Effect of Interferon-α on the Expression and Release of the CD23 Molecule in Hairy Cell Leukemia

By Elisabeth Genot, Marie Sarfati, François Sigaux, Elisabeth Petit-Koskas, Christian Billard, Claire Mathiot, Ernesto Falcoff, Guy Delespesse, and Jean-Pierre Kolb

Hairy cells are stimulated to DNA synthesis by low molecular weight B cell growth factor (LMW-BCGF) and this proliferative response is suppressed by interferon (IFN)-α, both in vitro and in vivo. The suggestion that the CD23 molecule (FcεRII receptor) might be involved in the signaling pathway of LMW-BCGF prompted us to study the expression of this molecule on hairy cells and its modulation by IFN-α. By flow cytometry and direct binding experiments with anti CD23 monoclonal antibodies, the presence of the CD23 antigen was detected in 7 of 12 cases tested, on variable percentages of cells, ranging from low to medium expression. In vitro incubation of hairy cells with IFN-α, which elicits a suppression of the proliferative response of these cells to LMW-BCGF, induced a parallel significant reduction of CD23 expression in only three cases. Similarly, a transient in vivo decrease of CD23 expression, concomitant with an inhibition of the LMW-BCGF response, could be detected in only one of three patients injected with IFN-α. Soluble scCD23/IgE-binding factor (BF) was quantitated in the serum from six other patients with hyperleukocytic hairy cell leukemia (HCL) undergoing a clinical trial of IFN-α therapy. Before treatment, these patients presented higher concentrations of the cleaved soluble form of the CD23 molecule than normal controls. Within a few weeks of IFN-α administration, these levels markedly decreased, paralleling a diminution of blood leukemic cells. Of interest, no such diminution was noticed for another patient resistant to IFN-α therapy. These results show that the proliferative response of hairy cells to LMW-BCGF is not linked to the expression of the CD23 marker. Besides, when the latter molecule was present, its decrease following IFN-α treatment, which could be detected in some cases, was not necessarily required for the suppression of the LMW-BCGF response and is thus not mandatory for the therapeutic efficacy of IFN-α. Our results point out that quantitation of serum scCD23/IgE-BF, whether related to a process of autocrine proliferation or not, is a parameter of potential importance for therapy monitoring.

Hairy cell leukemia (HCL) is a chronic B cell malignancy, where the tumor cells have been characterized at a preplasma cell stage of differentiation in the B lymphoid lineage.1 Hairy cells have undergone a functional rearrangement of both light and heavy chain immunoglobulin genes2 and frequently display multiple immunoglobulin isotypes at their membrane,3 suggesting they are blocked in a stage of isotype switch during the B cell differentiation pathway.

Most hairy cells can proliferate in vitro with low molecular weight B cell growth factor (LMW-BCGF),4 a lymphokine known to be involved in the G1—S transition in normal B cells activated by a first signal; however, although they express the CD25/Tac antigen, they do not respond to interleukin 2 (IL-2).4 Hairy cells produce a growth factor similar to the cytoplasmic precursor of LMW-BCGF,5 suggesting the existence of an autocrine loop of LMW-BCGF in these cells.

Treatment of HCL with interferon (IFN)-α results in significant clinical improvement in a high percentage of cases,4 but the mechanism of IFN action is not yet entirely understood. Paganelli et al4 and we5 have shown that in vitro treatment of hairy cells with IFN-α induces an inhibition of the proliferative response to LMW-BCGF. Furthermore, we have observed that in vivo injection of IFN-α to patients with HCL results in a transient and reversible inhibition of LMW-BCGF-dependent proliferation of the leukemic cells. We have thus proposed that the therapeutic effect of IFN-α in HCL patients may be due, in part, to its ability to block an autocrine loop of LMW-BCGF in these cells.6

The CD23 molecule, originally described as an activation marker of B cells9 has been identified as the second type of IgE receptor and cloned.10-12 This low affinity receptor for IgE has been detected on T and B lymphocytes, eosinophils, platelets, and monocytes.13-16 On B lymphocytes, its expression is increased following mitogenic stimulation17 or treatment with IL-4.18 Furthermore, the CD23 molecule has also been postulated to serve as (or be closely associated to) the receptor of LMW-BCGF.18-20 The soluble cleaved portion of this molecule or sCD23, also called IgE-binding factor (IgE-BF) since it retains the capacity to bind IgE, plays a role in the regulation of IgE production21 and can be readily detected in supernatants of mononuclear cells cultures and in biologic fluids.22,23 In addition, soluble scCD23/IgE-BF has also been reported to display autocrine growth activity on normal and tumoral B cells.24,26

In the present work, we have studied the presence of the CD23 marker on hairy cells and investigated the effects of IFN-α, both in vitro and in vivo, on the expression and release of this molecule, by comparison with normal resting or activated B cells. Most strikingly, the in vivo levels of scCD23/IgE-BF in sera from patients with HCL, high when

References

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2455

2455
compared with sera from normal donors, were found to greatly decrease during the course of interferon administration in the patients responding favorably to IFN-α-therapy.

MATERIALS AND METHODS

HCL Patient Cells and Serums

HCL patients were selected on the basis of high number of peripheral blood hairy cells (>10 x 10^9 white cells/L). Immunofluorescence and binding studies were performed either on fresh or thawed hairy cells isolated from the blood or spleen of untreated HCL patients by Ficoll-Paque density gradient centrifugation. In some instances, hairy cells were collected just before and shortly after patients received a test injection of IFN-α (3 x 10^6 IU subcutaneously [SC]).

Quantification of soluble sCD23/IgE-BF was performed in serum collected from patients who had received weekly injections of IFN-α (3 x 10^6 IU SC) for 3 to 4 weeks. Sera were harvested before the first injection and 6, 12, 24, and 48 hours later, then immediately before the following administrations of IFN.

Interferons and Growth Factors

Interferons. The recombinant human IFNs used in the in vitro studies were derived from Escherichia coli by recombinant DNA technology, with a purity of more than 99% and were provided by Schering (IFN-α2; Kenilworth, NJ) and Roussel-Uclaf (IFN-γ; Romainville, France). Their specific activities were 2 x 10^8 and 2 x 10^7 U/mg, respectively. They were kept frozen at -80°C and thawed once just before use.

LMW-BCGF or 12 Kd BCGF. Natural LMW-BCGF (12 Kd-BCGF) was purchased from CTI (Cytokine Technology International, Buffalo, NY). Before use, the various batches of growth factor were checked for the absence of IL-2 reactivity on the murine CT-1-L1 line and their B cell supporting activity was determined by a test of restimulation of human B cell blasts, preactivated by a 3-day culture in the presence of insolubilized anti-mu antibody (10 μg/mL; Biorad, Richmond, CA), as previously reported. Recombinant human LMW-BCGF, a generous gift from S. Sharma (Providence, RI) was prepared and used as described.

Antibodies

The preparation, characterization and purification of the 4.3/79 (IgG1-λ), 135, 45, and 30 monoclonal anti CD23 antibody have been reported previously. The other monoclonal antibodies (MoAbs) used in the flow cytometry study were: KB 61 (a kind gift from K. Pulford), which recognizes hairy cells and peripheral B cells but not B cells from the germinal centers; B1 (CD20-pan B) and B4 (CD 19-pan B) from Coulter (Coultronics, Margency, France); OKT3 (CD3-pan T), OKT4 (CD4), and OKT10 (CD38), purchased from Ortho Diagnostic (Raritan, NJ); and 2.7 (IgG1 anti-arsenotic control antibody), a kind gift from S. Amigorena (Institut Curie, Paris, France).

In Vitro Treatment of Hairy Cells With IFNs

Hairy cells were adjusted at 10^6 cells/mL of RPMI-1640 medium (GIBCO; Paisley, Scotland) containing 10% fetal calf serum (FCS) and incubated with or without 10^4 IU/mL of IFN overnight at 37°C in a humidified atmosphere of 5% CO2 in air. They were then washed and tested in parallel for LMW-BCGF proliferative response and for expression of the CD23 marker.

Immunofluorescence Study

Hairy cells (0.5 to 1 x 10^6 cells) were incubated with an appropriate dilution of the various MoAbs for 30 minutes on ice. After washing with isotonic phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA) and 0.1% NaN3, 40 μL of a fluorescein conjugated (dichlorotriazinyl amino fluorescein)-affini-pure F(ab')2 fragment goat anti-mouse IgG (H+L) (Immunotech, Marseille) was added and incubated for an additional 45 minutes on ice. After washing, the cells were analyzed by flow cytometry with an EPICS V fluorescence-activated cell sorter (Coultronics, Margency, France), gating on the viable B cell populations. Data analysis was performed on reading 5,000 cells per sample and results are expressed as percent of the CD23-bearing cells after subtraction of background fluorescence (cells incubated with PBS containing 1% BSA followed by incubation with the fluoresceinated second antibody).

Binding Study

Labeling of the MoAb 4.3/79 with 125I was performed with the use of chloramine T. Briefly, 10 μg of antibody in 10 μL of PBS were diluted with 40 μL of PBS and 50 μL of K2HPO4/KH2PO4, 500 mmol/L buffer, pH 7.5. Five microliters of 121I NaI (1 mCi) were added with 20 μL of chloramine T (2 mg/mL) for 90 seconds at room temperature. The reaction was stopped by the addition of 100 μL of sodium metabisulphite (2.5 mg/mL) and 30 μL of a 0.1% solution of KI. Removal of free iodine was achieved by filtration through a column of Sephadex G-25 previously equilibrated in PBS + 0.1% BSA. The specific activity of the recovered labeled antibody was 96 Ci/mmol.

Hairy cells were washed twice and resuspended at 20 x 10^6 cells/mL in RPMI-1640 medium containing 5% FCS. Aliquots of 50 μL (10^5 cells) were dispensed in Eppendorf tubes and 50 μL of serial dilutions of labeled 4.3/79, in the presence of a 100-fold excess of unlabeled 4.3/79 antibody or not, were incubated on ice for 1 hour. At the end of the incubation time, the cells were placed over 200 μL of a 1/1.1 mixture of di-octyl and di-butyl phlatate in an Eppendorf microtube and centrifuged at 12,000 x g for 2 minutes. Free radioactivity was estimated by counting a 50 μL aliquot of the supernatant while the radioactivity bound to the cells was determined by cutting the tube through the oil phase after freezing and counting the pellet. Non-specific binding radioactivity was calculated from the results of experiments performed in the presence of an excess of unlabeled 4.3/79 antibody.

Hairy Cells Proliferation

Hairy cells were resuspended at 10^6 cells/mL of RPMI-1640 medium (GIBCO) containing 10% FCS in microtitration plates (100 μL/well) in presence of LMW-BCGF (4 U/mL) or not. Cultures were carried out at 37°C for 3 days and the cells were labeled with 1 μCi/well (37 kBq/well) of (1H)-tritiated thymidine (TMM 79B-CEA-Gif sur Yvette; specific activity: 25 Ci/mmol) for the last 16 hours of the incubation time. Cells were harvested onto glass microfiber filters with a multiple automated sample harvester (Skatron, Flow Labs, Lier, Norway). After drying, filters were put in vials with 2 mL of scintillation liquid (Econofluor) and counted in a beta spectrometer (Rackbeta-IKB, Sweden). Stimulation index was defined as the ratio of cpm in LMW-BCGF culture/cpm in control culture and considered significant above 2.

The percentage of inhibition of the proliferative response of hairy cells to LMW-BCGF, either incubated in vitro with IFN-α or collected from the blood of patients receiving an injection of IFN-α, was calculated as follows:

\[
\text{% Inhibition} = \frac{\Delta \text{cpm in control cells} - \Delta \text{cpm in IFN-α-treated cells}}{\Delta \text{cpm in control cells}} \times 100
\]

where \(\Delta \text{cpm} = (\text{cpm with LMW-BCGF}) - (\text{cpm with medium})\).
IFN-α AND CD23 IN HAIRY CELL LEUKEMIA

Release of sCD23/IgE-BF In Vitro by Normal Peripheral Blood Mononuclear Cells or B Cells and Hairy Cells

Peripheral blood mononuclear cells (PBMC) were prepared from normal blood samples by Ficoll density gradient centrifugation. B cells were purified by two cycles of rosetting with 2-AET-treated sheep erythrocytes, followed by Ficoll centrifugation. PBMC, either unstimulated or stimulated with PHA-P 1% (Wellcome Laboratories, Beckenham, UK) or recombinant IFN-γ (Green Cross Corporation, Osaka, Japan), were incubated or not with various concentrations of IFN-α. The supernatants were collected after 3 days of incubation and the amount of sCD23/IgE-BF released was determined by radioimmunoassay as already described.23

Similar measurements were performed with B cells, either unstimulated or stimulated with a supernatant from activated T cells. This supernatant was obtained by culture of 2-AET erythrocytes rosetting T cells for 48 hours at 10^6 cells/mL in serum-free defined medium HB 101 (Hana Biol) in the presence of PHA-P 1%. The cell-free supernatant was reconstituted 100 times before use.

The presence of sCD23/IgE-BF in supernatants of hairy cells was similarly tested after 3 days of culture of the leukemic cells (1 to 5 x 10^6/mL) with IFN-α (10^5 IU/mL), IFN-γ (10^5 IU/mL), natural LMW-BCGF (1/10 vol/vol), or control medium.

Radioimmunoassay for Detection of sCD23/IgE-BF

Radioimmunoassay was performed according to the technique previously described.23 Briefly, 96-well microtiter plates were coated with 100 μL of MabERα, blocked with Hank’s balanced salt solution (HBSS) containing 10% BSA, and finally washed with PBS. Test serum or supernatant (75 μL) was added to the wells, and after 4 hours at room temperature plates were washed and supplemented with 75 μL 125I-MabER 135 (2 to 3 x 10^6 cpm/75 μL, specific activity: 2 to 3 x 10^6 cpm/ng). After overnight incubation, wells were washed and counted in a gamma counter. All samples were tested in duplicate, with interassay variation below 10%. The assay was calibrated by reference to a standard preparation of supernatant from RPMI 8866 cells given an arbitrary titer of 10^5 U/mL.

RESULTS

Expression of CD23 on Hairy Cells and Effects of IFN-α Treatment In Vitro

Leukemic cells from 12 hyperleukocytic HCL patients (OKT3+, B4+, and KB 61+ cells) were tested for expression of the CD23 marker by flow cytometry. The results of these experiments are summarized in Table 1.

In 7 of the 12 samples, leukemic cells were found to display significant amounts of the CD23 marker (>10% labeled cells). All hairy cells responded to LMW-BCGF by a marked increase in tritiated thymidine (3H-TdR) incorporation, with stimulation index ranging between 2.1 and 11.3, with the exception of patient no. 9, whose cells were not stimulated by LMW-BCGF; of interest, this patient was found to be resistant to IFN-α therapy.

Since natural commercial LMW-BCGF is not purified to homogeneity and contains other lymphokines, we checked the response of hairy cells from one patient (no. 2) to recombinant LMW-BCGF. As seen in Table 2, a marked increase in H-TdR incorporation was obtained in the presence of the recombinant interleukin, whereas recombinants IL-2, IL-4, and IL-5 were devoid of mitogenic effect.

When incubated with IFN-α, hairy cells displayed an impaired proliferative response to natural LMW-BCGF, with inhibitions in the range 34% to 92%; yet, only in three of the seven CD23 positive samples could we detect a significant decrease (>20%) of marker expression after treatment with IFN-α (Table 1).

These results were confirmed when binding experiments were performed with iodine-labeled 4.3/79 anti CD23 antibody. A slight reduction in the number of antibody molecules bound per cell could be detected only for some hairy cells (two of five cases tested) after treatment in vitro with IFN-α, but not with IFN-γ (data not shown).

Thus, although for some tumors, an IFN-α–induced decrease of CD23 expression can be found, associated with a suppression of the LMW-BCGF response, this is not a general phenomenon.

Effect of In Vivo Administration of IFN-α to HCL Patients on Hairy Cell CD23 Expression

Three patients with hyperleukocytic HCL received a single injection of IFN-α (3 x 10^6 IU subcutaneously) and blood samples were collected just before (time 0) or 6, 12, and 24 hours after injection. The expression of the CD23 marker on the leukemic cells and their proliferative response to LMW-BCGF were tested. For all three patients, CD23 could be readily detected on hairy cells collected before treatment and after injection of IFN-α, whereas recombinants IL-2, IL-4, and IL-5 were devoid of mitogenic effect.

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Table 1. Expression and In Vitro Modulation by IFN-α of the CD23 Antigen on Hairy Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cell Origin</th>
<th>% CD23+ Cells</th>
<th>Response to LMW-BCGF</th>
<th>% Inhibition by IFN-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spleen</td>
<td>1.2</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>Spleen</td>
<td>10.3</td>
<td>6.2</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>Spleen</td>
<td>0.8</td>
<td>NT</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>Spleen</td>
<td>32</td>
<td>32</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>Blood</td>
<td>0.5</td>
<td>0.5</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>Blood</td>
<td>6.6</td>
<td>2.5</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>Spleen</td>
<td>31.4</td>
<td>35.3</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>Spleen</td>
<td>32.1</td>
<td>26.6</td>
<td>3.4</td>
</tr>
<tr>
<td>9</td>
<td>Blood</td>
<td>1.7</td>
<td>NT</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>Spleen</td>
<td>15</td>
<td>14</td>
<td>2.7</td>
</tr>
<tr>
<td>11</td>
<td>Blood</td>
<td>57</td>
<td>55</td>
<td>11.3</td>
</tr>
<tr>
<td>12</td>
<td>Blood</td>
<td>58.7</td>
<td>35</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Hairy cells were incubated overnight with medium or IFN-α (10^5 IU/mL), then washed and tested for their proliferative response to LMW-BCGF and for the expression of the CD23 marker. Control SI is the stimulation index of hairy cells pre-incubated with medium alone. Abbreviation: NT, not tested.

Table 2. Stimulation of Hairy Cells by Recombinant LMW-BCGF

<table>
<thead>
<tr>
<th>Interleukin</th>
<th>3H-TdR Uptake (cpm ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>7,249 ± 2,273</td>
</tr>
<tr>
<td>rLMW-BCGF (1/10)</td>
<td>143,193 ± 28,792</td>
</tr>
<tr>
<td>rIL-2 (200 U/mL)</td>
<td>2,509 ± 310</td>
</tr>
<tr>
<td>rIL-4 (200 U/mL)</td>
<td>3,420 ± 2,517</td>
</tr>
<tr>
<td>rIL-5 (200 U/mL)</td>
<td>4,368 ± 616</td>
</tr>
</tbody>
</table>

Hairy cells from patient no. 2 were incubated for 3 days in the presence of the various recombinant interleukins, and 3H-TdR uptake was estimated by a pulse of 1 μCi/well during the last 16 hours of the culture.
administration of the therapy (Table 3). As reported previously,8 IFN-α injection elicited in hairy cells an inhibition of the subsequent LMW-BCG F–dependent proliferation. For patient B, this suppressive effect was associated with a significant transient decrease of CD23 expression: by flow cytometry, the percentage of CD23+ cells dropped from 18% (before IFN-α injection) to 5% (6 hours later) and 3% (12 hours later), then reincreased to reach 14% 24 hours after IFN administration. No such modulation, however, could be observed for the two other patients tested.

**Anti-CD23 Antibodies Do Not Block or Enhance the Proliferative Response of Hairy Cells to LMW-BCGF**

Some anti-CD23 MoAbs are able to potentiate the proliferative response of B cells activated by tetradeoxyphol phorbol acetate (TPA) and LMW-BCGF.20 We have thus tested several anti-CD23 MoAbs for their effects on hairy cell proliferation, alone or in the presence of LMW-BCGF. The results of a representative experiment with the 4.3/79 antibody are presented in Fig 1, but similar data were obtained with other anti-CD23 MoAbs such as 45, 30, and 135 (data not shown). Over a wide range of concentration, anti-CD23 antibodies alone are devoid of growth promoting ability on hairy cells and they neither enhance nor inhibit the LMW-BCGF–induced 3H-TdR uptake in those cells.

**Comparison of sCD23/IgE-BF Release in Resting or Activated B Cells and in Hairy Cells: Effect of IFNs and LMW-BCGF**

Culture supernatants of peripheral blood mononuclear cells or purified B cells, either resting or stimulated by various procedures, were tested for their content in sCD23/IgE-BF. As shown in Fig 2, the amount of sCD23/IgE-BF released by the cultured cells is markedly reduced following incubation with recombinant IFN-α. This inhibition is dose-dependent: a significant reduction of sCD23/IgE-BF release is already achieved with low concentrations of IFN-α (10 to 50 IU/mL), while incubation with higher concentrations results in an almost total suppression of the release (Fig 3).

By contrast, the amount of sCD23/IgE-BF detected in culture supernatants of eight hairy cell samples (either

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### Table 3. Expression of the CD23 Antigen and LMW-BCGF Response in Hairy Cells From 3 Patients Treated In Vivo With IFN-α

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time After IFN-α Administration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>(A)</td>
<td>% CD23+ cells</td>
</tr>
<tr>
<td></td>
<td>3H-TdR uptake (cpm)</td>
</tr>
<tr>
<td></td>
<td>% Inhibition</td>
</tr>
<tr>
<td></td>
<td>LMW-BCGF</td>
</tr>
<tr>
<td>(B)</td>
<td>% CD23+ cells</td>
</tr>
<tr>
<td></td>
<td>3H-TdR uptake (cpm)</td>
</tr>
<tr>
<td></td>
<td>% Inhibition</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>(C)</td>
<td>% CD23+ cells</td>
</tr>
<tr>
<td></td>
<td>3H-TdR uptake (cpm)</td>
</tr>
<tr>
<td></td>
<td>% Inhibition</td>
</tr>
<tr>
<td></td>
<td>LMW-BCGF</td>
</tr>
</tbody>
</table>

Blood samples were collected from three patients with HCL either just before (time 0) or 6, 12, and 24 hours after injection of IFN-α (3 x 10⁴ IU subcutaneously). Hairy cells were separated and analyzed for expression of the CD23 marker and for their response in vitro to LMW-BCGF.
Soluble sCD23/IgE-BF in Serum From HCL Patients: Reduction After IFN-α Administration

Serum from six HCL patients was collected before or various times after weekly injections of IFN-α. Blood samples were harvested just before or 6, 12, 24, and 48 hours after the first administration of IFN and later on at weekly intervals, just before the next IFN injections.

Serum from HCL patients collected before any treatment was found to contain higher amounts of soluble sCD23/IgE-BF (mean, 37 U/mL; range, 16 to 97 U/mL) than serum from normal matched donors. By comparison, the mean value obtained for 37 normal volunteers (age range, 20 to 60 years) was 13.9 ± 1.8 U/mL (1 U/mL = 150 pg sCD23/IgE-BF/mL) and no influence of the sex was noted.

IFN-α administration to these patients resulted in a significant reduction in serum sCD23/IgE-BF, which is summarized in Fig 4. This reduction was already apparent for some patients 48 hours after the first injection (an early transient increase was sometimes noted). After the next IFN-α or IFN-γ, nor by the addition of LMW-BCGF (1/10 vol/vol) to the culture (not shown).

CD23⁻ or CD23⁺), collected from hyperleukocytic HCL patients, was very low (range, 0 to 3.4 U/mL), with the exception of one patient (32.1 U/mL). This level was not significantly modified by the presence of 10⁵ IU/mL of IFN-α or IFN-γ.
injections, sCD23/IgE-BF serum concentration in these patients continued to drop to reach the levels detected in normal sera; typical patterns of sCD23 evolution are presented for four patients in Fig 5. This decrease grossly paralleled a reduction in the blood hairy cell count of those patients.

By contrast, such a decrease of serum sCD23/IgE-BF was not detected for another patient with a “variant” HCL (whose hairy cells responded normally to LMW-BCGF in vitro) during the course of his treatment with IFN-α (Table 4). Of importance, this patient was found to be resistant to the therapy.

DISCUSSION

By flow cytometry, the presence of the CD23 molecule has been detected on about half of the hairy cells tested, either of spleen or blood origin. This result is in agreement with previous work by Freedman et al, who found a similar pattern of positivity in their HCL panel. Dadmarz and Cawley have shown a heterogeneity of CD23 and IFN-α receptor expression in B-chronic lymphocytic leukemia (CLL) correlated with stage, being high in more favorable disease, and they hypothesized that cell activation in CLL was a feature of such prognosis. This is not the case for HCLs, since most patients (95%) respond favorably to IFN-α therapy, although their leukemic cells display variable and sometimes undetectable amounts of membrane CD23.

All hairy cells, with one exception of a patient resistant to IFN-α therapy, were significantly stimulated to DNA synthesis by natural LMW-BCGF. Recombinant LMW-BCGF, but not IL-2, IL-4, or IL-5 was also highly efficient in inducing G1→S transition in hairy cells of one selected patient. As already reported in vitro incubation with IFN-α resulted in a marked reduction in LMW-BCGF responsiveness. This is at variance with other B cell malignancies such as B-CLL, where IFN-α alone stimulates DNA synthesis and does not suppress the LMW-BCGF response, even in some cases a synergistic effect of the two factors can be seen. After IFN-α treatment in vitro, a decrease in CD23 expression on hairy cells, paralleling the suppression of the LMW-BCGF–dependent response, was detected only in a few instances; CD23 down-expression is thus not mandatory for eliciting suppression of the LMW-BCGF–dependent proliferation. The same conclusions can be drawn from the three in vivo experiments where patients received a test injection of IFN-α. Here again, a reduction of CD23 expression was not an absolute requirement for observing an inhibition of the proliferative response to LMW-BCGF, measured subsequently in vitro, although such a decrease was apparent in one case.

**Fig 5.** The simultaneous determinations of serum sCD23/IgE-BF and blood hairy cell counts are presented for four of the six patients treated in vivo with IFN-α. Patients received subcutaneous injections of IFN-α (3 × 10⁶ IU) on the days indicated.
The presence of cell surface specific LMW-BCGF receptors on activated normal and tumoral B cells has been assessed by binding studies with labeled growth factor.

Several activation markers of B cells, including the CD23 molecule have been proposed as potential receptors for LMW-BCGF or molecules closely associated to the receptors and involved in the transduction pathway of this growth factor. Our results do not favor the idea that CD23 is the actual ligand-binding moiety of the LMW-BCGF receptor, in agreement with a report of Vazquez et al. However, it is quite possible that in CD23+ cells, the molecule could be associated with the LMW-BCGF receptor, since both LMW-BCGF and anti-CD23 antibodies have been reported to trigger the shedding of a soluble form of CD23. Furthermore, in a recent study comparing the expression on hairy cells of CD23 and B8.7, another B cell activation marker involved in LMW-BCGF responsiveness, we found, despite the lack of detection of CD23 on some leukemic cells, a positive correlation between the level of expression of this molecule and the intensity of the LMW-BCGF response. This would suggest that CD23, although not binding LMW-BCGF, might nevertheless be involved in its signalling pathway, at least in some CD23+ B cells.

The anti-CD23 MoAbs used in this study are devoid of any mitogenic effect on normal B or hairy cells and neither block nor potentiate an LMW-BCGF-driven response. However, it should be recalled that only some anti-CD23 antibodies have been reported to be able to trigger DNA synthesis in normal B cells, such as MHM6 or the LA1 and LA2 antibodies, which recognize a distinct form of CD23 only expressed after activation. Whatever the role of membrane CD23 in the recognition or transduction of the LMW-BCGF signal might be, its down-modulation by IFN-α would not be required, anyway, for inducing an inhibition of the proliferative response.

Monoclonal antibodies against CD23/RFcII cross-react with soluble IgE-BF, which are released from RFcII-bearing cells as breakdown products of the receptor. Cleavage products of RFcII of 25 Kd and 12 Kd can be detected in supernatants from cell lines and in biologic fluids by sensitive radioimmunoassays. These soluble CD23 molecules play an important role in the regulation of IgE synthesis. Moreover, it has been proposed that a complex of two polypeptides (25 Kd and 12 Kd), derived from a shed form of the CD23 molecule could be an autocrine B cell growth factor for normal and transformed B cells.

In the present work, we confirm that IFN-α is able to inhibit sCD23/IgE-BF synthesis and release by peripheral blood mononuclear cells and purified B cells. The other hand, with one exception, supernatants from CD23- or CD23+ hairy cells were found to contain very low amounts of sCD23, which were not modified by addition of IFN-α. Moreover, sCD23 release was not increased in the presence of LMW-BCGF, but such a LMW-BCGF-driven augmentation of sCD23 release is still a matter of controversy. Yet, the in vitro control of sCD23/IgE-BF synthesis and release, as well as its regulation by IFN-α, appear to be different in peripheral blood B cells and in hairy cells. This could be related to the existence in B cells of two species of FcRII, which may be differentially regulated in normal and tumoral B cells. It should also be recalled that, in addition to its release from the membrane form, a generation of sCD23 resulting from intracellular cleavage of newly synthesized molecule has recently been described.

Since IFN-α is highly efficient in the control of HCL we were interested in measuring the amount of sCD23/IgE-BF in the plasma of patients undergoing IFN therapy. In six of six cases studied so far, our results clearly indicate that a marked reduction in the level of circulating sCD23/IgE-BF was detected in the serum of the patients during the course of the therapy. The initial concentration of serum sCD23/IgE-BF, before starting the IFN-α injections to the patients, could not be related to the hairy cell count. However, since more than 90% of the mononuclear cells in these patients consisted of hairy cells, it is reasonable to assume that the sCD23/IgE-BF detected in the serum is derived from the leukemic cells. Such an elevation in serum of sCD23/IgE-BF has already been reported for patients with B-cell derived chronic lymphocytic leukemia, with levels 3 to 500-fold higher than in normal sera, thus generally higher than the values found in HCL sera. In addition, whereas sCD23/IgE-BF could readily be detected in supernatants from the CLL-B cells, this is not the case for hairy cells. This discrepancy may be explained by a higher CD23 membrane expression on CLL leukemic cells than on hairy cells, leading to an increased turnover and release of the soluble shed form, or by a difference in CD23 gene expression or processing.

Following the first IFN-α injection, a marked reduction in serum IgE-BF could be already detected 48 hours later (in some instances, a transient increase could be noted very early after the injection). This reduction was more pronounced when the patients received one, two, or three more weekly injections of IFN; 3 to 4 weeks after the first IFN-α administration, all six patients IgE-BF serum levels had decreased to the normal level detected in control donors (≈10 U/mL equivalent to 1,500 pg/mL). Of interest, such reduction was not observed for one patient who turned out to be refractory to IFN-α therapy, suggesting early monitoring of sCD23/IgE-BF after the beginning of the treatment may be valuable for estimating the reactivity of the patient to the therapy.

It is tempting to speculate that IFN-α-induced reduction of sCD23/IgE-BF, a molecule possibly endowed with a B cell growth promoting activity, would explain its therapeutic success in HCL. However, a similar decrease in the serum concentration of soluble CD25/IL-2 receptor (TAC or α chain) has been reported for HCL patients treated with IFN-α and has been inversely correlated to the natural killer activity displayed by the PBL of the patients. Hairy cells carry TAC receptors, which are apparently nonfunctional in vitro, since these leukemic cells do not proliferate in the presence of IL-2. These results suggest that an excess of soluble IL-2 receptors may interfere with the IL-2-induced activation of the natural killer system and a possible relationship between the quality of the response to IFN-α and the normalization of the plasma soluble IL-2 receptor concentration. Thus, the observed decrease in serum sCD23/IgE-BF may only reflect the reduction of the tumo-
ral mass secondary to IFN-α therapy, whatever its mechanism of action might be. Experiments are in progress to clarify that point and to test the effects of various cleavage products of the CD23 molecule on hairy cell multiplication.

In conclusion, measurement of serum sCD23/IgE-BF may prove an important clinical parameter for the evaluation of patients receiving IFN-α therapy or other forms of treatment (chloraminophen-deoxycoformycin) and may be of special interest for monitoring possible relapses in these patients.

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