4,168</td>
</tr>
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<td>IgG2</td>
<td>2,017*</td>
<td>1,655**</td>
<td>2,404</td>
</tr>
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<td>IgG3</td>
<td>369</td>
<td>328</td>
<td>441</td>
</tr>
<tr>
<td>IgG4</td>
<td>211</td>
<td>177</td>
<td>365</td>
</tr>
</tbody>
</table>

Values are geometric mean immunoglobulin concentration (µg/ml).

*P = .03 by paired t test comparing pre- and post-rIL-2 values.
† P = .026 by t test comparing post-rIL-2 values and normal control values.
However, no specific effects on IgG subclass production have been shown. Human IgG2 is functionally equivalent to murine IgG3, as T cell-independent antigens induce predominantly IgG2 in humans and IgG3 in mice. Thus, IFN-γ down-regulation of human IgG2 may be analogous to murine LPS-induced IgG3 inhibition. Finally, alternative explanations should be considered, as we have no direct evidence that IFN-γ mediates the effect. IL-2 could directly affect B-cell IgG2 production or perhaps regulate subclass selection through other cytokines elaborated by NK cells. Future studies will address the mechanism of rIL-2 regulation of human IgG2 subclass production using the reconstituted SCID model.

The clinical significance of rIL-2 infusions causing decreased IgG2 concentrations in patients receiving IL-2 is unclear. Low IgG2 concentrations have been documented to be a risk factor for polysaccharide-encapsulated pathogens in a variety of clinical settings. It has been shown that low IgG2 is associated with increased infections in infants and young children, IgG2 selective deficiencies, post-BMT, vaccine failures, and certain ethnic populations. Increased infections during rIL-2 treatments have been documented in several studies, including the largest review of 935 rIL-2 treatment courses. However, typical polysaccharide-encapsulated pathogens were not reported, and Staphylococcus aureus was the primary bacteremic isolate in this review. Low serum IgG2 concentrations have never been implicated as a risk for bacteremia with this pathogen.

In summary, rIL-2 results in significant decreases in serum IgG2 concentrations. We propose that increased NK cell numbers and increased IFN-γ production are the mediators of this effect. Future studies in our SCID mouse model and in patients receiving IL-2 will address the mechanisms and clinical significance of NK regulation on human IgG2 serum concentrations.

REFERENCES

Recombinant interleukin-2 infusions and decreased IgG2 subclass concentrations [see comments]

RJ Soiffer, C Murray, J Ritz, N Phillips, D Jacobsohn, S Chartier and DM Ambrosino