Chronic myeloid leukemia patients resistant to or intolerant of interferon α and subsequently treated with imatinib show reduced immunoglobulin levels and hypogammaglobulinemia

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Background and Objectives. Imatinib mesylate inhibits ABL tyrosine kinase. This protein serves a complex role in cell cycling and is important in lymphopoiesis. We describe the immunologic findings in patients with chronic myeloid leukemia resistant to or intolerant of interferon (IFN) who were treated with imatinib. This aspect could be of interest since patients with these characteristics may be exposed to this treatment for long periods.

Design and Methods. Immunologic and hematologic evaluation (including immunoglobulin levels and parameters of autoimmunity), immunophenotyping analysis of peripheral blood and bone marrow, and cytogenetic bone marrow analysis were performed at sequential time points of the treatment (0, 3, 6, and 9 and 12 months). The relationships among immunologic variables, and between the immunologic findings and response, were investigated.

Results. Hypogammaglobulinemia IgG, IgA and IgM developed in 28%, 14% and 22% of the patients, respectively. Lymphocyte counts decreased significantly along the treatment. No correlation was found between Ig levels and lymphocyte counts or CD4, CD8 or CD19 subpopulations in peripheral blood, nor between Ig levels and bone marrow B-lineage precursors. No autoimmune phenomena were detected. Hypogammaglobulinemia had no clinical repercussions in patients who developed it. The percentage reductions of IgG, IgA and IgM levels were higher in patients with major genetic response to imatinib.

Interpretation and Conclusions. Hypogammaglobulinemia can develop in as many as 20-25% of patients with chronic myeloid leukemia previously exposed to IFN α and who are then treated with imatinib. The reduction of Ig is greater in patients with a better cytogenetic response, perhaps reflecting that the efficacy of imatinib in blocking BCR-ABL kinase activity runs in parallel with ABL inhibition, leading to a dysregulation of B-lymphocyte function. Close immunologic evaluation is recommended in these patients.

Key words: imatinib mesylate, interferon α, immunoglobulin levels, lymphocytes.

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median age was 50 years (range 16–76). There were 24 males and 12 females. Seven of the patients had had a bone marrow transplantation (BMT) (1 allogeneic, 6 autologous). The median time from diagnosis to Imatinib treatment was 1323 days (354–5053). The median duration of IFN treatment was 1116 days (337–3817).

The patients were treated with imatinib mesylate at a median oral dose of 400 mg per day (100–400 mg). At the time of analysis, the patients had been followed for a median time of 507 days (330–663). Twenty-nine of them were followed for at least 12 months.

Hematologic and immunologic evaluation
Prospective studies. Patients were evaluated at 0, 3, 6, 9 and 12 months with complete blood cell counts, serum biochemistry, serum levels of IgG, IgA and IgM, percentages and absolute counts of CD4 and CD8 lymphocyte subpopulations. Several autoimmunity parameters were also prospectively studied: direct antiglobulin test and eluate, thyroid hormone levels and autoantibodies (ANA, anti-DNA, ENA).

Immunophenotyping
After finding that some patients had reduced levels of immunoglobulins, we serially determined peripheral blood CD19+ B–lymphocyte counts by flow cytometry. The study also included characterization of distinct subpopulations within bone marrow B-lineage cells.

Immunophenotypic analysis was performed every three months using a standard four-color flow cytometry approach with monoclonal antibodies directed against the following human surface antigens: CD45, CD19, CD20, CD4, and CD8 (all from Becton Dickinson, Mountain View, CA, USA). Briefly, 1×10⁶ nucleated unseparated cells were incubated for 15 minutes in the dark at room temperature with saturating amounts of the relevant fluorochrome-conjugated mouse anti-human monoclonal antibody.

Flow cytometric analysis was performed on a FACScalibur flow cytometer (Becton Dickinson, San Diego, CA, USA) after lysis of red blood cells. Data acquisition was performed using CellQuest software (Becton Dickinson). The data analysis was performed on the lymphoid population using Paint-A-Gate software (Becton Dickinson). Mature B-lymphocytes were identified as CD19+CD20bright−cells, and B precursors as CD19+CD20dimcells.

Cytogenetic and bone marrow analysis
Cytogenetic and fluorescence in situ hybridization (FISH) analysis were performed on bone marrow samples prior to treatment and at 3, 6, 9 and 12 months during the first year, and every six months thereafter.

Cytogenetic studies were done using standard G-banding with trypsin–Giemsa staining. At least 20 metaphases, if possible, were analyzed in each study. In some patients, particularly at the beginning of therapy, it was not always possible to find 20 metaphases for analysis.

FISH studies were performed using the LSI-BCR-ABL dual color fusion probe (Vysis, USA). Hybridization procedures were done according to the protocol supplied by the manufacturer. FISH images were captured and analyzed using Quips FISH imaging software (Vysis, USA). At least 500 cells were scored. The abnormal hybridization pattern observed with this probe is 1 red, 1 green, and 2 yellow signals (representing both the derivate chromosome 9 and the derivate chromosome 22). Observation of a single cell with this hybridization pattern is considered to be positive for the Ph rearrangement. Only karyotype was considered for classifying the response. Cytogenetic response was classified according to MD Anderson criteria.12,13

Statistics
Descriptive analysis. For the quantitative variables, medians and ranges, and means and standard deviations were calculated. For the qualitative variables, percentages and proportions were estimated. For the accumulated dependent variables contingency tables for categorical variables were developed (χ² and Fisher’s exact test). The comparison of means was made by two-tailed t-tests of quantitative variables. The relationship between two quantitative variables was studied by calculating Pearson’s linear correlation coefficient. The temporal evolution of the quantitative variables was studied by analyzing the variance of repeated measures. When an a posteriori comparison of the different subgroups was made, this analysis was adjusted by the Bonferroni method.

The whole analysis was conducted with the aid of the SPSS v.10 program (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered to be statistically significant.

Results
Response
After 3 months of treatment, 97% of the patients had a documented complete hematologic response (CHR), defined as a white blood cell count below 10×10³/mL and a platelet count below 450×10³/mL, with a normal differential. The median time to reach the CHR was 21 days (0–168). According to CTC criteria, grade 3–4 anemia, neutropenia, or thrombocytopenia was detected during the treatment in 1, 12 and 5 patients, respectively. These cytopenias were resolved with transient interruption and/or dose...
reduction of imatinib. Two patients required granulocyte colony-stimulating factor (G-CSF) for recovery of white blood cells and one patient was hospitalized because of febrile neutropenia, which resolved with G-CSF in three days.

At the time of this analysis and considering the best cytogenetic response, imatinib mesylate induced a major cytogenetic response (complete plus partial) in 67% of patients: the response was complete in 53%. In 14% of patients the response was minimal and 19% of patients showed no cytogenetic response. The correlation between karyotypic and FISH evaluation was 0.92, 0.9, and 0.92 at 3, 6 and 9 months (*p* < 0.001), although the values of Ph cells obtained by FISH-i were significantly inferior to those obtained by karyotype (*data not shown*).

**Ig levels**

The evolution of Ig levels is depicted in Table 1. Ig levels could be tested in 33 patients. Ig levels were not available immediately prior to imatinib in 3 patients: these patients were considered ineligible for this analysis.

**Baseline Ig levels**

Prior to imatinib therapy none of the patients had IgG hypogammaglobulinemia, and only one had low levels of IgA. However, 17 patients had IgM hypogammaglobulinemia before imatinib treatment.

There was no correlation between baseline Ig levels and duration of disease or duration of IFN treatment. There was no significant difference in the values of basal Ig between patients previously transplanted and those who were not (*data not shown*).

**Evolution of Ig levels while on imatinib**

The Ig levels diminished significantly during treatment with imatinib. The percentage reduction in the 6th month with respect to basal values was 24.8 ±16.7, 23.8 ±24.5 and 36.3 ±36.8, for IgG, IgA and IgM, respectively. The differences between the mean values of IgG, A and M were significant when

| Table 1. Median and range of immunoglobulin levels at 0, 3, 6, 9 and 12 months. |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| Ig levels       | Basal       | 3 months    | 6 months    | 9 months    | 12 months   |
|                 | n=33        | n=24        | n=26        | n=29        | n=20        |
| Ig G            | 1200 (823-3000) | 1030 (744-1880) | 932.5 (627-2110) | 866 (539-1950) | 845 (575-2170) |
| Ig A            | 236 (74-664) | 238 (55-512) | 194 (48-442) | 236 (66-389) | 149.5 (64-383) |
| Ig M            | 79 (34-287) | 57 (6-179) | 61.7 (8-150) | 58.8 (8-141) | 55.4 (8-106) |

Normal ranges: IgG: 800-1600 mg/dL; IgA: 100-300 mg/dL; IgM: 80-250 mg/dL.

**Figure 1. IgG levels in CML patients treated with imatinib at 0, 3, 6 and 9 months. (mean, 95% CI).**

**Figure 2. IgA levels in CML patients treated with imatinib at 0, 3, 6 and 9 months. (mean, 95% CI).**

**Figure 3. IgM levels in CML patients treated with imatinib at 0, 3, 6 and 9 months. (mean, 95% CI).**
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basal values were compared with 6th-month and 9th-month values, with \( p<0.05 \) in all the points (Repeated Measures test) (Figures 1, 2 and 3).

Among the patients with normal basal values of Ig, hypogammaglobulinemia IgG, IgA and IgM developed in 10/32 (28%), 5/30 (14%) and 8/14 (22%) during treatment. Considering all patients, 21 out of 33 reached low levels of IgM. Table 2 depicts the Ig levels of the patients who developed low levels of Ig. The duration of IFN treatment was slightly longer in patients who developed hypogammaglobulinemia but the difference was not significant (Table 3). There was no association between duration of disease or previous ABMT (data not shown).

Clinical correlation

There were no serious adverse clinical effects attributable to hypogammaglobulinemia. One of the patients developed lobar pneumonia, which was treated in the outpatient clinic. This patient was the only one to have previously received an allogenic BMT, and he had low levels of IgM prior to imatinib. We did not find a higher incidence of minor infections in patients with lower levels of Ig.

Autoimmune phenomena

The only abnormality detected was a low-titer of ANA without anti-DNA in two patients. It is interesting to note that in one patient who developed antiphospholipid antibodies while on IFN, these disappeared after 6 months. A similar case was observed in a patient with autoimmune thyroiditis, whose antibody levels fell to near-normal levels after 12 months of imatinib treatment.

Lymphocyte counts and lymphocyte subpopulations in peripheral blood and bone marrow

Lymphocyte counts in the peripheral blood diminished significantly during treatment (repeated measures test, Figure 4). Seven out of 36 patients (19.4%) had lymphopenia prior to therapy. Among the patients who had normal counts prior to therapy, 7 developed lymphopenia on some occasion during treatment. The counts prior to imatinib therapy
were $2.71 \pm 2.55 \times 10^9/L$ (mean $\pm$ SE) and at 12 months were $1.38 \pm 0.51 \times 10^9/L$ (Table 4 and Figure 4). The CD4 and CD8 proportions, however, remained fairly stable over time and the mean percentage was in the range of 40% and 33%, respectively.

The percentage of CD19 lymphocytes in peripheral blood appeared to increase during treatment although a statistical analysis of means could only be made in nine patients who had this count measured prior to imatinib therapy, and the variation did not reach statistical significance (Table 5).

The percentage of bone marrow lymphocytes increased slightly over time, but the difference did not reach statistical significance. Among the lymphoid population, the proportions of CD19CD20$^{bright}$ and CD19CD20$^{dim}$ appeared to increase with time, but this difference was not significant either (data not shown).

**Association between immunologic variables**

There was no correlation between Ig levels and lymphocyte counts or CD19 lymphocytes in peripheral blood. However, patients who developed IgG hypogammaglobulinemia had significantly higher counts of CD19CD20$^{bright}$ at 6 months ($36.9 \pm 19.1$ vs $14.2 \pm 9.8$, $p=0.006$). The same applied for patients who developed IgA hypogammaglobulinemia ($26.9 \pm 20.7$ vs $14.3 \pm 9.7$, $p=0.08$).

**Association between immunologic variables and response**

The percentage reduction of IgG, IgA and IgM levels at the 9th month was higher in patients with a major genetic response to imatinib ($p=0.07$, 0.03 and 0.02, respectively) (Table 6). Patients who achieved a major genetic response also had a higher proportion of CD19 lymphocytes in peripheral blood, and higher percentages of CD19CD20$^{bright}$ and CD19CD20$^{dim}$ cells in bone marrow at the 3rd and 6th months of therapy. In fact, there was a significant negative correlation between the percentage of peripheral blood CD19 lymphocytes at 3, 6 and 12 months and the percentage of Ph$^+$ cells in bone marrow at the same time-points ($r=-0.775$, $-0.614$, $-0.578$, with $p$ values=0.005, 0.003 and 0.049, respectively). This negative relationship was also found when correlating the percentage of bone marrow CD19CD20$^{bright}$ cells and the percentage of Ph$^+$ cells at 9 and 12 months ($r=-0.503$ and $-0.595$; $p=0.009$ and 0.009, respectively).

**Discussion**

Our results show that treatment with imatinib in patients previously exposed to IFN$\alpha$ is associated with a significant reduction in immunoglobulin lev-
els, an association that has not been reported before. Indeed, hypogammaglobulinemia IgG, IgA and IgM developed in 10/32 (28%), 5/30 (14%) and 8/14 (22%) of patients during treatment.

The reduction was gradual and did not reach severe ranges during the study period. We did not observe any clinical consequences of this abnormality, although it could be argued that the time of observation was relatively short.

Could the reduction in Ig levels during treatment with imatinib be explained by the disease itself or the treatment received before imatinib? We cannot rule out that the previous treatment with IFN, chemotherapy, or ABMT could favor the development of hypogammaglobulinemia in this kind of patient. Low baseline IgM values were seen in 47% of the patients, and this finding is consistent with the hypothesis.

However, we have three reasons for arguing against this possibility. First, we did not find any association between Ig levels and disease duration, duration of IFN treatment or previous BMT. Second, we could not find any report describing hypogammaglobulinemia either in CML patients treated with conventional or intensive chemotherapy or in patients treated with IFN. Finally, although a significant proportion of patients have IgM hypogammaglobulinemia before imatinib therapy, levels of IgG were normal before this therapy in all patients, and IgA levels were normal except in one case.

We postulate that our findings could be explained by quantitative or qualitative alterations in B cells induced by imatinib. The study of the lymphocyte counts and subpopulations has offered some hints on this. Absolute numbers of lymphocytes in the peripheral blood fell significantly during the observation period, but the reduction appeared to stop in the third month. One fifth of the patients developed lymphopenia, which was mild in all patients. Percentages of CD4 and CD8 did not vary. CD19 lymphocytes in peripheral blood and B-lymphoid precursors in bone marrow showed a certain tendency to increase with time, although this did not reach statistical significance. We did not find any clear-cut correlation between immunoglobulin levels, lymphocyte counts and B lymphoid cells. Nonetheless we did find a higher percentage of B cells and B precursors in patients with hypogammaglobulinemia IgG and IgA at 6 months of treatment.

In the light of our findings it is reasonable to hypothesize the existence of an altered immune responsiveness of B cells as a consequence of imatinib-mediated inhibition of physiologic ABL tyrosine kinase, as B lymphopoiesis is Ph-negative in most CML patients. It has been demonstrated that constructed mice homozygous for the c-abl mutation develop a running clinical picture, accompanied by T- and B-cell lymphopenia, thymic atrophy, spleen atrophy, and early death. In this model, there were no differences in the myeloid or erythroid lineages, and no clear deficiency was seen in bone marrow cellularity or bone marrow colonies induced by GM-CSF, M-CSF or IL-3. Immunoglobulin levels were not reported. Besides, c-abl null B cells show a weaker proliferative capacity in response to B-cell antigen receptor stimulation than do wild-type cells, thus suggesting a key role of c-abl protein on B cell proliferation downstream of the B-cell antigen receptor. At the same time, proliferation and differentiation of B-cell progenitors into IgM positive cells seem to be unimpaired in c-abl deficient mice, findings which agree with the conserved numbers of precursors and mature B lymphocytes in our patients.

Other results which reinforce our hypothesis are that patients with a major cytogenetic response had a more marked reduction of Ig levels than did patients who did not obtain such a response. Moreover, we observed a slight-moderate inverse correlation between peripheral CD19, bone marrow CD19 lymphoid precursors and Ph+ cells.

Alternatively, it must be kept in mind that in a minority of CML patients, bcr-abl gene could be present in B-lymphoid cells, and blocking the BCR-ABL protein could also affect Ph+ B-lymphoid homeostasis. If blocking the BCR-ABL protein is the sole cause of the hypogammaglobulinemia, one would expect recovery of Ph+ lymphopoiesis to restore Ig levels. However, in our series, we found just the opposite, and the percentage reduction of IgG, IgA and IgM levels was higher in patients with a major genetic response to imatinib. For this reason, we postulate that imatinib-mediated inhibition of physiologic ABL tyrosine kinase may be crucial in the immunoglobulin decrease.

Although we cannot provide a definitive explanation for these observations, one possibility might be that the efficacy in blocking BCR-ABL kinase activity, leading to suppression of Ph+ hemopoiesis, could run parallel to ABL inhibition leading to a reduction in immunoglobulin synthesis because of a functional alteration in B cells. It is important to point out that we have not yet found any clinical repercussions of these phenomena, but more time is required to make adequate observations.

In conclusion, our study shows for the first time that patients previously exposed to IFN and subsequently treated with imatinib show reduced levels of immunoglobulins, with roughly 20% of them developing hypogammaglobulinemia. The incidence of lymphocytopenia, which was mild, was the same. We cannot exclude a possible role of the previous treatment or the disease itself in the development of this phenomenon. It is possible that hypogammaglobulinemia might not develop in naive CML patients. For these reasons, a similar study in newly diagnosed patients would be extremely interesting. Experiments are in progress to characterize the biochemical and molecular bases of these findings.
Although the clinical repercussions seem to be minor, the short follow-up and the absence of a clear explanation for this phenomenon indicate that the immunologic profile of these patients should be closely monitored.

References

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