CONCISE REPORT

Human Leukocyte Interferon Preparation Blocks Granulopoietic Differentiation


Since interferon administration in the treatment of some malignant conditions has been reported to cause leukopenia, we studied the effects of human leukocyte interferon preparation (HLIF) on myelopoiesis in vitro. The continual presence of HLIF in semisolid agar cultures caused a progressive decline in colony and a rise in cluster incidence with increasing concentrations of interferon. The total plating efficiency, however, remained nearly constant, regardless of the HLIF doses used. Morphological examination of the clusters demonstrated a progressively increasing percentage of immature granulocytic precursors with increasing HLIF concentrations. This suggests that HLIF causes leukopenia by blocking differentiation of marrow myeloid precursors.

THE DEVELOPMENT of an in vitro agar clonal assay for the myeloid progenitor cell (CFU-C), the first cell committed to proliferation and differentiation into granulocytes and monocytes, has made it possible to study the effect of various macromolecules in the regulation of myelopoiesis. The first study linking interferon with CFU-C inhibition was reported by McNeill and Killen and subsequently has been confirmed in both mice and rabbits. Using a semisolid agar culture system, Fleming et al. demonstrated that interferon inhibited CFU-C proliferation. Based on findings of “abnormal multinucleated cells” in colonies, these authors also hinted that interferon might be involved in normal granulocytic differentiation; however, no details or direct evidence of such a regulatory role were presented. Recently, other investigators have reported the dose-related inhibitory effect of interferon on CFU-C and erythroid progenitor cells (CFU-E) without describing the exact mechanism involved. In this article, we describe findings that suggest human leukocyte interferon (HLIF) may play a role in the differentiation of granulocytic precursors.

MATERIALS AND METHODS

Acquisition of Marrow Specimens

Human marrow cells obtained from normal volunteers or patients with solid tumors who had no evidence of marrow or bone metastases, were collected in polystyrene tubes containing 0.3 ml preservative-free heparin and 1.7 ml phosphate-buffered saline (PBS).

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Cell Separation Procedures

Light-density cells were prepared on the basis of buoyancy of cells. The marrow samples were layered over a column of Ficoll-Hypaque solution (density 1.077 g/ml) and centrifuged at 400 g for 35 min. The interface cells were gently aspirated with a Pasteur pipette, washed twice with PBS, and washed again with α-MEM (alpha modification of minimum essential medium) mixed with 15% fetal calf serum (FCS). Subsequently, these cells were resuspended in α-MEM plus 15% FCS and used as target cells.

Culture Procedures

The cells were suspended in a mixture of equal volumes of double-strength α-MEM plus 30% FCS and 0.6% Difco agar (final concentrations of single strength α-MEM plus 15% FCS and 0.3% agar), giving a final cell concentration of $10^6$ or $2 \times 10^7$/ml. One milliliter of this cell suspension was layered over a 1-ml underlayer of α-MEM plus 15% FCS and 0.5% Difco agar per culture dish (35-mm diameter). Human placental-conditioned medium (HPCM) was used at a 20% concentration in underlayers as a source of colony-stimulating factor (CSF). The cultures were plated in triplicate for each observation and incubated at 37°C in a fully humidified atmosphere of air and 7% CO2. Cultures were scored after an 8-day incubation, using an Olympus dissecting microscope at 25-40 magnification. They were analyzed for total number of colonies (aggregates of $\geq$40 cells) and clusters (aggregates of 3–39 cells) per dish. The results were noted as the mean of triplicate readings ± standard deviation (SD).

Human leukocyte interferon was provided by Dr. Cantell9 as a solution in 1-ml vials containing $3 \times 10^6$ international reference units (IRU) and 4 mg of protein. For use, a whole vial was thawed and stored at 4°C as a stock solution in α-MEM at a concentration of 10 IRU/ml for up to 2 wk. Prior to use, the stock solution was serially diluted to the desired concentration and incorporated in the target cell layer.

Colony and Cluster Morphology

Representative clusters from each agar culture dish containing different concentrations of HLIF were picked up with a Unopett pipette equipped with a fine capillary tube. They were placed on egg albumin-smeared microscopic slides and allowed to dry. Luxol Fast Blue (MBS) (Hartman-Ledden Co., Philadelphia, Pa.) was used to stain the clusters. After 2 hr of staining, the slides were washed in distilled water for 2 hr and subsequently counterstained with Harris’ hematoxylin for 2 min. The clusters were then examined under a light microscope for cell composition. The percentage differential count was calculated from the cumulative data on differentials of 50 clusters from each culture dish.

RESULTS

The effects of the continual presence of HLIF were examined by its incorporation in the target cell layer of in vitro agar cultures in the presence of HPCM. Table 1 presents the data for four such experiments performed using marrow cells from different donors. In the first three experiments, a sharp decrease in the colony incidence was observed with HLIF concentration of 10 IRU/ml. With increasing concentrations of HLIF per culture dish, a further drop in colony incidence was noted in all three experiments, and at 1000 and 10,000 IRU/ml almost no cell aggregate of colony size was noted. When cluster incidence was scored, it was noted there was a progressive increase in the cluster incidence with increasing HLIF concentration. This resulted in a final plating efficiency that remained nearly constant regardless of the concentrations of HLIF used. In the fourth experiment, the marrow cells were found to be relatively resistant to the inhibitory effect of HLIF. Even at 10,000 IRU/ml, there was a drop in the colony incidence of only 63%. The total plating efficiency, however, remained constant at all HLIF concentrations. The exact cause for this variable sensitivity in marrow cells from different donors is not clear, although cell lines with variable sensitivity and development of resistance to interferon have previously been described.11,12

When HLIF was incorporated into target marrow cell layer without a source of
Granulopoietic differentiation block by HLIF

Average percentage from 50 clusters examined for morphology. Morphological examination of the colonies from dishes with and without interferon revealed normally differentiating cells.

Table 1. Effect of Human Leukocyte-Interferon on Colony and Cluster Formation When Incorporated in the Ager Overlayers With HPCM

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cell Aggregates Scored</th>
<th>Plating Efficiency Without Interferon (Control)</th>
<th>Number of Colonies and Clusters as Percent of Control at Various Concentrations of Interferon per Dish (IRU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>Colonies*</td>
<td>171.3 ± 3.5</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>Clusters†</td>
<td>370.0 ± 10.0</td>
<td>124.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>541.3</td>
<td>93.0</td>
</tr>
<tr>
<td>2</td>
<td>Colonies</td>
<td>124.3 ± 5.1</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Clusters</td>
<td>326.7 ± 15.0</td>
<td>135.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>451.0</td>
<td>103.0</td>
</tr>
<tr>
<td>3</td>
<td>Colonies</td>
<td>35.0 ± 4.6</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>Clusters</td>
<td>68.0 ± 6.5</td>
<td>139.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>103.0</td>
<td>97.0</td>
</tr>
<tr>
<td>4</td>
<td>Colonies</td>
<td>26.7 ± 4.0</td>
<td>87.2</td>
</tr>
<tr>
<td></td>
<td>Clusters</td>
<td>253.0 ± 5.8</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>279.7</td>
<td>101.3</td>
</tr>
</tbody>
</table>

Number of light density (< 1.077 g/ml) cells plated per dish was 2 x 10⁶ for the first two and 10⁷ for the last two experiments. The colony and cluster incidence is mean ± SD from triplicate culture dishes.

*Aggregates of ≥ 40 cells.
†Aggregates of 3-39 cells.

CSF, it did not stimulate colony or cluster formation (data not shown). The HLIF dose-related increase in the number of cluster-size aggregates associated with simultaneous progressive decrease in the colony-size aggregates suggested that HLIF did allow the CFU-C proliferation but limited their growth to colony size. This could be explained either by inhibition of the proliferation of granulocyte precursors at a late stage of maturation or prolongation of the generation time of each successive mitotic division. However, when the culture dishes were incubated for 12 instead of 8 days, no further increase in size of the aggregates was observed, suggesting that prolongation of the generation time either was not the cause of the observations made in this series of experiments or was so marked that it resulted in no significant further growth of clusters even after 4 more days of incubation.

To further evaluate these points, a morphological examination was performed on clusters from culture dishes with and without HLIF concentrations of 10, 100, 1000, and 10,000 IRU/ml. The data from one of two such experiments are shown in Table 2. Clusters from dishes incubated without interferon revealed no myelo-

Table 2. Effect of Human Leukocyte-Interferon on Cell Composition of Aggregates Grown in Agar Culture

<table>
<thead>
<tr>
<th>Interferon Concentration (IRU/ml)</th>
<th>Myeloblasts</th>
<th>Promyelocytes</th>
<th>Myelocytes</th>
<th>Metamyelocytes</th>
<th>Bands</th>
<th>Polymorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.7</td>
<td>22.8</td>
<td>23.0</td>
<td>48.5</td>
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<tr>
<td>10</td>
<td>3.0</td>
<td>10.0</td>
<td>27.0</td>
<td>27.2</td>
<td>22.4</td>
<td>10.4</td>
</tr>
<tr>
<td>100</td>
<td>9.6</td>
<td>19.2</td>
<td>27.0</td>
<td>27.8</td>
<td>10.6</td>
<td>4.8</td>
</tr>
<tr>
<td>1,000</td>
<td>6.0</td>
<td>12.7</td>
<td>37.0</td>
<td>30.0</td>
<td>9.2</td>
<td>4.1</td>
</tr>
<tr>
<td>10,000</td>
<td>8.4</td>
<td>16.2</td>
<td>40.8</td>
<td>28.0</td>
<td>6.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Average percentage from 50 clusters examined for morphology. Morphological examination of the colonies from dishes with and without interferon revealed normally differentiating cells.
blasts or promyelocytes. Most of the cells were polymorphonuclear cells (48.5%) and bandforms (23.0%). The metamyelocytes accounted for only 22.8% of the cells. However, a progressive left shift was noted in the clusters from cultures containing increasing concentrations of interferon. At a concentration of 10 IRU/ml, there were 13% myeloblasts plus promyelocytes, 27% myelocytes, 27.2% metamyelocytes, and 32.8% bandforms and polymorphonuclear cells, while at a concentration of 10,000 IRU/ml, myeloblasts and promyelocytes accounted for a significantly higher proportion of cells (24.6%) and no polymorphonuclear cells were noted. These data confirmed that high concentrations of HLIF interfere with the normal differentiation process in a subtle manner that results in maturational arrest and a lack of differentiation of granulocyte precursors to polymorphonuclear cells.

DISCUSSION

The results of our experiments suggest that HLIF does allow the proliferation of myeloid progenitor cells, but only up to a certain stage in the maturation process. This results in the formation of cluster-size cell aggregates of predominantly granulopoietic precursors.

We postulate that as a myeloid progenitor cell proliferates and differentiates, successive generations become more sensitive to the effects of HLIF, with a maximum effect being noticeable at myelocyte level. Since such a mechanism would allow the differentiation of early granulopoietic precursors to continue, although at a relatively slower rate, they would not accumulate in large numbers. This would, however, result in a gradually increasing accumulation of successively more differentiated progeny, constituting the late mitotic pool of the myelopoietic hierarchy. According to our postulate, these more differentiated progeny of the mitotic pool, e.g., first and second generation myelocytes, are also the ones most sensitive to the antimitotic and/or antidifferentiation effects of HLIF and, therefore, would divide or differentiate slowly. The net result would be small aggregates of cells belonging to the late mitotic pool. At higher concentrations of HLIF, this effect would become manifest at even earlier stages of the maturation process.

Other investigators have noted decrements in the colonies,5,7 however, they have failed to note the rising cluster incidence in these cultures and make no mention of the composition of clusters. As the effect of interferon is dose-related and can be counteracted by CSF (unpublished data), interferon might be functioning as an inhibitory arm of the myelopoietic regulatory system, which, unlike prostaglandin E, does not inhibit colony formation at the granulocyte-macrophage progenitor cell level13 but blocks proliferation and differentiation, thus leading to maturational arrest.

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