AOP ID and Title:

SNAPSHOT

Created at: 2017-06-08 09:41

AOP 154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response

Short Title: Immunosuppression

Authors

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Status

Author status	OECD status	OECD project	SAAOP status
Open for comment. Do not cite	EAGMST Under Review	1.38	Included in OECD Work Plan

Abstract

Calcineurin is a type of protein phosphatase that is known to impair immune function when phosphatase activation is inhibited. The relationship between calcineurin and immune functions are well understood, and immunosuppressants that work by inhibiting calcineurin have been developed.

This AOP describes the linkage between the impairment of T-cell dependent response and immunosuppression that occurs due to inhibition of calcineurin.

Calcineurin activity is inhibited when stressors bond with immunophilins, which interferes with the nuclear localization of nuclear factor of activated T cells (NFAT), a substrate of calcineurin. As a result, the formation of functional NFAT complexes that bind at the site of IL-2 and other cytokine promoters is reduced, thereby suppressing production of these cytokines. Thus, T-cell dependent antibody response (TDAR) is impaired by the suppression of production of IL-2, IL-4, and other types of cytokines, which affects the proliferation and differentiation of B-cells.

We have identified a number of key events from within this pathway, and based on these key event relationships, created an AOP for inhibition of calcineurin activity leading to impaired T-cell dependent antibody response.

Calcineurin expresses in cells among vast variety species, because of which, this AOP is applicable to many mammal species, including humans and rodents.

Background

Although there are numerous stressors that inhibit calcineurin activity, this AOP is based on an understanding of immunosuppression caused by FK506 and FKBP12 complexes, on which a significant body of scientific literature has been published.

We look forward to future amendments to this AOP with up-to-date information on other stressors, which will clarify the linkage between inhibition of calcineurin activity and T-cell dependent antibody response.

Summary of the AOP

Stressors

Name	Evidence
Tacrolimus	
Cyclosporin	

Molecular Initiating Event

Title	Short name
Binding, Immunophilins (https://aopwiki.org/events/1201)	Binding, Immunophilins

1201: Binding, Immunophilins (https://aopwiki.org/events/1201)

Short Name: Binding, Immunophilins

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	MolecularInitiatingEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
Macaca mulatta	Macaca mulatta	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9544)
Macaca fascicularis	Macaca fascicularis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9541)

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Mixed	Strong

FKBP is found in a wide variety of organisms, from prokaryotes to multicellular organisms. (Siekierka et al. 1989a) Multiple subfamilies of FKBP have been reported, with at least eight types having been found in mammals. FKBP12 is reported to be expressed in B-cells, Langerhans cells, and mast cells as well as in T-cells of humans, mice, and other mammalian species.

Cyclophilins have been found in mammals, plants, insects, fungi, and bacteria. They are structurally conserved throughout evolution and all have PPIase activity. (Wang P et al. 2005)

How this Key Event Works

Immunophilins are a general class of proteins that exhibit peptidyl-propyl isomerase (PPIase) activity, such as FKBP (FK506-binding protein) or cyclophilin. (Barik. 2006) FKBP and cyclophilin bind with calcineurin-inhibitors FK506 and cyclosporin A to form complexes, which inhibit calcineurin activity. (Barik. 2006) While FKBP12, FKBP12.6, FKBP13, and FKBP52 are all part of the FK506-binding FKBP family, FKBP12 has a significant involvement in the mechanism of action for FK506-induced immunosuppression. (Siekierka et al. 1989, Kang et al. 2008) FKBP12 has an FK506-binding domain (FKBD) that comprises 108 amino acids. FKBP12 is expressed in T-cells, B-cells, Langerhans cells, and mast cells. (Siekierka et al. 1990, Panhans-Gross et al. 2001, Hultsch et al. 1991)

How it is Measured or Detected

The binding of cyclosporin A with cyclophilin can be detected using an ELISA kit.

Microtiter plates precoated with BSA and conjugated to cyclosporin are incubated with cyclophilin. Bound cyclophilin is then revealed by incubation with anti-cyclophilin rabbit antiserum followed by incubation with anti-rabbit globulin goat IgG coupled to alkaline phosphatase. (Quesniaux et al. 1987)

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Key Events

Title	Short name
Inhibition, Calcineurin Activity (https://aopwiki.org/events/980)	Inhibition, Calcineurin Activity

Title	Short name
Interference, nuclear localization of NFAT (https://aopwiki.org/events/979)	Interference, nuclear localization of NFAT
Reduction, NFAT complex formation (https://aopwiki.org/events/981)	Reduction, NFAT complex formation
Suppression, IL-2 and IL-4 production (https://aopwiki.org/events/1202)	Suppression, IL-2 and IL-4 production

980: Inhibition, Calcineurin Activity (https://aopwiki.org/events/980)

Short Name: Inhibition, Calcineurin Activity

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	KeyEvent

Stressors

Name
Tacrorimus
Cyclosporin

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus rattus	Rattus rattus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10117)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

Calcineurin is broadly distributed T-cells, B-cells, and throughout the body. The structure of CnA and CnB is highly conserved from yeasts to humans. Also highly conserved are the amino acid sequences of the catalytic and regulatory domains of calcineurin A isoforms from different organisms. (Kincaid. 1996)

How this Key Event Works

Calcineurin is a heterodimer that comprises a catalytic subunit (CnA), which handles phosphatase activity as well as calmodulin binding, and a Ca-binding regulatory subunit (CnB), which regulates intracellular calcium as well as CnA (Klee et al. 1988, Zhang et al. 1996). CnA, a 59kDa protein, has a serine-threonine phosphatase domain. A immunophilin binds directly to CnA in the cell, causing steric hindrance of substrate binding to calcineurin, which inhibits the phosphatase activity of calcineurin (Schreiber and Crabtree 1992, Liu et al. 1993, Bierer et al. 1993, Bram et al. 1993, Rao et al. 1997, Liu et al. 1991).

How it is Measured or Detected

Phosphatase activity can be measured using a phosphatase assay. Calcineurin, calmodulin, FK506, and FKBP are incubated together, and the phosphatase activity is measured at various concentrations of FKBP. Kinetic analysis of FKBP12 concentration-dependent phosphatase activity and calculation of Ki inhibition of calcineurin by the FKBP12-FK506 complex are conducted. (Bram et al. 1993). Phosphatase activity of calcineurin in the presence of CsA and cyclophilin can also be determined in the manner described above.

References

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979: Interference, nuclear localization of NFAT (https://aopwiki.org/events/979)

Short Name: Interference, nuclear localization of NFAT

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	KeyEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

NFAT expresses in B cells, mast cells, neutrophil granulocytes, dendritic cells, macrophages, and natural killer cells as well as T cells from humans, rodents, and other mammalian species. (Rao et al. 1997)

How this Key Event Works

The nuclear factor of activated T cells (NFAT) is a substrate of calcineurin (Rao et al. 1997). A NFAT has an N-terminal with a plurality of SP motifs rich in serine and proline, which are controlled by means of phosphorylation and dephosphorylation. There is a nuclear localization signal (NLS) held between these SP regions as well as a nuclear export signal (NES) in the N-terminal adjacent to the SP motifs (Beals et al. 1997, Zhu and McKeon 1999, Serfling et al. 2000). SP motifs ordinarily phosphorylate, which covers the NLS and leaves NES exposed, so NFAT localizes in cytoplasm. When calcineurin activates through stimulus from outside the cell, it binds directly to the N-terminal of NFAT in cytoplasm, after which SP motifs dephosphorylate to expose NLS and cover NES, thereby promoting nuclear localization of NFAT (Matsuda and Koyasu 2000, Zhu and McKeon 1999). When T-cell activation takes place, T-cell receptor (TCR)-mediated stimulus increases the intracellular concentration of calcium and activates CnB, which subsequently induces CnA phosphatase activation, leading to dephosphorylation of NFAT followed by nuclear localization. Calcineurin inhibitor-immunophilin complexes inhibit calcineurin phosphatase activation, thereby interfering with NFAT nuclear localization (Bhattacharyya et al.2011).

How it is Measured or Detected

Interference with translocation of NFAT to the nucleus can be detected using a gel mobility shift assay to test nuclear or cytoplasmic extracts. (Flanagan et al. 1991)

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981: Reduction, NFAT complex formation (https://aopwiki.org/events/981)

Short Name: Reduction, NFAT complex formation

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	KeyEvent

Biological Organization

Level of Biological Organization
Cellular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

FK506-induced interference with NFAT/AP-1 complex formation at the promoter site of the IL-2 gene might be in common among mammalian T cells including humans and rodents (Flanagan et al. 1991).

How this Key Event Works

Activated NFAT that has localized to the nucleus binds cooperatively at the site of the Interleukin-2 (IL-2) promoter with activator protein AP-1, which is a heterodimer comprising a Fos and a Jun protein, (Schreiber and Crabtree 1992, Jain et al. 1992) thereby inducing transcription of IL-2. (Jain et al. 1993) FK506 hinders the formation of the functional NFAT complexes necessary to binding at the site of IL-2 promoters by interfering with nuclear localization of NFAT. (Flanagan et al. 1991)

NFAT is known to bind cooperatively at the sites of both IL-2 and IL-4 promoters.

How it is Measured or Detected

Reduction of NFAT/AP-1 complex formation can be detected using a gel shift assay (Jain et al. 1992) to test nuclear extracts from either stimulated or unstimulated Ar-5 T cells with radio-labelled murine NFAT oligonucleotide.

References

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1202: Suppression, IL-2 and IL-4 production (https://aopwiki.org/events/1202)

Short Name: Suppression, IL-2 and IL-4 production

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	KeyEvent

Biological Organization

Level of Biological Organization
Cellular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Calcineurin inhibitors suppress production of IL-2, IL-3, IL-4, IL-5, IFN-γ, GM-CSF, and other cytokines, as induced by CD2/CD3 or CD3/CD26 stimulation, in human peripheral blood mononuclear cells (PBMC). (Sakuma et al. 2001a) Also, calcineurin inhibitors suppress production of IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ, and GM-

CSF, as induced by CD3/PMA stimulation, in human PBMC. (Dumont et al. 1998) Calcineurin inhibitors exhibit suppression of IL-2 production induced from mixed lymphocyte reactions in mice and humans. (Kino, T et al. 1987a)

How this Key Event Works

NFAT that has localized to the nucleus binds cooperatively at the site of the Interleukin-2 (IL-2) and interleukin-4 (IL-4) promoters with activator protein AP-1, which is a heterodimer comprising a Fos and a Jun protein, (Schreiber and Crabtree 1992, Jain et al. 1992) thereby inducing transcription of IL-2 (Jain et al. 1993). Calcineurin inhibitors, by interfering with NFAT nuclear localization, hinder the formation of the functional NFAT complexes necessary to binding at the site of IL-2 promoters. (Flanagan et al. 1991) Transcription of IL-4 is also inhibited by calcineurin inhibitors in the same manner as IL-2.

How it is Measured or Detected

Quantitation of cytokine content was done on appropriately diluted samples, run in duplicate, using Sandwich ELISA kits to test matched Ab pairs with biotin-horseradish peroxidase (HRP)-streptavidin detection and DAKO TMB substrate (Carpinteria, CA). ELISA plates were scanned in a Molecular Devices UVmax plate reader (Menlo Park, CA), using SOFTtmax software (Molecular Devices). (Dumont et al. (1998))

Total RNA was extracted using RNeasy mini kit (Qiagen, Chatsworth, CA) and quantitated by absorbance at 260 nm. Cytokine mRNAs were detected using a RiboQuant MultiProbe RPA system (PharMingen, San Diego, CA). Riboprobes were 32P-labeled and hybridized overnight with 10 to 30 mg of the RNA samples. The hybridized RNA was treated with RNase and purified according to the RiboQuant protocol. The samples were then electrophoresed in 6% polyacrylamide-Tris-borate-EDTA-urea gels using the Seqi-Gen GT Nucleic Acid Electrophoresis Cell (Bio-Rad, Hercules, CA), or minigels (Novex, San Diego, CA). The gels were dried, exposed and quantitated in a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using the ImageQuant software (Dumont et al. (1998)).

References

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2. Flanagan, W.M., Corthésy, B., Bram, R.J. and Crabtree, G.R. (1991). Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. Nature 352 (6338): 803-7.
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Adverse Outcomes

Title	Short name
Impairment, T-cell dependent antibody response (https://aopwiki.org/events/984)	Impairment, T-cell dependent antibody response

984: Impairment, T-cell dependent antibody response (https://aopwiki.org/events/984)

Short Name: Impairment, T-cell dependent antibody response

AOPs Including This Key Event

AOP ID and Name	Event Type
154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	AdverseOutcome

Biological Organization

Level of Biological Organization
Individual

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

In vitro experiments showed that treatment with FK506 of peripheral blood mononuclear cells from blood-bank donors suppressed the production of immunoglobulin (Ig) M and G antibodies specific to T-cell dependent antigens. (Heidt et al, 2009) Also, in human PBMC cultures, FK506 suppressed the production of IgM antibodies in the presence of T-cell activation. (Sakuma et al. 2001b) Oral administration of FK506 to mice for 4 days impaired the response of plaque forming cells (PFC) in splenocyte after intravenous immunization with sheep erythrocytes. (Kino et al. 1987) Oral administration of FK506 to rats over a four-week period reduced production of both anti-KLH-IgG and IgM antibodies after subcutaneous immunization with KLH. (Ulrich et al. 2004)

How this Key Event Works

Calcineurin inhibitors are known to impair T-cell dependent antibody response, but have not been shown to affect antibody production in B-cells directly.
FK506 suppresses the production IL-2, IL-4, and other classes of cytokines in T cells. IL-2 stimulates B cells to proliferate through surface IL-2 receptors. IL-4 stimulates B-cells to proliferate, to switch immunogloburin classes, and to differentiate into plasma and memory cells. Suppressing the production of these B-cell-related cytokines appears to be the main factor in impairment of TDAR by FK506 (Heidt et al, 2009). Cyclosporine A, which is also a calcineurin inhibitor, exhibits the same effects as FK506.

How it is Measured or Detected

In vitro: T cells and B cells isolated from human PBMC were co-cultured with a calcineurin inhibitor (CNI) for nine days in the presence of polyclonal T cell stimulation, after which supernatants were tested for immunoglobulin IgM and IgG levels using a Sandwich ELISA kit. (Heidt et al, 2009) SKW6.4 cells were cultured with anti-CD3/CD28 antibody-stimulated PBMC culture supernatant. After culturing for four days, IgM produced in the culture supernatants was measured using an ELISA kit. (Sakuma et al. 2001b) In vivo: Rats were repeatedly administered FK506 orally and immunized with KLH, after which the serum was examined for T cell dependent, antigen-specific IgM and IgG levels using a Sandwich ELISA kit. Mice were repeatedly administered CNI orally and immunized with SRBC, after which spleen cells were examined using a plaque forming cell assay. (Heidt et al, 2009, Kino et al. 1987, Ulrich et al. 2004) Class switching: T cells derived from human PBMCs were cultured with CNI, and cytokine mRNA levels of IFN-gamma, IL-2, IL-4, IL-5, IL-10, IL-13, and other B cell stimulatory cytokines produced in T cells were measured by quantitative PCR. (Ulrich et al. 2004)

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Scientific evidence supporting the linkages in the AOP

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Binding, Immunophilins	directly leads to	Inhibition, Calcineurin Activity	Strong	Strong
Inhibition, Calcineurin Activity	directly leads to	Interference, nuclear localization of NFAT	Strong	Strong
Interference, nuclear localization of NFAT	directly leads to	Reduction, NFAT complex formation	Strong	Strong
Reduction, NFAT complex formation	directly leads to	Suppression, IL-2 and IL-4 production	Strong	Strong
Suppression, IL-2 and IL-4 production	directly leads to	Impairment, T-cell dependent antibody response	Strong	Strong

Binding, Immunophilins leads to Inhibition, Calcineurin Activity (https://aopwiki.org/relationships/1253)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**directly leads to**	**Strong**	**Strong**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Term	Scientific Term	Evidence	Links
Macaca mulatta	Macaca mulatta	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9544)
Macaca fascicularis	Macaca fascicularis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9541)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

FKBP is found in a wide variety of organisms, from prokaryotes to multicellular organisms (Siekierka et al. 1989). Multiple subfamilies of FKBP have been reported, with at least eight types having been found in mammals. FKBP12 is reported to be expressed in B-cells, Langerhans cells, and mast cells as well as in T-cells of humans, mice, and other mammalian species.

Cyclophilins have been found in mammals, plants, insects, fungi, and bacteria. They are structurally conserved throughout evolution and all have PPIase activity. (Wang P et al. 2005)

Calcineurin is broadly distributed throughout the body, including T- and B-cells, and the structure of CnA and CnB is highly conserved from yeasts to humans. Also highly conserved are the amino acid sequences of the catalytic and regulatory domains of calcineurin A isoforms from different organisms. (Kincaid. 1996)

How Does This Key Event Relationship Work

Immunophilins are a general class of proteins that exhibit peptidyl-propyl isomerase (PPIase) activity, such as FKBP (FK506-binding protein) or cyclophilin. (Barik. 2006) FKBP and cyclophilin bind with calcineurin-inhibitors FK506 and cyclosporin A to form complexes, which inhibit calcineurin activity. (Barik. 2006)

While FKBP12, FKBP12.6, FKBP13, and FKBP52 are all part of the FK506-binding FKBP family, FKBP12 has a significant involvement in the mechanism of action for FK506-induced immunosuppression. (Siekierka et al. 1989, Kang et al. 2008)

FKBP12 is a 12-kDa protein localized in cytoplasm and has been isolated from Jurkat T-cells as a receptor that binds with the calcineurin inhibitor FK506. (Bram et al. 1993) FKBP12 has an FK506-binding domain (FKBD) that comprises 108 amino acids, and is expressed in T-cells, B-cells, Langerhans cells, and mast cells (Siekierka et al. 1990, Panhans-Gross et al. 2001, Hultsch et al. 1991).

Cyclophilin and FKBP both exhibit PPIase activity, but no structural similarities have been found between them. Additionally, while immunophilin complexes formed with either substance do inhibit CN phosphatase activity, the PPIase activity and the inhibition of activity that they indicate are unrelated to CN regulation.

Calcineurin is a heterodimer that comprises a catalytic subunit (CnA) and a Ca-binding regulatory subunit (CnB). CnA handles phosphatase activity as well as calmodulin binding, and CnB regulates intracellular calcium and CnA. (Klee et al. 1988, Zhang et al. 1996) CnA is a 59kDa protein with a serine-threonine phosphatase domain. A FK506-FKBP complex binds directly to CnA in the cell, causing steric hindrance of substrate binding to calcineurin, which in turn inhibits phosphatase activity of calcineurin. (Schreiber and Crabtree 1992, Liu et al. 1993, Bierer et al. 1993, Bram et al. 1993, Rao et al. 1997, Liu et al. 1991) Cyclophilin-CsA complexes also function in the same manner, binding directly to CnA in the cell, which in turn inhibits CN phosphatase activity.

Weight of Evidence

Biological Plausibility
Immunophilins are a general class of proteins that exhibit PPIase activity, but modification of these functions are unrelated to inhibition of CN activity, which is thought to arise in the molecular structure of the complexes.

FKBP12 is expressed in several types of immune cells, where it functions as an accessory molecule to classes of intracellular calcium channels, namely IP3 receptors and ryanodine receptors (RyRs) (Schreiber and Crabtree 1992, Cameron et al. 1997). In addition, FKBP12 binds to transforming growth factor-β receptor 1 (TGF-βR1) and acts as a natural ligand to TGF-β.

Empirical Support for Linkage
There is no evidence of any relationship between FKBP12 KO and the immune system in the FKBP12 knockout mouse model.

Uncertainties or Inconsistencies
Not specified.

Quantitative Understanding of the Linkage
In the phosphatase assay for calcineurin, addition of 1 molar equivalent of FKBP-FK506 complex inhibited over 80% of the calcineurin protein phosphatase activity. (mixed with 300 nM calmodulin-calcineurin and 30 µM FK506) (Liu et al. 1992) Dose-response analysis of the effects of FK506 on calcineurin-mediated phosphatase activity in KiSVMC4W cells showed that increased expression of FKBP12 resulted in a greater than ten-fold increase in the sensitivity of the KiSV-MC4W cells containing human FKBP12 cDNA to FK506-mediated inhibition of calcineurin phosphatase activity, as indicted by an IC50 value of ~3 nM (Fruman et al.1995). The phosphatase assay showed that FK506 inhibition of calcineurin activity was concentration-dependent and that IC50 values for calcineurin inhibition were approximately 0.5 nM for FK 506 (Fruman et al.1992).

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Inhibition, Calcineurin Activity leads to Interference, nuclear localization of NFAT (https://aopwiki.org/relationships/1508)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**directly leads to**	**Strong**	**Strong**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculoides	Mus musculoides	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=60742)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

NFAT expresses in B cells, mast cells, neutrophil granulocytes, dendritic cells, macrophages, and natural killer cells as well as T cells from humans, rodents, and other mammalian species. (Rao et al. 1997)

How Does This Key Event Relationship Work

The nuclear factor of activated T cells (NFAT) is a substrate of calcineurin (Rao et al. 1997). When calcineurin activates through stimulus from outside of the cell, it binds directly to the N-terminal of NFAT in cytoplasm, after which dephosphorylation of SP motifs exposes NLS and covers NES, thereby promoting nuclear localization of NFAT. (Matsuda and Koyasu 2000, Zhu and McKeon 1999). When T-cell activation takes place, T-cell receptor (TCR)-mediated stimulus increases the intracellular concentration of calcium and activates CnB, which subsequently induces CnA phosphatase activation, leading to dephosphorylation of NFAT followed by nuclear localization.

When CN activity is inhibited by the binding of immunophilin complexes, dephosphorylation does not occur in NFAT, thereby interfering with nuclear localization.

Weight of Evidence

Biological Plausibility

A NFAT has an N-terminal with a plurality of SP motifs rich in serine and proline, which are controlled by means of phosphorylation and dephosphorylation. There is a nuclear localization signal (NLS) held between these SP regions as well as a nuclear export signal (NES) in the N-terminal adjacent to the SP motifs. (Beals et al. 1997, Zhu and McKeon 1999, Serfling et al. 2000) SP motifs ordinarily phosphorylate, which covers the NLS and leaves NES exposed, so NFAT localizes in cytoplasm. Immunophilin complexes such as FK506-FKBP12 inhibit calcineurin phosphatase activation, thereby interfering with NFAT nuclear localization.

Empirical Support for Linkage
Nothing in particular

Uncertainties or Inconsistencies
Nothing in particular

Quantitative Understanding of the Linkage
Interference with translocation of NFAT to the nucleus is detected using a gel mobility shift assay to test nuclear extracts and cytoplasmic extracts. (Flanagan et al. 1991) NFAT translocation has been found to be regulated by the concentration of CNI additives.

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Interference, nuclear localization of NFAT leads to Reduction, NFAT complex formation (https://aopwiki.org/relationships/1017)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**directly leads to**	**Strong**	**Strong**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Unspecific	

NFAT expresses in B cells, mast cells, neutrophil granulocytes, dendritic cells, macrophages, and natural killer cells as well as T cells from humans, rodents, and other mammalian species. (Rao et al. 1997)

How Does This Key Event Relationship Work

T-cell receptor (TCR)-mediated stimulus increases the intracellular concentration of calcium and activates CnB, which subsequently induces CnA phosphatase activation, leading to dephosphorylation of NFAT followed by nuclear localization (Matsuda and Koyasu 2000, Zhu and McKeon 1999).

Nuclear localization of NFAT results in the NFAT binding with AP-1 at the IL-2 promoter region, (Schreiber and Crabtree 1992; Jain et al. 1992 (file:///C:/Users/Kushi/AppData/Local/Temp/Temp2_AOPwiki.bhk.zip/KER3_KE2%20to%20KE3(2).bhk.docx#_Jain,_J.,_McCaffrey,)) and induces transcription of IL-2. (Jain et al. 1993 (file:///C:/Users/Kushi/AppData/Local/Temp/Temp2_AOPwiki.bhk.zip/KER3_KE2%20to%20KE3(2).bhk.docx#_Jain,_J.,_Miner,)) In addition to IL-2, NFAT localized in the nucleus of T cells also binds to the promoter region of the other classes of cytokines including IL-4 and IL-13.

Weight of Evidence

Biological Plausibility
Interference with the nuclear localization of NFAT in T cells leads to a reduction in the formation of NFAT complexes, thereby suppressing transcription of IL-2, IL-4, and a number of other cytokines.

In contrast to T-cells, B-cell receptor (BCR)-mediated increases in intracellular concentration of calcium in B cells leads to NFAT nuclear localization, thereby producing some classes of cytokines in the same manner as T-cells (Bhattacharyya et al.2011). However, there has been no report of any evidence that CNI acts directly on B cells to effect antibody production.

Empirical Support for Linkage
The following phenotypes have been observed in NFAT knockout mice: moderate hyperproliferation with splenomegaly, reduced proliferative responses by T cells, mild hyperactivation of peripheral T cells. (Macian. 2005)

This can be understood as an indication that NFAT has no direct relation to the division of T cells and that NFAT is related to the production of some albeit not all classes of cytokines. Additionally, it is conceivable that CNI suppresses some induction of transcription related to NFAT.

Uncertainties or Inconsistencies
Nothing especially

Quantitative Understanding of the Linkage
inhibitor concentrations up to 1 µM (1000 nM). Higher concentrations induced cellular toxicity and resulted in cell death. Dose-dependent interference of nuclear NFAT1 translocation per calcineurin inhibition was also observed in CD4+ T cells from healthy donors, again at maximal concentrations of 1 µM. (Maguire et al. 2013).

Nevertheless, there are no reports of direct evidence that interference with the binding of NFAT–AP-1 complexes at the site of cytokine promoters is dependent on CNI dosage.

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Reduction, NFAT complex formation leads to Suppression, IL-2 and IL-4 production

(https://aopwiki.org/relationships/1509)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**directly leads to**	**Strong**	**Strong**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

In addition to data on suppression of cytokine production by CNI in rodents, the following is known from the use of human cells. That is to say, FK506 inhibited expression of both IL-2 and mRNA in human anti-CD3/PMA-activated cells. (Dumont et al. 1998) Also, FK506 suppresses expression of IL-2R (CD25) and costimulatory molecules CD80 (B7.1)/CD40 in human Langerhans cells. (Panhans-Gross A et al. 2001)

How Does This Key Event Relationship Work

NFAT–AP-1 complexes appear to bind to promoter or enhancer regions of cytokine genes in T cells. There are reports that production of IL-2 after activation of T cells is suppressed by calcineurin inhibitor treatment in vitro and in vivo as the result of interference to nuclear translocation of NFAT. (Dumont et al. 1998) Production in T cells of IL-4 and other classes of cytokines is also suppressed in the same manner as IL-2.

Weight of Evidence

Biological Plausibility

In addition to NFAT–AP-1 complexes, NFAT forms complexes at the site of IL-3 and IL-4 enhancers with avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF), early growth response 1 (EGR1), early growth response 4 (EGR4), interferon-regulatory factor 4 (IRF4), octamer-binding transcription factor (OCT), and other transcriptional partners to induce transcription of a variety of cytokines. (Macian 2005) The production of cytokine induced by these transcriptional partners also suppressed by CNI.

FK506 suppresses expression of IL-2R (CD25) and costimulatory molecules CD80 (B7.1)/CD40 in Langerhans cells. (Panhans-Gross A et al. 2001)

In human NK cells, FK506 suppresses IL-2 responsive proliferation and cytokine production as well as lowers cytotoxicity directed toward K562 tumor cells. (Kim et al. 2010) FK506 suppresses IL-2 production of NKT cell line DN32.D3 induced by stimulus from phorbol 12-myristate 13-acetate (PMA)/calcium-ionophore. (van Dieren et al. 2010)

The relationship between the above mechanisms and NFAT is unclear.

Empirical Support for Linkage

There is no evidence of a relationship between FKBP12 KO and the immune system in the FKBP12 knockout mouse model.

The following phenotypes are observed in NFAT knockout mice: moderate hyperproliferation with splenomegaly; moderately enhanced B- and T-cell responses, with bias towards Th2-cell responses; decreased IFN-γ production in response to TCR ligation; reduced proliferative responses by T cells; impaired repopulation of the thymus and lymphoid organs; impaired Th2-cell responses and IL-4 production; grossly impaired T-cell effector functions, with profound defects in cytokine production and cytolytic activity; B-cell hyperactivity; impaired development of CD4 and CD8 single-positive cells, with increased apoptosis of double-positive thymocytes; and mild hyperactivation of peripheral T cells. (Macian, 2005)

Uncertainties or Inconsistencies
Nothing in particular

Quantitative Understanding of the Linkage
FK506 suppressed, in a dose-dependent (1.2 to 12.5 nM) manner, production of IL-2, IL-4, and IFN-γ by human T cells stimulated with anti-CD3 mAb in the presence of PMA, as well as inhibited, also in a dose-dependent (10 nM) manner, expression of IL-2, IL-4, and IFN-γ mRNA in anti-CD3/PMA-activated cells. (Dumont et al. 1998)

In addition to the above quantitative expressions of cytokine production in T cells, it is possible to measure quantitatively expression of IL-2 receptors and IL-2 production in other immune cells per CNI. That is to say, FK506 suppresses, in a dose-dependent (0.1 to 1000 nM) manner, expression of IL-2R (CD25), as well as suppresses, also in a dose-dependent (10 nM) manner, expression of costimulatory molecules CD80 (B7.1)/CD40 in Langerhans cells. Thus, FK506 is roughly 100 times more potent than β-Methasone valerate at inhibiting LC stimulatory function. (Panhans-Gross A et al. 2001)

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Suppression, IL-2 and IL-4 production leads to Impairment, T-cell dependent antibody response (https://aopwiki.org/relationships/1510)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**directly leads to**	**Strong**	**Strong**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Mixed	Strong

The effects of FK506 on production of IL-2, IL-4, and other B-cell stimulating cytokines as well as production of T-cell dependent antibodies have been demonstrated in vitro and in vivo using mice and rats. Also, FK506 contributes to the regulation of cytokine secretion in normal human T cells. (Dumont et al. 1998) FK506 strongly suppressed humoral immunity (antibody production) with a marginal decrease in the number of spleen cells. FK506 showed higher activity than CS on PFC response in mice. (Kino, T et al. 1987a, 1987b)

How Does This Key Event Relationship Work

Experiments have shown that IL-2 mRNA expression in T cells stimulated with anti-CD3/ anti-CD28 antibodies decrease after treatment with FK506. (Dumont et al. 1998). Additionally other experiments have shown that mRNA levels of IL-2, IL-4, and other B cell stimulatory cytokines decreases in T cells stimulated with anti-CD3/anti-CD28 antibodies after treatment with FK506. These results suggest that FK506 is a potent inhibitor of T cell-dependent antibody production. (Heidt, S. et al. 2009) Thus, after treatment with FK506, production of IL-2, IL-4, and other cytokines decreases in T cells, reducing stimulation of B cells as well as proliferation, activation, and class switching, and leading to impairment of TDAR.

Weight of Evidence

Biological Plausibility
In humoral immunity, calcineurin inhibitors (CNI) do not affect B cells directly but rather indirectly through T cells. (Heidt et al, 2009) CNI suppress cytokine mRNA expression levels in T cells that stimulate proliferation of B cells as well as B cell activation and class switching induced by IL-2 and IL-4. IL-2 stimulates B cells to proliferate through the surface IL-2 receptors. IL-4 stimulates B cells to proliferate, to induce class switching, and to differentiate into plasma and memory cells. Calcineurin inhibitors inhibit transcription of IL-2, IL-4, and other cytokines to suppress production of T-cell dependent antibodies. In tests examining antibody production in blood samples obtained from blood-bank donors, PBMC treated with FK506 suppressed the production of immunoglobulin (Ig) M and G antibodies to T-cell dependent antigens. (Heidt et al, 2009)

Empirical Support for Linkage
FK506 is capable of inhibiting immunoglobulin production when B cells are cultured with non-pre-activated T cells, but FK506 fails to inhibit immunoglobulin levels when pre-activated T cells are used to stimulate B cells. The inhibition of B cell response by FK506 appears due solely to inhibition of T helper cells. (S.Heidt et al. 2009)

Quantitative Understanding of the Linkage
FK506 suppressed, in a dose-dependent (1.2 to 12.5 nM) manner, production of IL-2, IL-4, and IFN-γ in human T cells stimulated with anti-CD3 mAb in the presence of PMA, and inhibited, in a dose-dependent (10 nM) manner, expression of IL-2, IL-4, and IFN-γ mRNA expression in anti-CD3/PMA-activated cells. (Dumont et al. 1998).

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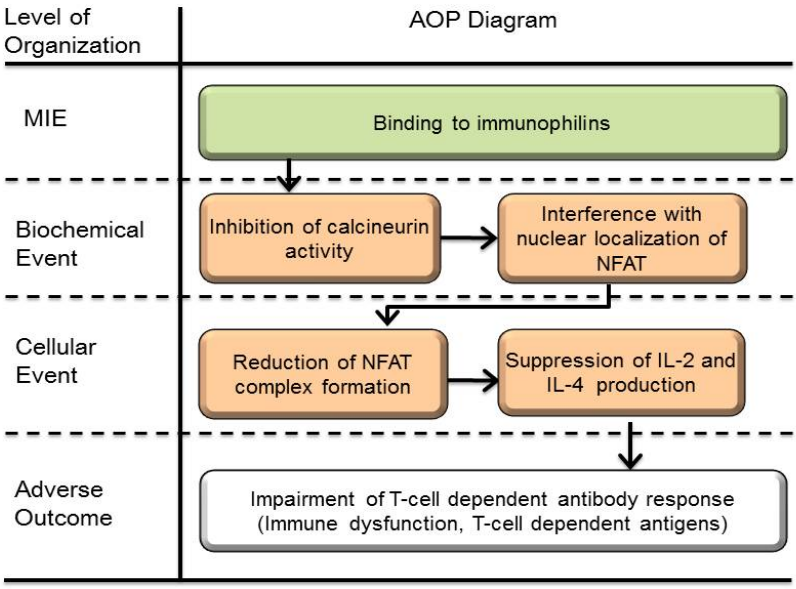
Graphical Representation



Overall Assessment of the AOP

Calcineurin activity is inhibited when stressors bond with immunophilins, which interferes with the nuclear localization of nuclear factor of activated T cells (NFAT), a substrate of calcineurin. As a result, the formation of functional NFAT complexes that bind at the site of IL-2 and other cytokine promoters is reduced, thereby suppressing production of these cytokines. Thus T cell dependent antibody response (TDRA) is impaired by the suppression of production of IL-2, IL-4, and other types of cytokines, which affect the proliferation and differentiation of B-cells. We have identified a number of key events from within this pathway, and based on these key event relationships, created an AOP for inhibition of calcineurin activity leading to impaired T cell dependent antibody response. There are many varieties of calcineurins, because of which, this AOP is applicable to many mammal species, including humans and rodents.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Macaca fascicularis	Macaca fascicularis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9541)
Rattus norvegicus	Rattus norvegicus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Sex Applicability

Sex	Evidence
Mixed	Strong

The proposed AOP of inhibited immunophilin activation leading to immunosuppression is not associated with life stage-, sex-, or age-dependency. The relevant life stages for the AOP are from child to adult, and since tacrolimus ointment (Protopic) is approved for pediatric atopic dermatitis, the MOA for immunosuppression appears to be applicable to all of life stages. Since FK506-induced outcomes in humans are mimicked by similar responses in a variety of animal models, immunosuppression induced by immunophilin-calcineurin inhibitor complexes are considered to be preserved across a variety of mammalian species.

Essentiality of the Key Events

In calcineurin subunit A knockout (CnA-/-) mice, T cell proliferation in response to ovalbumin stimulation is lower than for wild-type mice and is not complemented by normal antibody producing cells.1 In addition, when stimulated with ovalbumin, CnA-/- mice produce less IFN-g, IL-2, and IL-4 than wild-type mice.1 However, primary antibody response in CnA-/- mice is normal in response to TNP-ovalbumin.1

There is no evidence of a relationship between FKBP12 KO and the immune system in the FKBP12 knockout mouse model.

The following phenotypes are observed in NFAT knockout mice2: moderate hyperproliferation with splenomegaly; moderately enhanced B- and T-cell responses, with bias towards Th2-cell responses; decreased IFN-γ production in response to TCR ligation; reduced proliferative responses by T cells; impaired repopulation of the thymus and lymphoid organs; impaired Th2-cell responses and IL-4 production; grossly impaired T-cell effector functions, with profound defects in cytokine production and cytolytic activity; B-cell hyperactivity; impaired development of CD4 and CD8 single-positive cells, with increased apoptosis of double-positive thymocytes; mild hyperactivation of peripheral T cells.

The study of NFAT-/- mice shows that NFAT is involved in a wide range of immune-cell phenomenon, and some of these phenomenon are known to be regulated by calcineurin. This indicates that the production of T-cell derived cytokine is regulated by CN-NFAT.

Weight of Evidence Summary

The inhibition of CN phosphatase activity due to the formation of immunophilin-CN ihibitor complexes, which are CN stressors, is well known as an effect of CsA-Cuclophilin complexes orFK506-FKBP12 complexes. The information in this AOP concerns FKBP12. Immunophilins are a general class of proteins that exhibit peptidyl-propyl isomerase (PPIase) activity, but there is no commonality between their two amino acid sequences. The three-dimensional structure of immunophilin complexes is essential to inhibition of CN phosphatase activity, but it is known that these enzyme activities are not related to inhibition of CN.

CN is expressed in immune cells and other tissue cells throughout the body, and experimentation with T cells indicates that TCR stimulation brings about intracellular increases in concentrations of Ca2+, which triggers CN activity, thereby inducing nuclear localization per dephosphorylation of its substrate NFAT, which forms complexes with AP-1 at the site of T-cell cytokine promoters and induces production of these cytokines.

It is also well known that one of the effects on immune function when FK506 forms complexes with immunophilins and inhibits CN activity is the suppression of IL-2 and other T-cell derived cytokine production. It is further well known that inhibition of CN leads to suppression of T-cell dependent antibody production because IL-2, IL-4, and other cytokines promote the proliferation, class switching, differentiation, and maturation of B-cells.

Furthermore, CN-NFAT also exists in B-cells, and although it has been reported that CN inhibitors do suppress production of certain cytokines, at the time of our review of the literature, we did not find any reports of a direct effect on B-cells that affected the proliferations, class switching, differentiation, or maturation of B-cells.

Also, although CN-NFAT is known to exist in dendritic, NKT, and other types of cells, where it regulates the expression of IL-2 receptors, there are no reports of effects on the production of antibodies.

Quantitative Consideration

Binding, immunophilins

The binding of CsA with cyclophilin can be detected quantitatively using ELISA kits.

Inhibition, calcineurin activity

Phosphatase activity of CN can be measured quantitatively using a phosphatase assay.

Interference, nuclear localization of NFAT

Interference with the translocation of NFAT to the nucleus can be detected quantitatively using a gel mobility shift assay.

Reduction, NFAT complex formation

Reduction in generation of NFAT/AP-1 complexes can be detected using a gel shift assay. Cytokine mRNA levels after NFAT complex formation can be measured using RNase protection assay in vitro and ex vivo.

In so far as the formation of NFAT/AP-1 complexes during T-cell cytokine production is dependent on the quantity of NFAT that undergoes nuclear localization, however, there would appear to be no meaning to measuring false formations of NFAT/AP-1 complexes.

Suppression, IL-2 and IL-4 production

Quantification of cytokine content can be measured using a Sandwich ELISA kit, and cytokine mRNA levels can be determined using a RiboQuant MutiProbe RPA system (PharMingen, San Diego, CA).

Impairment, T-cell dependent antibody response

Total IgM and IgG levels as well as antigen-specific antibodies can be determined in vitro and in vivo. The effects on immunoglobulin class switching can also be evaluated in vitro. These all can be determined quantitatively.

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