Human recombinant interferon alpha-2C enhances the expression of class II HLA antigens on hairy cells

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Human Recombinant Interferon α-2C Enhances the Expression of Class II HLA Antigens on Hairy Cells


Isolated splenic hairy cells from three untreated patients were cultured in presence of recombinant human interferon alpha-2C (IFNa). Ultrastructural cytochemistry and immunophenotype analysis with a large panel of monoclonal antibodies were performed to study cellular modification induced by IFNa. Hairy cells showed a typical phenotype: SmIg+, B1+, BA1+, anti-Tac+, OKDR+, Leu-M5+, HC2+, TRAP+, myeloperoxidase-. Under our experimental conditions, we found no direct cytotoxic effects or significant variations in morphology, cytochemistry, and percentage of reactivity with the tested monoclonal antibodies.

After culturing in the presence of different doses of IFNa, we observed a significant enhancement of the expression of class II HLA antigens on hairy cells as demonstrated by increased fluorescence for OKD1, OKDr, Leu-10 at fluorescence-activated cell sorting analysis. In agreement with this finding IFNa-treated hairy cells showed an increased stimulatory capacity v allogeneic T cells in one-way mixed lymphocyte reaction. To our knowledge this is the first report describing the induction of class II HLA antigens on hairy cells by IFNa.

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samples selected for immunoutrastructural investigations because previous immunofluorescence screening had presented a high percentage of cells in all the cases, whereas OKM1 positivity was observed only in case 3. No significant variations in viability, cell number, morphology at light microscopy, and cytochemical pattern were observed at any culture time point, irrespective of the presence or amount of IFNα. No CyIg or M rosettes were detected either before or after culture.

After culture with or without IFNα, ultrastructural morphological changes appeared in the cells, indicating increased intracellular protein synthesis. More than 50% of the cell population had a large Golgi apparatus, numerous short strands of endoplasmic reticulum, and a very high number of cytoplasmic vesicles and pale granules. The Graham and Karnovsky myeloperoxidase reaction continued to be negative (Fig 1B through D).

We did not find any significant variation in percentage positivity for the various surface antigens investigated except in case 2. In all cultures of cells from this patient, we observed increased positivity to OKM1, which went from a low basal level (6%) to 50% and 60%, respectively, for cells exposed and unexposed to IFNα. On the other hand, at ultrastructural analysis, we observed persistent myeloperoxidase negativity as well as low expression (<5%) of other mAbs with known macrophage–monocyte specificity, such as Leu-M1 and FMC32 (Fig 1C).

The only IFN-induced variation we observed was in intensity of expression of class II HLA antigens. In all three cases studied, incubation of spleen hairy cells with IFNα clearly enhanced the intensity of surface expression antigens detected by OKDR, OKIa1, and Leu-10. This effect was

**RESULTS**

Changes in morphology, cytochemistry, and immunophenotype in splenic hairy cells cultured with IFNα. After separation, the recovered cell fraction consisted mainly of mononuclear cells in which typical ultrastructural features of hairy cells could be found. At TEM, more than 80% of the cells had oval and deeply indented nuclei, numerous cytoplasmic vesicles, mitochondria, and characteristic narrow-based long projections at the periphery (Fig 1A). Graham and Karnovsky’s myeloperoxidase reaction was negative. The cell surface antigen expression detected by mAb in fresh spleen cells from our patients is reported in Table 2. All three cases displayed a B cell phenotype; a high percentage of cells expressed monoclonal-type SmIg, B1 antigen, and class II HLA antigens. Intensity of staining with Leu-10 was weaker than that with OKDR and OKIa1 but present on most cells. Positivity with Leu-10 in hairy cells was also less intense than that usually observed in normal circulating B lymphocytes. Leu-M5 staining was strongly positive in most hairy cells in contrast to BA1 antigen expression, which varied in the three cases. Positivity to anti-HC2 and anti-Tac was weak but detected in a high percentage of cells in all the cases, whereas OKM1 positivity was observed only in case 3.

Statistical analysis. The statistical significance of data was evaluated with Student’s t test.

**Table 1. Antibodies and Their Specificity**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>OKIa1</td>
<td>Human la antigens, B lymphocytes, monocytes, activated T lymphocytes</td>
<td>Ortho Pharmaceutical Corp, Raritan, NJ</td>
</tr>
<tr>
<td>OKDR</td>
<td>HLA-DR monomorphic antigens on B lymphocytes, monocytes, activated T lymphocytes</td>
<td>Ortho Pharmaceutical Corp, Raritan, NJ</td>
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<tr>
<td>OKM1</td>
<td>Monocytes, granulocytes, natural killer cells</td>
<td>Ortho Pharmaceutical Corp, Raritan, NJ</td>
</tr>
<tr>
<td>BA1</td>
<td>B lymphocytes, non-T/non-B ALL, pre-B ALL, CLL, malignant lymphoma, granulocytes</td>
<td>Hybritech Inc, San Diego</td>
</tr>
<tr>
<td>B1</td>
<td>B lymphocytes</td>
<td>Coulter Immunology, Hi- aleah, Fla</td>
</tr>
<tr>
<td>Leu-1</td>
<td>Peripheral T cells, thymocytes, B CLL</td>
<td>Becton Dickinson, Sunnyvale, Calif</td>
</tr>
<tr>
<td>Leu-2a</td>
<td>T suppressor/cytotoxic cells</td>
<td>Becton Dickinson, Sunnyvale, Calif</td>
</tr>
<tr>
<td>Leu-3a</td>
<td>T helper/inducer cells</td>
<td>Becton Dickinson, Sunnyvale, Calif</td>
</tr>
<tr>
<td>Leu-7</td>
<td>NK and K cells</td>
<td>Becton Dickinson, Sunnyvale, Calif</td>
</tr>
<tr>
<td>Leu-10</td>
<td>HLA-DC/DS antigens on B lymphocytes, B cell lines</td>
<td>Becton Dickinson, Sunnyvale, Calif</td>
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<tr>
<td>Leu-11a</td>
<td>NK and K cells, neutrophils</td>
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<tr>
<td>FMC32</td>
<td>Monocytes, macrophages</td>
<td>Gift of H. Zola, Bedford Park, Australia</td>
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<tr>
<td>αHC2</td>
<td>Hairy cells, leukemic myeloblasts, some lymphomas</td>
<td>Gift of D. Posnett, Rockefeller University, New York</td>
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<tr>
<td>Leu-M5</td>
<td>Hairy cells, monocytes</td>
<td>Gift of H. Stein, Freie Universität, Berlin</td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>Membrane receptor for interleukin 2 on malignant and activated T cells</td>
<td>Gift of T.A. Waldmann, National Cancer Institute, Bethesda, MD</td>
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</table>
Fig 1. Splenic hairy cell suspension. (A) Oval and indented nuclei, cytoplasmic vesicles, and ribosome-lamella complexes are typical ultrastructural features of hairy cells (PbUs; original magnification ×10,000; current magnification ×7,700). (B) After culture in presence of IFN. The cells are positive for Leu-M5 (single arrow) at immunogold staining (PbUs; original magnification ×10,000; current magnification ×8,100). (C) After culture in presence of IFN. The cells are myeloperoxidase negative with Graham and Karnovsky’s method (unstained; original magnification ×7,000; current magnification ×5,390). (D) Detail of cultured hairy cells strongly positive for Leu-M5 (single arrow). The increased number of cytoplasmic vesicles suggests active cell synthesis (PbUs; original magnification ×26,000; current magnification ×20,800).
shown by fluorescence microscopy, and even more clearly by cytofluorimetric analysis, and was detectable already at 48 hours' culture and with IFN concentrations as low as 20 IU/mL. Possibly due to some degree of activation induced by FCS, intensity of expression of these surface antigens was slightly increased in hairy cells cultured in the absence of IFN compared with freshly stained cells. However, this enhancement was significantly lower than that observed in the presence of IFN. FACS histograms derived from case 2 hairy cell analysis (Fig 2) show the characteristics of enhancement of class II HLA antigen expression, which were almost identical to those of cases 1 and 3 (not shown). The addition of a neutralizing concentration (1 μg/10 IU IFN) of an anti-IFNα mAb (EBI1, kindly provided by Dr G.R. Adolf, Ernst Boehringer Institut, Vienna) at the start of cultures of case 2 hairy cells with 100 IU/mL IFN was found to inhibit the enhancement of class II HLA antigen expression significantly compared with hairy cells cultured with IFNα and without EBI1. This enhancement was not significantly modified when hairy cells from case 2 were preincubated with anti-Tac for one hour at 4°C.

Stimulatory capacity in MLR of spleen hairy cells cultured with IFNα. The increase in class II HLA antigens expression after IFN treatment in vitro was accompanied by enhancement of the stimulatory activity of spleen hairy cells against allogeneic purified peripheral T lymphocytes in one-way MLC. The responsiveness of these T cells was previously shown to be in the normal range (mean ± SD of 3H-thymidine incorporation: 22 ± 3 x 103 cpm) in conventional MLC with T-depleted allogeneic peripheral mononuclear cells from normal subjects.

After 48 hours' culture in presence of 100 IU/mL IFNα, the stimulatory capacity of the hairy cells of both the cases studied was markedly increased (Fig 3), the difference being statistically significant at the lowest cell concentrations. At the highest cell concentrations, the MLR was no longer proportional to the number of stimulator cells added. As shown in Fig 3, at the highest cell concentration, the increase of stimulatory capability after exposure to IFNα was significant only in case 3 hairy cells v T4 cells.
sis. As this finding was detected in presence or absence of constant ultrastructural features of increased protein synthesis after exposure of hairy cells to chemical inducers.

hairy cells were unaffected by culturing. We only observed than in normal peripheral B lymphocytes, we detected both expressed differentially on normal hemopoietic and leukemic cell origin (SmIg, BI, BAt', OKIa1, OKDR). Positivity for anti-Tac, which binds interleukin 2 (IL 2) receptor, was initially reported in activated and malignant T cells. Recently, however, IL 2 receptor has also been identified on hairy cells from the three cases we studied all expressed a typical immunophenotype consistent with their proposed B cell origin (SmIg+, B1+, BA1+, OKIa1+, OKDR+). Positivity for anti-Tac, which binds interleukin 2 (IL 2) receptor, was initially reported in activated and malignant T cells. It is interesting that in this study, human recombinant IFN alpha-2C enhanced the expression of class II HLA antigens in hairy cells. This phenomenon was accompanied by increased stimulatory activity of hairy cells in MLC, functional behavior well known to be closely HLA-DR-linked. This effect was more evident at the lowest hairy cell concentrations, probably due to a functional limit of cultured T cells' responder activity. The increased expression of class II HLA antigens can be attributed to a direct action of IFN, since it was neutralized by the addition of anti-IFNα mAb at the start of culture. No differences were seen when hairy cells were pretreated with anti-Tac; this supports the hypothesis that the IL 2 receptor is not involved. IFNα was active in vitro at a low concentration (20 IU/mL), comparable to that reached in vivo with the administration of therapeutic doses (5 × 10^6 IU/d).

It is known that IFNs can modify the membrane behavior of cells exposed to their action and, particularly, HLA antigen and Fc receptor expressions. In fact, enhancement of class II HLA antigens has been shown on human monocyte cell lines and on various murine cell lines of hemopoietic, lymphoid, macrophage, fibroblast, and neuronal origin in presence of recombinant or highly purified IFNγ. An increase of mRNAs codifying these HLA antigens has been demonstrated by other authors in IFN-treated human lymphoblastoid and melanoma cell lines.

The biological meaning of these effects is still discussed. The induction of Ia antigens, particularly on B lymphoma and macrophage cell lines, may result in a functional enhancement of antigen-presenting capability. Another hypothesis is that hemopoiesis may be modulated by IFN through Ia-restricted communication with regulatory T cells. Finally, it is conceivable that this class II HLA antigen modulation is an expression of differentiation in normal hemopoietic cells and their neoplastic counterparts. The fact that IFNα and β are less effective in inducing class II HLA antigens than IFNγ, and that the latter is normally released by activated T cells, seems to indicate that IFNγ plays a more important role in the physiologic regulation of these antigens.

Hokland et al., using partially purified IFNα, demonstrated an increase of HLA antigen expression detected by β₂-microglobulin on peripheral blood mononuclear cells and in various human non-T lymphoblastoid cell lines. Nevertheless, in the same experimental conditions, the HLA-DR-related Ia antigens were unaffected. Our study shows that hairy cells are sensitive to IFNα, as indicated by the increase of HLA-DR and DR-linked antigen expression. This sensitivity was particularly notable, since we failed to observe such a phenomenon on cells from four cases of B-CLL. Moreover, in OKDR+ Leu-10+ cells from ALL (two cases of

**Fig 3. Increase of stimulatory capacity of IFN-treated splenic hairy cells in MLC.** Splenic hairy cells were cultured with (G) or without (E) 100 IU/mL IFN for 48 hours, irradiated with 3,000 rad, and then cocultured at three concentrations (2.5, 5.0, and 10 × 10^6 cells/200 μL as shown on the abscissa) with 10^5 purified T cells from four healthy donors (T1, through T4). The mean ± SD of 3H-thymidine uptake of purified T, through T4 cells in conventional MLC with non-T peripheral mononuclear cells from allogeneic donors was 22 ± 3 × 10^3 cpm. Cultures were done in quintuplicate and results are expressed as cpm ± SD. (A, P < .05; B, P < .02; C, P < .01).
n-ALL), one case of c-ALL), IFNα treatment did not induce either DR antigen expression enhancement or positivity to Leu-10 (data not shown).

IFNα did not seem to have a differentiating action on hairy cells, since we did not observe any other immunocytomorphological modification, indicating cellular maturation. On the other hand, HLA antigens are known to be involved in cell-mediated cytotoxicity virus-infected and possibly tumor cells. In particular, class II HLA antigens have been implicated in the sensitization phase of cell-mediated cytosis and as restriction antigens in cytotoxicity mediated by T4+ clones. This cytotoxic mechanism has been demonstrated also in interactions between cytotoxic effector lymphocyte clones and autologous B lymphoblastoid lines transformed by Epstein-Barr virus. It may be speculated that the selective enhancement of class II HLA antigens shown in our study, by potentiating a cytotoxic mechanism, contributes to the antileukemic activity IFNα exerts in vivo. Our data may therefore help to elucidate the multifactorial mechanism of therapeutic effect of IFNα in HCL.

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