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Serum levels of soluble interleukin-2 receptor in hairy cell leukaemia: a reliable marker of neoplastic bulk

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Summary. In hairy cell leukaemia (HCL), the strong membrane expression of the Tac antigen, corresponding to the p55 chain of the interleukin-2 receptor (IL-2R), is associated with the presence in the serum of high levels of a soluble form of the same molecule (sIL-2R). Previous observations that therapy-induced clinical and haematologic improvement in HCL is accompanied by a progressive decrease of sIL-2R suggest a possible correlation between sIL-2R levels and tumour burden. To verify this hypothesis, we monitored the variation of sIL-2R values in 13 non-splenectomized HCL patients admitted for treatment with recombinant interferon alpha-2. The data were correlated with the estimated weight of bone marrow (BM) and spleen infiltrations, which in these patients almost entirely account for the tumour mass. The regression analysis test showed a direct correlation between sIL-2R values and both BM neoplastic involvement ($r=0{\cdot}63$) and spleen tumour mass ($r=0{\cdot}76$). In addition, the correlation was further improved ($r=0{\cdot}86$) when sIL-2R values were correlated with the total neoplastic mass, as calculated by the sum of spleen and BM neoplastic tissue weight. These data indicate that the detection of sIL-2R in HCL is a reliable non-invasive marker of tumour burden, which can be regarded as an additional useful tool for monitoring treatment response.

Hairy cell leukaemia (HCL) is a chronic lymphoproliferative disorder of B type, characterized on clinical grounds by pancytopenia, prominent splenomegaly without significant lymphadenopathy, and the presence of recognizable circulating cells with cytoplasmic projections ('hairy' cells: HC) (Golomb *et al*, 1978; Bouroncle, 1979; Westbrook *et al*, 1984; Cawley & Worman, 1985). The neoplastic HCs, which in the majority of cases are relatively scanty in the peripheral blood (PB), massively infiltrate the red pulp of the spleen and the bone marrow (BM), which shows a typical histologic pattern of neoplastic replacement associated with pronounced fibrosis (Westbrook *et al*, 1984).

The surface phenotype of HCs not only demonstrates the features of mature B cells (Catovsky *et al*, 1974; Jansen *et al*, 1982; Worman *et al*, 1983) but is also characterized by the strong expression of the so-called Tac antigen (Uchiyama *et al*, 1981; Korsmeyer *et al*, 1983), detectable by a number of monoclonal antibodies (MoAb) recognizing the CD25 molecule (McMichael *et al*, 1987), corresponding to the p55 chain of the interleukin-2 receptor (IL-2R) (Teshigawara *et al*, 1987). This molecule is expressed on the membrane of some normal haemopoietic cells upon stimulation (Uchiyama *et al*, 1981; Waldmann *et al*, 1984; Tsudo *et al*, 1984; Herrmann *et al*, 1985; Waldmann, 1986), as well as on malignant cells in different haematologic neoplasia, including the large majority of cases of HCL (Uchiyama *et al*, 1981; Korsmeyer *et al*, 1983; Waldmann *et al*, 1984; Pizzolo *et al*, 1984; Lantz *et al*, 1985). The membrane-bound IL-2R molecule can be released in a soluble form (sIL-2R) both *in vitro* and *in vivo* conditions, increased levels being observed in several disorders (Rubin *et al*, 1985; Greene *et al*, 1986; Chilosi *et al*, 1987; Pizzolo *et al*, 1987a, b; Semenzato *et al*, 1987).

The serum level of sIL-2R in HCL at diagnosis is extremely high (Greene *et al*, 1986; Chilosi *et al*, 1987) and can be regarded as a useful diagnostic marker in the context of the clinical picture of the disease (Pizzolo *et al*, 1987a). Interestingly, in HCL patients successfully treated with alpha interferon (alpha-IFN) a decrease in serum sIL-2R level parallels the improvement in haematologic parameters and the reduction of splenomegaly and of BM HC infiltration (Chilosi *et al*, 1987; Steis *et al*, 1988).

This observation suggests the possibility that sIL-2R levels in HCL are directly dependent on the neoplastic bulk, which

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in the majority of patients is mainly represented by BM and spleen infiltration, since the HC PB count is usually low and the involvement of other organs quite negligible. To examine this possibility, we have followed the serum levels of sIL-2R in 13 non-splenectomized patients with HCL admitted for alpha-IFN treatment, looking for a possible correlation with treatment-induced variations in tumour mass, as evaluated by quantifying the BM HC infiltration and the splenic neoplastic mass.

The presence of such an easily detectable correlation would be of help in the therapeutic management of HCL patients.

MATERIALS AND METHODS

We studied 13 patients with HCL, one female and 12 males, aged 44–74 years (median age 60 years). The diagnosis was based on established clinical, cytomorphologic, histologic, cytochemical and immunophenotypic criteria. None of the patients had been splenectomized nor received any other specific treatment for HCL.

The main clinical and haematologic pre-treatment data are summarized in Table I. Eight out of the 13 patients were severely neutropenic (neutrophil count $< 0{\cdot}5 \times 10^9$/l) with a previous history of serious infections. Severe anaemia (Hb <8 g/dl), requiring repeated blood transfusions, had occurred in five cases. Splenomegaly was present in all patients but one.

Between October 1984 and March 1988 all patients entered a therapeutic protocol with recombinant IFN alpha-2 (rIFN alpha-2) (Schering Corp., Kenilworth, N.J.). The treatment schedule consisted of 2×10^6 IU/m^2 of rIFN alpha-2 injected subcutaneously three times a week for 12 months. Ten patients completed the 12 months of treatment; three are still on rIFN alpha-2. The response to treatment was defined as complete remission, partial remission, minimal response, and no remission (Consensus Resolution, 1987). So far, only one patient has been submitted to a second course of rIFN alpha-2, 14 months after the end of the first one.

During and after rIFN alpha-2 treatment the main clinical and haematologic parameters were strictly monitored in each patient. In particular, a physical examination and full blood count were carried out at least every month for the first 6 months, and every 2 months subsequently. Overall, this evaluation was performed 88 times. At the same time, samples of serum for sIL-2R determination were collected from the patients and kept frozen. A BM trephine biopsy was obtained before treatment and after 3 and/or 6 months in all cases, and after 12 months of therapy in 10 patients. BM samples were evaluated both by histologic and immunophenotypic methods, in order to characterize the extent of BM infiltration more precisely and also to detect minimal residual disease (Chilosi *et al*, 1983). For this latter purpose, BM cryostat sections were immunostained with MoAbs to CD25, CD22 and CD11c (Schwarting *et al*, 1985; McMichael *et al*, 1987). The extent of BM involvement was expressed as the HC index (HCI), calculated by multiplying the percentage of infiltrating HC by the percentage of BM cellularity and dividing by 10000 (Golomb & Vardiman, 1983). The calculation of HCI allowed the estimation of the mass in grammes of neoplastic BM involvement. This was obtained by multiplying the HCI by the putative normal total BM mass of each patient calculated as 3·5% of body mass (Wintrobe *et al*, 1974).

At the same time that BM biopsies were taken, spleen mass was also evaluated. For this purpose spleen diameters (longitudinal: d_1; antero-posterior: d_2; transversal: d_3) were measured by ecothomography. From a geometric point of view, the spleen is comparable to a hemiellipsoid and we have calculated its volume according to the following formula: $d_1/2 \times d_2/2 \times d_3 \times 2/3$. Spleen mass was derived by multiplying the volume by putative density (1·020) and its neoplastic

Table I. Main clinico-haematological data in 13 patients with HCL as evaluated before starting rIFN alpha-2 therapy

Case	Age/sex	Hb (g/dl)	WBC $\times 10^9$/l	PMN $\times 10^9$/l	HC $\times 10^9$/l	Plt $\times 10^9$/l	Spleen*	HCI†	sIL-2R (U/ml)
1	44/M	11·3	3·84	0·35	3·0	35	13	0·80	37980
2	64/M	10·8	1·59	0·18	0·32	60	2	0·72	13370
3	64/F	13·3	1·04	0·27	0·49	144	0	0·21	26680
4	70/M	8·9	1·40	0·20	0·28	30	7	0·54	20500
5	74/M	10·7	10·0	0·22	8·72	38	10	0·48	38100
6	51/M	10·8	3·49	0·44	2·50	53	13	0·48	26070
7	52/M	7·7	2·02	0·85	0·56	143	4	0·72	48090
8	70/M	11·7	2·14	0·62	1·09	82	6	0·40	16080
9	60/M	10·9	2·48	0·16	1·50	52	14	0·12	41430
10	70/M	13·8	1·94	0·84	0·80	47	5	0·10	11680
11	48/M	7·4	2·80	0·18	0·95	55	18	0·16	40000
12	65/M	8·1	2·70	0·58	2·00	54	4	0·48	22280
13	74/M	11·5	5·30	0·72	4·90	104	4	0·12	10020

*cm below the costal margin.
†Hairy cell index.

component was calculated by subtracting the mean normal spleen mass (150 g).

Serum levels of sIL-2R were determined by an ELISA test (Cellfree, T Cell Science, Cambridge, Mass.), based on the use of two MoAbs recognizing different epitopes on the p55 chain of the IL-2R complex. Briefly, the IL-2R available in the test samples or in the standards binds to the polystyrene microtitre wells which have previously been incubated with 100 μl anti-Tac equivalent MoAb (1 μg/ml). A horseradish peroxidase conjugated anti-IL-2R MoAb directed against a second epitope on the IL-2R molecule binds to the IL-2R captured by the first antibody and completes the sandwich. After washing to remove the unbound enzyme-conjugated anti-IL-2R MoAb, a substrate solution is added to the wells. The reaction is then stopped and the absorbance determined at 490 nm. A standard curve is prepared from four IL-2R standards. The IL-2R standard used was the cell-free supernatant obtained from phytohaemagglutinin *in vitro* stimulated T cells which was assigned a value of 1000 IL-2R U/ml. Normal values of sIL-2R serum levels, as detected in a series of 54 healthy donors, never exceeded 500 U/ml, the mean value (±SEM) being 256±15·2 U/ml.

The correlation between serum sIL-2R levels of the patients in different phases (before, during and after rIFN alpha-2 therapy) and the corresponding data of spleen, BM and spleen+BM neoplastic masses was evaluated by the Pearson product-moment correlation (r) and t test analyses.

RESULTS

In all patients rIFN alpha-2 treatment produced a consistent clinico-haematologic improvement, although a complete remission, i.e. normalization of clinical parameters and complete disappearance of HCs in the blood and BM, has never been observed. We obtained a partial remission in six cases and a minimal response in four. Three patients have not yet been evaluated for definite clinical response.

Variations of the main clinico-haematologic parameters during and after rIFN alpha-2 treatment (mean values ±SEM), as compared with serum sIL-2R levels, are shown in Fig 1. Platelet count and spleen size improved more rapidly (already at the first month of rIFN alpha-2 treatment) in comparison with the remaining parameters which recovered later.

Serum levels of sIL-2R, which were extremely high before therapy (mean±SEM: 26 963±3284, range 10 020–48 090 U/ml), showed a rapid and consistent decrease, paralleling the clinical response to treatment in most cases and reaching the minimum values at the twelfth month (mean 1810±483, range 690–6230 U/ml). In the four patients with a minimal response, the decrease in sIL-2R level was less pronounced (minimal values ranging from 2760 to 6230 U/ml) as compared to partial responders (mean 1158±299, range 690–2176 U/ml). The progressive worsening of clinico-haematologic parameters observed in most cases after

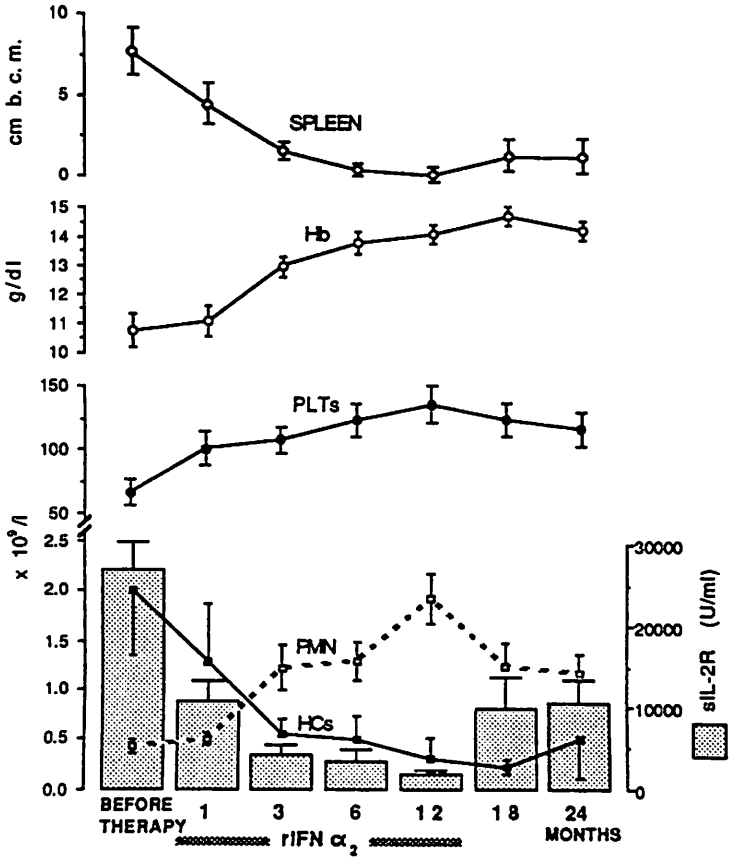

Fig 1. Variations of sIL-2R serum levels and of main clinical and haematological parameters in 13 patients with hairy cell leukaemia treated with rIFN alpha-2 (mean±SEM).

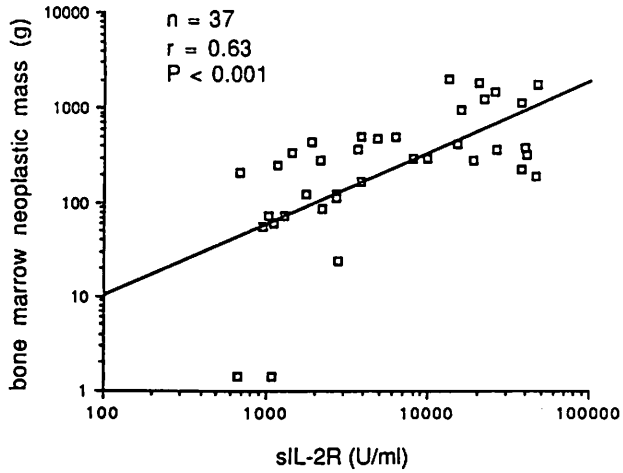

Fig 2. Correlation between sIL-2R serum levels and bone marrow hairy cell mass detected before, during and after rIFN alpha-2 therapy.

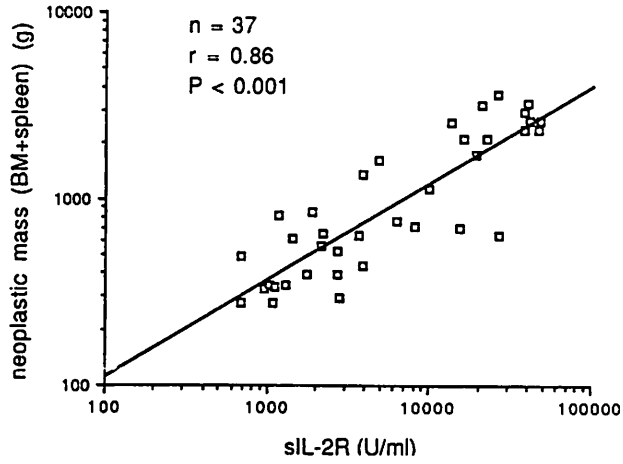

Fig 4. Correlation between sIL-2R serum levels and total neoplastic mass, calculated on the basis of estimated mass in grammes of spleen plus BM neoplastic tissue.

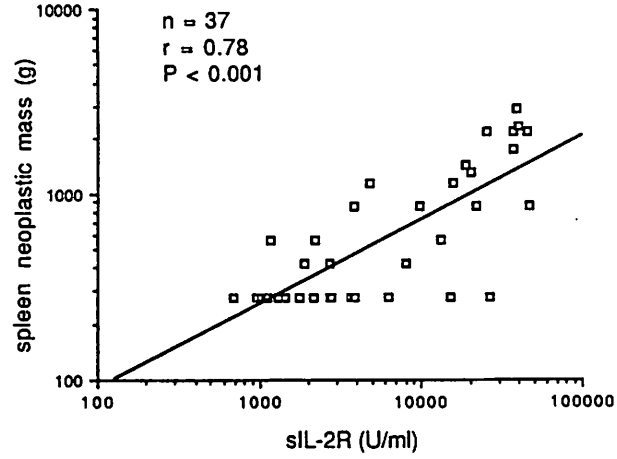

Fig 3. Correlation between sIL-2R serum levels and spleen neoplastic mass.

discontinuation of rIFN alpha-2 was associated with an increase in sIL-2R levels (mean values at twenty-fourth month: 10 487±7485, range 3880–21 250 U/ml). In six cases the progressive increase of sIL-2R values after rIFN alpha-2 therapy was observed 2–22 months (mean 5 months) before any noticeable change in other haematological parameters.

Data related to the correlation analysis between sIL-2R values and the figures of the investigated parameters were as follows: sIL-2R *v.* BM neoplastic mass: $r=0{\cdot}63$ ($P<0{\cdot}001$; Fig 2); sIL-2R *v.* spleen neoplastic mass: $r=0{\cdot}78$ ($P<0{\cdot}001$; Fig 3); sIL-2R *v.* total neoplastic mass (spleen + BM): $r=0{\cdot}86$ ($P<0{\cdot}001$; Fig 4). On the other hand, no correlation was observed between BM neoplastic weight and spleen neoplastic mass ($r=0{\cdot}30$).

DISCUSSION

This study demonstrates that in patients with HCL the serum levels of sIL-2R closely parallel the clinical response to rIFN alpha-2 therapy and are directly correlated with the amount of neoplastic mass. This statement is based on a number of observations. First, the treatment-induced normalization of clinico/haematologic parameters was associated with the progressive decrease of sIL-2R values, the lowest figures being observed after 12 months of therapy (Fig 1). A progressive increase of sIL-2R occurred when therapy was stopped, along with the reappearance of clinical signs of the disease. Second, the regression analysis test showed a direct correlation between sIL-2R levels and the estimated weight of BM (Fig 2), spleen (Fig 3) and BM plus spleen (Fig 4) neoplastic infiltration. Since evidence has definitely been provided that in HCL the increased sIL-2R values depend on the release of membrane-associated IL-2R molecules by HCs (Semenzato *et al.* 1988), our results indicate that the actual levels of sIL-2R reflect variations in the amount of tumour mass induced by treatment.

This last statement could give rise to some concern since in our study the calculation of tumour mass has been based on indirect measurements. However, no alternative methods other than HCI seem to be available to improve the quantification of BM infiltration and, hence, the BM neoplastic mass. In addition, preliminary data obtained in four patients who underwent splenectomy showed a good correlation between the actual spleen weight and the spleen mass calculated on the basis of ecothomographic parameters, the observed differences being between 2% and 16% (data not shown). The data showing a possible reduction of sIL-2R levels following splenectomy in these four cases is not available since they were not included in the present study.

Although BM and spleen infiltration does not account for the totality of tumour cells in HCL, it represents the largest part of neoplastic bulk in the majority of cases. In fact, HC involvement of other organs is quite negligible, with the exception of the liver, and circulating HCs are usually rather scanty (Golomb *et al.* 1978; Bouroncle, 1979; Westbrook *et al.* 1984; Cowley & Worman, 1985). None of our cases had a high number of circulating HCs (Table I).

The close correlation we observed between sIL-2R levels and tumour burden makes quite unlikely the alternative possibility that a reduction in membrane-bound IL-2R or a decreased shedding of the same molecule induced by rIFN alpha-2 therapy plays a major role in the treatment associated fall of sIL-2R levels.

In agreement with a previous observation (Golomb & Vardiman, 1983), the lack of correlation between BM neoplastic mass and spleen tumour mass ($r = 0{\cdot}30$) suggests their independent contribution, variable from case to case, to the total tumour burden, and therefore to the levels of sIL-2R. However, spleen neoplastic mass shows a better correlation than BM with sIL-2R levels ($r = 0{\cdot}78$ *v.* $0{\cdot}63$), suggesting that spleen itself represents the major bulk of the disease in non-splenectomized patients.

Interestingly, the normalization of sIL-2R levels has never been observed in our cases, thus supporting the view that the achievement of a true complete remission is a rare event after rIFN, since a minimal residual disease is almost invariably detectable at BM level. (Golomb, 1987), in spite of the disappearance of circulating HCs and normalization of all clinico/haematologic parameters.

All these considerations taken together, our data suggest that the detection of sIL-2R values in patients with HCL admitted to rIFN alpha-2 treatment can be utilized as a disease marker which may prove of clinical utility in the management of these patients.

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