Cytokine Deregulation in Hematological Malignancies: Clinical and Biological Implications

Razelle Kurzrock

Abstract

Endogenous cytokines are aberrantly produced in many cancers and serve as autocrine growth factors or indicators of immune response to the tumors. Hence, cytokine deregulation is likely to participate in the development or evolution of the malignant process. Over the last few years, we have performed a series of studies, with the objective of measuring cytokine levels in tumors and correlating endogenous levels with phenotypic manifestations of cancer and with prognosis. Here, we present our analysis of serum interleukin 6 (IL-6) levels in lymphomas. We demonstrate that IL-6 levels are elevated in both relapsed and newly diagnosed Hodgkin's and non-Hodgkin's lymphoma and that these levels correlate with established prognostic features. Furthermore, in diffuse large cell lymphoma, IL-6 is an independent prognostic variable for both complete remission and failure-free survival. The molecular mechanisms underlying cytokine deregulation are now being investigated, with preliminary data from our laboratory suggesting heterogeneous genetic driving forces. In some cases, oncogene aberrations, particularly in the RAS system, may be responsible.

Introduction

It is now well established that cytokines are deregulated in a variety of hematological disorders (1–11). In addition, recent data indicate that many of these molecules, despite being designated as “hematological” growth factors, are also aberrantly expressed in solid tumors (12–14). To date, these phenomena are, however, best studied in the leukemias and lymphomas (Table 1).

Over a period of several years, we have conducted a series of studies investigating the role of cytokines in protean aspects of the biology of various neoplastic disorders (3–10, 15–18). Herein, we will review our recent data, using lymphoma as a paradigm, to demonstrate that endogenous cytokine levels can be exploited as powerful prognostic markers.

Cytokines in Relapsed Hodgkin's and Non-Hodgkin's Lymphomas

During the 1980s, we noted that patients who were treated with recombinant IFN-γ developed fever and drenching night sweats that were reminiscent of B symptoms (19, 20). Because it seemed reasonable to presume that B symptoms were due to endogenous cytokine release, we measured the level of IFN-γ in the serum of 57 relapsed lymphoma patients with and without B symptoms and compared these levels to those of a group of normal volunteers (n = 19; Ref. 15). Disappointingly, serum IFN-γ levels were not elevated in patients suffering from B symptoms. Furthermore, phytohemagglutinin-stimulated T lymphocytes from these individuals were actually deficient in IFN-γ production.3 These preliminary results suggested that IFN-γ was an unlikely cause of B symptoms.

TNF-α4 and IL-1β were investigated next because both of these molecules are known to be phlogistic mediators (1, 2, 21). Again, elevation of serum levels in our lymphoma patients with B symptoms was not observed (15).

IL-6 has potent growth and differentiation effects on lymphoid tissue, is pyrogenic, and promotes cachexia (22–24). Using an ELISA with a lower limit of sensitivity of 22 pg/ml, we recently found that our relapsed lymphoma patients with B symptoms had significantly higher serum IL-6 levels (median, 28.9 pg/ml) than did normal volunteers or lymphoma patients without B symptoms (median, undetectable; P < 0.001; Ref. 15). Because B symptoms are known to be prescient of a poor prognosis, we next examined the correlation between IL-6 and survival in the 26 relapsed Hodgkin’s lymphoma patients within our study cohort. Patients with higher IL-6 levels (defined as >22 pg/ml, the lower limit of sensitivity of the ELISA) had a median survival of <1 year, whereas those with low IL-6 levels had not reached their median survival after a median follow-up of >3 years (P < 0.05, logarithmic rank test; Ref. 15).

The results of this study suggested that IL-6 may be a mediator of B symptoms in individuals with relapsed lymphoma and, furthermore, might be exploitable as a prognostic factor. However, the study suffered from several flaws. First and foremost was the heterogeneity of the patients. In addition, the number of individuals analyzed was small, and they were not treated in a uniform fashion. We, therefore, sought to evaluate the prognostic value of serum IL-6 levels in a more homogeneous group of lymphoma patients.

IL-6 in DLCL

In our next investigation (17), we elected to study patients with DLCL for several reasons, including that serum samples


2 To whom requests for reprints should be addressed, at Department of Bioimmunotherapy, Box 302, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 794-1226; Fax: (713) 734-2374.

3 R. Kurzrock, unpublished data.

4 The abbreviations used are: TNF, tumor necrosis factor; IL, interleukin; DLCL, diffuse large cell lymphoma; CR, complete remission.
from these patients had been routinely frozen at the time of initial diagnosis and referral to the M. D. Anderson Cancer Center and that all of these patients had been treated in a uniform fashion using a protocol consisting of alternating, triple, multiagent chemotherapy (M-BACOS, MINE).

Serum samples from 58 newly diagnosed DLCL patients with at least one adverse prognostic variable were available. All patients were untreated at the time that serum was stored. A much more sensitive ELISA (lower limit of detection, 0.35 pg/ml) was used for this study. Again, the serum IL-6 levels in the lymphoma patients were significantly higher (median, 4.3 pg/ml; range, undetectable–110 pg/ml) than those in the normal volunteers (n = 33; median, undetectable; range, undetectable–1.89 pg/ml; P < 0.01). High IL-6 levels (defined as those above the upper limit of normal, i.e., ≥1.9 pg/ml) correlated with canonical, poor prognostic features such as B symptoms, a high β2-microglobulin level, and an unfavorable International Index (all P < 0.05; Ref. 17). In addition, using the Spearman rank correlation test, IL-6 levels were noted to directly correlate with leukocyte count, platelet count, and erythrocyte sedimentation rate and to inversely correlate with serum albumin (17). These results are of interest because IL-6 is a known hemopoietic growth factor, and this molecule increases hepatic fibrinogen synthesis (fibrinogen is the main component of erythrocyte sedimentation) and decreases hepatic albumin synthesis (24).

With regard to outcome, high IL-6 levels were associated with inferior failure-free and overall survival rates (all P < 0.05). At 90 weeks, the failure-free survival in patients with low IL-6 levels (<1.9 pg/ml) was 76% versus 44% for patients with high IL-6 levels (≥1.9 pg/ml; P < 0.05). Overall survivals at 90 weeks were 92% for patients with low IL-6 levels and 44% with patients with higher levels (P < 0.05; Ref. 17).

These observations indicate that high IL-6 levels are frequently present in patients with newly diagnosed DLCL and correlate with established poor prognostic variables such as B symptoms, elevated β2-microglobulin levels, and an unfavorable International Index. Importantly, there is a significant correlation between high IL-6 levels and low failure-free and overall survival rates.

Nevertheless, this study was not large enough to perform a multivariate analysis. Such an analysis was, therefore, our next goal.

### Multivariate Analysis of IL-6 in DLCL

The objective of our most recent study has been to expand the number of DLCL patients analyzed for IL-6 levels to determine whether IL-6 is an independent prognostic factor for this disease (18). We have now measured serum IL-6 levels in 118 patients with newly diagnosed DLCL. For the most recent study, the IL-6 values were converted to the NIBSCIWHO standard. This conversion requires multiplying the value obtained from the ELISA by a constant. We found that, in the 118-patient cohort with DLCL, the median IL-6 level was 4.6 pg/ml (range, undetectable–224.8 pg/ml), whereas the median value for our healthy controls (n = 45) was undetectable (range, undetectable–4.3 pg/ml; P < 0.009). [If the upper limit of normal of 1.89 pg/ml found in the previous study (17) is converted to the NIBSCIWHO standard, it closely approximates the 4.3 pg/ml upper limit of normal in this study.] High serum IL-6 levels were associated with virtually all well-established poor prognostic variables: B symptoms, elevated β2-microglobulin levels, bulky disease, high lactate dehydrogenase levels, advanced age (≥60 years), Ann Arbor stage of >2, and an unfavorable International Index (all P < 0.01).

Elevated IL-6 levels predicted a lower CR rate (66% for patients with higher IL-6 levels versus 95% for those with low IL-6 levels; P < 0.001; Ref. 18). Failure-free survival was considerably shorter in patients with an elevated IL-6 level (P = 0.00001), as was overall survival (P = 0.000001). The 3-year survival of patients with an elevated serum IL-6 level was 46% (95% confidence interval, 31–61%) whereas that for patients with a high IL-6 level was 90% (95% confidence interval, 82–98%; Ref. 17). A univariate analysis demonstrated that age, Ann Arbor stage, lactate dehydrogenase, β2-microglobulin, International Index, IL-6, and disease bulk were all important prognosticators for failure-free survival (all P < 0.02). A multivariate analyses was then performed using both backward and forward methodologies, with IL-6 dichotomized at 4.3 pg/ml (the upper limit of the NIBSCIWHO standardized normal range). In the logistic regression for CR, IL-6 was the only parameter selected (P = 0.013). The Cox regression analysis for failure-free survival also selected only IL-6 (P = 0.004). International Index, with a P of 0.08, would have been the next variable selected. The Cox regression analysis for overall survival selected only International Index (P = 0.008). IL-6 would have been the next variable selected. Therefore, our group of DLCL patients with high IL-6 levels do poorly, both because they fail to achieve CR and because they are at higher risk for relapse, and IL-6 appears to be the most important independent prognostic factor for both CR and failure-free survival.

Finally, because International Index is generally considered

### Table 1: IL-6 as a prognostic factor

<table>
<thead>
<tr>
<th>Disease</th>
<th>Comment</th>
<th>Ref.</th>
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<tr>
<td>Relapsed non-Hodgkin's lymphoma</td>
<td>High serum IL-6 levels correlate with poor prognostic features</td>
<td>15</td>
</tr>
<tr>
<td>Relapsed Hodgkin's disease</td>
<td>High serum IL-6 levels correlate with poor prognostic features and inferior survival</td>
<td>15</td>
</tr>
<tr>
<td>Newly diagnosed Hodgkin's disease</td>
<td>High IL-6 levels correlate with poor prognostic features</td>
<td>16</td>
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<tr>
<td>Newly diagnosed DLCL</td>
<td>High IL-6 levels correlate with poor prognostic features and inferior CR, failure-free survival, and overall survival rates; high IL-6 levels are an independent prognostic factor for CR and failure-free survival in multivariate analysis</td>
<td>17, 18</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>IL-6 levels correlate with tumor burden, clinical disease status, and survival</td>
<td>12</td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>High serum IL-6 levels are an adverse prognostic factor in metastatic renal cell carcinoma</td>
<td>13</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>High serum IL-6 levels correlate with other laboratory parameters reflecting disease morbidity</td>
<td>14</td>
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Plausible. Hence, both autocrine and paracrine functions for IL-6 are demonstrated that 89% of patients with non-Hodgkin’s lymphoma, we sought to determine whether IL-6 could further stratify this index. We found that high IL-6 levels predicted for poor failure-free and overall survival rates (Fig. 1), despite a favorable International Index. In addition, within the cohort with an unfavorable International Index, there appeared to be a subgroup of patients with low IL-6 levels who had a significantly better prognosis (all $P < 0.05$; Fig. 1).

Cellular Source of IL-6

Although our studies have not addressed the question as to which cells are producing IL-6, i.e., the lymphoma cells or the immune accessory cells, this issue has been researched by other investigators. For instance, Voorzanger et al. (25) recently demonstrated that 89% of patients with non-Hodgkin’s lymphoma show IL-6 positivity in their tissue by immunohistochemistry. Both tumor cells and macrophages express this molecule. Hence, both autocrine and paracrine functions for IL-6 are plausible.

Mechanism of Cytokine Deregulation

Normal lymphocytes do not produce IL-6 unless they are induced by an exogenous stimulus (26). Therefore, a molecular, IL-6-inducing alteration must exist in lymphoma cells that supplements the need for an exogenous stimulus. To elucidate the nature of this alteration, we have recently examined two lymphoma cell lines (Ly-3 and Ly-12) that express high levels of IL-6 and use this cytokine as an autocrine growth factor (11). IL-1α, TNF-α, and TNF-β (lymphotoxin) were produced by Ly-3 cells, although neither secreted nor cellular IL-1β was detected. Neutralizing antibodies to IL-1α reduced IL-6 concentrations in a dose-dependent manner. (Neutralizing anti-TNF antibodies had no effect.) These results suggest that, in this cell line, IL-1α is responsible for induction of IL-6. In contrast, Ly-12 cells produced IL-1α and TNF-α (but not IL-1β or TNF-β), yet neutralizing anti-IL-1 and anti-TNF antibodies had no effect on IL-6 production. It appears, therefore, that more than one mechanism serves to promote aberrant IL-6 expression: induction by IL-1 or induction that is independent of IL-1 (or TNF). Our current investigations are focused on identifying the molecular defect(s) responsible for deregulation of IL-1 in hematological malignancies. Preliminary data from our laboratory suggests that activation of RAS signaling pathways, either directly, because of RAS mutation, or indirectly, by other genetic defects, may be operative. As a result of these events, the normal ligand, receptor, signal transduction, and transcriptional activation pathway can be circumvented. Because RAS mutations are ubiquitous in hematological malignancies in which IL-1 is deregulated (as well as in many solid tumors), this genetic defect may account for many (but not all) cases of autocrine IL-1 production.

Therapeutic Implications

An inchoate understanding of the molecular pathways responsible for autocrine cytokine production is emerging, and this knowledge has potential for clinical exploitation. It is plausible that autocrine growth of tumor cells could be attenuated by soluble receptors or receptor antagonists that inhibit cytokine action directly (4, 5). Alternatively, interruption of upstream signaling pathways is possible. In this regard, RAS is an apt target for a novel class of molecules, farnesyl transferase inhibitors, which disrupt a posttranslational modification of RAS requisite for functional activation (27).

Summary

To date, prognostic variables for lymphomas (as well as for the majority of other cancers) have been clinical features that do not participate in the development of the disease process. They therefore represent surrogate markers for underlying biological variables. In contrast, there are several lines of evidence that support a pathogenic role for IL-6 in lymphoma. First, overexpression of IL-6 in transgenic mice is associated with the development of lymphomas (28). Second, high IL-6 levels corre-

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Footnote:

late with an increased risk of lymphoma in AIDS patients and in renal transplant recipients (29, 30). Third, transfection of IL-6 into EBV-transformed B lymphocytes confers a growth advantage in soft agar cultures and tumorigenicity in nude mice (31). Finally, IL-6 can serve as an autocrine growth factor for some lymphoma cell lines (11). Taken together, these data indicate that IL-6 represents a new class of prognostic factors, one with a pathogenic role. Exploration of the role of this cytokine in other hematological malignancies and in solid tumors is underway. In addition, the molecular defects that are responsible for cytokine activation are just beginning to be identified. Interference with these molecular oncogenic changes may be exploitable as a new form of gene-directed therapy.

References
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R Kurzrock


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