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Serum Interleukin-10 in Plasma-Cell Dyscrasias

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Serum levels of interleukin-10 (IL-10) were measured by enzyme-linked immunosorbent assay in 115 patients with multiple myeloma (MM) in various phases of the disease (68 at diagnosis, 22 in plateau phase, 22 in relapse), in 71 individuals with monoclonal gammopathy of undetermined significance (MGUS), and in 53 normal volunteers. Detectable levels of serum IL-10 were found in 24 myelomas (20.9%), in 7 cases of MGUS (9.9%), and in 4 normal subjects (7.5%) (P = NS, χ^2 test). In patients with MM, cytokine was detected with a comparable frequency in all pathologic stages and phases of the disease: 4/19 in stage I, 6/26 in stage II, 5/23 in stage III, 4/22 in plateau phase, and 5/25 in progressing or relapsed disease. IL-10 concentrations did not differ significantly between controls and patients with plasma-cell dyscrasia, between patients with MGUS and those with MM, between early vs. advanced MM, or between patients in different phases of the disease. In 36 patients with MM in whom IL-10 was measured serially, no significant changes were observed over the course of the disease. Also, when comparing the outcomes of individuals with detectable or undetectable IL-10 in single stages or in the whole myeloma group, no differences were revealed. Our results do not support an apparent involvement of IL-10 in the pathogenesis of MM in vivo. However, further studies are required to define the exact role of this cytokine within the complex cytokine network of this neoplastic disorder. Am. J. Hematol. 54:335–337, 1997

INTRODUCTION

Recent observations suggest the involvement of IL-10 in multiple myeloma (MM). This molecule has been shown to be the most potent inducer of immunoglobulin (Ig) secretion in various systems of B-cell activation. In particular, in the CD40 system IL-10 promotes IgA synthesis and selects for the secretion of IgG_1 and IgG_3, inducing transient proliferation of activated naive (surface IgD^+) B cells followed by a complete differentiation of these cells into mature plasma cells [1]. It is also the main cytokine responsible for the terminal differentiation of plasma cells in the EL-4 T cell-dependent culture system [2]. Furthermore, one recent report indicates that IL-10 can stimulate the growth of malignant plasmablastic cells in vitro in the absence of IL-6 [3]. In this same study, high serum IL-10 concentrations were found in the majority of patients with plasma-cell leukemia, which led to the hypothesis that this cytokine might be involved in the late phases of MM in vivo [3].

These findings prompted us to investigate IL-10 levels in the sera of individuals with plasma-cell dyscrasias to define IL-10's relationships, if any, with presentation features, tumor mass, and disease course.

PATIENTS AND METHODS

Patients

From April 1990–October 1995, we collected sera from 186 patients with plasma-cell dyscrasia: 71 with monoclonal gammopathy of undetermined significance (MGUS), 68 with newly diagnosed MM, 22 with myeloma in plateau phase, and 25 in relapse. MGUS patients (39 men and 32 women, median age 63 years, range 39–85 years) showed a stable M component for at least 2 years. There were 41 men and 27 women (median age 61 years, range 44–83 years) in the untreated group. According to the staging system of Durie and Salmon [4], 19 were stage I, 26 were stage II, and 23 were stage III; 6 were substage B. Forty-eight patients were positive for IgG, 12 for IgA, 6 for Bence-Jones myeloma, and 2 for nonsecreting myeloma. Patients in plateau phase were considered to be those with partial (M component decrease >50%) or complete (complete disappearance of M component) response after chemotherapy and a stable disease for at least 6 months. The median age of this

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group was 62.5 years; at time of diagnosis, 8 were stage I, 8 were stage II, and 6 were stage III. The median age of relapsed patients was 64 years; 7 were originally stage I, 10 were stage II, and 9 were stage III. The median time to relapse or progression was 14 months (range, 6–27 months). Controls were 53 apparently healthy subjects comparable for median age and sex distribution with our patient population.

IL-10 Assay

Measurements of IL-10 were carried out by enzyme-linked immunoassay (ELISA) with a commercially available test kit produced by Bouty (Milan, Italy), following standard procedures described previously [5]. The sensitivity limit was found to be 1 pg/ml, with highest intra-assay coefficient of variability (CV) of 4.3%, and highest interassay CV of 7.2%. As reported by the manufacturer, this ELISA is specific for human IL-10 and does not crossreact with other known cytokines.

Statistical Analysis

Statistical evaluation was performed using the procedures of the Winstat® package (Kalmia Co. Inc., Cambridge, MA) on an IBM computer. The significance of cytokine levels in different subsets of patients was assessed by Student's t-test, analysis of variance (ANOVA), or the χ^2 test. Correlations of IL-10 with other laboratory parameters were calculated by Spearman's rank correlation coefficient. Survival curves were plotted by the method of Kaplan and Meier. For comparison of overall and progression-free survival between patients with high and undetectable IL-10, the log-rank (Mantel-Haenszel) test was applied. $P \leqslant 0.05$ was designated statistically significant. All P values are two-tailed.

RESULTS

Testing results for IL-10 are shown in Figure 1. Detectable levels of serum IL-10 were found in 4/53 (7.5%) normals, and in 7/71 (9.9%) patients with MGUS, as compared to 24/115 (20.9%) myelomas (P = NS, χ^2 test). It is noteworthy that in MM, cytokine was detected with a comparable frequency in all pathologic stages and phases of the disease: 4/19 in stage I, 6/26 in stage II, 5/23 in stage III, 4/22 in plateau phase, and 5/25 in progressing or relapsed disease. Statistical analysis (using Student's t-test) revealed no significant differences between controls and patients with plasma cell dyscrasia, between patients with MGUS and those with MM, between early vs. advanced MM, or between patients in different phases of the disease (onset vs. relapse/progression, onset vs. plateau phase, or relapse/progression vs. plateau phase). Also, no relationship was found between elevated IL-10 and the Ig isotype. In 36 myelomas, IL-10 measurements were performed serially. IL-10 was found elevated at diagnosis in 7 cases, with 4 of them retaining elevated levels at time of remission. Sixteen of these patients could also be evaluated at time of progression or relapse: IL-10 was detected in 3 cases, of whom 2 already had elevated levels at time of diagnosis. No significant correlation was found between IL-10 and β_2-m, neopterin, and lactate dehydrogenase (LDH). These markers confirmed their value as reliable indicators of tumor mass and disease activity, with highly increased levels in patients with advanced-stage MM and relapsed disease.

When comparing the outcomes of individuals with detectable and undetectable IL-10 in single stages or in the whole myeloma group, no differences were observed. Median survivals of patients with detectable IL-10 levels relative to those with undetectable cytokine concentrations were: 28 vs. 38 months in the untreated stage I–III groups; 27 vs. 24 months in the plateau-phase group; 13 vs. 16 months in the relapse or no response group. Analysis by the method of Kaplan and Meier revealed these differences not to be statistically significant. Likewise, estimates of progression-free survival did not differ significantly between IL-10+ and IL-10− cases (median, 19.6 vs. 21.3 months, P = NS).

DISCUSSION

The identification of the biologic factors that influence the prognostic parameters of MM has great relevance in clinical practice. IL-10 might be a particularly interesting molecule in this regard, because recent in vitro experiments indicate that this cytokine can stimulate the proliferation of freshly explanted myeloma cells [3]. IL-10 might be produced as a paracrine factor by cell types such as monocytes/macrophages [6]. It was therefore important to explore the pattern of endogenous IL-10 concentrations in patients with MGUS and MM, as previous studies have reported that the circulating levels of cytokines such as IL-6, M-CSF, or G-CSF, involved with a paracrine mechanism in the pathogenesis of plasma-cell dyscrasias, show a correlation with clinical and laboratory parameters of disease activity (reviewed in Klein [7]).

Our results do not demonstrate statistically significant elevations of IL-10 in serum samples from patients with MM relative to those with MGUS or controls. Also, IL-10 levels were not correlated with a particular clinical presentation or treatment outcome. Lu et al. [3] recently reported their data about serum IL-10 measurements in patients with MM and plasma-cell leukemia. Using an assay with a reported sensitivity of 1 pg/ml, IL-10 was detected in only 3 of 89 patients with MM (3.4%), in 12 of 20 patients with plasma-cell leukemia (60%), and in no controls. Methodological differences may partly explain the discrepancies with our results, because using an

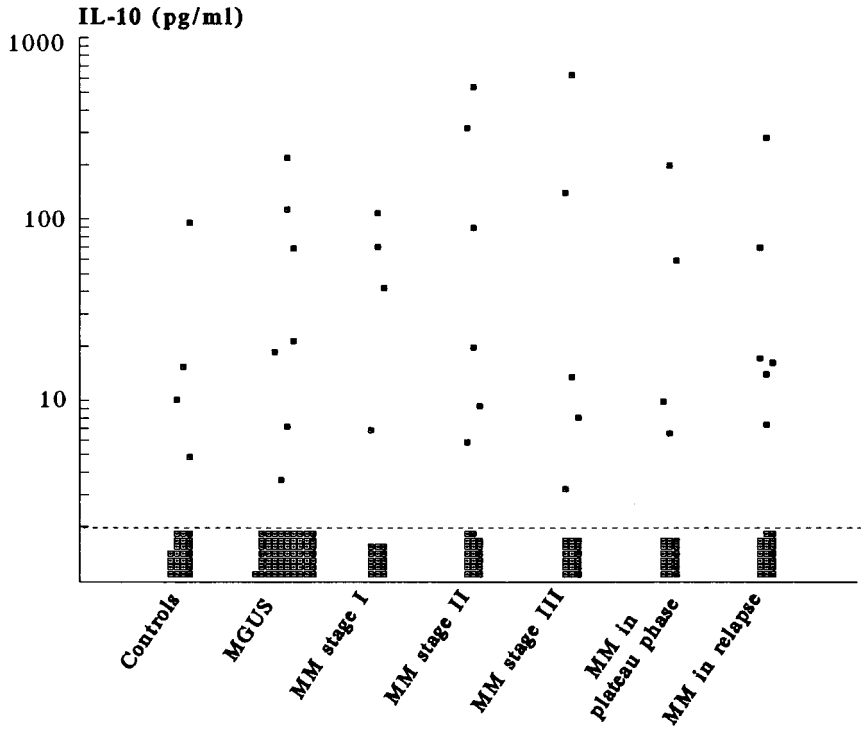

Fig. 1. IL-10 concentrations in patient groups and controls. Dashed line, limit of detection.

assay with lower sensitivity, the same group had previously described elevated IL-10 levels in 20% of early-stage MM [8]. Besides, in other studies detectable levels of IL-10 were found in 4–7% of apparently healthy individuals [9,10], and in 16% of patients with MM [10].

Our findings do not support a role for IL-10 in the pathogenesis of multiple myeloma. Plasma-cell leukemia may represent an unusual variant, especially in later phases of each patient's disease, and on which we have no data. Because our study showed similar levels of IL-10 at diagnosis with different extents of disease, during remission, and at relapse, this cytokine does not appear to play a major part in the progression of the disease.

REFERENCES

1. Rousset F, Garcia E, De France T, Péronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Bancherau J: Interleukin-10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 89:1890, 1992.
2. Werner Favre C, Matthes T, Barnet M, Zubler RH: High IgE secretion capacity of human plasma cells. Eur J Immunol 23:2038, 1993.
3. Lu ZY, Zhang XG, Rodriguez C, Wijdenes J, Gu ZJ, Morel-Fournier B, Harousseau JL, Bataille R, Rossi JF, Klein B: Interleukin-10 is a proliferation factor but not a differentiation factor for human myeloma cells. Blood 85:2521, 1995.
4. Durie BGM, Salmon SE: A clinical staging system for multiple myeloma: Correlation of measured myeloma cell mass with presenting features, response to treatment, and survival. Cancer 36:842, 1975.
5. Stasi R, Zinzani PL, Galieni P, Lauta VM, Damasio E, Dispensa E, Dammacco F, Tura S, Papa G: Detection of soluble IL-2 receptor and IL-10 in the serum of patients with aggressive non-Hodgkin's lymphoma: Identification of a subset at high risk of treatment failure. Cancer 74:1792, 1994.
6. De Waal-Malefyt R, Abrams J, Bennett B, Figdor C, de Vries JE: IL-10 inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J Exp Med 174:1209, 1991.
7. Klein B: Cytokine, cytokine receptors, transduction signals and oncogenes in human multiple myeloma. Semin Hematol 32:4, 1995.
8. Merville P, Rousset F, Bancherau J, Klein B, Bataille R: Serum interleukin-10 in early stage multiple myeloma. Lancet 340:1544, 1992.
9. Cortes JE, Talpaz M, Cabanillas F, Seymour JF, Kurzrock R: Serum levels of interleukin-10 in patients with diffuse large cell lymphoma: Lack of correlation with prognosis. Blood 85:2521, 1995.
10. Klouche M, Wilhelm D, Kirchner H: Cytokines, immunoglobulins, and IgG subclasses in patients with IgG plasmacytomas. Immun Infekt 22:149, 1994.