Use of *iLite*™ Human Interferon Alpha Cell-Based Assay Kit and an anti-IFNAR2 neutralizing antibody in rapid activity determination of interferons secreted from poly I:C-stimulated A549 cells

Background

In recent years, it has become clear that the mammalian innate immune system responds to numerous pathogen-associated molecular patterns (PAMPs) produced by many infectious agents. Namely, retinoic acid inducible gene 1-like receptors (RLRs) and Toll-like receptors (TLRs) have emerged as powerful components that recognize and initiate the host immune response to limit pathogen infection and dissemination. Among these, nucleic acids have been shown to be highly immunostimulatory, promoting inflammatory cytokine production including interferons. Specifically, double-stranded RNA (dsRNA) is recognized by several proteins including protein kinase R, 2'5'–oligoadenylate synthetase retinoic acid inducible gene 1 (RIG-1) and Toll-like receptor 3 (TLR3). Many viruses produce factors to block this host dsRNA response. Consequently, interest in this area of research has increased dramatically. Therefore, improved methods are needed to more rapidly determine the effects of PAMPs on host control of infection.

One of the classic methods for determining interferon activity units per ml (U/ml) in treated cells has been the cytopathic effect assay (CPE). In this method, cell culture supernatants are collected from treated cells and added to naïve cells. Following and incubation period, cells are then challenged with virus. The virus will infect control cells resulting in cell lysis. If interferon is present, the cells are protected from lysis in a dose-dependent manner. When run in parallel with and compared to international interferon standards, the assay effectively determine the unit level of interferons present in the cell culture supernatant (U/ml). The drawbacks of this approach are the need for continuous cell culture, highly skilled technicians, and maintaining virus stocks. In addition, these assays often take >72hrs to complete.

Recently, PBL has introduced the *iLite*™ human IFN-α cell-based assay kit (PBL product #51100-1). Similar to the CPE assay, the *iLite*™ kit provides the researcher with a U/ml determination of interferon in a tissue culture or serum sample. In contrast, this assay does not require continuous cell culture, is simple to perform, and does not require the handling of biohazardous virus stocks. Importantly, it can be completed in <24hrs.

This present study was performed to demonstrate the utility of the *iLite*™ kit when studying PAMPs, such as dsRNA. It also includes specific conditions for a receptor-neutralizing antibody that researchers in this and similar areas should find useful when designing their studies.

In either the CPE or *iLite*™ assay format, it is important to determine the contribution of type I IFNs to the antiviral or the reporter gene expression, respectively. Type I IFNs seen in stimulated cells include alpha, beta, kappa, and omega, all of which can inhibit viral replication or induce type I IFN-mediated reporter gene expression. A researcher has many options to determine the presence (or absence) of type I IFNs present in the treated cell supernatant. These might include quantitative RNA detection assays, classical ELISA kits or using neutralizing antibodies directed against each individual type I IFN. However, each of these methods has limitations. For example, several studies have shown that mRNA levels do not always correlate effectively with IFN protein levels. Individual ELISA assays and neutralizing antibodies to each type I IFN proteins work well, but are laborious and may not be necessary if type IFNs were not produced during the cell treatment. Likewise, performing all of the multiple assays can use up precious amounts of limited supernatants or serum samples.

The study presented here is designed to use small quantities of sample in a rapid assay designed to determine if type I IFNs are present in an experimental sample. Instead of using neutralizing antibodies to each type I IFN, a single neutralizing antibody that blocks receptor binding of all type I IFNs was utilized to provide a rapid yes/no answer regarding the presence of type I IFNs in a treated cell line.

Materials and Methods

TLR3 Stimulation

Human A549 cells (human lung carcinoma) were transfected with lipid complexes containing increasing concentration of the synthetic dsRNA analogue polyinosinic:cytidilic acid (poly I:C). Six hours after addition of the transfection mix, cells were washed, and complete growth media (DMEM with 10% FCS) was added and cells were incubated an additional 16hrs. Cell culture supernatants were collected for use in the *iLite*™ Human Interferon Alpha Cell-Based Assay (PBL product #51100-1).

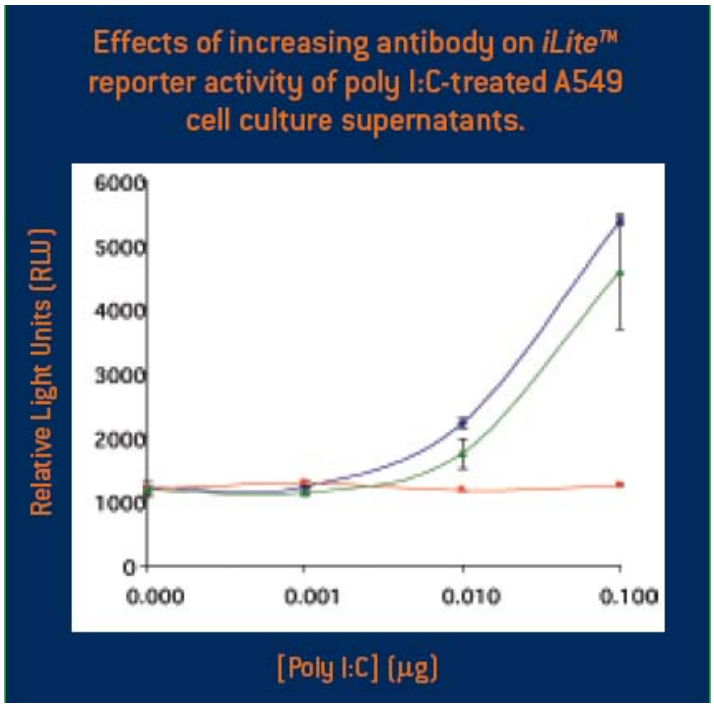
iLite™ *Assay*

The *iLite*™ assay was performed according to the kit protocol. Cell culture supernatants were split into three groups. Group 1 supernatants received no additional treatment. Group 2 supernatants were mixed Mouse anti-Human IFN-α/β R2 receptor antibody (PBL product #21385-1). Group 3 supernatants were mixed with mouse antihuman Anti-IFN-γ R1 antibody (PBL product #21580-1).

Results

The addition of poly I:C in transfection complexes to A549 cells resulted in a dose-dependent increase of RLU activity in the *iLite*™ assay suggesting that type I interferons were present in the supernatants. To confirm the RLU activity was attributable to type I interferons, a separate aliquot of the cell culture supernatants were mixed with a monoclonal antibody that recognizes the type I interferon receptor chain

2 and neutralizes signaling. Separately, the type II receptor chain 1 antibody was added in a similar fashion. The anti-IFNAR2 neutralizing antibody showed near complete inhibition of the RLU signal demonstrating that the activity in the cell culture supernatants was due to type I interferon activity (Figure below).



Increasing amounts of poly I:C (blue circles) produced increased Relative Light Units (RLUs). The addition of anti-human anti-γR1 antibody inhibited RLU levels at all poly I:C test concentrations [red squares]. Non-specific anti-IFN-γ R1 [green triangles] showed results similar to samples where no antibody was added.

Discussion

Type I IFNs are expressed at varying levels in many cell types in response to a wide variety of stimuli. Furthermore, there are many aspects of immune function that do not involve type I IFNs. Consequently, one cannot predict *a priori* if a given stimulus will or will not produce type I IFN production.

This present study showed that a receptor neutralizing antibody used in conjunction with a rapid cell based assay (*iLite*^{TM} kit) can quickly and quantitatively determine the levels of type I IFNs in treated cell supernatants. Based on these results, more in-depth studies such as ELISA and/or CPE assays with individual IFN type neutralizing antibodies are warranted to discern specifically which type I IFNs are present.