Binding of $^{125}$I-Gamma Interferon to Cultured Human Keratinocytes

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Gamma interferon (IFN-γ), a product of activated lymphocytes, influences keratinocyte proliferation and differentiation. Recombinant gamma interferon (r-IFN-γ) was radiiodinated using the Bolton-Hunter reagent and retained 90% of its biologic activity as determined by induction of HLA-DR expression. The biochemical ligand-binding properties of iodinated IFN-γ to cultured human keratinocytes revealed a plateau of binding at 150 min at 4°C, and a single class of specific high affinity receptors (kD = $1.3 \times 10^{-10}$ m; 2200 sites/cell). The binding of human IFN-γ, to keratinocytes was inhibited by human r-IFN-γ, but not by either murine recombinant gamma interferon or human recombinant beta interferon (r-IFN-β). The presence of high affinity receptor sites on human keratinocytes assures the reception of appropriate immunologic signals in lymphocyte-keratinocyte interactions. J Invest Dermatol 89:132–135, 1987

We have previously studied the interaction of gamma interferon (IFN-γ), a product of activated T lymphocytes, with cultured human keratinocytes and made the following observations: (1) Recombinant gamma interferon (r-IFN-γ) induces the synthesis and expression of HLA-DR on keratinocytes [1,2]; (2) r-IFN-γ inhibits the growth of iodinated keratinocytes in a concentration-dependent fashion [3]; (3) r-IFN-γ induces ultrastructural changes in keratinocytes suggesting an influence on differentiation [4]; and (4) during allogeneic mixed lymphocyte reactions, IFN-γ is produced and may modulate lymphocyte-keratinocyte reactions [5].

These results indicate that IFN-γ can bind to and interact in a biologically relevant fashion with keratinocytes. This report will characterize the binding of iodinated r-IFN-γ ($^{125}$I-IFN-γ) to cultured human keratinocytes and correlate this binding to its biologic activity.

MATERIALS AND METHODS

Cell Culture Single-cell suspensions of normal skin from face lift skin was prepared as previously described [3]. The keratinocytes were grown on collagen coated Petri dishes (Lux, Flow Laboratories, Naperville, Illinois) with Dulbecco's modified Eagle’s medium (DMEM) containing 1.4 mm calcium, supplemented with 10% heat inactivated fetal calf serum and 2 mm glutamine.

Interferons Lyophilized recombinant human and murine IFN-γ was kindly provided by Dr. Michael Shepard (Genentech, Inc., South San Francisco, California). The specific activity of human r-IFN-γ, purified to homogeneity, was $2 \times 10^7$ units/mg as determined by virus inhibition plaque assay. Recombinant human beta-ser IFN was kindly provided by Dr. T. Basham (Stanford University, Stanford, California).

$^{125}$I Iodination of r-IFN-γ The iodination procedure utilized was that previously reported by Ayer et al with slight modification [6]. Briefly, 500 mCi of dried $^{125}$I-diiodo-Bolton-Hunter [7] reagent (4250 Ci/mmol; purchased from Amersham Corp., Arlington Heights, Illinois) was incubated with 10 μg of freshly reconstituted r-IFN-γ in 100 μl of 0.1M sodium borate, pH 8.5 on ice for 2 h. The reaction was stopped by the addition of 100 μl of 0.2 M glycine in 0.1M sodium borate pH 8.5. The mixture was then placed on Sephadex G25-M column (packed, PD-10 columns from Pharma, Inc., Piscataway, New Jersey) equilibrated with 0.1M sodium phosphate buffer, pH 7.5, containing 0.25% (w/v) gelatin (swine skin, 300 Bloom, purchased from Sigma Chemical Co., St. Louis, Missouri). Fractions (0.5 ml) were collected; fractions 6 and 7 contained the greatest amount of $^{125}$I-IFN-γ and were stored at 4°C. Greater than 90% of the radioactivity in these fractions was precipitable with 10% trichloroacetic acid (TCA). The $^{125}$I-IFN-γ obtained by this procedure had a specific activity of 6.6 μCi/μg, based on a molecular weight of r-IFN-γ of 34,000 daltons. The iodinated r-IFN-γ retained its ability to induce HLA-DR and inhibit the proliferation of cultured keratinocytes. The induction of the HLA-DR expression was studied as below, but the antiproliferative effect (300 U/ml) was only determined by phase microscopy without further quantitation.

Keratinocyte HLA-DR Staining Keratinocyte HLA-DR staining was performed as previously described [5]. Briefly, either iodinated r-IFN-γ or noniodinated r-IFN-γ was preincubated with keratinocytes at the indicated concentration for 3 days. Next, the keratinocytes were trypsinized (0.3% trypsin, 1% EDTA) into single-cell suspension and indirectly stained using anti-HLA-DR (L243; Becton-Dickinson, Mountain View, California) and fluo- resein isothiocyanate-conjugated goat antimouse IgG (Becton-
specific binding averaged 35% of the total binding at 37°C and 25% of the total binding at 4°C.

**Table 1. Inhibition of 125I-Gamma Interferon (IFN-γ) (100 pmol) Binding to Keratinocytes by "Cold" Interferons**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Specific Binding (cpm)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1520</td>
<td>0</td>
</tr>
<tr>
<td>r-IFN-γ (10^3 pmol)</td>
<td>1220</td>
<td>19</td>
</tr>
<tr>
<td>r-IFN-γ (10^4 pmol)</td>
<td>973</td>
<td>26</td>
</tr>
<tr>
<td>r-IFN-γ (10^5 pmol)</td>
<td>213</td>
<td>86</td>
</tr>
<tr>
<td>Murine r-IFN-γ (10^5 pmol)</td>
<td>1498</td>
<td>1.5</td>
</tr>
<tr>
<td>Murine r-IFN-γ (10^6 pmol)</td>
<td>1511</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Human r-IFN-β (10^5 pmol)</td>
<td>1486</td>
<td>2</td>
</tr>
<tr>
<td>Human r-IFN-β (10^6 pmol)</td>
<td>1493</td>
<td>2</td>
</tr>
</tbody>
</table>

r-IFN-γ = recombinant gamma interferon.

r-IFN-β = recombinant beta interferon.

The estimated keratinocyte density for this calculation was 1 × 10^7 cells/ml. The standard error of the mean for the ligand binding studies was approximately 15%.
Figure 4. Scatchard analysis of ligand binding data from Fig 3. A linear plot was obtained (regression coefficient = 0.9). The kD was $1.3 \times 10^{-10}$ M with 2200 receptor sites/keratinocyte.

### RESULTS

#### Biologic Activity of $^{125}$I-IFN-γ

The retention of biologic activity of the labeled r-IFN-γ recovered following radioiodination was determined by measuring the induction of keratinocyte HLA-DR expression. Figure 1 reveals that using “100” and “300” U/ml (based on weight) of r-IFN-γ after the iodination process, approximately 90% of the biologic activity was retained when compared with the same amount of noniodinated r-IFN-γ.

#### Time-Course of IFN-γ Binding

$^{125}$I-IFN-γ bound to specific receptors on cultured keratinocytes in a time- and temperature-dependent fashion (Fig 2). At 4°C, the steady state was achieved after 150–180 min, but at 37°C, no saturation was observed after 5 h incubation. The lack of saturation at 37°C could be due to the internalization of IFN-γ [9]. Three different preparations of radiolabeled r-IFN-γ produced similar values.

#### Specificity of IFN-γ Binding

Binding site competition between $^{125}$I-IFN-γ and unlabeled r-IFN-γ are shown in Table I. The r-IFN-γ, but not the r-IFN-β or murine r-IFN-γ inhibited the binding of $^{125}$I-IFN-γ to the keratinocytes. The competition data indicate that the binding sites are specific for r-IFN-γ.

#### IFN-γ Binding to Keratinocytes and Scatchard Analysis

Binding data utilizing increasing concentrations of $^{125}$I-IFN-γ and cultured keratinocytes are shown in Fig 3. Saturation of the receptor was achieved at the higher $^{125}$I-IFN-γ concentrations utilized. Scatchard analysis of the ligand binding data yielded a linear plot (regression coefficient = 0.9) indicating the presence of a single class of binding sites. The dissociation constant was $1.3 \times 10^{-10}$ M with 2200 receptors/keratinocyte (Fig 4).

### DISCUSSION

The utilization of the Bolton-Hunter reagent produced high specific activity radiolabeling of r-IFN-γ with good retention of biologic activity (Fig 1). The resultant high specific activity iodinated r-IFN-γ produced ligand binding results which indicate a single class of specific high affinity receptors for IFN-γ on cultured human keratinocytes. The data presented serve to define and partially characterize the IFN-γ receptor on cultured human keratinocytes. The plateau in binding at 4°C at 150 min (Fig 2) is similar to previous reports using other cell types [10,11]. The dissociation constant of $1.3 \times 10^{-10}$ M (Figs 3, 4) correlates well with our previous biologic studies [12] of the induction of HLA-DR and other proteins with a half-maximal response to r-IFN-γ between $10^{-9}$ to $10^{-10}$ M. Thus, while IFN-γ induces a wide spectra of biologic response in keratinocytes [1–5], the binding of IFN-γ to its cell surface receptor is the first step in all these reactions and the binding data agrees well with these biologic responses.

Table II summarizes the previously published IFN-γ receptor-binding characteristics on both mouse and human cells. As can be seen, there is a wide variety of reported values for hematopoietic and nonhematopoietic cells. The current data utilizing r-IFN-γ and cultured human keratinocytes revealed a kD which is in agreement with the more recent ligand-binding studies. The number of estimated receptor sites per keratinocyte (approximately 2200) is also within the reported range as seen in Table II. The most recent data utilizing high specific activity murine r-IFN-γ indicates a high affinity receptor which may have been missed in earlier studies utilizing iodinated IFN-γ of lower specific activity at significantly higher IFN-γ concentration. [6]. In this regard, the concentration range of biologic responses elicited by r-IFN-γ indicate that only approximately 5% occupancy of high affinity sites is required for 50% maximal induction of murine langerin expression [6].

There have been conflicting reports detailing the ability of IFN-β to compete for IFN-γ receptor sites. Two studies documented cross-reactivity using nonrecombinant IFN-β [9,11] whereas 3 other studies failed to demonstrate significant cross-reactivity with either nonrecombinant or recombinant IFN-β [6,11,18]. The lack of cross-reactivity between murine and human IFN-γ (Table I) suggests that the species specificity of the biologic activity of IFNs may reside at the receptor level.

The presence of high affinity receptors for IFN-γ on cultured keratinocytes provides additional support for the notion that lymphocyte products can biochemically interact with keratinocytes [19]. Thus, not only has nature provided the skin with unique immunocompetent lymphoid cells, but has endowed the keratinocyte with specific receptors for products of lymphocytes assuring the reception of appropriate immunologic signals [20]. Since the ability of r-IFN-γ to induce HLA-DR synthesis in keratinocytes continues to be of interest to investigative skin biologists [21], further work on the modulation of keratinocyte

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Cell Type</th>
<th>kD(M)</th>
<th>Receptors/Cell</th>
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</thead>
<tbody>
<tr>
<td>Anderson et al [10]</td>
<td>Human fibroblast</td>
<td>$1.5 \times 10^{-10}$</td>
<td>2,400</td>
</tr>
<tr>
<td>Sarkar et al [13]</td>
<td>WISH cells</td>
<td>$7.0 \times 10^{-9}$</td>
<td>50,000–70,000</td>
</tr>
<tr>
<td>Celada et al [14]</td>
<td>Murine macrophages</td>
<td>$8.3 \times 10^{-9}$</td>
<td>15,200</td>
</tr>
<tr>
<td>Littman et al [15]</td>
<td>Daudi cells</td>
<td>$3.7 \times 10^{-10}$</td>
<td>13,000</td>
</tr>
<tr>
<td>Finbloom et al [11]</td>
<td>HeLa cells</td>
<td>$6.3 \times 10^{-10}$</td>
<td>5,000</td>
</tr>
<tr>
<td>Orchansky et al [16]</td>
<td>Human monocytes</td>
<td>$2.5 \times 10^{-10}$</td>
<td>4,000</td>
</tr>
<tr>
<td>Rashidbaigi et al [17]</td>
<td>WISH cells</td>
<td>$6.8 \times 10^{-9}$</td>
<td>2,400 (multiple sites)</td>
</tr>
<tr>
<td>Aiyer et al [6]</td>
<td>Human fibroblasts</td>
<td>$1.4 \times 10^{-9}$</td>
<td>20,100</td>
</tr>
<tr>
<td>Current study</td>
<td>Human keratinocytes</td>
<td>$1.3 \times 10^{-10}$</td>
<td>2,200</td>
</tr>
</tbody>
</table>

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IFN-γ receptors may provide new insight into lymphocyte-keratinocyte interactions and ligand-binding techniques will allow quantitation of keratinocyte IFN-γ receptors in both normal and inflamed skin.

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REFERENCES