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Sounding Board

On the Mechanism of Action of Interferon-alpha on Hairy Cell Leukemia

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It is now clear that low dosage interferon-alpha (IFN-α) is an efficient therapy for hairy cell leukemia (HCL). Since the initial report of Quesada *et al.* [1], a number of trials have demonstrated that this therapy induces an overall response of around 90%, including 5–40% complete remissions (reviews in [2, 3]), whereas there was a median survival of 4 years before these trials [4]. Although the optimal dose, schedule and duration of treatment are still to be defined [5], it appears that the correction of cytopenias occurs in most cases [3] and that the quality of life of the patients even with minor responses is similar to that of complete responders (similar levels of hemoglobin and resolution of severe infections) [5]. Patients relapsing after interrupting the treatment can again respond to treatment with IFN-α [3, 5]. It seems that simply introducing small doses of IFN-α, producing low serum IFN levels (≤40 U/ml), is sufficient to improve gradually a physiological state towards normalization and that exogenous IFN-α could restore regulatory mechanisms occurring at the pre-plasma stage of B lymphoid maturation [6], corresponding to what seems to be a block differentiation in HCL [7, 8].

Interferons are natural growth inhibitors which are thought to be involved in the control of DNA synthesis, particularly during the differentiation of hematopoietic cells [9]. There is some evidence that this control may be due to their anti-growth factor properties [9]. In this respect, convergent data have shown that IFN-α can exert its therapeutic effects on HCL by acting directly on the hairy cells [10, 11] and that inhibition of the proliferative response of hairy cells to low molecular weight B cell growth factor (BCGF) might be one of the mechanisms involved in this action [12]. It is therefore conceivable that the loss of capacity to produce endogenous IFN-α in HCL, which has been already reported [13, 14], could result in a deficiency in negative controls of pre-plasma B-cell growth. Such a deficiency could be one of the events leading to the development of this malignancy. This hypothesis was suggested previously on the basis that monocytes, which are the major source of IFN-α, are either missing or greatly depressed in HCL [15].

However, interferons are only components of a complex network of cytokines. In the physiological setting, the cytokines act in combination, and it is likely that the growth and differentiation of pre-plasma B cells depend on interactions between several cytokines. Tumor necrosis factor-alpha (TNF-α) can be one of these cytokines since, as is IFN-α, it also derived from monocytes, and since its capacity to be produced is greatly reduced in HCL [16]. Recent results in our laboratory indicate that IFN-α administered *in vivo* no only up-regulates the levels of high affinity receptors for TNF-α in hairy cells, but also induces their expression when the receptors are not detectable in untreated patients [17]. The role of TNF-α as a growth inhibitor in HCL is under study. Recently, Cordingley *et al.* have reported that high concentrations of TNF (500–1000 U/ml) *in vitro* stimulate thymidine incorporation in hairy cells and in chronic lymphocytic leukemia (CLL) B cells, a neoplasia having only a low response rate to IFN-α [18]. Digel *et al.* have then shown that concentrations ≤20 U/ml can induce growth inhibition in CLL cells while higher doses enhanced thymidine incorporation in the same patients [19]. Opposite effects depending on

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the dose of TNF-α were also observed in HL-60 and ML-1 myeloid cell lines [20]. They were attributed to a bimodal binding of TNF: binding of low concentrations (from 0.1 to 100 U/ml) to high affinity receptors causes the growth inhibition and differentiation of both cell lines, while binding of high concentrations (500–1000 U/ml) to low affinity receptors abolishes these effects [20]. These data might explain the results of Cordingley *et al.* and it is reasonable to assume that only effects due to low concentrations of TNF-α occur in the physiological setting. Moreover, preliminary experiments seem to indicate that IFN-α2a can induce hairy cells to produce TNF cytotoxic activity *in vitro* [17]. Presently, no production of biologically active TNF has been detected in supernatants of hairy cell cultures or in the serum of untreated HCL patients [16–18, 21, 22]. It remains yet to be determined whether IFN-α therapy could stimulate *in vivo* the production of active TNF in the serum of patients, as previously found in mice [23]. Finding this to occur would favor the hypothesis of the involvement of TNF-α in the therapeutic effects of IFN-α on HCL.

Interleukin-6 (IL-6) is another cytokine which is known to play an important role in the growth and differentiation of B lymphocytes. First, IL-6 can stimulate terminal differentiation of B cells towards mature plasmocytes [24]. Second, the effects of IL-6 are not restricted to normal cells, since it was shown to be a growth factor in human multiple myeloma, a malignancy of plasma cells [25]. Recent results have indicated that IL-6 acts as a paracrine but not autocrine growth factor in this neoplasia [26]. *In vitro*, IL-6 is capable of inducing the proliferation of hairy cells and tumoral B cells from the lymph node of non-Hodgkin lymphomas ([27] and our unpublished results). This proliferative response is dose-dependent and is inhibited upon treatment with IFN-α2a. Furthermore, it seems that hairy cells and B lymphoma cells can secrete IL-6 *in vitro*, since, using the hybridoma growth factor activity assay described by Van Snick *et al.* [28], we have found around 200 U/ml of this activity in the supernatants of untreated cultures. This activity was markedly reduced when the cultures were treated with IFN-α2a.

From these data, two working hypotheses may be deduced. First, pre-plasma B cell growth can be under the control of two opposing mechanisms: a positive regulation depending on growth factors such as BCGF and IL-6, and a negative regulation due to monocyte inhibitory factors such as IFN-α and TNF-α. Monocytopenia could result in the loss of negative controls, which could be one of the events leading to the development of hairy cell leukemia. Second, therapeutic effects of IFN-α would imply the interactions with several cytokines: IFN-α therapy would restore antagonistic effects on growth factors (BCGF, IL-6), as well as it would enhance the sensitivity of hairy cells to TNF-α through up-regulation of its receptors. The antagonistic effects of IFN-α on IL-6 activity could also be achieved by inhibiting the secretion of this cytokine.

Therefore, we have to increase our knowledge of the interrelations between IFN-α and the other cytokines for a better understanding of the mechanism of action of IFN-α in hairy cell leukemia. Three levels of interactions seem to be of great interest: the effects of IFN-α on the synthesis and on the secretion of the different molecules as well as on the expression of their cell surface receptors. This will allow us to answer several questions such as: Does IFN-α modulate IL-6 receptors on hairy cells or inhibit IL-6 secretion or both? Is IFN-α action mediated partly by TNF-α? What is the mechanism allowing the inhibition of the response to BCGF (down-regulation of BCGF receptors, inhibition of BCGF synthesis)? Other factors could play a role in the effects of IFN-α on hairy cells. For instance, it would be interesting to know whether IFN-α exerts an effect on the 5–6 kD factor produced by hairy cells, which has been recently reported to inhibit granulopoiesis and erythropoiesis [22]. Finally, other types of mechanisms require further investigations, such as differentiating effects [29] and indirect effects mediated by stroma cells. It is indeed possible that different mechanisms can occur in the peripheral blood and in the bone marrow.

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