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The role of immunophilins in viral infection ☆

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ABSTRACT

Background: Tremendous progress has been made in the past 20 years in understanding the roles played by immunophilins, and in particular the cyclophilins, in supporting the replication cycles of human viruses. A growing body of genetic and biochemical evidence and data from clinical trials confirm that cyclophilins are essential cofactors that contribute to establishing a permissive environment within the host cell that supports the replication of HIV-1 and HCV. Cyclophilin A regulates HIV-1 replication kinetics and infectivity, modulates sensitivity to host restriction factors, and cooperates in the transit of the pre-integration complex into the nucleus of infected cells. Cyclophilin A is an essential cofactor whose expression supports HCV-specific RNA replication in human hepatocytes.

General Significance: Peptidyl-prolyl isomerase inhibitors have been used in clinical trials to validate cyclophilins as antiviral targets for the treatment of HIV-1 and Chronic Hepatitis C virus infection and as molecular probes to identify the roles played by immunophilins in supporting the replication cycles of human viruses.

Scope of Review: This review summarizes emerging research that defines the functions of immunophilins in supporting the replication cycles of HIV-1, HCV, HBV, coronaviruses, and other viral pathogens and describes new information that suggests a role for immunophilins in regulating innate immune responses against chronic viral infection.

Major Conclusions: The dependence on cyclophilins by evolutionarily distinct viruses for accomplishing various steps in replication such as viral entry, initiation of genomic nucleic acid replication, viral genome uncoating, nuclear import and nuclear entry, emphasizes the potential of cyclophilin inhibitors as therapeutic agents. This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

The regulatory approvals of cyclosporine A (Neoral, Sandimmune, Gengraf) in 1983 and FK506 (Tacrolimus, Prograf, Advagraf) in 1994 have unquestionably revolutionized the field of solid organ and tissue transplantation. The elucidation of the biological targets and mechanisms-of-action for cyclosporine A and FK506 led directly to the discovery of two multi-enzyme families that exhibit unique catalytic activities. The cyclophilins and the FK Binding Proteins share little sequence homology; however, they are both capable of catalyzing the interconversion of the two energetically preferred conformers (*cis* and *trans*) of the planar peptide bond preceding an internal proline residue. Each family of enzymes, referred to as peptidyl prolyl *cis*/*trans* isomerases or PPIases, catalyzes a reversible peptide bond isomerization in a non-covalent reaction that does not require the consumption of ATP but rather depends on energy derived from protein substrates. Collectively the cyclophilins and FK binding proteins are now referred to as immunophilins by virtue of their ability to bind these highly immunosuppressive agents. In contrast, the third and most recently discovered class of human PPIase enzymes, the parvulins, is not sensitive to inhibition by either cyclosporine A or FK506. Parvulins, originally described as a novel PPIase activity isolated from *Escherichia coli*, were shown to be present in higher organisms by their sequence homology to the yeast protein Ess 1 leading in turn to its own identification as a PPIase. The corresponding human ortholog, Pin 1, was identified in a two-hybrid assay as the *P*rotein *I*nteracting with *N*IMA and was later confirmed to be a PPIase by its ability to complement yeast Ess 1 deletion mutants. Unlike the cyclophilins and FK binding proteins,

Abbreviations: ATP, Adenosine triphosphate; PPIase, peptidyl-prolyl isomerase; HIV-1, human immunodeficiency virus type 1; HCV, hepatitis C virus; HBV, hepatitis B virus; AIDS, acquired immunodeficiency syndrome; N-MLV, N-tropic murine leukemia virus; Ref-1, restriction factor 1; TRIM5, Tripartite motif-containing protein 5; Lv-1, lentivirus susceptibility factor 1; NPC, nuclear pore complex; TRN-SR2, transportin SR2; TNPO3, transportin 3; CPSF6, cleavage and polyadenylation specific factor 6

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which are present in the human genome as multi-gene families and express multiple isoforms, parvulin-type PPIases are expressed from only two human genes. Immunophilins and parvulins are ubiquitously distributed throughout nature and have been identified in all organisms examined to date including bacteria, fungi, animals and plants.

Cyclosporine A and FK506 suppress immune function by binding to their respective intracellular targets, cyclophilin A and FK binding protein (FKBP). In a remarkable example of convergent evolution, the binary complexes formed by cyclosporine A/cyclophilin A and FK506/FKBP form high affinity ternary complexes with calcineurin. These ternary complexes block the intrinsic phosphatase activity of calcineurin, which in turn inhibits the de-phosphorylation driven nuclear translocation of members of the nuclear factor of activated T-cells (NF-AT) family of transcription factors. Inhibiting the nuclear translocation of NF-AT reduces the expression of many lymphokines including interleukin-4 (IL-4), which is required for B-cell help and interleukin-2 (IL-2), which is required for T-cell expansion and proliferation. The profound immunosuppressive activity exerted by cyclosporine A and FK506 has provided the rationale to investigate the use of these agents in a wide variety of conditions which are characterized by uncontrolled hyper-inflammatory responses including autoimmune diseases (muscular dystrophy, Crohn's disease, rheumatoid arthritis), ischemic reperfusion injury following myocardial infarction or stroke, ocular disease (uveitis, xerophthalmia), and dermatologic conditions (atopic dermatitis, severe recalcitrant plaque type psoriasis).

In general the therapeutic effects of cyclosporine A and FK506 for use in their primary indications (the prevention of allograft rejection), secondary indications (atopic dermatitis, xeropthalmia), as well as for investigational uses has been attributed to their ability to bind to their cognate immunophilins and to inhibit calcineurin phosphatase activity. Although a variety of methods have been developed in recent years to measure protein binding to proline rich regions including yeast two hybrid analysis, GST pull down, fluorescence anisotropy, circular dichroism, and nuclear magnetic resonance, these techniques have had limited success in identifying additional binding targets and therefore the roles played by immunophilins in the pathophysiology of other hyper-inflammatory or autoimmune conditions. The natural substrates for PPIases have been difficult if not impossible to identify because they typically exhibit binding constants in the micro-molar range easily escaping detection in protein-based capture techniques. The quantitative characterization of isomerization in native proteins represents a second major difficulty that has been successful only when applied to highly stable proteins such as RNase A and bovine pancreatic trypsin inhibitor. Assay methods have therefore relied on measuring proline isomerization in non-physiological substrates comprised of short chemically modified peptides. Because PPIases catalyze both the forward and reverse isomerization reactions protease coupling techniques have been employed to trap reaction “products” in order to assess unidirectional catalysis.

In contrast to autoimmune diseases and hyper-inflammatory conditions, tremendous progress has been made in the past 20 years in understanding the roles played by immnophilins, and in particular the cyclophilins, in supporting the replication cycles of human viruses. A growing body of genetic and biochemical evidence and data from clinical trials now confirms that cyclophilins are essential host cofactors that contribute to establishing a permissive environment within the host cell that supports the infectivity and replication of HIV-1 and HCV. Pull down experiments have been used to identify specific cyclophilin binding proteins from these viruses including the p24 capsid protein expressed by HIV-1 and the non-structural protein expressed by HCV — NS5A. The relatively greater binding affinity demonstrated by these virally-encoded proteins almost certainly reflects an evolutionary selective advantage on the part of the pathogen allowing it to outcompete the host for the recruitment of critical proteins. To some extent these observations constitute indirect evidence that cyclophilins are indeed essential cofactors necessary to support viral replication. Clinical proof of concept has been demonstrated using second-generation non-immuno suppressive analogs of cyclosporine A confirming that cyclophilins are valid biochemical targets for developing therapeutics for treating patients who are chronically infected with either HIV-1 or HCV. Based in part on these results sensitivity to cyclosporine A *in vitro* is now used as a surrogate marker to infer that cyclophilins play an obligate role in supporting viral replication. *In vitro* sensitivity to cyclosporine A has been observed for HBV, coronaviruses, influenza, cytomegalovirus and human papilloma virus.

This review will focus on summarizing the body of research that establishes the roles played by immunophilins in supporting HIV-1 and HCV infectivity and replication. In addition, emerging data describing the potential roles played by immunophilins in supporting the replication of other human viruses will be discussed together with new information suggesting that immunophilins may play a role in regulating innate immune responses against chronic viral infection.

2. HIV-1

During the 1980's when the pathogenesis of HIV-1 infection was poorly understood it was suggested that AIDS was characterized by a phase of disease progression whereby various types of activated lymphocytes participated in the destruction of healthy as well as HIV-1 infected cells drawing analogies between chronic HIV-1 infection and autoimmune cytopenias such as aplastic anemia. This concept of disease progression led many to speculate that the loss of CD4+ lymphocytes in HIV-1 infected individuals could be mitigated by treatment with immunosuppressive therapy. The recent successes of cyclosporine and FK506 in preventing allograft rejection was largely attributed to their ability to inhibit interleukin-2 dependent T-cell activation and proliferation — a step that was also recognized as an important aspect of HIV-1 replication. These observations prompted a pilot evaluation of cyclosporine in patients with advanced HIV-1 related disease [1]. Eight patients, all with evidence of either Pneumocystis carinii pneumonia or Kaposi's sarcoma, received cyclosporine at a total daily dose of 7.5 mg/kg given as a divided dose every 12 h. Doses were adjusted in order to maintain trough plasma concentrations of 100 to 150 ng/mL, which was the typical regimen for transplant recipients. Upon initiation of cyclosporine treatment all patients exhibited clinical signs (nausea, vomiting, fatigue) and laboratory evidence (declines in CD4+ and CD8+ lymphocytes and platelets) of accelerated disease progression. Paradoxically treatment with cyclosporine increased the efficiency with which virus was isolated from all patients. Cessation of treatment led to resolution of all exacerbated symptoms. The researchers concluded that cyclosporine-based immunosuppressive therapy in AIDS patients is not warranted. Nonetheless this work provided the impetus for further evaluations using cyclosporine as a mechanistic probe into the role of cyclophilins in HIV-1 infection and replication.

Initial reports of the *in vitro* antiviral activity of cyclosporine and FK506 described the ability of each compound to decrease the production of infectious HIV-1 by chronically infected T cells; however, no mechanistic data accompanied these reports [2]. Although the inhibition of the PPIase activities of cyclophilin and FKBP could not be excluded as accounting for the observed antiviral effects, it was speculated that T-cell activation through the calcineurin NF–AT pathway could potentially be exploited as a target for the discovery of anti-HIV-1 therapeutics. The application of yeast two-hybrid techniques revealed that two host proteins, cyclophilins A and B, bound specifically to the Gag polyprotein, $Pr55^{gag}$, and to p24 albeit with differing affinities [3]. These results were confirmed using GST-cyclophilin fusion constructs. The binding of HIV-1 $Pr55^{gag}$ and p24 capsid to cyclophilins A and B was inhibited by cyclosporine suggesting that the PPIase active site of both immunophilins was involved in binding viral proteins. The ability of GST-cyclophilin fusion constructs to capture calcineurin was dependent on the presence of cyclosporine; however, these same

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