Recombinant Interleukin-2 Infusions and Serum IgG Subclass Levels

To the Editor:

In a recent article, Soiffer et al reported a significant selective decrease of mean serum IgG2 concentrations after low-dose infusions of recombinant interleukin-2 (rIL-2) to patients with malignant neoplasms. They proposed that an increased number of natural killer (NK) cells may be the cause of IgG2 downregulation, possibly through the production of interferon-γ (IFN-γ). Such results are strikingly different from those we obtained in patients treated with higher doses of rIL-2.

Two groups of patients with metastatic melanoma were treated with high doses of rIL-2 (Roussel Uclaf, Paris, France) using the following regimens: in group A, 8 patients received 16 MUI/m²/d (6 patients) or 20 MUI/m²/d (2 patients) by continuous infusion during 5 days and then on days 15 to 19; in group B, 7 patients received 24 MUI/m²/d during 2 days and then every week during 5 weeks. Blood samples were collected just before and 8 to 12 days after the end of therapy for group A and the day before the fifth weekly infusion of IL-2 for group B patients (ie, after a total of 170 MUI/m² and 190 MUI/m² rIL-2 in groups A and B, respectively). IgG subclass levels were determined by a competitive immunoenzymatic assay using monoclonal antibodies (Unipath, Bedford, UK) from clones NL16 (IgG1), GOM2 (IgG2), ZG4 (IgG3), and R14 (IgG4), developed in our laboratory as previously described. IgA and IgM levels were determined by laser nephelometry. Lymphocyte immunophenotyping was performed on blood samples collected on EDTA; white blood cells were labeled with monoclonal antibodies to CD3, CD4, CD8, CD19, CD25, and CD56 (Becton Dickinson, Pont de Claix, France) as previously described.

As shown in Table 1, the absolute numbers of CD3, CD25, and CD56 increased in almost all cases after rIL-2 infusion, suggesting an elevation of activated T lymphocytes and NK cells. Proportions of CD4⁺ and CD8⁺ cells were not significantly modified (data not shown). In all tested patients (group B), the absolute number of circulating B cells (CD19⁺, see Table 1) increased.

Among Ig isotypes, only IgG1 levels in group A patients were
ent effects may be expected from treatments with low and high doses of rIL-2. However, the study of Soiffer et al.11 IgG2 levels marginally increased in 10 of the 15 patients (from 2.26 ± 1.28 to 2.54 ± 1.19 mg/mL; NS). Interestingly, IgG1 serum levels also increased, although not significantly, after treatment with low doses of rIL-2.9 Similar profiles of IgG subclasses are frequent in individuals undergoing normally repeated exogenous antigen stimulations, such as in the context of recurrent bacterial infections.1 Non-specific stimulation of B and T cells by rIL-2 might partially mimic such conditions.

Results of IgG2 serum levels are more controversial. Although different effects may be expected from treatments with low and high doses of rIL-2, it is worth noting that our patients also had increased numbers of CD56+ cells, which questions the role of NK cells in IgG2 deficiency pursued by Soiffer et al.11 A possible role of IFN-γ in IgG2 downregulation is even more unlikely in view of the recently described association of IFN-γ production defects and IgG2 deficiency.2 Comparisons with mouse models, in which IFN-γ plus lipopolysaccharide induce an inhibition of IgG3 production, is not contributive because it is now well established that human IgG2 cannot be considered an equivalent of mouse IgG3, from both structural and functional points of view.6

Measurements of IgG2 concentrations are prone to certain pitfalls that are not of common knowledge. In particular, we and others have clearly shown that the antibody HP6014, which is included in many commercially available kits, reacts up to ninefold more strongly with \( \lambda \) than with \( \kappa \) type IgG29; other factors such as the Gm(23) allotype and certain variable regions may also influence IgG2 level determinations using this reagent.9 Because Soiffer et al.11 used the antibody HP6014 in their study, it is conceivable that alterations of the expressed variable region repertoire or an increase of the \( \kappa:\lambda \) ratio induced by rIL-2 treatment may have resulted in biased IgG2 level determination. However, although we believe that such methodologic considerations are worth noting, differences in doses and protocols of rIL-2 may still explain the striking discordance between both studies.

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Table 1. Results of IgG Serum Levels (in Milligrams per Milliliter) and Peripheral Blood Mononuclear Cell Phenotypes (in Cells per Microliter)

<table>
<thead>
<tr>
<th>Patients</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA</th>
<th>IgM</th>
<th>CD19</th>
<th>CD3</th>
<th>CD56</th>
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<tr>
<td>1</td>
<td>6.4</td>
<td>2.5</td>
<td>0.49</td>
<td>0.4</td>
<td>2.6</td>
<td>5.9</td>
<td>ND</td>
<td>1,750</td>
<td>315</td>
<td>21</td>
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<td>2</td>
<td>12.5</td>
<td>4.5</td>
<td>0.82</td>
<td>0.78</td>
<td>4.1</td>
<td>7.9</td>
<td>ND</td>
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<td>3</td>
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<td>2.1</td>
<td>0.22</td>
<td>0.25</td>
<td>4</td>
<td>4</td>
<td>ND</td>
<td>1,300</td>
<td>1,570</td>
<td>57</td>
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<tr>
<td>4</td>
<td>6</td>
<td>2.45</td>
<td>0.36</td>
<td>0.25</td>
<td>3</td>
<td>5</td>
<td>ND</td>
<td>1,230</td>
<td>343</td>
<td>160</td>
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<tr>
<td>5</td>
<td>8</td>
<td>2.1</td>
<td>0.53</td>
<td>0.12</td>
<td>3</td>
<td>4</td>
<td>ND</td>
<td>777</td>
<td>518</td>
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<td>2.15</td>
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<td>55</td>
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<td>3.4</td>
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<tr>
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<td>0.33</td>
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<tr>
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<td>0.17</td>
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<td>ND</td>
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<tr>
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<td>0.19</td>
<td>3.4</td>
<td>2.3</td>
<td>8</td>
<td>656</td>
<td>66</td>
<td>66</td>
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<tr>
<td>15</td>
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<td>2.4</td>
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<td>0.22</td>
<td>2.2</td>
<td>1.2</td>
<td>276</td>
<td>3,600</td>
<td>3,459</td>
<td>721</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

Blood samples were collected before (upper lines) and after (lower lines) treatment with 170 MU/mL (group A, patients 1 through 8) or 190 MU/mL (group B, patients 9 through 15) of rIL-2.
REFERENCES


Response

Aucouturier et al’s letter cites an experience of 15 patients receiving different intermittent doses and schedules of rIL-2 and reports on IgG2 concentrations at 5 weeks after treatment. He reports that this group of patients did not show decreased IgG2 concentrations. Our study differed in that the patients received continuous treatment and we evaluated subclass levels 8 to 10 weeks later. We propose that the substantial differences in the study design may explain the differing results.

Aucouturier et al also suggests that our comments on murine IgG3 regulation are not relevant to our study as “it is now well established that human IgG2 cannot be considered an equivalent of mouse IgG3.” The reference he refers to does not support this statement; in fact, it states that murine IgG3 is the predominant subclass response to polysaccharides and the overrepresentation of IgG2 in human responses to polysaccharides is well recognized. This functional correlation between murine IgG3 and human IgG2 may well relate to common regulatory mechanisms such as interferon-γ suppression. In our report, we speculate that the decrease in IgG2 concentrations associated with rIL-2 treatment could result from interferon-γ suppression. However, Aucouturier et al points out a recent report that implicates interferon-γ deficiency in association with IgG2 deficiency. This study examines four IgG2 deficient patients’ in vitro response to mitogen. Although interesting, it is difficult to draw firm conclusions from these results on the effects of interferon-γ on serum IgG2 regulation. Further studies are needed to assess the role of interferon-γ and other cytokines on human IgG2 subclass regulation.

Finally, the use of clone HP6014 for determination of IgG2 concentration is questioned. Aucouturier et al notes that this monoclonal antibody “reacts more strongly with λ light chains” and suggests this may affect assay results. Clone HP6014 was used in the assay that we developed and standardized with the Centers of Disease Control (CDC). Using this assay, age-specific normal concentrations were established and the delay in maturation of IgG2 was noted. HP6014 antibody has a Ka for λ light chain of 2.9 × 10−7 L/mol which represents moderate to high affinity (personal communication, George Carlone, CDC) and the monoclonal antibody is used in excess. We therefore think that HP6014 is an appropriate reagent for determining IgG2 concentrations. We agree with Aucouturier et al that the differences in rIL-2 doses and the timing of IgG2 subclass measurement are the most likely reasons for the discrepant results.

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REFERENCES


Recombinant interleukin-2 infusions and serum IgG subclass levels [letter; comment]
P Aucouturier, JL Preud'homme, WH Fridman and C Mathiot