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Ligand-independent interaction of the type I interferon receptor complex is necessary to observe its biological activity

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ABSTRACT

Ectopic coexpression of the two chains of the Type I and Type III interferon (IFN) receptor complexes (IFN-αR1 and IFN-αR2c, or IFN-λR1 and IL-10R2) yielded sensitivity to IFN-alpha or IFN-lambda in only some cells. We found that IFN-αR1 and IFN-αR2c exhibit FRET only when expressed at equivalent and low levels. Expanded clonal cell lines expressing both IFN-αR1 and IFN-αR2c were sensitive to IFN-alpha only when IFN-αR1 and IFN-αR2c exhibited FRET in the absence of human IFN-alpha. Coexpression of RACK-1 or Jak1 enhanced the affinity of the interaction between IFN-αR1 and IFN-αR2c. Both IFN-αR1 and IFN-αR2c exhibited FRET with Jak1 and Tyk2. Together with data showing that disruption of the pre-association between the IFN-gamma receptor chains inhibited its biological activity, we propose that biologically active IFN receptors require ligand-independent juxtaposition of IFN receptor chains assisted by their associated cytosolic proteins.

1. Introduction

Type I interferons (IFNs), released in response to the detection of a viral infection, promote a virus-resistant state in cells and assist the immune system to detect and eliminate viruses and virus-infected cells. All activities of Type I IFNs are mediated by their interaction with the Type I IFN receptor complex [1,2], also known as the IFN-α or IFN-α/β receptor. The IFN-αR2c (also known as IFNAR-2C) chain interacts with not only the tyrosine kinase Jak1 but also the transcription factor Stat2. The IFN-αR2b splice variant encodes a transmembrane receptor chain with a truncated intracellular domain that binds neither Stat2 nor Jak1. The IFN-αR1 (also known as IFNAR-1) chain interacts with the tyrosine kinase Tyk2.

An important question is whether the Type I IFN receptor complex in its resting state is a preassembled entity or is dissociated prior to its nucleation by Type I IFN. Based on crosslinking studies, immunoprecipitations, and studies with soluble receptors, it is currently accepted that the Type I IFN receptor complex is dissociated until ligand is added, and IFN-αR1 and IFN-αR2c interact only indirectly through the ligand [3–7]. these studies are described in depth in Supplementary Text 2. Because these studies require analyzing the receptor outside of its native environment (where bias favoring high-affinity interactions that are environmentally insensitive is introduced), we wanted to use technologies that remove this bias by analyzing receptor structure in their physiological environment (i.e., within intact, where environmentally-sensitive and low-affinity interactions are maintained) in order to obtain more physiologically accurate data.

We successfully utilized fluorescence resonance energy transfer (FRET) to noninvasively probe the structure of the IFN-γ and the interleukin-10 receptor complexes. Observing FRET between IFN-γR1 and IFN-γR2 and between IL-10R1 and IL-10R2 in the absence or presence of ligand, we concluded that the IFN-γ and the IL-10 receptor chains interact in the absence of ligand, inferring that conformational changes accompany the activation of the receptor complex by ligand [8–10]. We confirmed the specificity of these interactions by coexpressing receptor chains from different receptor complexes and by mutating the IFN-γ receptor chains to inhibit biological activity; FRET between receptor chains was reduced

Abbreviations: CiFP, enhanced citrine fluorescent protein; ECFP, enhanced cyan fluorescent protein; EGFP, enhanced green fluorescent protein; EYFP, enhanced yellow fluorescent protein; FRET, fluorescence resonance energy transfer; IFN, interferon; IFN-α, interferon-alpha; IFN-γ, interferon-gamma; IFN-λ, interferon-lambda; OFP, orange fluorescent protein; RACK-1, receptor for activated protein kinase-1; StFP, strawberry fluorescent protein.

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substantially in most cases, providing evidence that mutations that eliminate biological activity also inhibit the interaction between IFN-γR1 and IFN-γR2.

Whether IFN-α and IFN-λ receptor complexes are similarly associated in a ligand-independent manner is the subject of these current studies.

2. Materials and methods

2.1. Genetic engineering and modification of proteins

All restriction endonucleases and DNA-modifying enzymes were obtained from New England Biolabs and used according to the manufacturer's instructions. Primers were obtained from Integrated DNA Technologies (Coralville, IA) and were used as provided. All primers in this manuscript are written in the 5′–3′ orientation. The synthesis of the various cDNA's used in this manuscript is described in Supplementary Text 1 of this manuscript and elsewhere [11].

2.2. Interferons

Human IFN-α2a (also known as IFN-αA, #11100-1) and human IFN-γ (#11500-1) were provided by PBL Biological Laboratories (Piscataway, NJ). The biological activity of human IFN-λ3 (recombinant, from conditioned medium of 293T cells, a gift from PBL Biomedical Laboratories) was assayed by the cytopathic effect inhibition assay in HepG2 cells challenged with EMCV. This assay was validated with human IFN-λ2-His (#11820-1). From these assays, half-maximal cytopathic effect inhibition is defined as one unit/mL (U/mL).

2.3. Cell culture

Cell lines were grown in HEPA-filtered incubators at 37 °C, 5% CO_2, and 95% relative humidity. CHO-q3 cells [8] were grown in F12 medium containing 10% fetal calf serum (FCS), while 293T cells were grown in DMEM medium containing 10% FCS and 4 mM glutamine.

To obtain stably transfected cell lines, geneticin (350 μg/mL) was added to the medium three days after transfection to select for transfected cells harboring genomically integrated plasmid until expanded colonies were visible to the naked eye (two to three weeks). A sample of these cells were diluted to single cells in 96-well dishes to isolate clonal colonies. The clones were analyzed by light fluorescent microscope using the FITC and TR channels to visually identify colonies harboring GFP and OFP. The clones mentioned in this manuscript were green and orange when GFP or OFP were respectively directly excited (g + o), were weakly orange when OFP was directly excited (wo), or had such weak fluorescence that it was essentially nonfluorescent (nf). Selected clones were further amplified and screened for their ability to induce MHC Class I or be protected from viral cytotoxicity in response to human IFN-α2.

2.4. Confocal fluorescence spectroscopy

FRET between fluorescent protein donor:acceptor pairs ECFP and EYFP in intact cells was measured with an instrument in which a spectrometer is integrated into the sidebox of a confocal microscope, as described previously [9,11–13].

2.5. Data analysis

Spectral deconvolution (the mathematical separation of an observed emission spectrum into several spectra of homogeneous components) was described previously [10,12,13]. The improved analysis of deconvolved spectra, calculation of FRET efficiency, other cellular biophysical quantities, and the inter-FP distances are described elsewhere [11].

2.6. Interferon activity assays

The antiviral assay is based on cytopathic effect inhibition, and was performed as described previously with minor modifications [14,15]. Briefly, 50,000 cells are plated in each well of a 96-well plate. IFN is next added in twofold serial dilutions throughout a row, and is incubated with the cells for 24 h. Afterward, vesicular stomatitis virus (VSV, Indiana Strain) is added at 1250 pfu/well. The virus is incubated with the IFN-treated cells for 48 h or until nearly complete killing of negative-control cells (that were not incubated with IFN) but not of positive control cells (that were incubated with neither IFN nor virus) is observed. Media is removed by inversion of the plates, and cells that survived viral infection are stained with crystal violet. Results are scored visually.

IFN also induces MHC Class I surface antigen in a dose-dependant fashion. The induction of MHC Class I Surface antigen by IFN was performed as previously described [8,15–18].

2.7. Fluorescence-activated cell sorting

Flow cytommetry was performed with a Becton–Dickinson FACScan as previously described [13]. In two-color experiments, the FL1 (530/30 nm barrier emission filter) and FL3 (650 long-pass emission filter) channels were compensated against the FL2 (585/42 barrier emission filter) channel prior to data collection to minimize the contribution of EGFP fluorescence in the FL2 channel and the contribution of StFP fluorescence into the FL1 channel. A positive gate on a forward scatter:side scatter dotplot was used to isolate intact cells.

Cells expressing StFP-labeled or EGFP-labeled receptors that were not treated with antibodies were identified and gated in a FL1:FL2 dotplot as events that were found at different dispersion angles from cells exhibiting only endogenous fluorescence. Gates were drawn to fully exclude cells that contain only endogenous fluorescence, accepting that cells with insignificantly low levels of acceptor and donor fluorescence will also be excluded with this negative gate. These gates were not changed among the various transfected populations. Because CHO-q3 cells constitutively express human Class I MHC surface antigen HLA-B7 on their cell surface, cells treated with primary anti-MHC Class I antibodies and FITC-conjugated secondary antibodies were shifted considerably along the FL1 axis whether they responded to IFN or not; induction by IFN further shifted only the subpopulation that is sensitive to IFN. To score for induction of MHC by IFN, a gate was created so that the left edge of the gate was positioned between the densest part of the IFN-inducible population and the densest part of the uninduced population, where the event density was minimal. The gate was held stationary between untreated and treated cells, and the percentage of cells within each gate was measured. The fraction of cells that induced MHC Class I surface antigen was taken as the difference in the percentage of gated cells in the ligand-treated and ligand-untreated populations. Data were collected and pre-processed with the CellQuest 3.3 software package (Becton–Dickinson) and processed with WinMDI2.9 (Joseph Trotter, The Scripps Research Institute, La Jolla, CA USA) for presentation in this manuscript.

2.8. Transfections

Several transfection systems were employed in this report, depending on the most cost-effective or favored transfection

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