Eosinophils Express a Functional Receptor for Interferon \( \alpha \): Inhibitory Role of Interferon \( \alpha \) on the Release of Mediators

By Delphine Aldebert, Bouchaib Lamkhioued, Corinne Desaint, Abdellah Soussi Gounni, Michel Goldman, André Capron, Lionel Prin, and Monique Capron

Recent reports describe the beneficial use of alpha interferon (IFNa) for the treatment of idiopathic hypereosinophilic syndrome (HES) unresponsive to conventional therapy. A clinical improvement associated with a rapid decrease of peripheral blood eosinophils suggested possible direct effects of IFNa on eosinophils through the presence of IFNa receptors (IFNaR). Reverse transcriptase-polymerase chain reaction (RT-PCR) and cytochemistry were used respectively to detect the presence and define the distribution of IFNaR on enriched eosinophil preparations purified from blood cells. IFNaR was found on eosinophils collected from patients with various eosinophilic disorders. In addition, IFNa inhibited the release of eosinophil granule proteins such as eosinophil cationic protein (ECP), neurotoxin (EDN), or interleukin-5 (IL-5). Moreover, antiparasite cytotoxicity was also strongly reduced in a dose-dependent manner by IFNa. These results provide the first evidence that human eosinophils express a functional receptor for IFNa and represent a potential basis for the beneficial effects of IFNa in patients with hypereosinophilic syndromes.

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Table 1. Summary of the Characteristics of Eosinophil Donors and Cell Purification

<table>
<thead>
<tr>
<th>Donors</th>
<th>Diagnosis</th>
<th>% Eosinophils</th>
<th>Cell Purification Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HES C</td>
<td>96.5</td>
<td>Metrizamide: CII</td>
</tr>
<tr>
<td>2</td>
<td>HES A</td>
<td>100</td>
<td>MACS</td>
</tr>
<tr>
<td>3</td>
<td>HES A</td>
<td>89</td>
<td>MACS</td>
</tr>
<tr>
<td>4</td>
<td>HES A</td>
<td>89</td>
<td>MACS</td>
</tr>
<tr>
<td>5</td>
<td>P</td>
<td>99.5</td>
<td>Metrizamide: CIII</td>
</tr>
<tr>
<td>6</td>
<td>HES A</td>
<td>100</td>
<td>MACS</td>
</tr>
<tr>
<td>7</td>
<td>HES C</td>
<td>93.5</td>
<td>Metrizamide: CII + CIII</td>
</tr>
<tr>
<td>8</td>
<td>HES C</td>
<td>98</td>
<td>MACS</td>
</tr>
<tr>
<td>9</td>
<td>HES A</td>
<td>91</td>
<td>Metrizamide: CV</td>
</tr>
<tr>
<td>10</td>
<td>HES A</td>
<td>100</td>
<td>MACS</td>
</tr>
<tr>
<td>11</td>
<td>Allergy</td>
<td>94.5</td>
<td>Metrizamide: CV</td>
</tr>
<tr>
<td>12</td>
<td>HES A</td>
<td>98</td>
<td>MACS</td>
</tr>
<tr>
<td>13</td>
<td>HES A</td>
<td>70</td>
<td>Metrizamide: CIII</td>
</tr>
<tr>
<td>14</td>
<td>HES A</td>
<td>98.5</td>
<td>Metrizamide: CV</td>
</tr>
<tr>
<td>15</td>
<td>HES A</td>
<td>92</td>
<td>Metrizamide: CIII</td>
</tr>
<tr>
<td>16</td>
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<td>91</td>
<td>Metrizamide: CV</td>
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<td>Allergy</td>
<td>55</td>
<td>Metrizamide: CV</td>
</tr>
<tr>
<td>18</td>
<td>HES A</td>
<td>99</td>
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</tr>
<tr>
<td>19</td>
<td>P</td>
<td>99.5</td>
<td>MACS</td>
</tr>
<tr>
<td>20</td>
<td>HES C</td>
<td>100</td>
<td>MACS</td>
</tr>
<tr>
<td>21</td>
<td>HES A</td>
<td>98.5</td>
<td>MACS</td>
</tr>
<tr>
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<td>HES B</td>
<td>96.5</td>
<td>MACS</td>
</tr>
<tr>
<td>23</td>
<td>HES A</td>
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<tr>
<td>24</td>
<td>HES A</td>
<td>92</td>
<td>Metrizamide: CV</td>
</tr>
<tr>
<td>25</td>
<td>HES B</td>
<td>94</td>
<td>Metrizamide: CV + CV</td>
</tr>
</tbody>
</table>

Abbreviations: HES A, patients without clinical complication; HES B, evident visceral involvement; HES C, malignant HES; P, parasitic infection.

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IFNα RECEPTOR ON EOSINOPHILS

Fig 1. Detection of IFNαR in human eosinophils. (A) RT-PCR analysis on purified human eosinophils (patients 5 through 10) (lane 5-10), on positive cell controls: peripheral lymphocytes obtained from two HES patients (lane 1-2) and human Daudi B lymphoblastoid cell line (lane 3), and on negative control (without cDNA) (lane 4). Primer 1: nucleotides 136-160; Primer 2: 532-552; 416-bp). (B) Enzymatic restriction analysis on purified eosinophils with SSP1 (lane 1) confirmed that the amplified band was specific 231bp for IFNαR. Lane 2 represents amplified band before enzymatic restriction.

significance of these data is discussed in the context of improved therapeutic strategies for HES treatment.

MATERIALS AND METHODS

Patients. A total of 25 different patients with hypereosinophilia of various etiologies (parasitic infections, hypereosinophilic syndrome, allergic diseases, hematologic malignancies) were selected for this study after informed consent. The characteristics of the eosinophil purification procedure for each patient are summarized in Table 1.

Reagents. Purified interferon alpha 2b (IFNα2b 2.10^6 U/mL) and Introma were obtained from Schering-Plough, (Levallois Perret, France). Micromagnetic beads bound to anti-CD16 monoclonal antibody (MoAb) and magnetic-activated cell sorter (MACS) columns were purchased from Miltenyi Biotec GmbH, (Bergish Gladbach, Germany). Human IgE was a generous gift from Dr H. Spiegelberg (University of California, La Jolla, CA). Secretory IgA was purchased from Sigma, (St Louis, MO) and antihuman IgA and antihuman IgE from Immunotech, (Marseille-Luminy, France). Biotin N-hydroxysuccinimide ester were obtained from Calbiochem, (Mézières, France). Streptavidin-phycocerythrin and streptavidin alkaline phosphatase were respectively obtained from Jackson Immunoresearch Laboratory, (West Grove, PA) and Sigma Laboratory. p-nitrophenylphosphate was obtained from Sigma. A rat antihuman or mouse IL-5 and a biotinylated rat antihuman IL-5 were obtained from Pharmingen, (San Diego, CA). Rabbit IgG antibodies directed to intracellular domain of IFNαR were obtained from TEBU (Le Perray, Yvelines, France).

Cell preparations. Eosinophils were isolated from the venous blood of patients with various diseases by centrifugation through metrizamide discontinuous gradients, according to previously described techniques. Using this separation procedure, distinct eosinophil populations, i.e., eosinophil populations that sediment in fractions of low density (hypodense eosinophils in 20% to 23% metrizamide solutions, corresponding to layers CII-CII1), intermediate density (24% metrizamide solution, layer CIV) or high-density zone (25% metrizamide solution, layer CV) were collected. A second technique using MACS was also used to increase the degree of purity. After blood centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), granulocytes were obtained by dextran sedimentation. Eosinophils were separated from neutrophils by negative immunomagnetic selection using anti-CD16 coated microbeads and the MACS system. Isolates consisting of >91% eosinophils were routinely obtained. Not all parameters could be evaluated on the same eosinophil preparations due to insufficient numbers of eosinophils purified from each patient.

Peripheral blood lymphocytes were obtained from HES patients after Ficoll-Paque density gradient centrifugation. The lymphoblastoid cell line Daudi routinely cultivated in the laboratory was used as a positive control.

RT-PCR. Total DNA isolated from purified eosinophil populations (donors 1 through 12, Table 1) by the guanidium isothiocyanate cesium chloride procedure was reverse transcribed in a 20 μL.

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Fig 2. Analysis of IFNαR expression. Purified eosinophils were permeabilized with methanol 70% and stained for intracellular IFNαR expression using (-) antibodies to IFNαR or (—) an isotype matched nonbinding control IgG antibody. Five thousand cells were analyzed by flow cytometry and representative histograms were shown: IFNαR expression on human eosinophils (no. 21 through 23) (A, B, and C) and on human lymphocytes from one HES patient (no. 21) (D).

Flow cytometry analysis. Eosinophils were purified from the blood of seven HES patients (no. 8, no. 18 through 23, Table 1) according to the two methods described above. Cell suspensions (10^7 cells/mL) were fixed with 0.25% paraformaldehyde for 15 minutes at room temperature. After washing, we added 1 mL of cold 70% methanol for 60 minutes at 4°C. The cells were then incubated with anti-IFNαR rabbit antibodies for 30 minutes at 4°C and labelled with FITC conjugated antirabbit IgG sheep antibodies. Rabbit IgG antibodies with a different specificity were used as isotype negative control for 30 minutes at 4°C. Cells were analyzed by flow cytometry using Epics Profiles, (Coultronics, Hialeah, FL). The gating on the combination of two parameters, forward scatter and logarithm of side scatter, was used to exclude lymphocytes from granulocytes.

Cytochemistry. Cytocentrifuged preparations of purified blood eosinophils (donors 1, 8, 10, 11, and 14 through 17, Table 1) were incubated overnight at 4°C with purified IFNα that had been biotinylated with biotin N hydroxysuccinimide ester (1 mg/mL, buffer pH 8,5). The preparations were then washed in Tris buffer saline (TBS), and streptavidin alkaline phosphatase was added. After incubation for 30 minutes at room temperature, slides were briefly washed in TBS and developed with new Fuschin, DAKO, (Gloshup, Denmark). Nuclei were colored with glychemalin (Rhé Poulenc, Villers St Paul, France).

Eosinophil activation. Highly purified eosinophils (91% to 100%) from five patients with eosinophilic disorders (donors 8 and 13 through 16, Table 1) were preincubated with RPMI 1640 supplemented with 10% fetal calf serum (FCS) for one night at 37°C and 5% CO₂ in the presence of different concentrations of interferon alpha 2b (INTRONA). We then added secretory IgA or IgE, respectively, at a final concentration of 20 µg/mL and anti-IgA or anti-IgE at a final concentration of 20 µg/mL for 3 hours. The supernatants were collected and frozen at −20°C until the measurements could be performed.
IFNa receptor on eosinophils

Cytotoxicity assay. Schistosoma mansoni schistosomula prepared according to the skin penetration procedure were the targets of IgE-mediated eosinophil-dependent cytotoxicity as previously described. Eosinophils purified from three different patients (8 and 24 through 25, Table 1) were preincubated for 30 minutes with different concentrations of IFNa. Eosinophils were then added to schistosomula in the presence of IgE rich immune sera from S. mansoni infected patients (unheated, final dilution 1/32) at a ratio of 5,000 effector cells to one target. Results were given as percent dead larve microscopically estimated after a 24-hour incubation at 37°C. Background cytotoxicity in negative control wells (containing normal human serum) was inferior at 10%.

Statistical analysis. The significance of differences in cytotoxicity and mediator release inhibition was determined using a Student’s t-test.

RESULTS AND DISCUSSION

Detection of IFNaR on human eosinophils. To determine whether human eosinophils could express a receptor for IFNa (IFNaR), RNA isolated from highly purified eosinophils from 12 patients (% of purity of eosinophils ranging from 91% to 100%) was analyzed by RT-PCR with constructed probes from the published sequence of DNA corresponding to IFNaR, a protein of 63 kD. Eosinophil RNA was compared with positive control samples from either Daudi cells or lymphocytes, which constitutively express IFNaR. Similarly-sized bands were observed for eosinophils from all patients tested except for one patient (no. 9) (Fig 1A). Negative control tests showed that no signal was observed in the absence of cDNA. Enzymatic restriction analysis (Fig 1B) confirmed the specificity of the amplified fragments. These results suggest that human blood eosinophils purified from patients with various hyper eosinophilic disorders could express mRNA encoding for IFNaR, except for one patient, suggesting either a possible defect or a process of mRNA regulation.

To exclude the hypothesis that cells contaminating the eosinophil preparations could represent only cell sources of IFNaR in the RT-PCR analysis, we performed flow cytometry analysis (Fig 2). Six of seven patients (except no. 20) gave positive results confirming the existence of a receptor for IFNa. The percentage of positive cells was variable according to individual patients (range, 20% to 86%). Cytchemistry was also used to identify eosinophil morphology (Fig 3). Staining of eosinophil cytocentrifugated preparations with biotinylated IFNa was shown by reaction with streptavidin alkaline phosphatase. Five of the eight patients tested were positive (no. 1, no. 11, no. 14 through 16). Variations in the expression of IFNaR among eosinophils from different patients may suggest a possible modulation of these receptors. Indeed, a low-density expression of IFNaR has also been described on lymphocytes.

The presence and/or the absence of IFNaR proteins might give evidence for a restrictive surface expression on a selective eosinophil subpopulation. Alternatively, such results could be related to a modulated expression of IFNaR by inducible factors, which remain to be defined, including cytokines. The binding affinity of IFNa is dependent on the molecular form of IFNaR, which presents a multimeric structure. Low or undetectable IFNaR expression might be also related to the release of a soluble form, as sIFNaR has
Fig 4. Inhibition by IFNα of eosinophil mediator release. Eosinophils were preincubated with different doses of IFNα and then stimulated with IgE or IgA immune complexes. Two representative experiments of EDN, ECP, and IL-5 release in the presence of increasing doses of IFNα are presented.
IFNα strongly decreased the release of IL-5 by purified eosinophils. As previously shown, IL-5 is the main factor of terminal eosinophil differentiation. It also participates in chemotaxis and activation of eosinophils. Our data demonstrate that IFNα can regulate the autocrine, as well as the paracrine, effects of IL-5 on eosinophil lineage.

Antibody-dependent mediated cytotoxicity towards parasite targets, namely S. mansoni schistosomula, is one of the most largely studied effector functions of eosinophils. This mechanism requires various antibody isotypes including IgE and IgA, as well as activated eosinophils. Effector molecules include the basic granule proteins, also involved in host tissue damage. This in vitro procedure therefore represents a useful tool to evaluate the release of functionally cytolytic mediators. Incubation of eosinophils with varying concentrations of IFNα led to a dose-dependent inhibition of parasite killing with a significant inhibition for the concentration of 1000 U/mL of IFNα (P < .05), in three representative experiments shown in Fig 5. Interestingly, this inhibitory effect was only observed when eosinophils had been briefly presensitized with IFNα, confirming the requirement of binding to a membrane receptor. These findings demonstrate that IFNα can exert direct inhibitory effects on cytolytic mediator release by human eosinophils, at least in vitro.

Our results indicate the existence of a functional IFNαR on human eosinophils. IFNα was shown to inhibit the release of cytotoxic proteins, such as ECP and EDN, as well as molecules involved in eosinophil differentiation and activation, such as IL-5. One of the main effector functions of eosinophils, namely cytotoxicity to parasites, is also strongly inhibited. These findings are relevant with the beneficial effects of IFNα in treated HES patients. Favorable clinical results are associated with a rapid decrease in blood eosinophilia, sCD23, and sCD25 levels. Indeed, IL-5 has the ability to induce sCD25 release from murine the B-cell surface. The inhibitory effects of IFNα on IL-5 release by eosinophils might be responsible for the decrease, not only in eosinophil count, but also in sCD23 and sCD25 levels.

The inhibitory decrease of sCD25 expression on eosinophils might be responsible for the decrease, not only in eosinophil infiltration and in tissue damage in patients with eosinophilic heart disease after treatment with IFNα could result from the inhibitory effects of IFNα on the release of highly cytolytic mediators by eosinophils, such as ECP and EDN. Finally the refractory state of some HES patients observed after IFNα therapy could be related to variable expression of IFNαR by eosinophils in this heterogeneous group of diseases.

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REFERENCES

human interferon α receptor into mouse cells: Cloning and expression of its cDNA. Cell 60:225, 1990


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