Utility and Application of the *VeriKine*™ Non-Human Primate IFN-α ELISA Kit

A pbl interferonsource White Paper

Abstract

Although non-human primates (Cynomolgus and Rhesus macaques) are used as important pre-clinical models for testing the safety and efficacy of immunomodulatory agents, such as Toll-Like Receptor (TLR) agonists and antagonists, in a variety of diseases, research is currently hindered by the limited availability of reagents for sensitively detecting interferon-alpha (IFN-α) production in these species. IFN-α is produced by many cell types, including plasmacytoid dendritic cells and macrophages, in response to TLR ligation by bacterial and viral components, and plays pivotal roles in both protective and pathologic immunity. Clinically, maintenance of serum IFN-α levels within a range that is advantageous for limiting viral infections, while simultaneously avoiding the onset of autoimmunity, is crucial. As an example, for autoimmune diseases such as rheumatoid arthritis (RA), antagonists to TLRs 7 and 9 are being developed as novel therapeutics to dampen excessive IFN-α production. In contrast, TLR 7 and 9 agonists are being evaluated for their ability to induce IFN-α and enhance protective immunity against Hepatitis C viral infection. However, both of these strategies necessitate carefully monitoring of IFN-α levels to protect against the development of immunocompromise or detrimental side effects. Use of pre-clinical macaque models therefore affords the unique opportunity to test novel therapeutics for efficacy, potency, safety, toxicology, and off-target side effects prior to beginning Phase I trials in humans. In the absence of a rapid, reliable method for detecting IFN-α in macaque samples, researchers are forced to use labor- and time-intensive biological activity assays, resulting in increased project costs and lengthened evaluation periods. In response to these needs, PBL InterferonSource has recently developed a simple, sensitive sandwich ELISA that accurately detects bioactive levels of macaque and other non-human primate IFN-α. Potential applications of the *VeriKine*™ Non-Human Primate Serum ELISA kit (catalog # 46100-1) will be discussed below.

Non-human primates in pre-clinical research

Two main species of non-human primates are used in the pre-clinical research setting: Cynomolgus (*Macaca fascicularis*) and Rhesus (*Macaca mulatta*) macaques. During the development of novel therapeutics for human diseases and infections, the majority of early research and development is performed in small animal models, such as rodents. However, because of the inherent differences between rodent and human organ, tissue, and cellular components together with differences in receptor sequences and other factors that limit the relevance of findings and impair translatability of novel therapeutics into the clinic, the use of macaques is becoming a critical pre-clinical step in evaluating the dose requirements and toxicologic effects of biotherapeutics and pharmaceuticals. Due to their genetic and physiologic similarities with humans, macaques comprise a unique and highly relevant model organism for evaluating the safety, efficacy, pharmacokinetics, and pharmacodynamics of novel therapeutics.

IFN—α and TLR therapeutics

Some of the most exciting new drugs currently in development are small molecule agonists and antagonists of TLRs (Toll-Like Receptors). TLRs are highly conserved receptors of the innate immune system that recognize pathogen-associated molecular patterns (PAMPs). These short recognition sequences are found in bacterial and viral components ranging from bacterial lipopolysaccharide (LPS) and flagellin to double stranded RNA and unmethylated CpG DNA motifs. Ligation of those TLR receptors that bind dsRNA, LPS, ssRNA, or CpG DNA (TLRs 3, 4, 7, 8, and 9, respectively) initiates signaling cascades that culminate in the production of type I IFNs, such as IFN-α, as well as proinflammatory cytokines and chemokines[1,2] (Figure 1). Current studies are focused on the use of TLR agonists as adjuvants in vaccines for cancers and infectious diseases, and TLR antagonists for treatment of SLE[3-5]. Many TLR agonists and antagonists have progressed to clinical trial testing for safety and efficacy (*www.clinicaltrials.gov*).

Because IFN-α production is a major downstream readout for TLR activation, it is frequently utilized in the research setting and in clinical trials as an indicator of TLR agonist potency *in vitro* (tissue culture media) or *in vivo* (serum or plasma). This is particularly true for studies on novel TLR-based therapies that employ or inhibit IFN-α production as an intended component of their mechanisms of action. Of particular relevance are several preclinical studies and clinical trials involving novel therapeutic TLR7 agonists that induce IFN-α to protect against Hepatitis C infections and TLR 7/8 antagonists that reduce IFN-α levels for the treatment of rheumatoid arthritis[6,7].

IFN—α and hepatitis infections

IFN-α, either as a single agent or in combination with other anti-viral drugs, is currently used to treat patients with chronic hepatitis B and hepatitis C infections[8, 9]. The response rate to IFN-α-based therapies is far from optimal, and significant side effects are common. These include exacerbation of preexisting autoimmune disorders or de novo induction of autoimmunity[10, 11]. Both of these side effects are attributable to the pro-inflammatory properties of type I IFNs. As a possible solution, current drug development is aimed at turning on a more "natural" level of endogenous IFN-α production through the administration of immunomodulatory molecules. The Non-Human Primate IFN-α Serum ELISA is one of the first assay systems available that allows researchers the ability to sensitively quantify IFN-α levels in macaque samples, whether generated in vivo (ex: plasma) or in vitro (ex: culturing of cells *ex vivo*). Accurately detecting cynomolgus and rhesus IFN-α to 25 pg/ml, this *VeriKine™* ELISA is a highly sensitive tool for researchers investigating novel therapeutics for hepatitis and other viral infections in non-human primate models.

IFN—α and rheumatoid arthritis

Rheumatoid arthritis (RA) is a progressive, immune-mediated joint disorder that can lead to bone destruction and loss of function in afflicted joints. RA is characterized by the production of pathogenic antibodies against normal self proteins. These antibodies accumulate in joints and trigger local inflammation involving IFN production as part of an ongoing feedback loop that sustains the inflammatory response over a period of years. Currently, therapies for RA include biologics that deplete B cells, so as to remove the source of arthritogenic antibodies, or that block the Tumor Necrosis Factor (TNF) pathway, so as to diminish the inflammatory response[12, 13]. However, a large number of patients fail to respond to these treatment options, and alternative therapeutic approaches are under investigation.

IFN–α is emerging as an important mediator of RA pathogenesis and predictor of patient responsiveness to therapy. It was recently demonstrated that RA patients with high serum levels of IFN–α responded poorly to treatment with a B cell-depleting antibody, whereas those with low serum IFN–α levels responded favorably[14]. These data suggest that monitoring IFN–α levels may be crucial for identifying the subset of patients who will respond positively to novel and existing RA therapies, and that IFN–α may play a fundamental role in the pathogenesis of RA. In other studies, synovial monocytes and dendritic cells from RA patients produced IFN–α in response to TLR 3 and TLR7 stimulation[15], again illustrating the potential key role of IFN–α proteins in promoting local joint inflammation. Novel therapeutics that target the TLR 7 and TLR 9 pathways are being investigated as a means to inhibit overproduction of IFN–α[16]. Additionally, therapeutics previously developed to inhibit TNF production in joints may need to be re-evaluated for their off-target effects on IFN–α levels[7]. As research into the role of IFN–α in RA progresses into pre-clinical trial testing, users of macaque models will be able to rapidly and accurately evaluate IFN–α levels using the *VeriKine™* Non-Human Primate IFN-α Serum ELISA kit.

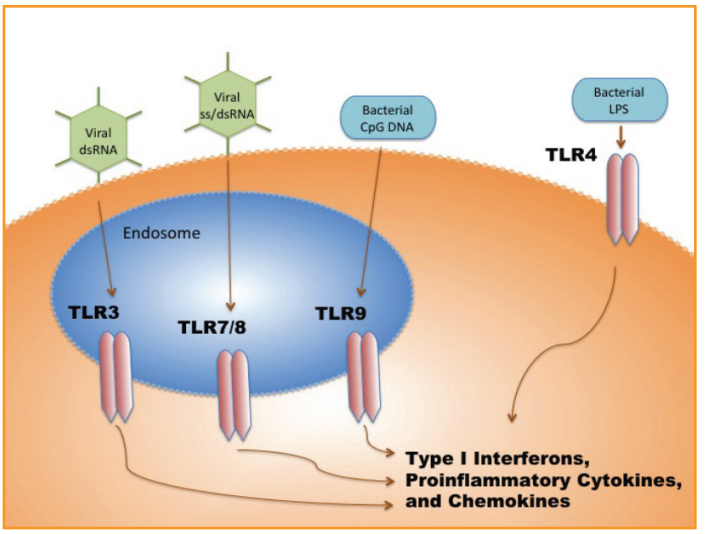

Figure 1. TLR signal transduction. TLR signaling in response to pathogens results in activation of the innate immune system.

Benefits of the *VeriKine™* Non-Human Primate Interferon-Alpha ELISA kit

The Non-Human Primate IFN-α Serum ELISA is an invaluable tool for investigators who use macaque models for preclinical research. ELISA is a powerful technique that combines the specificity of antibodies with the sensitivity of a simple enzyme assay for the rapid detection and quantification of IFN–α. All *VeriKine™* ELISA kits use the 'sandwich' strategy in which antigen is captured by a plate-bound antibody and detected by a second antibody that recognizes specific epitopes in the IFN-α proteins. Because both antibodies are specific for the target protein, this method can increase assay specificity several fold over other direct or indirect detection methods, thereby eliminating the need for sample purification prior to analysis. Thus, this ELISA is suitable for analyzing nonhuman primate samples with complex matrices such as tissue culture medium, serum, plasma, and potentially cerebrospinal fluid and urine. This serum ELISA is also a sensitive assay, as it can reproducibly detect IFN-α protein levels to 25 ng/ml. Using an ELISA to detect IFN-α affords many advantages over performing biologic assays; these include ease of use, the ability to analyze multiple samples simultaneously, reproducibility of results, and reduced hands-on time in the lab. All of these factors translate into decreased Research and Development costs for investigators moving novel therapeutics into Phase I clinical trial testing. Collectively, these advantages make the Non-Human Primate IFN-α Serum ELISA an excellent choice for researchers.

Clearly, IFN-α plays a pivotal role not only in the protective responses to many infections and diseases, but also in the generation of side effects and pathological sequelae when it is produced unchecked. The ability to precisely and comprehensively quantify the production of this family of cytokines is required not only in the clinical setting, but also for drug discovery and basic research. The

VeriKine™ Non-Human Primate IFN-α serum ELISA kit is therefore one of the most rapid, reliable, and cost-effective methods for detecting IFN-α during the course of preclinical non-human primate studies.

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